**GENERAL INFORMATION**

**Office**

The Conference Office is located in the Sterling Hall of Medicine, Mezzanine. Messages may be left with the Yale University Conferences Services, 203/432-0465, FAX: 203-432-7345.

**On-Site Registration**

The scientific registration fee includes meals, entrance to the symposia and poster sessions and receipt of the Program/Abstract Volume. Guest registrants receive dinner Saturday through Tuesday only and are not entitled to breakfasts or lunches.

**Registration/Conference Office Locations and Hours**

\begin{itemize}
  \item **Holiday Inn at Yale:**
    \begin{itemize}
      \item Saturday, June 25
      \item 6:30 pm–8:30 pm
    \end{itemize}

  \item **Yale Univ., Sterling Hall of Medicine**
    \begin{itemize}
      \item Saturday, June 25
      \item 12:00 Noon–6:00 pm
      \item Sunday, June 26
      \item 8:00 am–5:30 pm
      \item Monday, June 27
      \item 8:30 am–5:30 pm
      \item Tuesday, June 28
      \item 8:30 am–1:30 pm
    \end{itemize}
\end{itemize}

**Shuttle Bus Service**

Shuttle bus service will be available from the Holiday Inn at Yale Hotel and Calhoun College (dormitories) to the Commons Dining Hall and Sterling Hall of Medicine (Harkness Auditorium). The Commons Dining Hall is approximately 6 city blocks from the Sterling Hall of Medicine. Buses will pick-up at the main entrance of each facility promptly at the times indicated below.

**Saturday**

\begin{itemize}
  \item 6:15 pm Hotel to Calhoun to Harkness
  \item 9:45 pm Harkness to Hotel
  \item 9:15 pm Harkness to Calhoun
\end{itemize}

**Sunday**

\begin{itemize}
  \item 7:15 am Hotel to Commons
  \item 8:45 am Commons to Harkness
  \item 5:00 pm Harkness to Commons
  \item 7:15 pm Commons to Harkness
  \item 10:15 pm Harkness to Calhoun to Hotel
\end{itemize}

**Monday**

\begin{itemize}
  \item 7:15 am Hotel to Commons
  \item 8:45 am Commons to Harkness
  \item 5:45 pm Harkness to Calhoun to Hotel
  \item 6:45 pm Hotel to Commons
\end{itemize}

**Tuesday**

\begin{itemize}
  \item 7:15 am Hotel to Commons
  \item 8:45 am Commons to Harkness
  \item 5:00 pm Harkness to Calhoun to Hotel
  \item 6:15 pm Hotel to Calhoun
  \item 10:00 pm Calhoun to Hotel
\end{itemize}

**Meals**

Campus Dining Staff will require attendees to surrender vouchers at each meal. Registrants should pick-up meal tickets at the Conference Office, Sterling Hall of Medicine, Mezzanine. Please refer to the program for the location and time of each meal.

**Program/Abstract Volume**

The June issue of *The Physiologist* contains the contributed and speaker abstracts and program for the conference. Advance registrants were sent a pick-up card which may be exchanged at the Registration Desk for a copy of the volume. Replacement copies may be purchased for $20.00.
PROGRAM

APS Conference

Physiology of the Activity and Release of Cytokines

Daily Schedule

Saturday, June 25

Social
1.0 Welcoming Remarks and Buffet Supper
Chair: John T. Stitt
6:30 PM—Harkness Lawn

Plenary Lecture
2.0 The Ubiquity and Diversity of Cytokines in the Body
8:30 PM—Harkness Auditorium
Speaker: Charles A. Dinarello, Tufts University

Sunday, June 26

Breakfast
7:30 AM—Commons Dining Hall

Symposium
3.0 Cytokines and Homeostatic Mechanisms
Chair: Matthew J. Kluger, The Lovelace Insts.
9:00 AM—Harkness Auditorium

9:00 Cytokines and Hormone Interactions. Adriana Del Rey. Philipps Univ., Marburg, Germany.
9:40 Cytokines and Behavior. Robert Dantzer. INSERM, Bordeaux, France.
10:20 Break.

Lunch
12:30 PM—Harkness Cafeteria

Symposium
4.0 Mechanisms of Cytokine Regulation
Chair: Gordon W. Duff. The Hallam Royal Hospital, Sheffield, UK.
1:30 PM—Harkness Auditorium

2:50 Break.
4:00 Rearrangement of T-cell Receptor Genes: Regulation and Mechanism. Scott Durum. NCI, NIH, Frederick, MD.

