THE CELLULAR AND MOLECULAR REGULATION OF THE ACUTE INFLAMMATORY RESPONSE

Organizers: Heinz Baumann, Jack Gauldie and Carl Richards

February 7 - 12, 1994; Tamarron, Colorado

Sponsored by Abbott Laboratories and SmithKline Beecham Pharmaceuticals

Plenary Sessions *Page*

Poster Sessions

The Cellular and Molecular Regulation of the Acute Inflammatory Response

Initiation of the Acute Phase Reaction

O 001 THE MULTI-ENZYME PROSTAGLANDIN BIOSYNTHETIC PATHWAY, David DeWitt, Odette Lanueville, Marc Lecomte, and William L. Smith, Michigan State University, East Lansing MI, 48824.

The prostaglandin endoperoxide H (PGH) synthase, also known as the cyclooxygenase enzyme, is the first and central enzyme in the biosynthetic pathway leading to prostaglandin production. This enzyme is also the primary site of action of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). Mammalian cells contain two related, but unique, forms of the PGH synthase enzymes, referred to as the PGH synthase-1 (PGHS-1) and PGH synthase-2 (PGHS-2) isozymes. Although these two enzymes are only about 60% identical, they catalyze conversion of arachidonate to PGH_2 with about the same affinity (Km) for arachidonic acid and the same maximal velocity (Vmax). Our present knowledge suggests that one major difference between these two isozymes involves their dissimilar regulation and expression. PGHS-1 is a constitutively expressed enzyme that is present in most tissues and in platelets, while PGHS-2 is absent in most unstimulated cells and tissues. PGHS-2 is expressed, however, during inflammation or following exposure to mitogenic stimuli. For example, growth factors, phorbol esters and IL-1 induce PGHS-2 in fibroblasts and endothelial cells; LPS stimulates PGHS-2 expression in monocytes and macrophages, PGHS-2 increases in synoviacytes during inflammation, and PGHS-2 increases in rat follicles preceding ovulation, an event that has been likened to a local inflammatory response. Transcription of PGHS-2 is controlled, at least in part, by NF-IL6 elements in the PGHS-2 promoter. Like IL-6 and other similarly IL-6 sensitive proteins, transcription of the PGHS-2 gene is also inhibited by glucocorticoids; *in vivo* PGHS-2 expression is also tonically suppressed by endogenous glucocorticoids. Our current hypothesis is that PGHS-1 produces prostaglandins that are involved in "housekeeping functions", such as producing prostaglandins in response to circulating hormones, while PGHS-2 produces prostaglandins that are involved in inflammation and mitogenesis. Work in this laboratory has demonstrated that the two PGH synthase isozymes are pharmacologically distinct, and thus it may be possible to identify inhibitors that are specific for each isozyme. A selective inhibitor of the PGHS-2, for example, might be a more effective anti-inflammatory agent and lack the side-effects of the non-steroidal anti-inflammatory commonly used today.

O 002 NEUTROPHIL ATTRACTANT PROTEIN-1 (INTERLEUKIN-8/NAP-1) AND MONOCYTE CHEMOATTRACTANT-1 (MCP-1), Edward J. Leonard, Alison Skeel and Teizo Yoshimura, Laboratory of Immunobiology, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702.

In the relatively short time since their purification to homogeneity, NAP-1/IL-8 and MCP-1 have been subjects of intense scrutiny because of their postulated role in inflammation. The National Library of Medicine data base for the past 4 years lists 365 publications in which NAP-1/IL-8 is the major subject; and the more recently discovered MCP-1 has stimulated comparable investigative effort. Summaries of recent information are published in several reviews. These mediators are members of a family of host defense proteins that are grouped together because of 4 half-cystine residues that are in register when the protein sequences are suitably aligned. The first two half-cystines are adjacent (C-C), as in MCP-1, or separated by a single residue (C-X-C) as in NAP-1. Most of the proteins in the family are chemoattractants; other actions have also been reported. In contrast to many previously studied attractants, such as C5a, fMLP and LTB_4, NAP-1 attractants neutrophils and not monocytes; and the converse is true for MCP-1. Concentrations that elicit an optimal chemotactic response by human cells are 10 nM for NAP-1 and 1 nM for MCP-1. Two classes of human neutrophil high affinity receptors for NAP-1 have been reported; and labeled MCP-1 binds with high affinity to human monocytes. These attractants are secreted by many different cells in response to inflammatory stimuli. MCP-1 mRNA or protein has been found in macrophage-containing lesions of atherosclerosis, pulmonary fibrosis, cardiac allografts, experimental allergic enceophalomyelitis, rheumatoid arthritis, and neoplasms. It is important to elucidate the stimulus sequences that lead to secretion of NAP-1 and MCP-1 in disease. In this regard, we are studying an in vitro model of delayed cutaneous hypersensitivity, in which stimulation of lymphocytes leads to secretion of MCP-1 by monocytes. IL-2 caused relatively little secretion of MCP-1 by isolated lymphocytes or monocytes (1 pmole/10^6 cells), whereas 17 pmoles were secreted by the cell mixture. Experiments are in progress to determine if this synergy requires cell-cell contact or is due to elaboration of a soluble mediator. We have found NAP-1-IgG and MCP-1-IgG immune complexes in serum of normal human subjects and have shown that NAP-1-IgG does not bind to neutrophils. The results suggest that NAP-1 and MCP-1 are elaborated in normals, and that autoantibody serves as a molecular trap that prevents interaction with target cells in the circulation.

Progression of the Acute Phase Reaction-Local Tissue Response

O 003 THE ROLE OF CHEMOKINES IN LEUKOCYTE RECRUITMENT, WOUND REPAIR, AND HEALING, Steven L. Kunkel, Peter Polverini, and Robert M. Strieter*, Departments of Pathology and *Internal Medicine, Division of Pulmonary and Critical Care Medicine, The University of Michigan Medical School, Ann Arbor, Michigan 48109-0602

The evolution of inflammation involves a dynamic series of cellular events controlled by specific signals which are important to the initiation, maintenance, and final resolution of the inflammatory response. The various phases of inflammation are influenced via the expression and subsequent regulation of a number of key mediators which play a predominant role during each particular stage of the developing lesion. For example, the initial elicitation of blood born leukocytes to an inflamed area is a complex system that is dependent upon the expression of early response cytokines. These proximal mediators, including both interleukin-1 (IL-1) and tumor necrosis factor (TNF), are important in the initiation phase by activating both immune and non-immune cells and establishing a series of cytokine networks. Thus, the above proximal mediators can stimulate endothelial cells, epithelial cells, smooth muscle cells, and fibroblasts to generate more distal cytokines. These latter cytokines include interleukin-8 (IL-8), macrophage inflammatory protein-1 (MIP-1), and monocyte chemoattractant protein-1 (MCP-1). The importance of the above chemokines can be found in the role they play in leukocyte elicitation, as well as wound repair. Numerous investigations have shown the diverse cellular sources of IL-8 and the ability of IL-8 to elicit neutrophils in vitro at pM to nM concentrations; however, only recent studies have shown an additional role for IL-8 during the resolution phase of the inflammatory process. Using both rabbit and rat corneal pocket models of angiogenesis/neovascularization, IL-8 was found to be a potent angiogenic factor at concentrations ranging from 2-40 ng/ml. Sequential fluorescein angiograms demonstrated that IL-8 induced neovascularization by 14 days, which regressed by 6 weeks. In further analyses, both conditioned media recovered from either inflamed human rheumatoid synovial tissue macrophages or LPS-stimulated peripheral blood monocytes were found to possess potent angiogenic activity, which was effectively blocked by neutralizing antibodies directed against human IL-8. In addition, an IL-8 antisense oligonucleotide blocked the expression of a functionally active angiogenic factor from LPS treated monocytes. The above data demonstrate that IL-8 has multifunctional activities during the initiation, maintenance, and resolution of a local inflammatory response.

O 004 FIBROBLAST ACTIVATION, TIMP-1 AND CYTOKINE EXPRESSION, Carl D. Richards, Department of Pathology, McMaster University, Hamilton, Ontario, Canada.

Acute and chronic inflammation involves the dynamic interaction between various cell types. Immediate local events including tissue macrophage, mast cell or endothelial cell activation recruits the participation of circulating cells such as monocytes, granulocytes and lymphocytes, through the release of mediators such as IL-1, TNF and chemokines. Structural connective tissue cells, such as fibroblasts, also participate at the local level primarily in response to the mediators produced by macrophages and granulocytes. Activated human fibroblasts release eicosanoids such as PGE_2, matrix metalloproteinases (MMPs) which modulate extracellular matrix, as well as significant amounts of cytokines such as IL-8 and IL-6. This then contributes to local and systemic levels of these bioactive molecules. The role of other cytokines in inflammation, such as oncostatin M (OM), is not clear.

Tissue remodelling that occurs in inflammation is dependent partly on the balance of active MMPs and their specific inhibitor TIMP-1, all of which can be regulated in fibroblasts by cytokines. We have been examining the activation of fibroblast populations of lung and synovial origin and comparing IL-1-responses to responses induced by Oncostatin M, a T cell and monocyte product, and its activity-related cytokines IL-6 and LIF. IL-1 stimulated fibroblast expression of the MMPs collagenase and stromelysin as well as TIMP-1, whereas OM selectively enhanced TIMP-1 expression but not the MMP. OM had greater effects in this regard than IL-6 or LIF. We have also examined the regulation of CAT in fibroblasts and HepG2 cells transfected with TIMP-1 promoter/CAT constructs utilizing murine TIMP-1 gene sequences. Constructs containing -90 to +47 TIMP-1 promoter sequence (which contains AP-1 and PEA-3 response elements) were OM and IL-1 responsive, and both elements appear to play a role in TIMP expression in these cells.

IL-1 stimulated IL-8, GM-CSF and IL-6 production by synovial or lung fibroblasts in vitro, while OM had little effect. When OM was added simultaneously with IL-1α or IL-1β, IL-6 production was synergistically enhanced. Expression of luciferase in a stably-transfected fibroblast cell line that contained a rat IL-6 promoter/luciferase construct, showed that OM stimulation resulted in synergistically enhanced luciferase expression. This construct contained sequences from -585 to +14 of the rat IL-6 gene, and the results suggest that OM induced transcriptional regulation. Analysis of IL-8 and GM-CSF expression revealed that OM co-addition to IL-1-stimulated cells resulted in a marked decrease of fibroblast output of IL-8 and GMCSF at both the protein and mRNA level. Interestingly, IL-6 or LIF coaddition did not have similar effects. The profile of mediator products produced by fibroblasts can thus be modified by oncostatin M, and this suggests a complex interaction of cytokines occurs in the regulation of mediators produced by cells at local sites of inflammation. Supported by MRC and Arthritis Society of Canada.

O 005 CHEMOKINE RECEPTOR-LIGAND AND INTRACELLULAR SIGNALING INTERACTIONS IN THE REGULATION OF MONONUCLEAR CELL RECRUITMENT, Thomas J. Schall, David DiGregorio, Kuldeep Neote, and John Mak, Department of Immunology, Genentech, Inc., S. San Francisco, CA 94080.

Leukocyte trafficking *in vivo* is likely to be mediated through the coordinated actions of adhesion molecules and chemoattractant substances. The *chemokines,* a bipartite superfamily of chemoattractant proteins, are prime candidates to play a central role in the leukocyte trafficking process, since they exhibit the unusual capacity to discriminate between leukocyte subsets in inducing pro-adhesive and pro-migratory effects. For example, we have shown that the C-C chemokines RANTES, MIP-1α and MIP-1β all induce the selective migration of monocytes and different subpopulations of T lymphocytes. It has also been shown that these molecules promote the adherence of the same T cell populations to endothelium. We have undertaken to study the complex functions of the chemokines by investigating C-C chemokine receptor-ligand interactions. Our approach has been to first predict the number and type of chemokine receptors on target cells using signal transduction and direct ligand binding and displacement studies; second, to clone chemokine receptors using molecular techniques, and, third, to examine the signaling properties of cloned chemokine receptors in transfected cells. Using these approaches the following postulates have emerged: 1) chemokines are likely top bind to an array of shared and specific receptors on target leukocytes; 2) the spectrum of chemokine receptors displayed by a leukocyte is a function of the activation state of the cell; 3) single receptor components exhibit complex behaviors in terms of binding and signaling capacities; 4) chemokine receptor-ligand interactions could be regulated at several levels including the presence of a red cell chemokine clearance receptor, the polymerization state of the ligand, and the types of receptors displayed simultaneously on a given cell. Since the number of chemokine proteins identified currently far exceeds the number of receptors isolated by molecular cloning, these postulates may provide clues as to how a large number of chemokines could function through a limited number of receptors to effect the recruitment of leukocytes in inflammation and immunoregulation.

Progression of The Acute Phase Reaction-Systemic Activation of Inflammatory Mediators

O 006 FEVER, Matthew J. Kluger[1,2], John Klir[1,2], Jinfang Liao[1,2,4], Lee Morrow[2,3], Jennifer McClellan[2], Carole Conn[1,2], Joan Keiser[4], and Wieslaw Kozak[1,2], [1]Institute for Basic and Applied Medical Research, The Lovelace Institutes, Albuquerque, NM 87108, [2]The University of Michigan Medical School, Ann Arbor, MI 48109, [3]University of Iowa School of Medicine, Iowa City 52242, [4]Warner-Lambert Parke-Davis, Ann Arbor, MI 48105.

Contact with pathogenic organisms or exposure to psychological stress (e.g. for rats an "open-field") often results in a regulated rise in body temperature - fever. We have been trying to determine the roles of cytokines and other mediators in fevers and other pathophysiological responses induced by the injection of lipopolysaccharide (LPS) into rats. Using an isolated-perfused liver preparation, we have found that the liver is a major source of the cytokines TNFα and IL-6, which are known to influence body temperature. Based on experiments using the protein synthesis inhibitor, anisomycin, the release of these cytokines into the circulation is due to new synthesis. At low concentrations, the stress hormone corticosterone facilitates this release of cytokines; at high concentrations (within physiological range), this hormone suppresses the release of cytokines. Experiments are currently in progress to determine the role of catecholamines in release of pyrogenic cytokines from the liver.

Experiments using blocking antibodies in rats whose body temperature is monitored by biotelemetry have shown that the cytokine IL-1β is involved in LPS-induced fever. Based on antibody and other studies, evidence is accumulating that TNFα is an endogenous antipyretic or cryogen during a variety of experimental paradigms (i.e. injection of LPS, exposure to psychological stress, during the growth of an MCA sarcoma). We have evidence that IL-1β is inducing fever via the release of IL-6 within the anterior hypothalamus. This is based on data showing that during fevers induced by LPS injected ip, the concentration of IL-6 within the anterior hypothalamus rises. When rats, which are not injected with LPS, are infused into the anterior hypothalamus with a dose of IL-6 that mimics that seen during LPS-induced fevers, fevers are produced. Furthermore, when antibody to IL-1β is infused into the anterior hypothalamus, this not only suppresses fever, but also blocks the rise in hypothalamic IL-6. Experiments are currently in progress to determine whether similar results are found during an actual infection -- bacterial peritonitis.

Fevers are modulated by at least two peptides, α melanocyte stimulating hormone, and arginine vasopressin. Glucocorticoids, which modulate the release of cytokines from the liver into the circulation, also modulate LPS-induced fevers (as well as fevers due to psychological stress). Adrenalectomized rats develop much larger fevers, and administration of the type II glucocorticoid receptor antagonist, RU38486, results in larger fevers in normal (non-adrenalectomized) rats. The site of the glucocorticoid negative feedback on LPS-induced fevers appears to be the anterior hypothalamus; the site of the negative feedback on fevers due to psychological stress appears to be the hippocampus (based on transection of the fornix).

Thus, fever is a complex process, which is induced (and in some cases suppressed) by cytokines, and modulated by a variety of hormones.
This research was supported by NIH AI27556 and MH48609.

O 007 REGULATION OF INTERLEUKIN-6 EXPRESSION AND FUNCTION, Pravin B. Sehgal[1], Lester T. May and Ling Wang, Departments of Microbiology & Immunology, and [1]Medicine, New York Medical College, Valhalla, NY 10595.

Interleukin-6 (IL-6) synthesis and secretion is rapidly induced in diverse cell types in response to bacterial and viral infection, other inflammation-associated cytokines, and tissue injury. Several different transcription factors including C/EBP isoforms and NF-κB contribute to the upregulation of IL-6 transcription in a combinatorial and a cell-type and inducer-specific manner. Steroid hormones modulate IL-6 gene expression through mechanisms that include direct binding to the IL-6 promoter DNA and protein-protein interactions with relevant transcription factors.

In vivo, in man, circulating IL-6 exists at high concentrations largely in complexes with its soluble receptor and with other proteins. In this "chaperoned" state IL-6 is unavailable for function in the hybridoma growth factor (HGF) assay nor is it detectable in conventional ELISAs. High levels of chaperoned IL-6 (range 100-1000 ng/ml) in complexes of mass 150-200 kDa are observed in a sustained manner over several months in cancer patients subjected to regimens that include active immunization. Although this IL-6 is unavailable *ex-vivo* in the hybridoma growth assay, marked elevations in the levels of circulating C-reactive protein suggest that this IL-6 is bioavailable *in vivo*. The administration of anti-IL-6 mAb into experimental animals (mice, baboon) provides an experimental model to explore the relationship between IL-6 in the chaperoned state and its function *in vivo*. In mice administered "neutralizing" anti-IL-6 mAb together with an IL-6 inducer, there occur marked elevations in circulating HGF-assay bioactive IL-6 in the intravascular compartment but an inhibition of the levels of plasma fibrinogen. Paradoxically, complexes of IL-6/anti-IL-6 mAb at a molar ratio of 1:5 to 1:1 lead to an enhancement of *in vivo* fibrinogen response induced by IL-6 even though the *ex-vivo*-measured HGF activity is inhibited. The new data point to a new level of control of IL-6 function in the intact animal -- that of its bioavailability.

The ability of IL-6-type cytokines to enhance a fibrinogen promoter construct in hepatoma cells is modulated by the transcription factor p53. Wild-type p53 inhibits cytokine (IL-6 or LIF) and C/EBPα, β or δ-activation of the IL-6-response element in the β-fibrinogen gene promoter [the βFib(2xIL-6RE)/CAT construct). Mutations in p53, in particular Phe-132 in murine p53, upregulate cytokine-mediated activation of this fibrinogen reporter. These data suggest that p53 impinges on the IL-6-induced signal transduction pathway to hepatic plasma protein gene promoters. Mutations in p53 may alter the response of particular hepatoma cells to cytokines.