Dinner
5:30 PM—Commons Dining Hall

Poster Sessions
Posters are on display 8:30 AM Sunday through 6:00 PM Monday. Authors are in attendance for one hour at the times indicated.

5.0 Cytokines and Host Defense, Inflammation and the Acute Phase Response
7:30 PM—Harkness Lounge

Board #

1 7:30 5.1 Human Fcγ receptor II regulates IL-1 receptor antagonist production. C.B. Marsh, C.L. Anderson, and M.D. Wewers. Ohio State Univ.
3 7:30 5.3 Interactions of nuclear factor IL-6 with the long terminal repeat of the human immunodeficiency virus type 1. V.M. Tesmer and M. Bina. Purdue Univ.


Detection of endothelin peptide and receptor gene expression in bovine cornual epitheli-
11:00 Possible Involvement of IL-1α and IL-1β in Arthritis—Studies in Animals. Ivan G. Otterness. Pfizer Inc.


Lunch
12:30 PM—Harkness Lounge

Poster Sessions
Posters are on display 8:30 AM Sunday through 6:00 PM Monday. Authors are in attendance for one hour at the times indicated.

9.0 Cytokines and Metabolic/Endocrine Interactions Including Reproduction

7:30 PM—Harkness Lounge

Board #

22 1:30 9.3 Production of IL-1β and TNFα by PBMC does not decline with age. R. Roubenoff, T.B. Harris, J.G. Cannon, L. Abad, P.W.F. Wilson, and C.A. Dinarello. USDA at Tufts Univ., NIA/NIH, and Framingham Heart Study, Framingham, MA.
26 1:30 9.7 Regulation of insulin-like growth factor content and binding proteins by IL-1β. C.H. Lang, A.G.S. Baillie, J. Fan, and M.C. Gelato. SUNY, Stony Brook.

10.0 Cytokines and the Central Nervous System

7:30 PM—Harkness Lounge

Board #

29 2:30 10.2 Central injection of IL-1α increases both hepatic glucose production and peripheral glucose uptake. P.E. Molina, N.N. Abumrad, and C.H. Lang. SUNY, Stony Brook.
31 2:30 10.4 Sleep patterns in healthy and influenza-infected mice are correlated with alleles of the If-1 gene. L.A. Toth. St. Jude Children’s Hosp., Memphis.
33 2:30 10.6 Inhibition of T cell function in patients with glioblastomas: a selective impairment of the IL-2 system. C. Brodie, A. Tsukerman, E. Ashkenazi, M. Deutsch, R. Tiros, and A. Weinreh. Bar-Ilan Univ, Ramat-Gan, Israel.
11.0 Methodological Issues in Cytokine Measurement

1:30 PM—Harkness Lounge

**Board #**

38 1:30 11.1 Pro-IL-1β is released from monocytes in vitro in a form that is resistant to processing by IL-1β converting enzyme. **M.D. Wewers and H.A. Pope.** Ohio State Univ.


**Workshop**

12.0 Cytokine Workshop

Chair: **Joseph G. Cannon.** Penn State Univ.

3:30 PM—Harkness Auditorium

3:30 Measuring Cytokines: The Jurassic Park Syndrome. **Joseph G. Cannon.** Penn State Univ.

4:30 IL-6 as a Marker for Treatment after Septic Shock. **Tom Emerson.** Miles Inc.

**Conference Banquet**

7:00 PM—Commons, Presidential Room

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**Tuesday, June 28**

Breakfast

7:30 AM—Commons Dining Hall

**Symposium**

13.0 Cytokine Networks in the Body

Chair: **Joseph G. Cannon.** Penn State Univ.

9:00 AM—Harkness Auditorium

9:00 Cytokines and the Pathogenesis of Fever. **Matthew J. Kluger.** The Lovelace Insts.

9:40 Transport of Cytokines across the Blood-Brain Barrier. **William Banks.** Tulane Univ.

10:20 Break.

11:00 Interactions Between Cytokines and Neuropeptides in the Brain. **Nancy Rothwell.** Manchester Univ., UK.

11:40 Cytokines/Hypothalamic-Pituitary-Adrenal Axis Interactions. **Akira Arimura.** Tulane Univ., Belle Chasse, LA.

**Lunch**

12:30—Harkness Lounge

**Symposium**

14.0 Inhibitors of the Actions of Cytokines

Chair: **Ivan G. Otterness.** Pfizer Inc.

1:30 PM—Harkness Auditorium

1:30 IL-1 Receptor Antagonist. **Robert Thompson.** Synergen Inc., Denver, CO.

2:10 Antisense Oligonucleotide Development as Therapeutic Agents. **Ben Tseng.** Genta Inc., San Diego, CA.

2:50 Break.

3:20 TNF Receptors and Antagonists. Perspectives of New Therapeutic Perspectives. **Werner Lesslauer.** Hoffmann-LaRoche Ltd., Switzerland.

4:00 Effects of Small Molecules on Cytokine Production and Responses. **Katherine L. Molnar-Kimber.** Wyeth Ayerst Res.

**Barbecue**

6:30 PM—Calhoun Courtyard

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**JUST APPROVED!**

1996 APS Conferences

pHysiology of Acid-Base Regulation: From Molecules to Humans

Neural Control of Breathing: Molecular to Organismal Perspective

1996 Intersociety Meeting

The Integrative Biology of Exercise
## Sessions with Contributed Abstracts

### Invited Speaker Abstracts

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### Poster Abstracts

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Cytokine and Hormone Interactions

Adriana del Rey
Institute of Physiology, Philippus University, Marburg, Germany.

Cytokines are considered at present among the most relevant mediators of immunologically-derived signals within the complex network of interactions between the immune and neuroendocrine systems. In fact, several cytokines behave as true hormones, and the possibility that they also play a rôle as neurotransmitters is being explored. Furthermore, other, non-immunological sources of cytokines have been identified. Apart from the important functions that cytokines exert during the development of an immune response, other effects, namely, the neuroendocrine and metabolic effects that they mediate, constitute an essential part of the resultant pattern of changes and alterations that are observed during pathological processes such as infections, inflammation and neoplasia. Although reports about non-immunological sources and non-immunological actions of many cytokines are rapidly cumulating, probably the most studied lymphokines and monokines in this respect are IL-1, IL-6, TNFα (for review see 1, 2).

The capacity of IL-1β, IL-6 and TNFα to stimulate the pituitary-adrenal axis is now well established. IL-1, for example, induces a quick and profound increase in ACTH and corticosterone blood levels without markedly affecting the blood levels of other "stress hormones" such as catecholamines, prolactin, growth hormone and α-MSH. These endocrine effects of IL-1 are integrated at the levels of the hypothalamus, since in vivo injection of IL-1 results in increased CRF release in the portal system and blockade of CRF release impedes IL-1 induced increase in ACTH and corticosterone blood levels. This effect could be triggered by the binding of IL-1 to its receptors in the CNS, or indirectly through the induction of other factors without the need of the presence of the cytokine in the CNS. Recently, we have found that blockade of IL-1 receptors in the brain by intracerebral ventricular injection of the IL-1 receptor antagonist protein (IRAP), significantly reduces the increase in corticosterone blood levels observed following systemic administration of the cytokine. Comparable results were obtained when LPS was used to induce the endogenous production of IL-1. These results indicate that the
functional presence of IL-1 in the brain is necessary to induce the mentioned endocrine change.

IL-6 and TNFα can also stimulate the hypothalamus-pituitary-adrenal (HPA) axis. However, higher doses of these cytokines are needed to induce an increase in glucocorticoid blood levels. Based on time kinetic studies, it is not possible to exclude that the effect of IL-6 and TNF is not secondary to the induction of IL-1 release.

In certain parameters, however, the effects of IL-1β and TNFα might be just opposing. For example, it has been reported that TNFα may increase insulin-resistance. Conversely, IL-1 is capable of inducing a profound and long lasting hypoglycemia, which is not due to insulin secretagogue actions of this cytokine, in normal and in insulin-resistant animals. No similar effects are observed following administration of IL-6. The decrease in glucose blood levels induced by IL-1 in insulin-resistant mice is paralleled by normalization of the altered noradrenaline levels in the brain and in the spleen that these genetically diabetic mice display. Furthermore, injection of IL-1 into normal mice followed by a glucose load, results also in hypoglycemia, showing that this cytokine is capable of affecting the set point of glucose homeostasis.