O 008 NEUROENDOCRINE AND INFLAMMATORY CONTROL MECHANISMS OF ANTERIOR PITUITARY FUNCTION Bryan L. Spangelo and Brian R. Bond, Department of Physiology, Medical University of South Carolina, Charleston, SC 29425.

Anterior pituitary (AP) hormone secretion is regulated by the peptides and biogenic amines produced by hypothalamic neurons. These hypothalamic factors may either facilitate or inhibit the secretion of specific hormones. In addition, the peripheral target tissues regulated by the pituitary gland typically release other mediators that either stimulate or block AP hormone secretion through feedback pathways. Because proper functioning of the immune system is dependent in part on circulating levels of certain AP hormones (*e.g.*, prolactin and growth hormone), it is hypothesized that the soluble mediators released by lymphocytes and macrophages may affect hormone secretion by either direct or indirect feedback pathways. We have investigated a possible neuroendocrine regulatory role for the inflammatory cytokine interleukin-6 (IL-6). Primary cultures of rat AP cells treated with IL-6 released increased amounts of prolactin, growth hormone and luteinizing hormone during a 30 min incubation. These effects of IL-6 were rapid ($\leq$2 min) and did not rely on the activation of adenylate cyclase or phosphoinositidase. Because circulating levels of this cytokine were considered too low to normally exert a direct effect on neuroendocrine cells, we hypothesized that IL-6 may be produced within the hypothalamic-pituitary axis where it acts in a paracrine fashion for the regulation of hormone secretion. A non-hormone producing cell subpopulation of the AP released IL-6 in response to IL-1α and IL-1ß as well as lipopolysaccharide (LPS). Interleukin-1 and LPS stimulated IL-6 release by the phospholipase A2 mobilization of arachidonic acid (from phosphatidylcholine) and its subsequent catabolism via 5-lipoxygenase. Only one hypothalamic releasing factor, vasoactive intestinal polypeptide (VIP), enhanced IL-6 release from pituitary cells. Because VIP activated adenylate cyclase and agents that increase intracellular cAMP (*e.g.*, forskolin, cholera toxin) also stimulated IL-6 release, at least two signaling pathways are apparently involved in the stimulus-secretion coupling mechanism of AP IL-6 release. In other experiments, the medial basal hypothalamus (MBH) and the neurointermediate pituitary lobe (NIL) also synthesized and released IL-6 in response to IL-1ß and LPS *in vitro*. The hormones oxytocin and vasopressin inhibited the stimulated release of IL-6 from NIL cells, indicating a possible feedback role for these neuropeptides *in vivo*. Thus, IL-6 release occurs in the MBH, NIL and AP, suggesting that this cytokine may alter hormone secretion in one of four ways: 1. MBH production of IL-6 affecting hypothalamic releasing factor secretion; 2. NIL production of IL-6 affecting AP hormone secretion after traversing the short portal vessels; 3. AP production of IL-6 affecting hormone secretion via a paracrine mechanism; 4. increased circulating levels of IL-6 during inflammation affecting pituitary hormone release directly or indirectly. To begin to evaluate the functional importance of any of these possibilities, we have utilized a LPS-tolerance model. Male rats injected with 100 µg/kg (Day 1) and 500 µg/kg (Day 2) LPS were rendered tolerant to a subsequent lethal dose of LPS (15 mg/kg) on Day 5. Tolerant rats injected on Day 5 with 500 µg/kg had no increase in serum IL-6. Peritoneal macrophages from tolerant rats did not release IL-6 in response to LPS. In contrast, LPS stimulated IL-6 release from AP cells obtained from tolerant rats. Thus, tolerance to LPS may not extend to the neuroendocrine system. The LPS tolerance model may be appropriate for the examination of the role played by peripheral and neuroendocrine IL-6 in the inflammation-driven release of AP hormones.

O 009 CYTOKINE EXPRESSION DURING ACUTE INFLAMMATION, Thomas R. Ulich, M.D., Amgen, Inc., Thousand Oaks, CA 91320.

Acute neutrophilic inflammation and edema are induced in the lung by the intratracheal injection of LPS. LPS in rats initiates a proinflammatory cytokine cascade with the expression of endogenous TNF, IL-1, and CINC (Cytokine-Induced Neutrophil Chemoattractant, a rat chemotactic molecule with significant homology to MGSA/gro), all of which as recombinant molecules themselves cause acute inflammation when injected intratracheally. TNF, IL-1, and CINC expression are temporally closely accompanied by expression of E-selectin, an inducible endothelial adhesion molecule for neutrophils. The expression of proinflammatory cytokines and of leukocyte adhesion molecules is closely coupled with the expression of endogenous molecules that downregulate inflammation including 1) The release of antiinflammatory cytokines (IL-6 and LIF) and glucocorticosteroids that downregulate the expression of proinflammatory cytokines, 2) The release of soluble cytokine receptors (e.g. soluble TNF and IL-1 receptors) and cytokine antagonists (IL-1 receptor antagonist) that can bind or complete with proinflammatory cytokines, and 3) Blockade of neutrophil margination to endothelial-bound E-selectin by circulating E-selectin. Acute inflammation is followed by repair of tissue injury with reconstitution of the integrity of the alveolar epithelial surface. Cytokines such as keratinocyte growth factor stimulate the proliferation of type II pneumocytes that replenish the type I pneumocyte alveolar lining.

The Cellular and Molecular Regulation of the Acute Inflammatory Response

Systemic Manifestation of the Acute Phase Reaction-
Recognition of the Inflammatory Mediators

O 010 INTERLEUKIN-1 SIGNAL TRANSDUCTION, Timothy A. Bird[1], Steven K. Dower[1], John E. Sims[2], Margit Gayle[2], Jennifer L. Slack[1], Judith Giri[1], Alison Milne[3], Heather D. Schule[1], and G. Duke Virca[3], Departments of [1]Biochemistry, [2]Molecular Biology and [3]Protein Chemistry, Immunex Corporation, Seattle, WA 98101.

Molecular cloning studies have revealed two types of IL-1 receptor, type I and type II, which differ primarily in the length of their cytoplasmic domains (200 *versus* 29 amino acid residues, respectively). Type I IL-1 receptors are the only type found on most connective tissue cells, whereas type II receptors are widely distributed and are the predominant form expressed by B cell lines and neutrophils. In some cell types, such as the hepatoma HepG2, type I and II receptors are co-expressed. While it has been clear for some time that type I IL-1 receptors are capable of transducing a biological signal, recent evidence suggests that the type II receptor does not participate in signalling events but is a precursor for a circulating 'soluble' form of receptor which is capable of binding IL-1 and thus potentially regulating the action of IL-1 *in vivo*. Ligation of the type I IL-1 receptor results in the early activation of a number of second-messenger-independent protein serine/threonine kinases. Those kinases which have been identified include (1) the mitogen-activated (MAP) kinases and their activators; (2) a kinase which phosphorylates the small heat shock protein hsp27 and which is itself activated upon phosphorylation by MAP kinase, and (3) a newly described kinase which is detected by its ability to phosphorylate casein in vitro. Whereas kinases (1) and (2) are activated as part of a common pathway shared by many other cytokines and growth factors, the only 'physiological' triggers for the casein kinase appear to be IL-1 and TNF, which share many biological effects. Interestingly, the only other activator of the casein kinase that we have found to date is okadaic acid, a potent inhibitor of protein serine/threonine phosphatases which has been shown to mimic some of the early biochemical events signalled by IL-1 and TNF. The properties and activation mechanism of the novel casein kinase will be discussed.

O 011 TNF RECEPTOR SIGNAL TRANSDUCTION, Mike Rothe[1] and David V. Goeddel[2], [1]Genentech, Inc., South San Francisco, [2]Tularik, Inc., South San Francisco.

The biological activities of tumor necrosis factor (TNF) are mediated by two specific cell surface receptors, TNF-R1 (55 kd) and TNF-R2 (75 kd). TNF-R1 mediates signals for cytotoxicity and many other TNF activities in diverse cell types. In these systems TNF-R2 contributes to the cytotoxic signal that is initiated through TNF-R1 by facilitating the binding of TNF to TNF-R1 in a non-signaling mechanism. Demonstration of direct signaling by TNF-R2 has so far been restricted to a small subset of TNF activities in some lymphoid cells such as the stimulation of thymocyte proliferation and proliferation of the murine cytotoxic T-cell line CT6.

To investigate the signaling mechanisms of both TNF receptors we have developed functional transfection based assays. Results obtained suggest that the aggregation of TNF-R1 intracellular domains, which are not associated in the absence of ligand, is an important component of the signal for cellular toxicity. Mutational analysis of the human TNF-R1 intracellular domain has defined a region near the C-terminus that transduces the signal for cytotoxicity. This "death domain" has a region of weak homology to the intracellular domain of the FAS antigen, which mediates a programmed cell death very similar to that signaled through TNF-R1. This result establishes a potential functional conservation between these two receptors. Surprisingly, when transfected into murine CT6 cells these two receptors elicit opposite biological activities: TNF-R1 induces cell proliferaiton whereas the Fas antigen causes cell death.

In a search for targets of TNF-R2 signaling we have shown that in CT6 cells this receptor activates the transcription factor NFκB and mediates the transcriptional induction of the GM-CSF gene. Interestingly, the same gene is induced through activation of TNF-R1 in fibroblasts. A mutational analysis of the intracellular domain of the human TNF-R2 will be presented.

O 012 PLASMA PROTEIN GENES AS TARGETS OF INFLAMMATORY MEDIATORS, Heinz Baumann[1], Karen K. Morella[1], Chun-Fai Lai[1], Sanja Pajovic[1], Susana P. Campos[2], Yanping Wang[2], Steven F. Ziegler[3], David P. Gearing[3], [1]Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263, [2]Children's Hospital, Division of Endocrinology, Buffalo, NY 14222, [3]Immunex Corp., Seattle, WA 98101.

The liver is a major contributor to the systemic manifestation of an acute inflammatory reaction. Along with many metabolic changes, the liver responds to inflammatory mediators by a coordinately increased production of a set of functionally essential plasma proteins, termed acute phase proteins (APP). Maximal transcriptional activation of individual APP genes is achieved by a specific combination of cytokines and endocrine hormones. Primary stimulatory action has been observed for IL-1-type (IL-1α, IL-1ß, TNFα) and IL-6-type cytokines (IL-6, IL-11, LIF, OSM and CNTF) and glucocorticoids. Various growth factors, including insulin, IGF-1, HGF, FGF, and TGFß, which are largely ineffective on their own, specifically modulate the cytokine effects on APP genes. The cytokine-mediated stimulation of most, but not all, APP genes is immediate, independent of *de novo* protein synthesis and involves specific combinations of transcription factors that include, among others, members of the C/EBP, Rel, AP-1, HNF and Ets families. The specificity by which several APP genes are regulated has been used as a marker for defining the cytokine receptor signaling mechanism in hepatic cells. By reconstituting receptor function in transiently transfected hepatoma cells, we determined the minimal cytoplasmic domain structure of the signal transducing subunits for IL-6-type cytokine receptors (LIFR, gp130, and G-CSFR) that is necessary for activation of IL-6-sensitive APP genes. Signaling by each of the subunits is dependent upon the cooperativity of three separate sequence motifs. The two membrane-proximal motifs represent box 1 and box 2 known to be sufficient to deliver a proliferative signal in pre-B-cell lines. The current goal is the identification of the component of the hepatic signal transduction machinery that interacts with the activated IL-6-type cytokine receptors.
(Supported in part by grants CA26122, DK33886, Buffalo Foundation, and Women's and Children's Health Research Foundation)

The Cellular and Molecular Regulation of the Acute Inflammatory Response

Systemic Manifestation of the Acute Phase Reaction-
Regulation of Gene Expression by Inflammatory Mediators

O 013 REGULATION OF THE LIVER TRANSCRIPTION FACTOR HNF-3β by CYTOKINES SUGGEST INVOLVEMENT IN THE ACUTE PHASE RESPONSE, Robert H. Costa, Uzma Samadani, Xiaobing Qian, Derek E. Clevidence, David G. Overdier, and Anna Porcella. Department of Biochemistry (M/C 536), University of Illinois College of Medicine, Chicago, IL. 60612-7334.

Three distinct hepatocyte nuclear factor 3 (HNF-3) proteins (α, β, γ) are known to regulate the restricted expression of several liver genes, a subset of which include the acute phase response genes. The HNF-3α and -3β proteins are also important regulators in the lung and are expressed in early embryos (6.5 to 7.5 P.C.) suggesting that they play a more extensive role during development.The HNF-3 proteins share 95% homology in their DNA binding domain and possess amino acid conservation in their activation domains located at both amino and carboxy termini. The HNF-3 proteins bind to DNA as monomers using a novel winged helix motif which consists of a modified helix turn helix motif. The HNF-3 DNA binding domain also shares homology with the *Drosophila* intestinal differentiation factor, forkhead (fkh). Additional HNF-3/fkh-related proteins are known to be required for determination events during embryogenesis in *Drosophila* and *Xenopus*. An extensive family of HNF-3/fkh homologs (HFH) genes have also been isolated from non-hepatic rodent tissues which exhibit cell-type-specific expression patterns. The HFH DNA binding specificity differs from the liver HNF-3 proteins and therefore they regulate the transcription of different sets of genes.

The expression of acute phase proteins in the liver acts to change the serum protein composition which is critical for recovery from injury or infection. The C/EBPβ and the C/EBPδ transcription factors are stimulated rapidly after interleukin-6 (IL-6) and IL-1 receptor occupancy and mediates acute phase promoter activation through binding of C/EBP recognition sites. The HNF-3β genomic structure is 5 Kb in length containing two small introns allowing for rapid induction at the transcriptional level in response to environmental signals. The HNF-3β promoter activity requires recognition of four DNA sites by nuclear factors, one of which is the HNF-3 protein and serves an autoregulatory function. A second site binds the C/EBP protein family which confers IL-1 and IL-6 regulation to the HNF-3β promoter region. In addition, the HNF-3β promoter also contains an interferon stimulatory response element (ISRE) which mediates transcriptional induction by the cytokine interferon-γ (IFN-γ). These cytokines also increase the expression of the endogenous HNF-3β gene but not the other HNF-3 family members in human hepatoma (HepG2) cells. The fact that many acute phase genes contain HNF-3 binding sites within their promoter regions suggests that the HNF-3β is involved in mediating the cytokine induction of acute phase gene transcription.

O 014 THE C/EBP FAMILY OF NUCLEAR TRANSCRIPTION REGULATORY PROTEINS, Joel F. Habener[1], Mario Vallejo[1] and David Ron[2], [1]Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA, [2]Department of Cell Biology, New York University Medical Center, New York, NY.

The CAAT box/enhancer binding-proteins (C/EBP isoforms α, β, γ, δ) are a subfamily of the bZIP transcription factors (Jun, Fos, CREB, ATFs), so named because of their similar DNA-binding domains that consist of a basic region (b) involved in DNA recognition and a dimerization domain with heptad repeats of leucine (leucine zipper, ZIP). During the hepatic acute phase response induced by injection of LPS into rats or treatment of hepatic cell lines with cytokines (IL-1, IL-6), levels of C/EBPα fall, and C/EBPβ (and NFkB) increases concomitant with a marked induction of the expression of the angiotensinogen gene through binding to the acute phase response element (APRE). To identify additional members of the C/EBP family of DNA-binding transcriptional proteins that may participate in the interplay of factors involved in the regulation of genes in the acute phase response, a mouse adipocyte cDNA expression library was screened with a ^{32}P-labeled peptide probe consisting of the DNA-binding and dimerization domain (bZIP) of C/EBPβ. Two unique recombinant proteins that dimerize with the bZIP probe were identified. One protein (C/ATF) is a new member of the CREB/ATF family of bZIP proteins that dimerizes with C/EBPs α and β. C/ATF enhances gene transcription by binding to cAMP-response elements but suppresses C/EBP mediated activation of transcription by inhibiting C/EBP binding to the APRE. The other protein is a C/EBP-related bZIP protein, CHOP10 (CHOP), identical to GADD-153, a protein induced in cells by growth arrest and DNA-damage. CHOP has an unusually structured DNA-binding basic region, with amino acid substitutions at three positions known to be critically required for DNA recognition and binding. As a consequence of the intact leucine zipper domain and apparently defective basic region, CHOP readily dimerizes with C/EBPs α and β, but abrogates binding of CHOP-C/EBP heterodimers to C/EBP binding sites in gene promoters, e.g., the APRE of the angiotensinogen gene. Co-transfection of C/EBP and CHOP in HepG2 cells inhibits transactivation from an APRE-CAT reporter plasmid. Thus both C/ATF and CHOP function as dominant negative inhibitors of C/EBP-mediated transactivation of transcription on C/EBP binding sites (e.g. the APRE) by inhibiting C/EBP binding to DNA. During the acute phase response, the expression of CHOP increases with that of C/EBPβ. Thus, CHOP is envisioned to be a negative regulator of C/EBP functions that may modulate over-expression of C/EBPβ during critical stages in the acute phase cascade of gene expression. The increase in levels of C/EBPβ, however, greatly exceed those of CHOP and, as a consequence, the binding of C/EBPβ to the APRE is only partially perturbed. Therefore, it is possible that CHOP-C/EBP heterodimers bind to a specialized subset of DNA elements distinct from the conventional C/EBP binding sites and thereby may exert unique controlling influences on a subset of genes during the acute phase response apart from the inhibitory effects of CHOP on C/EBP.

Resolution of the Acute Phase Reaction

O 015 MECHANISMS OF CHRONIC INFLAMMATION, Jack Gauldie, Zhou Xing, Isao Ohno, Kevin Driscoll* and Manel Jordana, Department of Pathology, McMaster University, Hamilton, Ontario, Canada and *Proctor and Gamble, Cincinnati, Ohio, USA

The immediate response of the body to trauma or infection is the Acute Phase Reaction (APR) involving the release from the tissue of a variety of mediators and the subsequent accumulation in the tissue of various inflammatory cells. The most prevalent outcome of the APR is repair of the damaged tissue and return of the organism to normal function. Examination of *in vivo* models of inflammation demonstrates that macrophage/monocyte activation and release of cytokines such as IL1 and TNF are very early events in the response with tissue accumulation of leukocytes following shortly thereafter mediated by different chemokines. Resolution of the tissue and return to normal function are sequential events in the acute phase response. However, examination of two different experimental models of acute lung injury indicate quite different outcomes. Intratracheal instillation of LPS induces acute inflammation that is limiting and leads to resolution, while CdCl2 treatment mediates similar acute inflammation but converts to a chronic response with extensive tissue remodelling and fibrosis. Granulocytes that enter the tissue in response to chemokines become themselves potent cytokine effector cells and appear to contribute to the chronic stage of disease. The sequential appearance of further mononuclear cell chemokines and stromal cell growth factors are associated with conversion to chronic inflammation. Moreover, while regulatory cytokines of T cell origin may mediate this conversion stage, the remodelled tissue involved in the chronic stage of inflammation has an equally important role to play in the propagation of the response. Characterization of the cells and cytokines involved at each stage of disease progression is necessary to adequately define intervention strategies to reverse chronic inflammation and initiate resolution. (Supported in part by MRC Canada and AB Draco Sweden)

O 016 NATURAL AND RECOMBINANT SOLUBLE INTERLEUKIN-1 RECEPTORS AS IL-1 ANTAGONISTS, John E. Sims, Judith G. Giri, Margit A. Gayle, Jennifer Slack, Mark R. Alderson, Timothy A. Bird, Cindy A. Jacobs, Melanie K. Spriggs, and Steven K. Dower. Immunex Research and Development Corporation, Seattle.