The data discussed shows that cytokines, apart from playing a crucial role during the immune response, can affect neuroendocrine mechanisms essential for general homeostasis. However, most of the studies dealing with neuroendocrine and metabolic effects of cytokines are based on the exogenous administration of these substances. In the majority of the cases, it still remains to be shown that, under more physiological conditions or during naturally occurring pathological situations, these cytokines are in fact the true mediators of these changes. Now that cytokine antagonists, blockers and soluble receptors are becoming available, we believe that this type of studies will comprise a major part of the work to be performed in the near future in this field.

Non specific symptoms of infection include fever and profound psychological and behavioural changes. Sick individuals experience fatigue, malaise, listlessness and inability to concentrate. They also show hypersomnia, anorexia, depressed activity and loss of interest in their surroundings and social environment. These non specific changes are collectively termed "sickness behaviour" and, like fever, they play a crucial role in the adaptation of the host to infection (4). Using pharmacological tools in the form of recombinant cytokines and their antagonists, we have demonstrated that sickness behaviour is mediated by the central effects of proinflammatory cytokines that are released by activated accessory immune cells during infection and inflammation (7). Furthermore, using biochemical and physiological approaches, we have obtained evidence suggesting that the development of sickness behavior is dependent on the induction of cytokines in the brain in response to peripheral immune stimuli.

Peripheral or central injections of interleukin-1 (IL-1α, IL-1β) and tumor necrosis factor (TNFα) induce sickness behaviour in mice and rats, in the form of reduced social interest and decreased food-motivated behaviour (7). In general, smaller quantities of recombinant cytokines are necessary to induce these behavioral changes when the injection is made directly into the lateral ventricle of the brain (icv) than when it is given at the periphery (ip). Although this difference in the range of active doses is usually interpreted in terms of a central site of action for the treatment under investigation, it is important to note that depression of social behavior and reduction of food-motivated behavior do not share the same time course in response to centrally injected IL-1. Depression of social behavior appears earlier and lasts for a shorter time in response to icv IL-1 than in response to ip IL-1. In contrast, reduction of food-motivated behavior takes longer to appear after icv than after ip injection of IL-1. These findings already indicate that these two aspects of sickness behaviour are mediated by different mechanisms. This is further substantiated by the demonstration that icv administration of a specific antagonist of IL-1 receptors, IL-1ra, at a dose that antagonizes the depressing effects of icv IL-1 on social exploration and food-motivated behavior, fully blocks the effects of ip IL-1 on social behavior but attenuates only partially the effects of ip IL-1 on food-motivated behavior (6). Another piece of evidence for the involvement of different mechanisms in the effects of cytokines on social exploration and food-motivated behavior comes from the demonstration that ip IL-1ra blocks the depressing effects of the active fragment of endotoxin, lipopolysaccharide (LPS), on social behavior but has no effect on LPS-induced decrease in food-motivated behavior (1, 5).

The demonstration that blockade of central receptors of IL-1 by icv IL-1ra is able to antagonize the effects of ip injected IL-1 can be interpreted to suggest that IL-1 acts centrally to depress social behavior. However, this result by itself does not allow to trace the origin of centrally active IL-1. IL-1, like other cytokines is not supposed to enter the brain, except in a very limited manner at places where the brain lacks a blood-brain barrier (7). This is the case for circumventricular organs like the organum vasculosum of the lamina terminalis which has been proposed to be the main site of action for the pyrogenic activity of cytokines. Another possibility is the existence of specialized transport systems that enable cytokines to cross the blood-brain barrier. This last possibility is mainly based on pharmacokinetic data but the chemical nature of specific transport systems remains to be determined.

Based on the fact that most cytokines act as local communication signals rather than as hormones and cytokines are expressed in the brain both constitutively and in response to peripheral immune
stimuli, as demonstrated by molecular biology techniques (8) and immunocytochemistry, we proposed that peripheral immune stimuli cause sickness behavior by inducing the synthesis and release of cytokines in the brain (4, 7). Although this hypothesis is consistent with the observation that icv injection of IL-1ra blocks the behavioural effects of peripherally injected IL-1, it gives no indication of the way peripheral immune stimuli are transmitted to the brain. An obvious pathway of communication from the immune system to the brain is represented by primaryafferent nerve endings which are known to transmit to the brain the two main sensory components of inflammation, *dolor* and *calor* (7). If peripheral immune stimuli are transmitted to the brain via activation of primary afferent nerve endings, they should increase the expression of sensory neuropeptides in the corresponding neural pathways. This was found to be the case since ip injection of a behaviorally active dose of LPS in mice increased the levels of substance P, neurokinin A and calcitonin gene related peptide in the spinal cord and this increase was abrogated by pretreatment with indomethacin, at a dose which blocked the behavioral effects of LPS (3).