The cytokines IL-1α and IL-1β interact with two different cell-surface receptors, type I (≈80kd) and type II (≈60kd), which function independently rather than as subunits of a receptor complex. Responses to IL-1 are mediated via the type I receptor; the type II receptor has never been shown to signal. Instead, it appears to serve as a membrane-bound precursor of soluble receptor which is thought to function as an IL-1 antagonist. Shedding of type II receptor from neutrophils and other cells can be induced by agents such as TNF and endotoxin which produce high levels of circulating IL-1β. Consistent with this, serum levels of soluble type II receptor are significantly elevated in patients with septic shock. In vitro, the soluble type II receptor is capable of inhibiting biological responses to IL-1β. It is much less effective at binding IL-1α and IL-1ra. Confirmation that soluble type II IL-1 receptor can regulate IL-1 responses comes from studies with vaccinia virus, which encodes its own soluble type II receptor. Deletion of this gene has dramatic effects on viral pathogenicity. Recombinant soluble type I IL-1 receptor has also been shown to be an effective inhibitor of IL-1 activity in vitro and in vivo. In a clinical model of cutaneous allergy, soluble type I IL-1 receptor was able to eliminate the late phase response to several common environmental antigens. These results hold promise for treatment of autoimmune and allergic diseases such as asthma.

Genetically Engineered Mice as Models for Inflammation

O 017 IMMUNE DYSREGULATION IN THE TRANSFORMING GROWTH FACTOR-β1 (TGF-β1) DEFICIENT MICE, Ashok B. Kulkarni[1], Andrew G. Geiser[2], John J. Letterio[2], Marielle Christ[3], Nancy McCartney-Francis[3], Kathleen C. Flanders[2], Sharon M. Wahl[3], Anita B. Roberts[2], Michael B. Sporn[2], Jerrold M. Ward[2] and Stefan Karlsson[1], [1]National Institute of Neurological Disorders and Stroke, [2]National Cancer Institute, [3]National Institute of Dental Research, National Institutes of Health, Bethesda, MD.

TGF-β1 is a potent immunoregulatory molecule that contributes to initiation and resolution of multistep inflammatory events. To delineate specific biological roles of TGF-β1 we have generated TGF-β1 knockout mice by gene targeting. After normal growth for the first two weeks, the null mice develop a rapid wasting syndrome and die as early as three weeks after birth. Histopathological analysis revealed an excessive inflammatory response with massive infiltration of lymphocytes and macrophages in many organs especially in heart, lung, salivary gland and intestinal tract. Since this phenotype occurs in the absence of any detectable pathogens, potential immune disease mechanisms were investigated. Analysis of lymphoid cell populations revealed defective proliferative responses to mitogens in vitro and dysregulation of inflammatory cytokine production in the null mice. Aberrant immune function was apparent in both asymptomatic and symptomatic null animals. Major histocompatibility complex (MHC) expression was analyzed in the null mice. The levels of both the MHC class I and class II mRNA were found to be elevated in the tissues of the null mice. Cell surface expression of MHC molecules as detected by immunohistochemistry correlated with mRNA levels. This elevated expression was observed prior to any evidence of inflammatory infiltrates suggesting a causal relationship between elevated expression of MHC molecules and initiation of multifocal inflammatory events. These results suggest a prominent role for TGF-β1 in homeostatic regulation of MHC expression, immune cell proliferation and extravasation in tissues.

O 018 FUNCTIONAL ANALYSIS OF THE TRANSCRIPTION FACTOR IL-6DBP/C/EBPß BY GENE TARGETING IN THE MOUSE, Valeria Poli[1] and Frank Costantini[2], [1]Istituto di Ricerche di Biologia Molecolare P. Angeletti, Pomezia (Rome), Italy, [2]Dept. Genetics and Development, Columbia University, New York, N.Y.

Interleukin 6 is a potent inducer of liver protein synthesis during the acute phase response. The nuclear protein IL-6DBP (also known as LAP, NF-IL6, AGP/EBP, C/EBPß) was identified as one possible mediator of this transcriptional induction. IL-6DBP binding sites essential for IL-6 inducibility in human hepatoma cells have been identified on the promoter of several acute phase genes. Moreover, IL-6DBP trans-activating potential is induced by IL-6 treatment in Hep 3B cells, and its transcription increases in mouse tissues upon acute phase induction or IL-6 injection. IL-6DBP/NF-IL6 has also been implied in the regulation of the IL-6 gene itself. IL-6DBP is a member of a vast family of transcription factors related to C/EBPα; most family members have been shown to play a role in the regulation of liver-specific and fat-specific gene expression. At least one other member of the family, C/EBPδ, was shown to be induced by IL-6, and to be able to act in synergy with IL-6DBP to activate transcription. In order to analyse in vivo the functions of this transcription factor, IL-6DBP deficient mice were created by gene targeting. Mouse embryonic cell lines carrying a null mutation in the IL-6DBP gene were established, and chimeric animals able to transmit the mutation to the germ line were obtained. IL-6DBP deficiency impairs the viability of the mice, since at weaning age the percentage of homozygous mutant mice obtained from intercrosses between heterozygous animals was only 12% instead of the expected Mendelian ratio of 25%. The surviving mutant mice presented generalised plamacytosis, more dramatic when the animals were raised in a non germ-free facility. However, circulating levels of IL-6 were found to be normal. A preliminary analysis of the induction of several acute phase genes following LPS treatment did not show appreciable differences between wild type and homozygous mice. A more detailed analysis of the mutation's effects on liver-specific as well as on more general functions is in progress.

Initiation and Progression of the Acute Phase Response

O 100 SECONDARY CELL DEATH AND INFLAMMATORY REACTION AFTER SPINAL CORD LESION IN THE RAT, Deborah Bartholdi , Isabelle Dusart and Martin E. Schwab. Brain Research Institute, Univ. Zürich, August-Forel-Str. 1, CH-8029 Zürich, Switzerland.

Local spinal cord lesions are often greatly enlarged by secondary damage, a process which leads to a massive additional loss of tissue. The detailed cellular mechanisms of this process are poorly understood at present. In order to investigate the roles of the various tissue components and of the local inflammation we analyzed the events occurring at spinal cord lesion sites using cell type specific markers from 1 hour to 3 months after a partial transection. We show that secondary cell death occurs within a few hours, before inflammatory cells invade the tissue in large numbers. Cell death indiscriminately occurs for neurons, astrocytes, oligodendrocytes and microglial cells. A massive and short-lasting invasion of polymorphonuclear leukocytes is seen around 1 day after injury, and is followed by the appearance of macrophages. Whether these inflammatory cells lead to further damage ot the tissue is a matter of controversy at present. Interestingly, high bolus doses of methylprednisolone, which in clinical studies led to slightly improved neurological scores, failed to show a decrease in the early phase of secondary cell death but effectively inhibited the immigration of inflammatory cells into the area of the injured spinal cord. Detailed investigations of the expression of different cytokines (mRNA) by the various cell types in the area of lesion will help to further understand the complex cellular interactions of the inflammatory processes taking place at spinal cord lesion sites.

O 101 INVOLVEMENT OF HUMAN AIRWAY EPITHELIUM IN THE ACUTE INFLAMMATORY RESPONSE TO INHALATION OF ENDOTOXIN-CONTAMINATED GRAIN DUST. Susanne Becker[1], Jacqueline Quay[1], William.A. Clapp[2], and David A. Schwartz [2]. [1]TCR Environmental Corporation (Chapel Hill, NC), [2]University of Iowa (Iowa City, Iowa).

TNFα, IL-1β, IL-6, IL-8, and the secreted IL-1 inhibitor (sIL-1RA), are intimately involved in endotoxin- induced lung inflammation. We found significant increases in these proteins in the bronchoalveolar lavage fluid six hours after exposure to endotoxin-contaminated graindust (E-GD). Alveolar macrophages (AM) have long been considered the cell type responsible for producing these cytokines and only recently has it been realized that airway epithelial cells may also be involved in cytokine production. We have used a semiquantitative PCR technique to compare mRNA levels for IL-1β, IL-6, IL-8 and TNFα, and sIL-1RA in cells obtained by endobronchial brush biopsy (BE) and by bronchoalveolar lavage (BAL) from 14 grain handlers 6 hours after experimental inhalation of E-GD or saline. The composition of BAL cells after grain dust exposure was 20-30% AM and 60-80% neutrophilic granulocytes (PMN) while BAL cells after saline exposure were > 80% AM. The BE were > 90% epithelial cells with < 10% contamination by PMN in the grain dust- exposed samples. BE constitutively expressed low but detectable levels of IL-6, IL-8 and IL-1, but no sIL-1RA or TNF mRNA. With the exception of IL-8, cytokine mRNA levels were very low in control AM (< 0.2 mRNA molecule/cell). Six hours after exposure to grain dust, IL-1 and IL-8 mRNA in the BE were increased 3 and 2 fold respectively, while no induction was seen in IL-6, sIL-1RA and TNF mRNA expression. Comparing cytokine mRNA levels in BE and BAL after exposure to E-GD, both cell preparations expressed equal amounts of IL-8 ; IL-1 was 11 fold and IL-6 was 2.5 fold higher in BAL than in BE, while TNF and sIL-1RA were expressed exclusively in E-GD -exposed BAL cells. The contribution of the inflammatory PMN to the elevated mRNA levels in the BAL cells is presently under investigation. Based on these results we suggest that epithelial cells are major producers of IL-8 in the airways upon exposure to E-GD. These cells may also contribute IL-1β, although we have been unable to demonstrate release of IL-1 from LPS or TNF- stimulated primary airway epithelial cell cultures.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy

O 102 FUNCTIONALLY DISTINCT SPLENIC FIBROBLASTS CAN BE SEPARATED INTO SUBSETS ON THE BASIS OF THY-1 EXPRESSION, Melinda A. Borrello and Richard P. Phipps, Departments of Microbiology and Immunology, Pediatrics and the University of Rochester Cancer Center, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Fibroblasts have an important structural role in the spleen, as they provide a scaffold of extracellular matrix in which cells of the immune system reside and interact. Aside from their recognition as skeletal components of the spleen, splenic fibroblasts have not been characterized. A concept that has recently emerged is that fibroblasts are heterogeneous, dynamic cells which can secrete regulatory cytokines and interact with cells of the immune system. Our laboratory demonstrated that subsets of mouse lung fibroblasts exist which can be differentiated on the basis of morphology, collagen and cytokine production, as well as the ability to present antigen to T lymphocytes. Furthermore, much like subsets of T and B lymphocytes that can be distinguished immunologically based on their expression of surface antigens, lung fibroblast subsets can be delineated on the basis of Thy-1 expression. We have established fibroblast lines from mouse ($B6D2(F_1)$) spleen via tissue explant culture. Interestingly, 50-65% of the parental splenic fibroblasts express the Thy-1 antigen, while a subpopulation of Thy-1 negative fibroblasts exists. FACS sorting on the basis of Thy-1, as well as limiting dilution cloning, yielded stable lines and clones of Thy-1^+ and Thy-1^- splenic fibroblasts. Both subsets synthesize collagen and express Class I MHC and ICAM-1 constitutively. However, Thy-1^+ splenic fibroblasts produce significantly greater levels of IL-6 than do their Thy-1^- counterparts. Intriguingly, after treatment with IFN-γ (150 U/ml, 72-96 hours), Thy-1^- but not Thy-1^+ splenic fibroblasts express Class II MHC. This suggests that the Thy-1^- fibroblasts may present antigen to T lymphocytes *in vivo* under inflammatory conditions. In summary, splenic fibroblast subsets exist which can be differentiated immunologically. The functionally distinct cytokine and Class II MHC profiles expressed by the Thy-1^+ and Thy-1^- fibroblast subsets indicate a dynamic and unique role exists for each in the lymphoid microenvironment of the spleen. This research is supported by CA42739, CA11198, CA55305 and T32AI07285.

O 103 REGULATION OF MHC EXPRESSION BY ONCOSTATIN M

T. Joseph Brown, Philip M. Wallace and Paul Gladstone, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

The response of endothelial cells (EC) to lymphokines is thought to play an important role in the progression of acute and chronic inflammation. IFNγ has been demonstrated to dramatically induce MHC-I, MHC-II, and ICAM-1 expression on EC surfaces, thereby selectively increasing endothelial adhesivity for lymphocytes. We have previously shown that the lymphokine oncostatin M (OM) alters various EC properties such as morphology, proliferation, plasminogen activator activity and cytokine production. Since inflammatory cytokines such as IL-1 and TNFα can synergize with IFNγ to increase MHC-I and ICAM-1 antigen expression, we questioned whether OM might alter IFNγ responses in cultured EC. OM treatment alone had no effect on MHC- I (HLA-A,B,C) or MHC-II (HLA-DR,DP) antigen expression in either HUVEC, HAEC or HPAEC cell types as determined by FACS analysis. Interestingly, the level of induction of MHC-I and II antigens in HUVEC treated for 72 hours with either IFNγ or TNFα was reduced by 70% in the presence of OM (ED_{50}=20 pM). Similarly, OM decreased the expression level of IFNγ-induced MHC-II antigen in HAEC and HPAEC by 50-70 %. In contrast to its effects on MHC expression, treatment with OM alone elevated basal levels of ICAM-1 expression by 2-fold and stimulated ICAM-1 expression in combination with IFNγ in an additive manner. These results indicate that OM may play a role in selectively decreasing the antigen presenting capacity of vascular EC by downmodulating MHC expression, while at the same time allowing for continued lymphocyte adherence and extravasation mediated by ICAM-1.

O 104 EARLY DETECTION OF A CD4+ CELL CHEMOATTRACTANT IN BRONCHOALVEOLAR LAVAGE FLUID FROM ASTHMATICS FOLLOWING ANTIGEN CHALLENGE, Cruikshank W.W., Luis Teran*, Melissa Falvo, Pulmonary Center, Boston University, Boston, MA. 02118, *Southampton Hospital, Southampton, England.

Chronic and late phase asthma is characterized by a CD4+ cellular infiltrate, comprised mostly of eosinophils and lymphocytes. Mechanisms for recruitment of these cells has not been clearly defined. We have previously reported that a lymphocyte cell product, lymphocyte chemoattractant factor (LCF), induces cellular migration in CD4+ lymphocytes and eosinophils. LCF is generated following stimulation of peripheral human T cells with either histamine (10^{-6}M) or specific antigen and has been detected in vitro as early as 4hr post stimulation. To investigate the potential role of LCF in cell recruitment we analyzed BAL fluids from 14 asymptomatic asthmatics. The asthmatics were segmentally challenged with both saline and specific antigen, and BAL fluid collected 4 and 24hrs later. Individuals were bronchoscoped and 5ml saline was instilled into a lingular subsegment and 5ml solution of specific antigen was instilled under vision to effect a 50% narrowing of a similar right middle lobe subsegment. LCF detection was performed by bioassay (chemotaxis), ELISA and western blotting. It was determined that LCF was present in 10 of 14 BAL following antigen challenge and in only 1 of 14 saline challenge. The concentration of LCF in the positive samples ranged from 5-15ng/ml of BAL fluid. The presence of IL-3 and IL-5 were not detectable at the 4hr time point. In the 24hr time point LCF was detected in 5 of 14 BAL obtained post antigen challenge and 0 of 14 following saline challenge. Detectable levels of LCF had decreased to a range of 5-8ng/ml BAL fluid. At the 24hr time point there were detectable levels of IL-3 and IL-5 by ELISA. Since the elaboration of LCF by T cells can be elicited by histamine or antigen stimulation and is detectable by 4hr, LCF may function as a early factor in the overall mechanism of emigration of CD4+ cells from the circulation to sites of inflammation.

O 105 ROLE OF CRITICAL SUBSTRATES OF THE HEMATOPOIETIC CELL KINASE (*HCK*) IN MACROPHAGE ACTIVATION BY BACTERIAL LIPOPOLYSACCHARIDE (LPS). B. Keith English, Department of Pediatrics, The University of Tennessee, Memphis, 38103

Several *src*-related tyrosine kinases have been identified as key components of signaling pathways in hematopoietic cells. We have recently reported that the *hck* tyrosine kinase plays a critical role in the production of tumor necrosis factor (TNF) by the murine macrophage cell line, BAC1.2F5, in response to LPS (English, et. al., *J. Exp. Med.* 178: 1017, 1993).

We hypothesized that the tyrosine phosphorylation of certain key substrates is a critical step in macrophage activation by LPS. We compared the pattern of protein tyrosine phosphorylation induced by LPS in BAC1.2F5 parental cells, BAC1.2F5 subclones expressing an "activated" mutant of *hck* (Y501-F501), and BAC1.2F5 parental cells exposed to *hck*-specific antisense oligonucleotides.

Exposure of BAC1.2F5 macrophages to LPS resulted in the apparent tyrosine phosphorylation of several proteins; an especially prominent phosphoprotein of approximately 85 kD was noted. We found that this 85 kD protein was constitutively phosphorylated in BAC1.2F5 subclones which expressed an "activated" mutant of *hck*, and the LPS-induced phosphorylation of this protein could be inhibited by prior exposure of BAC1.2F5 parental cells to *hck*-specific antisense oligonucleotides or by exposure of the cells to herbimycin A.

These data suggest that the *hck* kinase mediates the LPS-induced tyrosine phosphorylation of this 85 kD protein in murine macrophages, and together with our previous observations indicates that this phosphorylation step may play an important role in macrophage activation by LPS. Ongoing studies in our laboratory aim to identify this 85 kD phosphoprotein.

O 106 THE NFβA BINDING ELEMENT, AND NOT AN OVERLAPPING NFIL-6 BINDING ELEMENT, IS REQUIRED FOR MAXIMAL INTERLEUKIN-1β GENE EXPRESSION. Matthew J. Fenton, Brian G. Monks, and Jon A. Buras, Department of Medicine, Boston University Medical Center, Boston, MA 02118.

Interleukin 1β (IL-1β) is a proinflammatory cytokine that plays an important role in many biological processes. Genomic sequences controlling human IL-1β gene expression include both a distal LPS-inducible enhancer element (positions -3757 and -2729 relative to the cap site), and cap-site proximal (CSP) promoter sequences (positions -131 to +11). We previously showed that the novel nuclear factor NFβA is required for trans-activation of the IL-1β CSP promoter by the human cytomegalovirus immediate early 1 gene product. We have further defined the functional role of NFβA in RAW264.7 monocytic cells using transient transfection analysis. We have found that NFβA is able to activate transcription from a heterologous promoter in a dose-dependent manner. NFβA appears to function in a positionally-independent manner within the IL-1β CSP promoter since moving the NFβA binding sequence from its native conserved position adjacent to the TATA box does not decrease promoter function. In addition, we have found that NFβA is required for maximal IL-1β gene expression directed by the upstream LPS-inducible enhancer element. Deletion of the NFβA binding sequence results in an 80% reduction in basal reporter gene activity and an 86% reduction in LPS-inducible reporter gene activity in constructs containing only the enhancer and CSP promoter. We observed that the capacity of NFβA to support transcription from this enhancer is not fully compensated by other regulatory elements located between the enhancer and the cap site. Other investigators have reported that IL-1β CSP promoter function was decreased by introducing multiple mutations within the NFβA binding sequence, and a putative overlapping NFIL-6 binding sequence. We have found that these mutations predominantly affect NFβA binding. Furthermore, we have determined that NFβA, and not NFIL-6, supports basal and LPS-inducible transcription from a minimal IL-1β CSP promoter (positions -58 to +11). Lastly, this promoter region does not appear to direct monocyte specific IL-1β gene expression since reporter constructs containing the IL-1β CSP promoter were also active in transiently transfected HeLa cells.

O 107 EFFECTS OF INHIBITION OF COMPLEMENT ACTIVATION USING RECOMBINANT SOLUBLE CR1 ON RELEASE OF IL8 AND NEUTROPHIL ACTIVATION IN A MODEL OF ACUTE INFLAMMATION, Adam Finn, B. Paul Morgan, Naomi Rebuck, Catherine A. Rogers and Neil Moat, Dept. Paediatrics, University of Sheffield, S10 2TH, Dept. Medical Biochemistry, University of Wales College of Medicine, Cardiff CF4 4XN and National Heart & Lung Hospital, London SW3 6HP, UK

The acute inflammatory syndrome induced by cardiopulmonary bypass includes activation of complement and induction of several cytokine and neutrophil activation pathways. A recombinant soluble form of complement receptor 1 (sCR1) was used as a specific inhibitor of complement activation in simulated cardiopulmonary bypass circuits containing human blood. Substantial complement activation was observed with progressive accumulation of both plasma C3a and terminal complement complex (TCC). sCR1 resulted in highly significant reduction in C3a but had no effect on TCC generation. A marked rise in neutrophil surface expression of CD11b/CD18 and simultaneous loss of L-selectin and a progressive accumulation of plasma elastase-α1antitrypsin occurred and were not affected by sCR1. Interleukin 8 (IL8) was generated in the circuits, a process significantly inhibited by sCR1 ($p < 0.01$). In a similar model using a different oxygenator type a modest rise in C3a occurred which was also inhibited by sCR1, but TCC and IL8 were not generated. Changes in neutrophil L-selectin expression were again not affected by sCR1. These results demonstrate two distinct patterns of complement activation and suggest that changes in neutrophil activation in both may not be induced by products of early complement activation. They also demonstrate the novel observation of inhibition of IL8 generation through specific inhibition of complement activation in human blood.

O 108 GENERATION OF NEUTRALIZING ANTIBODIES TO HUMAN INTERLEUKIN-8 (IL8) RECEPTORS, Mary Ellen Wernette Hammond, Guy T. Mullenbach, Susan R. Hilt, Carrie A. Gordon, Paul H. Feucht, Martin A. Giedlin, and Patricia Tekamp Olson, Chiron Corporation, Emeryville, CA 94608

Two receptors for interleukin-8 (IL8) are expressed on human neutrophils: IL8R1 shows specificity for IL8 while IL8R2 recognizes several related chemokines in addition to IL8. Polyclonal antisera were raised to synthetic peptide sequences corresponding to extracellular domains of IL8R1 and IL8R2. Peptide antisera, but not the preimmune control sera, labeled IL8 receptors on isolated human neutrophils as determined by FACS analysis. Recombinant human IL8 receptors were expressed on insect Sf9 cells by the baculovirus technology. Antibodies to one IL8R1 peptide inhibited binding of ^{125}I-IL8 to recombinant IL8R1, but not to IL8R2. Therefore these antibodies specifically neutralize IL8 binding to IL8R1. The inhibition was blocked by preincubation of the neutralizing antibodies with the peptide, confirming the specificity of the antiserum and identifying an epitope on IL8R1 that must be near to or overlapping the IL8 recognition locus. Similarly, antisera to IL8R2 peptides blocked binding of ^{125}I-IL8 to IL8R2 expressed on recombinant baculovirus-infected Sf9 cells, but not to IL8R1. Therefore these antibodies specifically neutralize IL8 binding to IL8R2 and recognize epitope(s) near to or overlapping the IL8 recognition sequence of IL8R2. The generation of specific, neutralizing antisera to each IL8 receptor will allow a determination of the relative roles of each receptor in biological responses to IL8 and related chemokines, and will allow a route for selective blockade of receptor function as a therapeutic strategy.

O 109 TISSUE DISTRIBUTION OF PROSTAGLANDIN G/H SYNTHASE-1 AND -2 (PGHS-1 AND PGHS-2) USING SPECIFIC ANTI-PEPTIDE ANTIBODIES, Kargman, S., Chan, C., Evans, J., Vickers, P., and O'Neill, G., Department of Pharmacology, Merck Frosst Centre for Therapeutic Research, Point Claire-Dorval, Quebec, H9R 4P8

Rabbit anti-peptide antibodies have been raised against specific peptide sequences of human prostaglandin G/H synthase-1 and -2 (PGHS-1 and PGHS-2). Affinity purification on peptide columns have yielded highly specific antibodies with ELISA titers of 1:4000 and 1:2000 for PGHS-1 (175AP) and PGHS-2 (168AP, 169AP), respectively. Western Blot analysis demonstrated that antibody 175AP recognizes recombinant PGHS-1 expressed in COS-7 cells as a triplet with approximate molecular weights of 74, 72 and 69 kD, presumably corresponding to the various glycosylated forms of PGHS-1. Western blot analysis demonstrated that 168AP and 169AP recognize recombinant PGHS-2 expressed in COS-7 cells as a triplet with similar molecular weights to PGHS-1. Neither antibody cross reacts by ELISA or western blot techniques with high concentrations of the other PGHS species. These specific antibodies also detect the analogous ovine or rat proteins. We have used these purified antibodies to examine the tissue distribution of the two enzymes and have demonstrated that PGHS-1 is present in high levels in rat testis, lung, kidney, large intestine, small intestine, stomach, pancreas, bladder and liver. Full length rat PGHS-2 is not detectable in these same resting tissues. Full length PGHS-2 was detectable in rat paws following injection with carrageenen, resulting in an inflammatory response. No immunoreactive PGHS-2 was detectable in the contralateral paw.

O 110 A SELECTIN PEPTIDE INHIBITS LEUKOCYTE MIGRATION IN A RAT PERITONITIS MODEL, Mark A. Nedelman, Eva Brunt, Marian Nakada and George A. Heavner, Centocor, Inc., 200 Great Valley Parkway, Malvern, PA 19355 USA

A family of adhesion molecules known as the selectins have been shown to play a role in the initial adhesive events thought to be necessary for leukocyte trafficking and migration. The objective of this study was to examine the ability of the selectin peptide, CEN 294, a peptide shown to inhibit selectin-dependent adherence *in vitro* with an IC_{50} of 10 μM to block leukocyte recruitment into the peritoneal cavity as a result of experimentally-induced peritonitis. Rats (250-350 grams) were administered 10 mLs of 2% oyster glycogen (OG) intraperitoneally. CEN 294 (approximately 0.6 mg/kg) was injected intravenously 5 minutes prior to OG. Animals were sacrificed 5 hours post-OG, the peritoneal cavity washed with HBSS/0.3% EDTA and leukocytes counted. Rats injected with OG alone (n=4) and with an intravenous vehicle control (n=4) demonstrated an approximate 10-fold increase in leukocytes in the peritoneal cavity from basal levels ($p < 0.05$). In contrast, animals treated with a single iv bolus of CEN 294 (n=4) exhibited near basal levels of leukocytes in the peritoneal cavity at 5 hours ($p < 0.05$ vs OG alone and vehicle control). Similar results were obtained when CEN 294 was administered post-OG. Thus, these results demonstrate that a peptide derived from the lectin domain significantly inhibits the migration of leukocytes in this model.

O 111 RECOMBINANT HUMAN PROSTAGLANDIN G/H SYNTHASE-1 AND -2: INHIBITION BY NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AND BIOSYNTHESIS OF 15-HYDROXY-EICOSATETRAENOIC ACID, G. P. O'Neill, J. A. Mancini, S. Kargman, J. Yergey, M. Kwan, J.-P. Falgueyret, M. Abramovitz, B. P. Kennedy, M. Ouellet, W. Cromlish, S. Culp, J. F. Evans, A. W. Ford-Hutchinson, and P. J. Vickers, Merck Frosst Centre for Therapeutic Research, Pointe Claire-Dorval, Quebec H9R 4P8, Canada

The rate-limiting step in the formation of prostanoids is the conversion of arachidonic acid to prostaglandin H_2 by prostaglandin G/H synthase (PGHS). Two forms of PGHS have been characterized: a ubiquitously expressed form (PGHS-1) and a recently described second form (PGHS-2) inducible by various factors including mitogens, hormones, and cytokines. Human PGHS-1 and PGHS-2 were expressed to high levels in COS-7 cells using a T7 RNA polymerase/vaccinia virus expression system. Enzymatically active recombinant PGHS-1 and PGHS-2 were present as glycosylated proteins in the microsomal fraction prepared from infected cells. The major products formed from arachidonic acid by recombinant PGHS-1 and PGHS-2 were prostaglandin D_2 and E_2, with lower levels of prostaglandins $F_{2\alpha}$ and 6-keto-$F_{1\alpha}$. In addition, recombinant PGHS-1 and PGHS-2 both produced 15- and 11-hydroxyeicosatetraenoic acid (HETE) from arachidonic acid with the 15-HETE production by PGHS-2 being stimulated 5-fold by preincubation with aspirin. Chiral phase HPLC analysis showed that aspirin-treated PGHS-2 produced 15(R)-HETE with no detectable 15(S)-HETE. A range of potencies was observed for various nonsteroidal anti-inflammatory drugs as inhibitors of PGE_2 synthesis by PGHS-1 and PGHS-2.

O 112 THE ROLE OF RANTES IN RENAL ALLOGRAFT REJECTION, James Pattison, Peter J.Nelson, Philip Huie, Irene von Luettichau, Christian Wiedermann, Richard K. Sibley and Alan M.Krensky, Department of Pediatrics, Stanford University Medical Center, Stanford, CA 94305.

RANTES is a potent and specific chemotactic agent for T lymphocytes, monocytes and eosinophils, and is likely to play a role in the pathogenesis of the cellular infiltrate present during renal allograft rejection. RANTES expression during acute cellular rejection was studied using in situ hybridization and immunocytochemistry. RANTES mRNA and protein were detected in infiltrating mononuclear cells, and renal tubular epithelial cells whilst RANTES protein was also localized on the endothelium of peritubular capillaries. A recombinant RANTES protein anti-RANTES monoclonal antibody complex added to biopsies from rejecting transplants, specifically bound to the endothelium, indicating the presence of a RANTES receptor on activated endothelium. Recombinant RANTES protein was shown in Boyden chamber assays to be a haptotactic agent for monocytes. During cellular rejection, RANTES is secreted by activated lymphocytes and stimulated tubular epithelial cells and binds to the activated endothelium where it can mediate the influx of mononuclear cells into the transplant. Potentially RANTES is a key mediator of inflammation in many disease states, and may be a target for therapeutic intervention.

O 113 INDUCTION OF NITRIC OXIDE SYNTHESIS IN MURINE LUNG FIBROBLAST SUBSETS: EFFECT ON IL-6 PRODUCTION, Richard P. Phipps*,†, Andreas K. Nussler‡, Kristin M. Fries*, David A. Geller‡, and Richard A. Willis*,†, University of Rochester Cancer Center* and Departments of Microbiology and Immunology†, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642 and the Department of Surgery, University of Pittsburgh, Pittsburgh, PA 15213‡

Previously, we isolated Thy1+ and Thy1- subpopulations of mouse lung fibroblasts, which differ in terms of cytokine production, morphology, response to cytokines and radiation, and ability to present antigen to T lymphocytes. When treated with the pro-inflammatory cytokines IFN-γ, TNF-α, and IL-1α, these fibroblast lines produce micromolar quantities of NO_2^- and NO_3^-, two stable end products of the NO pathway. A combination of all three cytokines provided the greatest induction, and there was no measurable production of NO in unstimulated cells. Thy1+ fibroblasts have fewer requirements for induction of NO production than the Thy1- line, in that NO production could be induced by only two of the above cytokines, where the Thy1- fibroblasts required all three. Inducible NO synthase (iNOS) mRNA was shown to be present by the reverse transcriptase-polymerase chain reaction (RT-PCR) as early as 2 hours after cytokine treatment in both cell lines. Addition of the NO synthase inhibitors N^G-monomethyl-L-arginine (NMA) and aminoguanidine (AG) inhibited production of NO_2^- and NO_3^-, but not iNOS mRNA. This inhibition was partially reversed by the addition of an excess of L-arginine. We have previously shown that IL-6 is an autocrine growth factor for mouse lung fibroblast subsets. Interestingly, inhibition of NO synthesis was shown to decrease IL-6 production by more than 50% in cytokine-treated Thy1+ fibroblasts. These results indicate for the first time that Thy1+ and Thy1- mouse lung fibroblast subsets have the capability to produce NO to differing extents in response to cytokines and may therefore play an important role in the inflammatory response in the lung as well as in the progression of lung disease. This research was supported by U.S.P.H.S. grants CA55305, CA11198 and RM-37753.

O 114 MECHANISM OF CHEMOKINE-INDUCED LEUKOCYTE EMIGRATION Antal Rot, Sandoz Research Institute, Brunnerstr. 59, Vienna, Austria, A-1235

The best known *in vivo* effect of chemokines is their induction of leukocyte diapedesis from blood into the tissues. This requires the action of chemokines in concert with the events of leukocyte-endothelial adhesion which, in turn, is achieved via involvement of different adhesion molecules. The first step of leukocyte adhesion to the endothelium is a selectin-mediated loose binding which results in leukocyte rolling on the endothelial surface. For the firm attachment a second, integrin-mediated, adhesion step is required. This step is induced by chemokines which rapidly activate leukocyte integrins. *In vivo*, integrins cannot initiate leukocyte adhesion. Unless preceded by the first adhesion step, the activation of leukocytes by a chemokine inhibits their subsequent adhesion. Thus, in the sequence of leukocyte-endothelial adhesion events the point of action of chemokines is critically positioned between the first, selectin-mediated and second, integrin-mediated steps. This is achieved by a mechanism whereby chemokines are acting not in solution, as it was postulated earlier, but while immobilized by the proteoglycan molecules on the endothelial cell surface. In this way the chemokines are presented only to those leukocytes which have already established their first adhesive interaction with the endothelial cell surface. Also the leukocyte responses to proteoglycan-bound chemokine dramatically differ from those elicited by the soluble molecule. Binding of IL-8 to proteoglycans dramatically enhances the neutrophil migration induced by this chemokine whereas proteolytic enzymes released from neutrophils are inhibited. Thus endothelial proteoglycan molecules can have dual function in leukocyte diapedesis: they bind chemokines and enhance haptotactic leukocyte transmigration by these molecule while protecting the tissue micro-environment from damage by lytic enzymes released from the migrating cells.

O 115 INDUCTION OF INTERLEUKIN 1 RECEPTOR TYPE I mRNA IN COCULTURES OF HUMAN KERATINOCYTES AND DERMAL CELL TYPES H. Smola[1,2], G. Thiekötter[1], T. Krieg[2], N.E. Fusenig[1]; [1]German Cancer Research Center, 69120 Heidelberg, FRG; [2]Department of Dermatology, University of Cologne, 50924 Cologne, FRG

Interleukin 1 (IL 1) plays a central role in regulating inflammatory processes. In skin, a common site of inflammation, IL 1 is synthesized by keratinocytes and monocytes, with dermal cell types readily responding to IL 1 by synthesizing e.g. IL 6 and collagenase. Many data are available concerning regulation of IL 1, in contrast, little is known about the IL 1 receptor type I (IL 1 R type I) in fibroblasts and dermal microvascular endothelial cells (MEC). We investigated regulation of IL 1 R type I expression in cocultures of epidermal and dermal cells in which IL 1 responsive gene products are readily detected. Total RNA of normal human skin and cultured cells (keratinocytes, fibroblasts and MEC) was analyzed for expression of IL 1 R type I mRNA and compared with cocultures of keratinocytes and dermal cells (monolayer as well as organotypic cocultures). In vivo, in normal human skin, only weak hybridization signals for IL 1 R type I mRNA transcripts were obtained. However, some cultured fibroblast strains (3 out of 5) expressed higher IL 1 R mRNA levels followed by less intense signals in microvascular endothelial cells. Keratinocytes were negative. Low IL 1 R type I mRNA levels in dermal cells (fibroblasts and MEC) were substantially stimulated by coculture with keratinocytes as was IL 6 mRNA. Correspondingly, the same was observed with three dimensional cultures consisting of keratinocytes cultured on top of a collagen gel populated with fibroblast or MEC at the air medium interface (organotypic cocultures). Here, cell-matrix interactions in control cultures (dermal cells in collagen gels but without keratinocyte influences) were sufficient to induce a slow but marked increase of IL 1 R mRNA levels which could be slightly stimulated by coculture with keratinocytes. IL 6 mRNA induction in organotypic cultures was dependent on *i.* IL 1 R mRNA levels in mesenchymal cells and *ii.* presence of keratinocytes. These findings suggest that IL 1 R type I mRNA is induced by epidermal-dermal cell as well as cell-matrix interactions. Furthermore, IL 1 R type I levels seem to regulate the sensitivity of dermal cells for proinflammatory IL 1 derived from activated keratinocytes and monocytes.