The role of this neural message in the induction of sickness behavior was further substantiated by the demonstration that in rats and mice, subdiaphragmatic section of the vagus nerve which represents the main afferent pathway from the abdominal cavity to the brain blocks the depressing effects of ip LPS on social behavior (2). This was not due to a decreased ability of the host to mount an inflammatory response to LPS since plasma and macrophage levels of IL-1 in LPS-treated animals were not altered by vagotomy (2). The possibility that the neural message conveyed to the brain by primary afferents is responsible for the increase in the expression of cytokines in the brain that is observed in mice treated ip with LPS (8) is currently under investigation. Microglia cells and meningeal and perivascular macrophages are likely to be the main sources of centrally produced cytokines in response to peripheral immune stimuli. Whether these cytokines act directly on neurons or indirectly, via other glial cells such as astrocytes, remains to be determined.

In conclusion, the mechanisms by which peripherally released cytokines affect brain functions and induce sickness behavior begin to be understood. Peripheral immune stimuli are transduced into a neural message which is conveyed to the brain by primary afferent nerves. This message leads to the local synthesis and release of cytokines. The way the specificity of the immune message is conserved during this entire process has still to be deciphered but it opens fascinating perspectives for the understanding of the communication pathways between the immune system and the brain.

References
CYTOKINES AND VASCULAR SMOOTH MUSCLE
Debbie Beasley, Ph.D., New England Medical Center, Boston, MA

Vasodilatation is a major feature of infection and inflammation, occurring systemically during sepsis, and locally at sites of infection or inflammation. The central hypothesis to be presented is that direct effects of the pro-inflammatory cytokine, interleukin-1 (IL1), on vascular smooth muscle cells (VSMC) mediate vasodilatation associated with septic shock, as well as local infection and inflammation. While IL1 can affect the release of vasodilatory mediators from the endothelium, vasodilatory effects of IL1 demonstrated on animal blood vessels in vitro are independent of the endothelium, suggest direct effects of IL1 on VSMC are important. There is evidence from animal studies supporting roles of VSMC-derived nitric oxide (NO) and prostanoids in IL1-induced vasodilatation. IL1 inhibits contraction of rat and rabbit aorta by a pathway which involves expression of inducible NO synthase activity resulting in prolonged activation of soluble guanylate cyclase, and elevation of cGMP (1-3). However, the hypotension induced by i.v. administration of IL1 in rabbits is dependent on prostanoids, and IL1 causes prostanoid-dependent relaxation of isolated rabbit mesenteric arteries, which is associated with increased production of PGI2 and PGE2 (4). Studies to be presented will address the effects of IL1 on vasodilatory mediators in human VSMC.

In vivo, VSMC function may be affected by IL1 produced and released by multiple cellular sources, including monocytes, macrophages, and endothelial cells. Circulating IL1 produced by blood monocytes may gain access to the VSMC at sites of endothelial denudation or damage. Macrophages which become activated during sepsis may cross the endothelium into the vascular wall and release IL1 locally. Alternatively, VSMC may be affected by IL1 from macrophages which are resident within atherosclerotic lesions. Endothelial cells may release IL1 into the medial layer of the blood vessel following activation by circulating IL1 or tumor necrosis factor (TNF), by bacterial products such as lipopolysaccharide (LPS), or by exposure to other stresses including hypoxia/reoxygenation or shear stress.
Vascular smooth muscle cells themselves also produce IL1. A second central hypothesis to be discussed is that IL1 produced by VSMC acts as an autocrine regulator of VSMC contractility via effects on both NO and prostanoid production. Studies which employ specific radioimmunoassays to characterize production of both members of the IL1 family of proteins (5), IL1α and IL1β, by VSMC will be discussed. In VSMC, as in other cell types, most of the IL1α produced remains in the cytosol, or is transported to the cell surface and associated with the membrane. IL1β, on the other hand, is released by the cell. The regulation of VSMC-associated IL1α production and its possible roles in VSMC function will be discussed. Finally, the IL1 family of proteins also includes IL1 receptor antagonist (IL1ra), a specific inhibitor of IL1 action. IL1ra is a secreted protein, which is produced by activated monocytes, macrophages and neutrophils (6). The role of IL1ra as a regulator of IL1 responses in VSMC will be discussed.