O 116 BRADYKININ IS AN INFLAMMATION INITIATOR; BRADYKININ ANTAGONISTS AS NEW DRUGS FOR INFLAMMATION. John M. Stewart, Department of Biochemistry, University of Colorado Medical School, Denver, CO 80262.

Recent data obtained principally by application of bradykinin (BK) antagonists indicate that BK is involved in initiation and early phases of most inflammatory responses and can evoke all the cardinal signs of inflammation. BK is the most potent pain-evoking substance known. It also lowers blood pressure. BK is produced by kallikreins activated directly by the "contact activation system" in tissue injury, and by low pH in tissue anoxia and infection. BK can stimulate release of most, if not all, later mediators of inflammation. In addition, prostaglandins and interleukins potentiate tissue responses to BK. The structure of BK is Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, and most responses to BK are mediated by "B2" receptors, which require the entire nonapeptide sequence. B2 receptors are involved in acute and early phase inflammatory responses. In chronic inflammation, a new class of BK receptors, "B1," is expressed; these bind BK(1-8) and not intact BK. BK(1-8) is produced when BK is inactivated by carboxypeptidase N, the chief plasma BK-degrading enzyme. Circulating BK is also inactivated by angiotensin converting enzyme (ACE), localized to vascular endothelium. In chronic inflammation, ACE is shed from the vasculature, allowing concentrations of BK and BK(1-8) to increase dramatically, with consequent production of shock. Total control of inflammation requires both B1 and B2 antagonists. Presently available BK antagonists are potent and long-lasting *in vivo*, and can prevent mortality due to the severe inflammation evoked by bacterial lipopolysaccharide, sepsis and acute pancreatitis. BK antagonists are now in human testing for septic shock, hantavirus pulmonary edema, acute pancreatitis, asthma and rhinitis and offer the potential of a new generation of antiinflammatory drugs. The early place of BK in the inflammation cascade makes such drugs particularly attractive. (Aided by NIH grant HL-26284)

O 117 INTERLEUKIN-10 MODULATES THE CHEMOKINE RECEPTOR EXPRESSION ON MONOCYTES

Wang JM, Xu LL, Oppenheim JJ*, and Kelvin DJ*
BCDP, PRI/Dyncorp, NCI-FCRDC; * LMI, BRMP, NCI-FCRDC

IL-10 is an acid-sensitive protein produced by different leukocyte populations under immune activation. While IL-10 exerts stimulatory properties on B lymphocytes, it is known as a potent inhibitor of monocyte/macrophage function. In this study, the effect of recombinant human IL-10 on chemokine responsiveness in monocytes was investigated. Purified human peripheral blood monocytes expressed receptors for macrophage inflammatory protien 1α (MIP1α) and migrated to MIP1α gradients in mirochemotaxis chamber assays. Incubation at 37°C caused a progressive reduction of monocyte binding for MIP1α associated with decreased chemotaxis. LPS further decreased MIP1α binding to monocytes. However, incubation with IL-10 maintained a high level of MIP1α binding capacity on monocytes compared to cells incubated with medium alone. IL-10-treated cells exhibited greater migration to MIP1α than untreated cells. Scatchard analyses revealed that monocytes incubated with IL-10 retained a high level of receptors rather than a change in affinity. Similar modulation by IL-10 of monocyte binding for monocyte chemotactic and activating factor (MCAF; MCP-1), another member of the chemokine family, was also observed. The results indicate that apart from its immuno suppressant activity, IL-10 was able to maintain the monocyte response to chemokines via prolonging the expression of chemokine receptors.

O 118 IN VIVO AND IN VITRO ACTIVITY OF A NOVEL PERIPHERALLY ACTING OPIOID RECEPTOR AGONIST/BRADYKININ (BK) ANTAGONIST HETERODIMER, Eric T. Whalley, John C. Cheronis, Val S. Goodfellow, Sharon D. Loy, Meighan Elder, Wendy L. Hanson and Manoj Marathe, Cortech Inc, 6850 N Broadway, Denver, CO 80221, USA.

The kallikrein-kinin system and neurogenic processes appear to be involved in a variety of inflammatory disorders including pain, edema, asthma and migraine. The novel homodimer BK antagonist CP-0127 (Bradycor) is at present undergoing clinical trials for sepsis and brain edema. CP-0127 has been shown to inhibit carrageenan paw edema, brain edema and burn edema in the rat and to be analgesic in the mouse formalin and kaolin tests and BK-induced pain in the rat. We describe here a series of combined BK antagonists (CP-0126 and CP-0347) coupled to the non-peptide opioid agonist, oxymorphone (OXY).

In vitro studies were performed on the rat uterus (RU) for BK antagonist activity and on the electrically -stimulated guinea-pig ileum (ESGPI) for opioid agonist activity.Monomer BK antagonists CP-0126 & CP-0347 were active on RU but not on ESGPI. The heterodimers CP-0494 ([CP-0126]-[OXY]) and CP-0499 ([CP-0347]-[OXY]) were active on both preparations. In the mouse formalin test, OXY produced a dose-dependent (0.3-0.9 umoles/kg s.c.) inhibition of the 1st (0-5min) and 2nd (15-30min) phase responses and at the highest dose (0.9 umoles/kg s.c.) caused marked behavioral obtundation. At equianalgesic doses, CP-0494 (0.1 umoles/kg,; 69% & 86% inhibition of 1st and 2nd phases respectively) was approximately ten times more potent than OXY (0.9 umoles/kg,; 72% & 96% inhibition of 1st & 2nd phases respectively) and, more importantly, had no observable CNS effects. The homodimer BK antagonist CP-0127 at 12.6 umoles/kg s.c. inhibited both the 1st (55%) and 2nd (58%) phases. In the rat blood pressure assay, CP-0494 but not OXY produced total blockade of BK-induced hypotension.

Compounds such as CP-0494 and CP-0499 may be useful in the treatment of disorders which 1) involve both a bradykinin and a neurogenic component and, 2) where peripheral selectivity is desired.

Systemic Manifestation of the Acute Phase Response

O 200 MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IS AN IMPORTANT MEDIATOR IN SEPTIC SHOCK

Jürgen Bernhagen, Thierry Calandra, Robert A. Mitchell, Anthony Cerami, and Richard Bucala, The Picower Institute for Medical Research, Manhasset, NY 11030

Inflammatory cytokines initiate the host response to endotoxemia, causing severe physiological and hemodynamic changes which may lead to septic shock. Among the regulatory systems that play an important role in controlling host inflammatory responses is the pituitary. It has been known for many years for example, that hypophysectomized animals are extremely sensitive to LPS lethality. While investigating the possibility that protective, pituitary mediators might explain this phenomenon, we identified the cytokine MIF to be a specific secretory product produced by pituitary cells *in vitro* and *in vivo* after LPS challenge. Analysis of serum MIF levels in control, T-cell deficient (nude), and hypophysectomized mice revealed that pituitary-derived MIF contributes significantly to the rise in serum MIF that occurs after LPS administration. Of note, pituitary MIF content (0.05% of total pituitary protein) and peak serum MIF levels (80-340 ng/ml) were determined to be within the range observed for other pituitary hormones that are released after pituitary stimulation.

To investigate a possible beneficial role for MIF in septic shock, we co-injected mice with purified, recombinant murine MIF (rMIF) together with LPS (15 mg/kg). Surprisingly, rMIF markedly potentiated LPS lethality compared to control mice that were injected with LPS alone (85% vs. 35%, P = 0.003). To confirm these results, mice were treated with anti-rMIF antibody prior to injection of a high dose of LPS (17.5 mg/kg). Anti-rMIF antibody fully protected mice against LPS lethality, increasing survival from 50% to 100% (P = 0.0004). Serum levels of TNFα, the first cytokine that appears in the circulation after LPS challenge, were reduced by 38.0 ± 9.5% in anti-rMIF-treated mice.

We conclude that MIF plays a critical regulatory role in the systemic host response to endotoxemia and septic shock. Pituitary-derived MIF contributes significantly to circulating MIF in the post-acute response in endotoxemia and may act in concert with other pituitary mediators to regulate both pro- and anti-inflammatory effects.

O 201 THE MACROPHAGE: AN UNSUSPECTED SOURCE OF MACROPHAGE MIGRATION INHIBITORYFACTOR (MIF)

Thierry Calandra, Jürgen Bernhagen, Robert A. Mitchell, Richard Bucala, The Picower Institute for Medical Research, Manhasset, NY 11030

For over 25 years, activated T lymphocytes have been considered to be the cellular source of MIF. We recently isolated and cloned the murine homolog of MIF after identifying the specific secretion of this protein by LPS-stimulated pituitary cells *in vitro* and *in vivo*. However, further experiments showed that MIF protein is detectable both in T-cell deficient (nude) and hypophysectomized mice, suggesting that additional cell types may contribute to MIF production *in vivo*.

Monocytes/macrophages play an essential role in the host inflammatory response to LPS stimuli, producing a range of pro-inflammatory cytokines such as TNFα, IL-1 and IL-8. We found that MIF is expressed constitutively in the murine macrophage-line RAW 264.7 and in thioglycollate-elicited peritoneal macrophages. Significant amounts of MIF mRNA (RT-PCR) and protein (Western blotting) were observed in cell lysates. In RAW 264.7 cells, MIF secretion was induced by as little as 10 pg/ml of LPS (*E.coli* 0111:B4), peaked at 1 ng/ml, but was not detectable at LPS concentrations ≥1 μg/ml. Similar data were obtained with elicited macrophages, but higher LPS concentrations were required, unless the cells had been preincubated with IFNγ. Production of MIF by LPS-stimulated (1 ng/ml) macrophages peaked at 12 hr. MIF secretion also was induced by TNFα (1 ng/ml) and IFNγ (100 IU/ml), but not by IL-1β and IL-6 (up to 10 ng/ml). LPS and IFNγ had additive effects in inducing MIF secretion. In separate experiments, macrophages stimulated with recombinant mouse MIF (1 μg/ml) were found to secrete bioactive TNFα (>750 pg/ml by L929 cytotoxicity), but not immunoreactive IL-1β (Western blotting).

We conclude that MIF is expressed constitutively by murine macrophages. MIF secretion is induced by LPS, TNFα and IFNγ, and MIF-stimulated macrophages secrete TNF. These data indicate that the macrophage is not only the cellular target of MIF, but also an important source of MIF *in vivo* after inflammatory stimulation.

O 202 REGULATION OF PROINFLAMMATORY CYTOKINE EXPRESSION IN HUMAN ENDOTHELIAL CELLS BY REDOX-SENSITIVE SIGNALS. Cecil C. Chen, Craig L. Rosenbloom, Thinh P. Nguyen, Alistar R. McNab, Donald C. Anderson, and Anthony M. Manning, Adhesion Biology, Upjohn Company, Kalamazoo, MI 49001. We tested the hypothesis that redox-sensitive signals regulate the expression of proinflammatory mediators in human umbilical vein endothelial cells (HUVEC), through the activity of transcription factor NFκB. Electrophoretic mobility shift assays showed that two structurally distinct antioxidants, N-acetyl cysteine (NAC) and pyrrolidine dithiocarbamate (PDTC), can block the activation of nuclear NFκB binding activity in HUVEC upon stimulation with TNFα. These compounds significantly inhibited (90% to 100%) the induction of interleukin-6 (IL6), interleukin-8 (IL8), and granulocyte-monocyte colony stimulating factor (GM-CSF) following treatment with either TNFα or bacterial lipopolysaccharide (LPS) in a dose-dependent manner. Inhibition was maintained for at least 24 hours following either treatment. Conditioned medium from antioxidant-treated HUVEC was less effective in activating neutrophils, as measured by upregulation of CD11b, indicating that lower levels of chemotactic cytokines were present. Northern hybridization analyses showed that NAC and PDTC exerted their inhibitory effects at the transcriptional level. These findings suggest that redox-sensitive mechanisms play an important role in the expression of proinflammatory cytokines in HUVEC, and that antioxidants inhibit the induction of these mediators via inhibition of NFκB activation.

O 203 UPSTREAM SEQUENCES INVOLVED IN DOWN-REGULATION OF RAT CYTOCHROME P450 (CYP2C11) GENE TRANSCRIPTION BY INTERLEUKINS 1 AND 6, Jinqiang Chen*, Anders Strom†, Jan-Åke Gustafsson† and Edward T. Morgan*, *Department of Pharmacology, Emory University, Atlanta, GA 30322 and †Department of Medical Nutrition, Karolinska Institute, F60 Novum, Huddinge University Hospital, 141 86 Huddinge, Sweden. It was previously shown that hepatic expression of the drug metabolizing P450 enzyme family is suppressed during an acute phase response. For one member of this gene superfamily, CYP2C11, we have shown that its specific expression in male rats is suppressed transcriptionally by endotoxin treatment. To investigate the molecular mechanisms underlying this phenomenon, we studied the effects of the inflammatory cytokines interleukin-1 (IL-1) and interleukin-6 (IL-6) individually, together, and in the presence and absence of glucocorticoid, on the transient expression of chloramphenicol acetyltransferase (CAT) reporter gene constructs containing 5'-flanking regions of the CYP2C11 gene. The CAT reporter constructs were transfected into primary hepatocytes by electroporation, which were then cultured on Matrigel in the presence or absence of cytokines for 48 h. In the absence of cytokine, CAT activity of cells transfected with a construct bearing 1300 bp of 5' flanking sequence,was reproducibly lower than that of a construct bearing only the proximal 200 upstream residues. CAT activity in cells transfected with the 1300 bp construct was reduced to less than 30% of control levels by treatment with IL-1, IL-6 or dexamethasone. No further suppression was observed with different combinations of these agents. Other, preliminary experiments indicate that at least part of these suppressive effects can also be observed with the proximal 200 bp construct, suggesting that sequences important for cytokine down-regulation lie within 200 bp of the transcription start site of the CYP2C11 gene. Supported by grant GM46897 from the National Institute of General Medical Sciences

O 204 REGULATION OF THE ACUTE INFLAMMATORY RESPONSE IN CATTLE: THE ROLE OF TNFα IN THE RESPONSE TO ENDOTOXIN FROM *PASTEURELLA HAEMOLYTICA*, P. David Eckersall[1], Ajantha Horadagoda[1], Neil Horadagoda[1], H. Alison Gibbs[1], Robert L. Davies[2] and J. Christopher Hodgson[3], Department of Veterinary Medicine[1], Department of Microbiology[2], Glasgow University, Glasgow, G61 1QH, UK, Moredun Research Institute[3], Edinburgh, EH17 7JH, UK.

Infection of cattle with the Gram negative bacterium *Pasteurella haemolytica* leads to an acute inflammatory response which stimulates hepatic synthesis and secretion of serum amyloid A (SAA), haptoglobin (Hp), fibrinogen (Fb). Many of the systemic effects of *P haemolytica* are likely to be caused by lipopolysaccharide (LPS) from the bacteria at the site of infection in the lung, stimulating cytokines such as tumour necrosis factor-α (TNFα). LPS from *Escherichia coli* has been shown to elicit the production of TNFα in cattle. In this investigation, calves (n=4) were injected with 0.7μg/kg of LPS, prepared by aqueous-phenol extraction, from field isolates of *P haemolytica* to assess whether the acute inflammatory response to this bacterium is related to LPS stimulation of TNFα production. LPS from field isolates of *P haemolytica* produced an increase in the serum TNFα concentration, which reached a peak 2 hours after injection, returning to undetectable levels by 6 hours post injection. Peak concentrations of TNFα ranged from 15 to 80 ng/ml. The serum concentration of SAA started to rise within 2 hours of the LPS injection and continued to increase for at least 30 hours. The serum Hp concentration showed a steady rise, reaching a peak 2 days after injection, while the plasma Fb concentration showed only a minor response to LPS injection. The regulation of the host response in the economically important disease of bovine pneumonic Pasteurellosis (shipping/transit fever) is likely to be dependent on LPS stimulation of the acute inflammatory response in which TNFα plays an integral role.

Acknowledgement: The generous provision of rbTNFα and antisera from Ciba-Geigy SA is gratefully acknowledged

O 205 THE ACTIVATION OF Aα FIBRINOGEN GENE BY IL-6 INVOLVES DEPHOSPHORYLATION OF A SPECIFIC DNA BINDING PROTEIN, Gerald M. Fuller and Zhiyong Liu, Department of Cell Biology, University of Alabama at Birmingham, Birmingham AL 35294

Fibrinogen is a complex dimeric glycoprotein comprised of three pairs of subunits linked by clusters of disulfide bridges. Each subunit is derived from a separate gene located in a 60kb cluster on chromosome 4 in man and 2 in rats. The promoter of each gene contains similar cytokine controlling domains, including C/EBP binding regions and an IL-6 responsive specific hexanucleotide (-CTGGGA-). Our studies have focused on the protein(s) that affect this latter binding region. Four different 30bp oligonucleotide constructs were made, containing either the intact hexanucleotide -CTGGGA- or specifically mutated forms of this region. Using mobility shift assays we could identify a specific binding protein at this site under control (unstimulated conditions). When the hepatocytes were treated with IL-6 there was a time dependent shift in the binding pattern creating a new specific band. The appearance of the new band was accompanied by a loss in density of the initial band or control band. We could create the IL-6 dependent shift by indirect phosphatase cleavage of the nontreated IL-6 treated extract proteins Southwestern blotting assays demonstrate that binding proteins that form the two distinct bands in the gel shift assay have the same molecular weight. These findings suggest that one essential event in IL-6 activation of the fibrinogen genes may involve a dephosphorylation of the specific protein that binds to the -CTGGGA- domain. Since fibrinogen is a constitutively expressed protein, we suggest that the up-regulation induced by IL-6 is the result of dephosphorylating an already existing DNA binding protein making the promoter more accessible to RNA polymerase II.

O 206 CYTOKINE REGULATION OF TYPE-1 PLASMINOGEN ACTIVATOR-INHIBITOR IN HUMAN HEPATOCYTES *IN VIVO* AND IN HUMAN HEPATOMA CELLS *IN VITRO*, T.D. Gelehrter, A.M. Healy, and A.J. Thornton, Departments of Human Genetics and Internal Medicine, University of Michigan, Ann Arbor, MI 48109-0618.

Type-1 plasminogen activator-inhibitor (PAI-1), the major regulator of fibrinolysis, is an important component of the acute phase (AP) response in humans. The source of plasma PAI-1 is unknown. To determine if human hepatocytes express the PAI-1 gene *in vivo*, we have performed *in situ* hybridization on liver samples obtained at autopsy and also from healthy donor livers not used for transplantation. PAI-1 specific transcripts were found in hepatocytes throughout the hepatic acinus in transplant donor liver, and, to an even greater extent, in liver from patients dying from sepsis. These observations are the first direct demonstration of human hepatocyte-specific PAI-1 gene expression *in vivo*, and suggest that the liver may be a major source of plasma PAI-1 during the AP response.

To characterize the regulation of PAI-1 gene expression by mediators of the AP response, we have analyzed the effects of the cytokines interleukin-1 (IL-1) and interleukin-6 (IL-6) in Hep G2 cells, a highly differentiated human hepatoma cell line that produces a broad spectrum of acute phase proteins. IL-1 caused a rapid and transient 30-fold induction of PAI-1 mRNA accumulation. Although IL-6 alone had only a modest effect on PAI-1 expression, in combination with IL-1, it caused a synergistic 100-fold induction of PAI-1 mRNA accumulation. Nuclear run-on experiments indicated that IL-1 alone, or in combination with IL-6, caused a 10- to 15-fold, transient stimulation of PAI-1 gene transcription. Concomitant protein synthesis was not required for these cytokine-induced effects; however, cycloheximide delayed, prolonged, and enhanced the induction of PAI-1 mRNA by IL-1 and IL-1 plus IL-6, and acted synergistically with IL-6 to induce PAI-1 mRNA accumulation.

These studies demonstrate that 1) human hepatocytes express PAI-1 *in vivo*, 2) PAI-1 gene expression is induced in human hepatoma cells by mediators of the AP response and, 3) suggest that this regulation may occur at both the transcriptional and post-transcriptional level.

O 207 APOLIPOPROTEIN J IS AN ACUTE PHASE PROTEIN, Carl Grunfeld, Ingibjörg Hardardóttir, Steven Kunitake and Kenneth R. Feingold, Metabolism Section Department of Veterans Affairs Medical Center, Department of Medicine, University of California, CA 94121

Previous studies by our laboratory have demonstrated that cytokine or LPS administration rapidly results in a two fold increase in serum VLDL levels which is due to increased hepatic production and secretion. Hepatic de novo fatty acid synthesis is increased by cytokines, and along with the increased delivery of fatty acids from peripheral tissues due to cytokine stimulation of lipopolysis, provides the substrate required for enhanced triglyceride secretion. However, despite the increase in lipoprotein secretion our laboratory and others have not demonstrated a concommittant increase in apolipoprotein (A1, B, CII and E) synthesis by the liver. In the present study, we demonstrate that endotoxin (LPS), tumor necrosis factor (TNF) and interleukin (IL)-1 increase hepatic mRNA and serum protein levels of apolipoprotein J (apo J) in Syrian hamsters. Hepatic apo J mRNA levels increased ten to fifteen fold with doses of LPS from 0.1 to 100 ug/100 g body weight by 4 h and were elevated for at least 24 h. Serum apo J concentrations were significantly increased by 16 h and were further elevated to 3.3 times that of control, 24 h following LPS administration. Serum apo J was associated with high density lipoprotein, increasing 5 fold in this fraction, by 16 h following LPS administration. Hepatic apo J mRNA levels increased 3.5 and 4.6 fold, with TNF and IL-1, respectively, and 8.2 fold with a combination of TNF and IL-1. Serum apo J concentrations were increased 2.3 fold by TNF, 79% by IL-1 and 2.9 fold with a combination of TNF and IL-1. These results demonstrate that apo J is a positive acute phase protein.

O 208 PHYLOGENETIC PERSPECTIVES OF THE INNATE HOST DEFENSE : A MOLECULAR ANALYSIS OF THE ACUTE PHASE RESPONSE OF *DROSOPHILA*, Jules A. Hoffmann, Jean-Marc Reichhart, Marie Meister, Christine Kappler, Philippe Georgel and Bruno Lemaître, Department of General Biology, University of Strasbourg, 67000 Strasbourg, France

Drosophila, like other insects, responds to the injection of bacteria by the rapid and transient response of a battery of peptides and polypeptides, some of which exhibit potent antibacterial activities. Structurally well characterized inducible antibacterial insect peptides are the cecropins, the insect defensins and proline-rich drosocins, which may have their counterparts in mammals. These peptides are synthesized in the fat body, a functional homologue of the mammalian liver, and in some blood cell types evocative of mammalian blood cells of the myeloid lineage. The *Drosophila* response is induced by a variety of stimuli, such as bacterial infections, lipopolysaccharide, or tissue injury; it shows acute phase kinetics and does not exhibit the hallmarks of the lymphocyte response, i.e. memory and specificity. The promoters of the genes encoding the inducible antibacterial peptides contain an impressive number of *cis*-regulatory sequences homologues to mammalian response elements involved in acute phase regulation e.g. binding sites for NF-κB, NF-IL6, NF-ELAM 1, etc. Footprint analysis and gel shift assays demonstrate that these sites are functional in bacteria-challenged *Drosophila*. The presentation will provide a functional dissection of these promoter sequences based on establishment of transgenic fly lines and a genetic and biochemical analysis of transcativating proteins. The phylogenetic perspectives of the insect host defense will be discussed.

O 209 ROLE OF IL-1ß, IL-6 AND CORTICOSTEROIDS IN THE REGULATION OF THE C/EBPA, ß AND ∂ AND THE ALPHA-1-ACID GLYCOPROTEIN GENES IN VIVO. Rosaria Ingrassia, Alba Magalini, Arrigo Caraffini, Gianfranco Savoldi, Alberto Albertini, Pietro Ghezzi*, and Diego Di Lorenzo, Institute of Chemistry, School of Medicine, University of Brescia, CNR-ITBA Sez. di Brescia, Italy and * Laboratory of Neuroimmunology "Mario Negri" Institute for Pharmacological Research, Milan, Italy.

We have examined the regulatory effects of interleukin-1ß (IL-1ß) and interleukin-6 (IL-6) on the activation of three different isoforms of the C/EBP family of transcription factors (α, ß and ∂), in hepatocytes of normal and adrenalectomized (Adx) rats. C/EBPα mRNA levels were not affected by either IL-1ß or IL-6, whereas those of C/EBPß and ∂ were enhanced by treatment with these interleukins in both normal and adrenalectomized rats. The magnitude of this effect was strikingly higher for C/EBP∂ in adrenalectomized animals treated with IL-1ß, indicating a suppressive effect of corticosteroids in this regulatory pathway.

The pattern of C/EBP protein synthesis reflected the mRNA findings except for the induction of C/EBP∂ in IL-1ß treated rats, which was not as pronounced as that of the corresponding mRNA and for the protein synthesis of the C/EBPα and C/EBPß which was much higher in IL-6 treated Adx rats. Hormonal modulation of C/EBP transcription factors was studied in parallel with the hormonal induction of the Alpha-1-Acid Glycoprotein (AGP) gene, which is highly induced in rat liver during the acute phase response. AGP, like C/EBPß and C/EBP∂, was strongly induced by IL-1ß and to a lesser extent by IL-6 in normal rats. In contrast to findings for C/EBP∂, IL-1ß failed to induced the same high level of expression of AGP in adrenalectomized rats.

This report also indicates an important role of the hypothalamus-pituitary-adrenal (HPA) axis in the expression of the C/EBPα, ß and ∂ and AGP genes during the acute phase response.

O 210 REGULATION OF C-REACTIVE PROTEIN (CRP) EXPRESSION IN HEP 3B CELLS, Irving Kushner, Shun-Lin Jiang, Dongxiao Zhang and David Samols, Depts. of Medicine (44109) and Biochemistry (44106), Case Western Reserve Univ., Cleveland, OH

Induction of CRP in Hep 3B cells has been found to require the cooperative effects of both IL-6 and IL-1. Previous studies in Hep 3B cells transfected with CRP-reporter constructs indicated that the effect of IL-6 was transcriptional, while the effect of IL-1 appeared to be post-transcriptional. To further elucidate this issue, we determined the effects of these cytokines on nuclear run-on (transcription) and mRNA accumulation of the endogenous CRP gene in kinetic studies over 48-72 h in Hep 3B cells. In the presence of dexamethasone (dex) and insulin, IL-1 (400 U/ml) alone had no effect on either run-on or mRNA levels, IL-6 (200 U/ml) caused a 38 fold increase in transcription (maximal at 12 h) and a 52 fold increase in mRNA abundance (maximal at 24 h), while the combination [IL-6 + IL-1] led to a 132 fold increase in transcription (24 h) and a 317 fold increase in mRNA levels (24 h). Thus, addition of IL-1 further enhanced transcription and mRNA accumulation by 3.5 and 6 fold respectively over the changes induced by IL-6 alone, indicating that the effect of IL-1 on CRP expression is exerted largely at the transcriptional level in this system. After 24 h of incubation, dexamethasone was found to enhance mRNA abundance induced by either IL-6 or by [IL-6 + IL-1] by about 25 to 35%, in the presence or absence of insulin. Insulin inhibited CRP mRNA induction by 75 to 85% whether or not dex was present. In these studies, [IL-6 + IL-1]-induced transcription was enhanced by dex in the presence or absence of insulin while insulin inhibited transcription in the presence or absence of dex. The effects of cytokines on the endogenous CRP gene were confirmed in studies in Hep 3B cells transiently transfected with CRP-CAT constructs containing 150 bp of 5' DNA and variable lengths of 5' nontranslated DNA. IL-1 led to no detectable CAT increase, IL-6 caused a 20 fold increase and [IL-6 + IL-1] about a 40 fold increase. The length of the 5' UTR did not influence the effect of either IL-6 alone or of [IL-6 + IL-1]. Insulin had no effect on CAT expression induced by cytokines. These findings indicate that the effects of IL-6, IL-1, dex and insulin on CRP expression in Hep 3B cells are exerted largely at the transcriptional level. In addition, the close proximity of the peaks of transcription and mRNA accumulation in kinetic studies suggests that CRP mRNA is relatively unstable. These conclusions differ from those for SAA in which similar studies suggest substantial participation of post-transcriptional mechanisms and a relatively stable message.

O 211 TRANSCRIPTIONAL ACTIVATION OF ALPHA-1 ACID GLYCOPROTEIN GENE BY YY1, Sheng-Chung Lee, Yu-May Lee, and Tzung-Horng Yang, Institute of Molecular Medicine, National Taiwan University and Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan.

Regulation of alpha-1 acid glycoprotein (AGP) gene expression involves both positive and negative transcription factors. We have previously identified a positive transcription factor, AGP/EBP, and a negative transcription factor, factor B-two dominant factors involved in regulating the expression of AGP and other acute phase response genes. We have shown that the transcription of AGP gene is positively regulated by a transcription factor, YY1. The activation of AGP gene by YY1 is mediated by a negative element B in the AGP promoter region. YY1 can also activate B motif linked to heterologous promoter. However, YY1 does not bind directly to the B element per se. Rather, it represses the negative function of factor B, thus resulting in the activation of AGP gene expression.

O 212 THE ACUTE PHASE RESPONSE PROTEIN SERUM AMYLOID A (apoSAA) ALTERS CHOLESTEROL FLUX
Jun-shan Liang and Jean D. Sipe. Department of Biochemistry.Boston University School of Medicine, Boston, MA 02118.
Serum amyloid A (apoSAA) synthesis is rapidly increased up to 1000 times basal levels during acute inflammation. ApoSAA isoforms are associated with high density lipoprotein (HDL), which plays a key role in reverse cholesterol transport. The direct effect of apoSAA on lipid transport and metabolism is unclear. We have investigated the effect of apoSAA on cholesterol(C) flux using recombinant human apoSAAp. Under physiological conditions, about 90% of apoSAAp (2 ug/ml in RPMI) is bound to plastic microtiter wells after 2h incubation at 37C; this binding is partially reversed by C at concentrations of 5-80ug/ml. Since this plastic bound apoSAAp can be partially released upon introduction of RPMI containing C, C may have a function in the physiological solubilization of apoSAAp. Affinity of apoSAAp for C has also been demonstrated using an exchange system in which 14C-C contained as a complex with HDL diffuses from a 3500 mw cut off dialysis membrane into a tissue culture well containing proteins in RPMI, C diffuses into apoSAAp in RPMI to a much greater extent that it does into apoAI, albumin or RPMI alone, suggesting there is higher affinity between C and apoSAAp than between C and apoAI. This is consistent with the observation of apoSAA displacing apoAI and apoAII from HDL in the acute phase response. The biological significance of these observations was supported by a dose dependent increase in 14C-C uptake by Hep G2 cells in the presence of apoSAAp at concentrations ranging from 2- 40 ug/ml. The enhancement by apoSAAp of C uptake by Hep G2 cells is completely blocked by antibodies to apoSAA. These data suggest that apoSAA may modulate reverse C transport during acute inflammation and apoSAA clearance may be linked to C transport. Supported by USPHS grant AG9006.

O 213 ACUTE PHASE RESPONSE IN UNDERNUTRITION. SERUM LEVELS OF IL-6, C REACTIVE PROTEIN AND PREALBUMIN IN UNDERNOURISHED CHILDREN.
Inés Malavé, María A. Vethencourt Raimundo Cordero and Moravia Pirela. Venezuelan Institute of Scientific Research (IVIC). Caracas 1020A-Venezuela.
We have quantitated levels of IL-6, C Reactive Protein (CRP) and Prealbumin (Pr) in serum from 110 clinically infected or non-infected undernourished children (CIU and CNIU) aged 3 months to 6 years and from their clinically infected or non-infected matched eutrophic controls (CIE and CNIE). Nutritional condition was evaluated by clinical and anthropometrical data and categorized by Score Z. Levels of IL-6 and CRP, both assessed by ELISA, were significantly increased in CIU as compared with CNIU and in CIE as compared with CNIE. No significant differences in the levels of IL-6 and CRP were observed between CNIU and CNIE as well as between CIU and CIE. Even CI children with moderate and severe undernutrition showed serum levels of IL-6 and CRP comparable to the CIE. Serum levels of Pr were quantitated by RID. Pr was significantly decreased in CNIU as compared with CNIE. However, Pr was similar in CIU and CIE. When children were divided according to the nutritional defect, each infected group showed significantly lower Pr levels than their uninfected reference group. A significant positive correlation was observed between Pr and the Score Z of weight for age in all non-infected children which dissapeared in the infected children. Pr also showed and inverse relationship with the levels of IL-6, whereas CRP correlated positively with IL-6. The results indicate that undernourished children had a preserved capacity to produce IL-6 and the major acute phase reactant CRP in response to infection, that serum levels of Pr diminished both by undernutrition and by infection with no additive effect probably because cytokines with antianabolic, procatabolic and anorexigen effects and increased energetic requirements in CIE induced some degree of secondary undernutrition not yet detectable by anthropometric measurements. Decreased levels of Pr in undernourished children could contribute to their high levels of IL1 which we have previously observed in this group because of the inhibitory effect of Pr on IL-1 secretion

O 214 REGULATION OF PROINFLAMMTORY CYTOKINES AND METALLOPROTEINASE INHIBITOR (TIMP-1) BY ONCOSTATIN M,
Najma Malik, Rosalind S. Chuang, Brad W. Greenfield, Alan Wahl, Bristol-Myers Squibb, PRI, 3005 First Ave., Seattle, WA 98121

Oncostatin M (OM) is a multifunctional cytokine produced by monocytes/macrophages and activated T-cells. In vitro studies were done to examine the effect of OM on key cell types participating in periodontal inflammation: primary human gingival fibroblasts and human endothelial cells (HUVEC). OM induced E-selectin, an adhesion molecule, in endothelial cells but did not induce proinflammatory cytokine interleukin-1α (IL-1α) in either fibroblasts or endothelial cells. Levels of the proinflammatory cytokine interleukin-8 (IL-8) were also reduced by OM in endothelial and in gingival fibroblast cells. Additionally, OM stimulated the levels of mRNA for metalloproteinase inhibitor (TIMP-1) in both endothelial and fibroblast cells. Effect of OM on metalloproteinase levels in both cells types was also determined. OM may down regulate the key components in the periodontal inflammatory process.

O 215 **Inhibition of serum amyloid A synthesis**
Raynes J.G., Bevan S., Hosp M., McAdam K.P.W.J. Molecular Immunology Unit, Dept. of Clinical Science, L.S.H.T.M., Keppel St. London, WC1E 7HT.

The acute phase response and in particular the induction of SAA is inhibited by hepatocyte growth factor (HGF) and epidermal growth factor (EGF) in human hepatoma cell lines. The SAA induction by IL-1 and IL-6 was inhibited by approximately 75% in the presence of 10 ng/ml EGF or HGF. Total secreted protein synthesis was reduced by HGF. Since HGF is found in elevated concentrations in response to liver, or kidney injury it may consequently have profound influences on hepatocyte protein synthesis and in particular the acute phase response to trauma. The effect of these growth factors on protein synthesis is not mediated by similar mechanisms to the mitogenic activity. SAA induction by IL-1 is mediated by the type I receptor since antibodies against human type I IL-1 receptor inhibited SAA secretion by >90% whereas antibody to type II receptor was without effect. The induction of other acute phase proteins (e.g. haptoglobin) was inhibited in a similar way.

O 216 REQUIREMENT FOR NF-κB P65 AND NF-IL6 BINDING ELEMENTS IN THE TNF RESPONSE REGION OF THE G-CSF PROMOTER, M. Frances Shannon, Stephanie M. Dunn, Leeanne S. Coles and Robyn K. Lang, Division of Human Immunology, Hanson Centre for Cancer Research, Institute of Medical & Veterinary Science, Frome Road, Adelaide, SA 5001 AUSTRALIA

Granulocyte-colony stimulating factor (G-CSF) is an important cytokine in myeloid haemopoiesis and in inflammatory reactions. Its expression is tightly controlled, being induced in mesenchymal and myeloid cell types by inflammatory mediators. Transcription mediated by the G-CSF promoter region mimics the endogenous gene response to inflammatory cytokines.

It has previously been shown that a region of the G-CSF promoter, (-200 to -165) containing the decanucleotide CK-1 element and two repeated sequences which resemble NF-IL6 binding sites, is required for activation of the G-CSF gene by tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). We now demonstrate that the NF-κB p65 protein can bind to and transactivate this TNF response region. There are several unusual features of this p65 interaction with the TNF response region. Firstly, NF-κB p65 but not NF-κB p50 can bind to the CK-1 element. Secondly, p65 transactivation and TNF/IL-1 activation are cell specific and finally p65 transactivation requires not only its own binding site but also the repeated NF-IL6 binding sites.

NF-IL6 also binds to the TNF response region of the G-CSF promoter and electrophoretic mobility shift studies show that p65 and NF-IL6 can bind cooperatively. The ability of this region to respond to TNF-α or p65 is correlated with the ability to form the p65/NF-IL6 ternary complex.

The interaction of NF-κB and C/EBP proteins appears to be a common feature of inflammatory cytokines such as G-CSF IL-8 and IL-6 and may lead to coordinate upregulation of these cytokines.