References

Effects of Cytokines on Sleep Patterns

James M. Krueger, Ph.D.
Department of Physiology and Biophysics
University of Tennessee, Memphis

Excessive sleepiness and fever are often experienced during systemic infections. Although fevers have been documented for many years, the measurement of sleep over the course of an infection was only recently reported. Inoculations of animals with bacterial, viral, protozoan and fungal organisms and man with virus result in complex sleep responses dependent upon the infectious agent and route of administration. The general response is characterized by an initial period enhanced sleep; this is followed 6-48 hr later by a period of reduced sleep. Bacterial products likely responsible for sleep and fever responses include muramyl peptides and endotoxin. Viral double-stranded (ds)RNA also induces sleep and fever in animal models. The exact mechanisms of how these structurally diverse microbial products elicit sleep and fever remain unknown, although these substances share the ability to enhance cytokine production. Several cytokines are somnogenic whether given intravenously (IV) or intracerebroventricularly (ICV); the list includes interleukin-1 (IL-1) α and β, tumor necrosis factor (TNF) α and β, and interferon-α (IFN-α). Other cytokines, IL-2, IL-6, and IFN-β do not affect sleep although IL-6 is pyrogenic. In rabbits the IL-1-IV dose required to elicit excess sleep is only about 15-20 times the effective ICV dose. In contrast, for muramyl peptides about a 5,000-fold excess is needed IV vs. ICV. This suggests the possibility that cytokines may be transported from blood to brain and/or that there is some type of amplification system at the blood-brain barrier. The major effect of cytokines is to enhance the duration of slow-wave sleep (SWS). The intensity of SWS is also increased as evidenced by enhanced amplitudes of EEG slow waves. Similar supranormal slow waves occur after sleep deprivation. Typically, after cytokine administration, excess SWS is observed for 2-10 hr. depending upon dose and route of administration; e.g., rabbits normally sleep about 45% of the time between 9:00 a.m. and 3:00 p.m.; after a 20 ng dose of IL-1 β SWS will occupy about 65% of this time. In contrast to SWS, rapid cyc movement sleep (REMS) is inhibited by high, but not low, somnogenic doses. Sleep and fever responses to cytokines can be separated, e.g., low doses of IL-1 elicit sleep but not fever in rats and antipyretics block IL-1-induced fevers but not sleep. There is also evidence that cytokines have a role in physiological sleep, thus, antibodies to IL-1β or TNFα, a soluble TNF receptor or the IL-1 receptor antagonist reduce normal sleep. Further, anti-IL-1β attenuates sleep rebound after sleep deprivation. The mechanisms by which cytokines elicit sleep remains unknown. The somnogenic actions of IL-1 are independent from prostaglandins, opioids and insulin. In contrast, corticotropin releasing hormone, α-melanocyte stimulating hormone (α-MSH) and inhibition of NO production block IL-1β-induced sleep. Finally, infection, endotoxin, IL-1 and TNF induce growth hormone (GH) release, probably via GH releasing hormone (GHRH). GH release is linked to SWS and GHRH is somnogenic in rats, rabbits and man. Anti-GHRH blocks IL-1-induced GH release and IL-1-induced sleep and fever responses. In conclusion, cytokines are likely key mediators of sleep and fever responses to infection. This microbial-cytokine altered sleep probably results from an amplification of physiological sleep mechanisms which include cytokines and several neuropeptides.

Reviews:


IDENTIFICATION OF NOVEL SIGNAL TRANSDUCTION PROTEINS OF MACROPHAGE ORIGIN. Bruce Beutler, M.D., Howard Hughes Medical Institute, 5323 Harry Hines Blvd., Dallas, TX 75235-9050.

Macrophages responses to LPS and other invasive stimuli depend upon activation of signalling pathways, the molecular details of which are, in most cases, still obscure. One endpoint of activation is cytokine gene expression. Regulation of tumor necrosis factor (TNF) biosynthesis has been intensively studied, and is known to involve both transcriptional and translational components. Certain drugs block either or both aspects of the pathway. For example, phosphodiesterase inhibitors, and other agents that elevate intracellular cAMP concentration, impose transcriptional blockade. Drugs of the pyridinyl imidazole class selectively block translation. Glucocorticoid agonists impede both transcriptional and translational activation, suggesting that they may exert their effects at a very early step in the signal transduction pathway.