O 217 INDUCTION OF METALLOTHIONEIN AND STOMATIN BY IL-6 AND GLUCOCORTICOIDS IN UAC CELLS, Luc Snyers and Jean Content, Institut Pasteur du Brabant (Department of Virology), B 1180 Bruxelles (Belgium)

The human amniotic cell line UAC has an increased number of IL-6 receptors after treatment by glucocorticoids (Snyers et al., Proc. Natl. Acad. Sci. USA, 1990, **87**: 2838-2842). To find a possible activity of IL-6 on these cells, a cDNA library of IL-6 + dexamethasone treated cells was screened with cDNA probes from induced versus non-induced cells. Two cDNAs showed a differential hybridisation signal. The first one corresponds to metallothionein (MT), a group of small cystein rich proteins thought to participate in the metabolism and storage of zinc and to protect cells against oxydative damages. A second cDNA corresponds to the recently cloned cDNA of "band 7 integral membrane protein" also called stomatin (ST). In hereditary stomatocytosis, absence of this protein on erythrocytes membranes is associated with high Na^+ and low K^+ concentrations (Stewart et al., Blood, 1992, **79**: 1593-1601). In UAC cells both MT and ST are induced by dexamethasone and IL-6 in an additive manner. Western blot analysis shows that ST protein is induced in a similar way as its mRNA. Possible implications of the regulation of this genes are discussed.

O 218 EXPRESSION OF CYTOKINE RECEPTOR GENES IN LPS-INDUCED ENDOTOXEMIA, Anna Stalder and Iain L. Campbell, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The LPS- induced acute inflammatory reaction is a model for sepsis in which the pivotal role of cytokines in the genesis of the disease is well established. During the host response to LPS a number of proinflammatory cytokines, including IL-1, IL-6, IFNγ and TNF are produced, whose actions depend ultimately on the qualitative and quantitative expression of cytokine receptors on target cells. Therefore, we investigated the regulation of cytokine receptor and cytokine genes in various organs in LPS- induced endotoxemia. For this study, we used two RNase protection assays that permitted the simultaneous analysis of multiple cytokine receptor and cytokine mRNAs respectively. In normal spleen, the TNFR p60, p80, IL-6 and the IFNγ receptor mRNAs were expressed at similar levels, whereas the IL-6R and IFNγR mRNAs were the predominant receptor gene products found in liver. In both these organs, expression of IL-1R p80 mRNA was very low. In brain and kidney, generally lower levels of the receptor gene products were found except for a strong predominance of the TNFR p60 mRNA in kidney. The IL-1R p60 mRNA was not detectable in normal organs. Following LPS injection significant induction of IL-1R p60 mRNA was seen in all organs, while the IL-1R p80 was markedly upregulated in liver. Upregulation of both TNF receptor mRNAs was seen in spleen and liver and less in brain and kidney. In all organs this response was maximal by 8h post- injection and remained elevated at 24h. The relationship between cytokine gene expression and the changes in cytokine receptor mRNA was also determined. Compared with controls, following LPS treatment in spleen and liver the levels of the IL-1α/β, TNFα/β and IFNγ mRNAs increased markedly and reached maximum by 4h post injection before returning to normal levels at 16h. In brain and kidney the same kinetics were observed but the levels of these mRNAs were somewhat less. In conclusion, LPS- induced endotoxemia is accompanied by significant, organ specific upregulation of some but not all cytokine receptor genes. In addition, the expression of cytokine receptor genes was delayed and prolonged as compared with that of the cytokine genes implicating a role for cytokines in the regulation of receptor gene expression.

O 219 CHARACTERIZATION OF THE ONCOSTATIN M (OSM)-SPECIFIC RECEPTOR, Bettina Thoma, Patrick R. Gearing* and Steven K. Dower, Departments of Biochemistry and Proteinchemistry*, IMMUNEX Corporation, Seattle, WA 98101

Two receptors for the growth inhibitor and acute-phase inducer Oncostatin M have been defined on the basis of their ability to bind leukemia inhibitory factor (LIF): the LIF/OSM receptor complex binds both ligands cross-competitively, whereas an OSM-specific receptor only binds OSM. Both receptors have been shown to be heteromeric structures, of which one component is the IL-6 receptor signal transducer, gp130. The second molecule of the LIF/OSM receptor is the LIF receptor. The analogous component of the OSM-specific receptor has not been characterized so far. Using cross-competitive binding behavior of OSM and LIF on the two types of receptors, we have defined two cell lines expressing either the LIF/OSM receptor complex (JAR) or the OSM-specific receptor (WI26-VA4). Crosslinking of ^{125}I-OSM to receptors on both cell lines resulted in receptor-ligand complexes, which were very similar in molecular weight. In both cases, the LIF receptor was not crosslinked to OSM, as the products could not be immunoprecipitated with anti-LIF receptor antibodies. Therefore, the crosslinking product of ^{125}I-OSM with the LIF/OSM receptor from JAR cells presumably consists of OSM and gp130. 2D-gel analysis of the ^{125}I-OSM crosslinking products from both cell lines revealed a difference in pI. Furthermore, digestion with N-glycanase or V8-protease showed that the ^{125}I-OSM receptor complexes formed either with the LIF/OSM receptor or the OSM-specific receptor are not identical. Thus, the crosslinking product from WI26-VA4 cells may not be gp130 but the second component of the OSM-specific receptor.

O 220 IMMUNOLOGIC MODULATION OF SLEEP DURING CANDIDA ALBICANS INFECTIONS IN RABBITS, Linda A. Toth and James M. Krueger, Departments of Comparative Medicine and Physiology and Biophysics, University of Tennessee, Memphis, TN 38163

Enhanced sleep, like fever, is a manifestation of the acute-phase response to microbial infections. The key inflammatory mediators and immune response modifiers interleukin-1 and tumor necrosis factor-α are somnogenic, suggesting a role for the immune/inflammatory response in the generation of altered sleep during infectious conditions.

To investigate this issue, we examined sleep during Candida albicans (CA) infections in rabbits treated with immune-modulating drugs. Intravenous administration of CA ($7.0 \pm 1.1 \times 10^7$ colony-forming units) to normal rabbits (n=14) increased the amount and intensity of sleep during the initial 10 h after inoculation and suppressed these parameters during h 20-46. Treatment with an anti-inflammatory and immune-suppressive dose of cortisone (20 mg/kg i.m.; n=12) attenuated all of these CA-induced alterations in sleep. Treatment with the immunosuppressive drug cyclosporine (25 mg/kg s.c.; n=11) did not alter the initial sleep enhancement caused by CA administration. However, during the sleep suppression phase, cyclosporine attenuated the CA-induced reduction in sleep time while potentiating the reduction in sleep intensity, as defined by the amplitude of electroencephalographic slow waves. In contrast to the effects of these immune-suppressive agents, two other treatments intended to enhance the immune response, prior immunization with killed CA (weekly i.v. administration for 8 weeks; n=8) or administration of incomplete Freund's adjuvant (0.25 ml/kg, s.c.; n=8), did not markedly influence the pattern of sleep alterations after CA inoculation.

These data indicate that sleep alterations after infectious challenge can be modulated by suppression of the immune/inflammatory response, and are consistent with the hypothesis that changes in sleep during infectious disease are mediated by immune-related factors.

Supported in part by NS-26429 and NS-25378.

O 221 DUAL REGULATORY ROLES FOR TRANSCRIPTION FACTOR NFIL-6 IN κB-DEPENDENT ACTIVATION OF INFLAMMATORY GENES, K.Yamamoto and H.Shimizu, Department of Molecular Pathology,Cancer Research Institute,Kanazawa University,13-1 Takaramachi,Kanazawa,Ishikawa 920,Japan

NF-κB and NFIL-6 are the members of the NF-κB/Rel/Dorsal(NRD) and C/EBP transcription factor families, respectively, and play major roles in the activation of diverse genes involved in host defense mechanisms including inflammation. In some of these genes such as interleukin-6 and serum amyloid A genes, binding sites for both of these factors are required for inducible expression by various inflammatory stimuli. Although NFIL-6 has been shown recently to interact directly with NF-κB, or its subunits(p50 and p65) in vitro, it is not clear how these factors function cooperatively in transcriptional activation in vivo. Here we show that NFIL-6 plays dual synergistic roles in κB-dependent transcriptional activation in vivo. It synergizes with transcriptionally-inactive p50 homodimers to induce low-degree transcriptional activation in the absence of NF-κB. However, in the presence of NF-κB that is a major positive NRD factor inducible in various types of cells,it antagonizes p50 homodimers which otherwise function as a physiological repressor for NF-κB in vivo. These synergistic activities of NFIL-6 for NF-κB are shared by other C/EBP family members. As C/EBP sites in conjunction of κB sites are found in several mammalian as well as insect genes important for host defense, this function of C/EBP family members may be physiologically as well as phylogenically important for host defense reactions.

Resolution of the Acute Phase Response and Genetically Manipulated Mice as Experimental Models

O 300 INHIBITION OF MOTILE RESPONSES IN B LYMPHOCYTES BY INTERLEUKIN-10.

Birgitta Clinchy, Pia Björck, Staffan Paulie and Göran Möller. Dept. of Immunology, Stockholm University, 106 91 Stockholm, Sweden.

Infiltration of lymphocytes and other blood cells is an important step during inflammatory reactions. The recruitment of lymphocytes to inflammatory sites can be greatly influenced by the presence of cytokines. We have earlier shown that Interleukin 4 (B cell stimulatory factor1) can induce migration in B lymphocytes. Here we report that the anti-inflammatory cytokine IL-10 inhibits motility of B lymphocytes induced to display motile morphology and active migration by IL-4 or by LPS. IL-10 inhibited locomotor responses to these two stimuli both when B cells of human or murine origin were used. In addition, other parameters of LPS stimulation, like cellular aggregation and proliferation, were supressed by IL-10. Exposure to IL-10 for 24 hours did not result in any changes in the surface expression of molecules involved in adhesion, such as CD2, CD11a/CD18, CD44, CD54 or L-Selectin on B lymphocytes.

O 301 TARGETED DISRUPTION OF THE INTERLEUKIN-6 GENE IN THE MOUSE GERMLINE.

Stacie A. Dalrymple, Linda A. Lucian, Stacy Fuchino, Dee Aud, Tom McNeil, Frank Lee, and Richard Murray. DNAX Research Institute, 901 California Ave, Palo Alto, CA 94304.

Interleukin (IL)-6 is a cytokine with multiple biological activies. Originally described as a B cell growth factor, IL-6 has subsequently been found to play a potential role in a wide variety of immune and inflammatory responses. These processes include the maturation of cytotoxic T cells, stimulation of immunoglobulin synthesis, stimulation of bone marrow colony formation, and acute phase protein induction. High levels of serum IL-6 are detectable after most traumatic injuries, inflammatory reactions, and immunological assaults. To elucidate the role IL-6 may have in these and other responses we have produced animals deficient in IL-6 production. The IL-6 gene was inactivated in the E14.1 ES cell line via homologous recombination, and these cells were capable of giving rise to germline chimeras. Mice homozygous for the IL-6 mutation appear healthy if unchallenged. Primary cultures of thymic, splenic, and bone marrow stromal cells, when stimulated by LPS, produce no detectable levels of IL-6. Most lineages of cells are present in normal numbers in the bone marrow, thymus, spleen, and lymph node. Thus these animals will serve as a good model to test immune cell function. Results of functional assays of T and B cells will be presented, as well as results of imflammatory challenges.

O 302 DETECTION OF AMNIOTIC FLUID INTERLEUKIN-10 (IL-10) IN WOMEN WITH PRETERM LABOR AND DURING LATE PREGNANCY. D.J. Dudley, C.L. Chen, C. Hunter, M.D. Mitchell, and M.W. Varner. Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, UT 84132.

Preterm labor and delivery accounts for the majority of perinatal deaths in the United States. Intrauterine infection has been associated with 10% to 30% of these preterm births. Since women with preterm labor associated with intrauterine infection have elevations of proinflammatory cytokines (IL-1β, TNF, IL-6, and IL-8) and prostaglandin E_2 in amniotic fluid, an important pathophysiologic event likely involves inflammation of gestational tissues including maternal decidua, fetal chorion, and fetal amnion. We have previously reported that decidua and chorion produce IL-6 and IL-8 in response to IL1β and TNF (J Clin Endocrinol Metab 74:884, 75:1081, 76:404). Also, we have found that amniotic fluid IL-6 increases with the onset of labor and correlates with cervical dilation (submitted). Moreover, cytokine mRNA isolated from gestational tissues indicates that IL-6 mRNA is expressed more commonly with the onset of labor. These findings suggest that inflammatory cytokine production by gestational tissues may be involved in both normal and abnormal parturition. Based on these studies, we hypothesized that IL-10 production by gestational tissues, as reflected by amniotic fluid concentrations, would be elevated in women in labor and thus could potentially exert cytokine inhibitory effects on gestational tissues. Amniotic fluid was collected at amniocentesis or amniotomy in women at term (not in labor), at term (in labor), preterm (not delivered within one week) and preterm delivered within one week. IL-10 was measured by ELISA (Pharmingen, San Diego, CA). Results are expressed as mean ± standard error (pg/ml).

Clinical scenario	n	#positive (%)	IL-10 (pg/ml)
Term, not in labor	42	29 (69%)	100.9 ± 23.1
Term, in labor	56	35 (63%)	108.6 ± 22.3
Preterm, undelivered	22	9 (41%)	50.4 ± 22.6
Preterm, delivered	34	23 (68%)	108.2 ± 22.2

There were no significant differences by Mann Whitney U test or chi square analysis. Two women with preterm labor and chorioamnionitis had marked elevations of amniotic fluid IL-10 (1406 pg/ml and 2497 pg/ml). These data show that there is no association of amniotic fluid IL-10 levels with the genesis or propagation of preterm or term labor. Further, the production of IL-10 by fetal or maternal tissues appears to be developmentally regulated. Lastly, gestational tissues responds to inflammatory stimuli with an increase in IL-10 production, but with a concomitant progression of labor. Studies are currently underway to determine if cultured decidua, chorion, and/or amnion produce IL-10 and if IL-10 production by these tissues is regulated by inflammatory stimuli.

O 303 SOME NATURAL PRODUCTS ISOLATED FROM KOREA MEDICINAL PLANTS SIGNIFICANTLY INHIBITED THE IL-8/CINC BIOSYNTHESIS IN LPS-STIMULATED RAT MACROPHAGE. Kim, Youngsoo,[1]* Gyeong-Im Lee,[1] Sam Sik Kang,[2] Jae Sue Choi,[3] and Kyung Rak Min[1]. Chungbuk National University,[1] Seoul National University,[2] and National Fisheries University of Pusan,[3] SOUTH KOREA

Cytokine-induced neutrophil chemoattractant(CINC), a member of IL-8 family, was originally identified in the culture supernatant of IL-1-stimulated rat renal epitheloid cells(NRK-52E). The CINC is now known to occur at high levels in culture fluids of IL-1 or TNF-stimulated rat fibroblasts(NRK-49F) and LPS-stimulated rat macrophages. CINC biosynthesis was quantitated in the LPS-stimulated rat macrophages by using a sensitive ELISA. Steroidal antiinflammatory drugs including dexamethasone and prednisolone at 1 μM exhibited strong inhibition on the LPS-induced CINC biosynthesis, but nonsteroidal antiinflammatory drugs (aspirin, indomethacin, ibuprofen, and piroxicam) at the same concentration did not. Inhibitory effects of natural products (59 herbal extracts, 39 terpenoids, 37 flavonoids, 4 coumarins, and other 87 natural products) isolated from korea medicinal plants on CINC production in the LPS-stimulated rat macrophage have been estimated and will be presented in this symposium.

O 304 THE EFFECT OF BACTERICIDAL/PERMEABILITY INCREASING PROTEIN (BPI) ON LIPOPOLYSACCHARIDE (LPS)- AND *E.COLI*-INDUCED ACUTE INFLAMMATION IN THE RAT, Fred R. Kohn, Kirsten Dumont, Robert Magee, Robert Peterson and Ada H. C. Kung, XOMA Corporation, Berkeley, CA 94710.

Polymorphonuclear leukocytes (PMN) play a critical role in the host defense against bacterial infection. Among the PMN's granular arsenal is a 55 kD cationic protein, BPI, which binds/neutralizes LPS and is bactericidal against Gram-negative microorganisms. We have tested a recombinant 23 kD N-terminal fragment of BPI ($rBPI_{23}$) for its ability to inhibit LPS- and *E. coli*-induced acute inflammatory responses in the rat 7-day subcutaneous air pouch. When administered into the pouch immediately after LPS, $rBPI_{23}$ inhibited PMN accumulation in a dose-dependent manner. A control protein of similar M_r and isoelectric point was ineffective. The effect of $rBPI_{23}$ was specific; it did not inhibit PMN accumulation induced by carrageenan. $rBPI_{23}$ also prevented the LPS-induced elevation of TNF-α and nitrite (an end-product of nitric oxide formation) in the inflammatory exudate. When $rBPI_{23}$ administration was delayed for 15 minutes after LPS instillation, an inhibition of PMN accumulation was not observed. However, $rBPI_{23}$ significantly inhibited TNF-α elevation when treatment was delayed for 30 minutes. Two different strains of *E. coli* were also used as inflammatory stimuli: O7:K1, a smooth, encapsulated strain sensitive to $rBPI_{23}$-killing and O111:B4, a smooth, unencapsulated strain relatively resistant to $rBPI_{23}$. $rBPI_{23}$ did not inhibit the PMN accumulation induced by live or formalin-killed O7:K1 or O111:B4. In contrast, $rBPI_{23}$ prevented the 2 hour TNF-α elevation induced by these bacteria (live and killed) and the 6 hour nitrite elevation induced by killed O7:K1. The results indicate that $rBPI_{23}$ can completely abolish the acute inflammatory response induced by LPS and can inhibit the *E. coli*-induced production of several potentially harmful inflammatory mediators without preventing the host defense response (i.e., PMN accumulation) against the bacteria.

O 305 ANTISENSE JUN-B COUNTERACT TGF-β-MEDIATED INHIBITION OF COLLAGENASE GENE EXPRESSION. Alain Mauviel, Wei Dong, Yue Qiu Chen, and Jouni Uitto. Department of Dermatology, Jefferson Medical College, Philadelphia, PA 19107

We have examined the molecular mechanisms by which TGF-β inhibits cytokine-induced collagenase gene expression in human dermal fibroblasts in culture. This effect takes place, at least in part, at the transcriptional level, by inhibition of collagenase promoter activity. Also TGF-β inhibits *trans*-activation of multimeric TRE constructs, which implies an interaction with the AP-1 *trans*-activation pathway. Northern analyses indicate that TGF-β induces the expression of *jun-B* proto-oncogene, whereas it has no effect on either basal or cytokine-induced *c-jun* expression. This differential effect leads to elevation of the ratio *jun-B/c-jun* in TGF-β-treated cells. We show that over-expression of *jun-B* mimics TGF-β action on collagenase gene expression and on AP-1 *trans*-activation induced either by cytokines or by over-expression of *c-jun*. On the other hand, antisense *jun-B* constructs abolish TGF-β inhibition of collagenase gene expression. These results establish a key role for *jun-B* proto-oncogene in mediating the inhibition of fibroblast collagenase gene expression by TGF-β. Also our data indicate potential therapeutic utility of *jun-B*, which may be a candidate for gene therapy of disease states characterized by excessive connective tissue degradation.

O 306 Cytokine regulation of endothelial cell effector functions against an intravascular parasite, *Schistosoma mansoni*. I.P. Oswald, I. Eltoum, T.A. Wynn, B. Schwartz, P. Caspar, D. Paulin, A. Sher and S. L. James. Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD; ENV Maison-Alfort, France, Institut Pasteur Paris, France.

Like many pathogens that undergo an intravascular stage of development, larvae of the helminth parasite *Schistosoma mansoni* migrate through the blood vessels where they are in close contact with endothelial cells. *In vitro* exposure of murine endothelial cells to various cytokines (IFN-γ, TNF-α and IL-1 α or β) resulted in their activation to kill schistosomula through an arginine-dependent mechanism involving production of nitric oxide (NO). Such cytokine-treated endothelial cells showed increased expression of mRNA for the inducible form of the NO synthase, and both NO production and larval killing were suppressed by treatment with the inhibitors N-monomethyl-arginine or Nω-nitro-arginine. The effector function of cytokine-treated endothelial cells was similar to that of activated inflammatory tissue macrophages, although activation and down-regulation appeared to be differentially regulated in these two cell types. Activated endothelial cells killed older (18 day) forms of the parasite, such as those currently thought to be a primary target of immune elimination in the lungs of mice previously vaccinated with radiation-attenuated cercariae, as well as newly transformed larvae. In C57BL/6 mice, which become resistant to *S. mansoni* infection as a result of vaccination with irradiated cercariae, prominent endarteritis and periarteritis associated with hypertrophy of endothelial cells was observed in the lung by one to two weeks after challenge infection. Similar endothelial cell changes were absent in P strain mice, which do not become resistant as a result of vaccination. Human endothelial cells were also capable of killing schistosomula upon cytokine activation, although as previously observed with human macrophages, cytotoxicity appears independent of NO production. Together, these observations indicate that endothelial cells, not traditionally considered to be part of the immune system, may play an important role in immunity to *S. mansoni*, and could serve as effectors of resistance to other intravascular pathogens.

O 307 MODULATION OF PULMONARY INFLAMMATION IN TRANSGENIC MICE EXPRESSING RABBIT CRP . David Samols*, Dongyuan Xia*, Rita Heuertz•, Robert O.Webster• *Dept of Biochemistry Case Western Reserve University, Cleveland, OH 44106, •Dept. of Internal Medicine, St. Louis University, St. Louis MI.63110

C-reactive protein (CRP) is a prototypic acute phase reactant in humans and rabbits whose serum concentration typically increases several hundred fold following modest to severe inflammatory stimuli. Although its evolutionary conservation, in vitro interactions with inflammatory cells and the magnitude of the CRP response all suggest that CRP plays an important role in the response to inflammatory stimuli, the precise function of CRP is unknown. CRP behaves as a major acute phase protein in many vertebrates but is present in only trace amounts in the mouse even following inflammatory stimuli. To evaluate the role of CRP *in vivo*, we have generated transgenic mice which express rabbit CRP under control of the phosphoenolpyruvate carboxykinase promoter, permiting induction of CRP by dietary manipulation. In previous studies we demonstrated that transgenic mice expressing high levels of CRP were relatively resistant to lethal challenges from mediators of septic shock. Here we employed the animals to assess the affect of CRP on experimentally induced alveolitis. Either 1μg/75 μl C5a des arg or 10 ng/10μl LPS was instilled intratracheally into the lungs of two groups of transgenic mice; those expressing CRP at a level of 99 +/- 11 μg/ml or littermates expressing CRP at a level of only 9 +/- 2 μg/ml. Neutrophil percentage in bronchoalveolar lavage fluids was determined 6 hrs after instillation. With both inflammatory agents, transgenic mice with high plasma levels of CRP showed a diminished percentage of neutrophils in the inflamed lung lavage fluid. With C5a des arg, the reduction was from 40% +/- 5% to 16% +/- 4% and with LPS from 55% +/- 4.5% to 37% +/-3.3%. In the C5a lavage fluids, a reduction in total protein levels was also observed in the high CRP expressing animals (1653 +/- 417 μg/ml compared to 307 +/- 39 μg/ml). These data support the hypothesis that CRP modulates the inflammatory response in this model with consequent reduced neutrophil influx and protein exudation.

O 308 IMPAIRED NEUTROPHIL MIGRATION IN IL-8 TRANSGENIC MICE, W. Scott Simonet, Tamar M. Hughes, Hung Q. Nguyen, Margaret DeRose, Babru Samal, Lisa Trebasky, Eugene Medlock and Dimitry M. Danilenko, Departments of Developmental Biology, Experimental Hematology and Experimental Pathology, Amgen Center, Thousand Oaks, CA 91320

The proinflammatory chemokine interleukin-8 (IL-8/NAP-1) has been implicated in the recruitment of neutrophils to sites of acute and chronic tissue inflammation. The ability of human IL-8 to influence targeted neutrophil migration in mice was investigated in transgenic mice expressing IL-8 in specific tissues. Quantitative analysis of human IL-8 protein revealed elevated serum IL-8 levels ranging from 0 to 118 ng/ml in heterozygous offspring from eighteen different transgenic lines. The elevated serum IL-8 levels was correlated with a proportional increase in circulating neutrophils, with the highest expressing lines having 8- to 10-fold higher levels of peripheral blood Gr-1$^+$ cells, which were also LFA-1$^+$ and Mac-1$^+$. Bone marrow cellular architecture appeared normal in all transgenic lines analyzed. Immunohistochemical staining for myeloperoxidase in tissues from transgenic mice and their littermates revealed that neutrophils were accumulating in the microcirculation of vascularized tissues, with no evidence of neutrophil extravasation into tissues in any of the lines. Endotoxin-mediated neutrophil migration was severly inhibited in IL-8 transgenic mice, but not in nontransgenic littermates, when intraperitoneal or intratracheal injections of lipopolysaccharide were administered. These results suggest a therapeutic role for IL-8, and perhaps analogs and homologs of IL-8, in patients with acute noninfectious inflammatory diseases.

O 309 DEXAMETHASONE DOWN-REGULATES THE 85 kDa PHOSPHOLIPASE A_2 AND THE SIGNAL TO ITS ACTIVATION IN MACROPHAGES, Roger Sundler and Karin Gäfvert, Department of Medical and Physiological Chemistry, Lund University, P.O. Box 94, S-221 00 Lund, Sweden

There is now considerable evidence that a single, intracellular, 85 kDa phospholipase A_2 (PLA_2-85) is responsible for the mobilization of the eicosanoid precursor arachidonate, in macrophages. Among such evidence are the very similar acyl chain selectivity observed in intact macrophages and with purified PLA_2-85, the similar calcium sensitivity and the correlation between phosphorylation and upregulation of the catalytic activity of PLA_2-85 and the release of arachidonate in response to both protein-kinase-C-dependent and -independent signalling.

It has also been known since long that glucocorticoids inhibit the formation of eicosanoids and it has been suggested that inhibition of arachidonate mobilization accounts for this effect. Treatment of mouse peritoneal macrophages with ≥ 10^{-8} M dexamethasone, a glucocorticoid analogue, led to a progressive reduction in the level of PLA_2-85, down to 30-40 % of controls in 20 h. In addition to this down-regulation, the ability of phorbol ester and zymosan to cause enhanced phosphorylation and upregulation of the catalytic activity of PLA_2-85 was virtually abolished. Thus, dexamethasone not only reduces the level of PLA_2-85 in macrophages, but also affects one or more components in the signal chain that normally leads to regulatory phosphorylation and activation of the enzyme. In contrast, the activation of PLA_2-85 brought about by the protein phosphatase inhibitor okadaic acid was unaffected, indicating that the effect of this agent was exerted down-stream of the dexamethasone-sensitive step(s).

In conclusion, several different levels of regulation appear to be utilized in the control of PLA_2-85 and the mobilization of eicosanoid precursor in macrophages.

O 310 **IS THE DEVELOPMENT OF ACUTE LUNG INFLAMMATION AND AIRWAY HYPERRESPONSIVENESS EXACERBATED BY THE AVAILABILITY OF FREE IRON?** IS Tepper, JR Lehmann, DW Winsett, DL Costa[1], and AJ Ghio[2]. ManTech Environmental, Research Triangle Park, NC, and [1]U.S, EPA, Research Triangle Park, NC, and [2]Duke University, School of Medicine, Durham, NC.

Previous results indicated that the concentration of surface complexed iron on a particle was associated with to the *in vitro* ability of the particle to support electron transfer and generate oxidants. We hypothesized that intratracheally instilled particles with differing amounts of surface complexed iron (Fe^{3+}) would increase lung inflammation and airway hyperresponsiveness. Initially, three ambient air pollution particles, with differing amounts of surface complexed iron, were instilled into the lungs of male, 60d, Sprague Dawley rats. In a second experiment, one of the particles was acid-washed to remove surface iron. A third experiment looked at instillation of an inert particle with and without iron added to the particle surface. A fourth experiment evaluated animals pretreated with different pharmacological interventions to reduce reactive oxygen species. Acute inflammation was assessed by measuring lavageable total protein, lactate dehydrogenase and lung cell differential. Airway reactivity was evaluated in anesthetized, ventilated rats challenged with doubling doses of iv acetylcholine. Acute inflammation and airway reactivity were tested 96h after intratracheal instillation of the respirable sized particles in 0.3 mL of saline. The results indicate that acute inflammation and the amount of airway hyperresponsiveness observed were in direct relation to the amount of surface complexed iron on the particle. Acid washing to remove surface iron reduced the airway hyperresponsiveness. Preliminary results indicate that pretreatment with the xanthine oxidase inhibitor, allopurinol, partially blocked the inflammation and airway hyperresponsiveness, while desferrioxamine and an antibody to rat neutrophils were ineffective. (This abstract does not necessarily reflect EPA policy).

O 311 THE PREDOMINANT ROLE OF TNF-α IN HUMAN MONOCYTE IL-10 SYNTHESIS. Chingchai Wanidworanun and Warren Strober. Mucosal Immunity Section, Laboratory of Clinical Investigation, NIAID, NIH, Bethesda, Maryland 20892, U.S.A.
In prior studies it has been shown that the bacterial endotoxin lipopolysaccharide (LPS) induces an initial burst of inflammatory cytokine synthesis in human monocytes which is followed by substantial IL-10 production; the IL-10 then down-regulates the inflammatory cytokine production as well as IL-10 production itself. Therefore, IL-10 may contribute to the resolution of inflammatory response. Here we tested the hypothesis that IL-10 production in human monocytes is under control of one of the cytokines induced by LPS. Accordingly, we co-cultured purified human peripheral blood monocytes with a panel of cytokines including TNF-α, IL-1α, IL-1ß, IL-6, GM-CSF, TGF-ß and IFN-α and then measured IL-10 mRNA production using a semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) technique. We found that TNF-α had a major effect on IL-10 mRNA production, inducing a 20-120-fold increase over baseline production. In contrast, IL-1α, IL-1ß, IL-6, GM-CSF, TGF-ß and IFN-α had little effect (<3-fold). The induction of IL-10 mRNA by TNF-α in monocytes was dose-dependent and began between 8-24 h following the addition of TNF-α; this suggests that the increased IL-10 mRNA level was due to *de novo* mRNA synthesis rather than mRNA stabilization; this latter finding was corroborated by actinomycin-D time-course studies, which showed that the half-life of IL-10 was less than 1 hr and was not significantly altered by TNF-α. These studies concerning IL-10 mRNA induction by TNF-α were corroborated by studies of IL-10 protein secretion: TNF-α alone, but not IL-1α, IL-1β or IL-6 induces substantial IL-10 secretion; furthermore, LPS induces large amount of IL-10 secretion which is largely inhibited (50-75%) by anti-TNF-α, but not by antibodies to other inflammatory cytokines; finally TNF-α augments LPS-induced IL-10 secretion. Taken together, these findings suggest that TNF-α is unique among the inflammatory cytokines in its role as an inducer of IL-10 in human monocytes, as such, it induces a molecule that provides negative feedback to its own production, and the resolution of inflammation.

Late Abstracts

RECOMBINANT HUMAN TYPE 5 ADENOVIRUSES EXPRESSING INTERLEUKIN 6 UPON INFECTION IN VIVO ENHANCE THE ACUTE PHASE RESPONSE, Todd A. Braciak, Frank L. Graham, Carl D. Richards, Jack Gauldie, Department of Pathology, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

Interleukin 6 is a pleiotropic cytokine involved in the regulation of host immune and inflammatory responses. We have constructed two recombinant Human Type 5 Adenoviruses containing the murine IL-6 cDNA in the E1 and E3 regions of the genome (Ad5E1mIL6 and Ad5E3mIL6 respectively). These recombinant viruses were used to infect mice by the intraperitoneal route and their effects on hepatic acute phase protein synthesis were determined. Serum IL-6 levels were significantly raised in both Ad5E1mIL6 and Ad5E3mIL6 immunized animals versus controls. Accompanying the elevated serum levels of IL-6 was a marked increase in acute phase protein production, with α_1 acid glycoprotein and haptoglobin levels being raised to levels equivalent to that induced by LPS. This use of the recombinant human type 5 adenoviruses to investigate the biological function of IL-6 confirms the major importance of this cytokine in the regulation of the acute phase response in a novel in vivo model approach. (Supported by MRC and NCI Canada)

Anthony J. Lawrence, Salah Chettibi and *Robin D. Stevenson

Department of Cell Biology, University of Glasgow, Glasgow G12 8QQ
*Department of Respiratory Medicine, Royal Infirmary, Alexandra Parade, Glasgow G31 2ES, Scotland

The mechanism for the principal anti-inflammatory action of glucocorticoids remains highly controversial. The currently accepted hypothesis is that steroids inhibit the production, release or action of pro-inflammatory cytokines or stimulate the production of lipocortins (a family of phospholipase A2 inhibitors). We have identified a peptide factor released by human monocytes under the influence of glucocorticoids that cause neutrophils to polarise, but differs from all known neutrophil activating factors and the lipocortin family of proteins by its ability to stimulate persistent dispersive locomotion, whilst decreasing their adhesion to both endothelial cell monolayers and protein-coated glass coverslips. All of the biological responses of neutrophils to this factor were inhibited by pertussis toxin.

REGULATION OF C5a-MEDIATED INFLAMMATORY ACTIVITIES BY SITE-DIRECTED NEUTRALIZING ANTIBODIES SPECIFIC FOR THE HUMAN C5a RECEPTOR. E. L. Morgan*, J.A. Ember*, M. Parimucha*, S. D. Sanderson#, R. Buchner*, R. D. Ye*, and, T. E. Hugli*. *The Scripps Research Institute, La Jolla, CA , #Univ. of Nebraska, Omaha, NE.

The receptor for C5a (C5aR) has been described on many cells including PMN and Mø. Structural analysis of the C5aR sequence suggested that this protein shared features with members of the rhodopsin family. Hydropathy analysis of the C5aR sequence predicted the N-terminus to be a hydrophilic region of the molecule. Based on these results, a 21 residue peptide was synthesized [C5aR(9-29)] and used to raise polyclonal and monoclonal antibodies (Ab). Results indicated that Ab specific for C5aR(9-29) bound to human cells reported to express the C5aR. Flow cytometric analysis of PMN, Mø, the U937 line, murine L cell C5aR transfectants (C5aR.neo), and the Hep.G2 line indicated that anti-C5aR antibodies bound these cell types in a specific manner. Binding inhibition studies indicated that anti-C5aR antibodies inhibited ^{125}I-C5a binding to normal PMN. Moreover, anti-C5aR neutralized proinflammatory and immunoregulatory activities induced by human C5a. Anti-C5aR(9-29) blocked: 1) C5a-induced PMN chemotaxis; 2) C5a-induced enzyme release from PMN; and 3) C5a-induced cytokine synthesis (IL-6 and IL-8) from human Mø in vitro. Control studies indicated that, by itself, anti-C5aR(9-29) did not activate either PMN or Mø. We postulate that the Ab to the extracellular N-terminal region of C5aR interferes with ligand-receptor interaction by preventing C5a from binding to the receptor. These data suggest that anti-C5aR Ab may be useful in inhibiting ligand-receptor interactions and may provide an avenue for ameliorating the effects of C5a in inflammatory and autoimmune diseases.

T CELL SIGNALING OF MACROPHAGE ACTIVATION: ROLE OF T CELL COGNATE SIGNALING OF MACROPHAGE TNF-α IN THE INDUCTION OF MACROPHAGE EFFECTOR FUNCTION. Jill Suttles• and Robert D. Stout§, Departments of Biochemistry• and Microbiology§, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614.

Previous studies have demonstrated a role for autocrine TNFα stimulation in LPS induction of effector function in rIFNγ-primed macrophages. The current study was undertaken to determine if cognate signaling by T cells could induce TNFα production in macrophages. Murine rTNFα can synergize with rIFNγ in the activation of macrophage effector function. Human rTNFα is not effective in this regard on mouse macrophages, indicating that it is the species specific p75 TNFα receptor that mediates this effect. TNFα is critically involved in the induction of macrophage cytostatic activity as evidenced by the ability of anti-TNFα antibody to cause a 3-4 fold reduction in macrophage activation by either LPS + rIFNγ or by paraformaldehyde-fixed Th2 cells + rIFNγ. This inhibition is not due solely to neutralization of membrane-bound TNFα on the fixed T cells since a Th2 clone which displays no TNF or LT activity in the L929 cytotoxicity assay is equally active in signaling macrophage activation as T cell clones which do display TNFα activity. Furthermore, paraformaldehyde fixed Th2 cells can, in the absence of IFNγ, induce macrophage expression of TNFα mRNA and induce secretion of TNFα by the macrophages. These studies indicate that although Th1-derived secreted cytokines (e.g., TNF and IFNγ) may provide a means for non-cognate activation of macrophages, an alternate mechanism involving cognate signaling also exists. The cognate signals can induce macrophage production and secretion of TNFα, thus allowing the establishment of autocrine TNFα stimulation of effector function in IFNγ-primed macrophages.