An early and important event in signal transduction is dependent upon the product of a single gene, known in mice as the Lps gene. Defective responses to LPS result from mutations at this locus, and a directed strategy toward isolation of the gene may yield important information about early events in signalling. As to late events, it is clear that NF-κB translocation must occur to allow TNF biosynthesis, and suggestive evidence implicates the ras/raf/erk pathway, in both transcriptional and translational responses.

Many other proteins, however, are undoubtedly involved in responses to LPS and other stimuli. In an effort to identify novel signal transduction proteins, we have employed a strategy based on selective hybridization screening. We noted that while RAW 264.7 mouse macrophages normally respond to LPS, hybrids created by fusing RAW 264.7 cells to NIH 3T3 cells fail to transduce the LPS signal. A discrete collection of genes, normally active in macrophages, are extinguished following fusion. We reasoned that among the population of genes that are extinguished, several genes encoding signal transduction components might be identified.

From a screening of 20,000 cDNA clones plated from a RAW 264.7 cell library, we identified 66 clones that were suppressed on fusion with NIH 3T3 cells. Of these, 26 were identified by pilot sequence analysis. 15 of the identifiable clones encoded structural proteins or enzymes, many of which are known to be highly represented in macrophages. The remaining seven known clones encoded signalling proteins or cytokines.
Among 40 clones that could not be identified by pilot sequence analysis, eight were sequenced in entirety. Four encoded proteins with no known structural homology. However, one entirely new cytokine was identified, as well as three candidate signalling molecules. The new cytokine cDNA encodes a close homologue of MIP-1α, MIP-1β and C10, all members of the C-C chemokine group. The signalling candidates encompass a novel G-protein, a methyltransferase, and a zinc finger protein, none of which have close homologues in vertebrates.

Interestingly, while cytokine mRNAs typically exhibit enhanced expression following LPS activation of the cell, mRNAs encoding the candidate signal transduction molecules are rapidly suppressed in response to LPS. This may correspond to the observation that macrophages become refractory to LPS challenge following initial exposure to the agent.

Using antisense RNA expression, we are currently attempting to study the importance of these proteins in LPS signal transduction.
CYTOKINE GENETICS

Gordon W. Duff MD PhD
Department of Molecular Medicine
University of Sheffield, UK

Cytokines are extracellular signalling molecules that influence the proliferation, migration and behaviour of many cell types. More than 100 cytokines have been characterised and they are (glyco)proteins within the size range Mr 5-50kD approximately. Cytokines act on cells via surface receptors of which there are several families that share common features. The physiological role of the cytokine system seems to be in host defence. Thus cytokines control the development of leukocyte lineages, activate inflammatory/immune mechanisms and participate in the repair and remodelling of damaged tissue.

It has now been established that cytokines also play important pathological roles in infectious, inflammatory and immune diseases. For example, in rheumatoid arthritis Interleukin 8 (IL8) seems to be a major chemotactic factor in recruiting inflammatory cells into the joint. IL1 and TNF activate pro-inflammatory enzyme systems (cyclo-oxygenase, nitric oxide synthase) and cause the breakdown of collagen and proteoglycan in cartilage and bone. With IL6 and LIF, IL1 and TNF also stimulate fever by a CNS mechanism and the acute phase response in liver cells leading to the rapid elevation of acute phase proteins in blood.

In health, the actions of these powerful mediators are held in check by endogenous inhibitory molecules such as the IL1 receptor antagonist and soluble cytokine binding proteins derived from cell surface receptors. In diseases, the actions of some cytokines appear to be unopposed, giving rise to the idea that cytokine inhibitors control the progression to chronic inflammation.

Recent work from several laboratories has shown that cytokine genes are polymorphic in animals and man. Several such polymorphisms have been characterised at the level of DNA sequence and include variable tandem repeat sequences and single base substitutions in flanking DNA and within
the introns and exons of cytokine genes. With these genetic markers it has been possible to test associations between cytokine genes and some of the common inflammatory diseases.

For example, TNF alpha is highly associated with the MHC haplotype A1, B8, DR3, DQ2 on chromosome 6. The TNFα polymorphism linked with this haplotype is a single-base transition at 308 from the transcription start site.

The rarer allele, TNF2, is associated with the same diseases as DR3 because of the haplotypic phenomenon but TNF2 may also play some independent role in disease pathogenesis since reporter gene assays show that it can function as a much stronger transcriptional promoter than TNF1.

On chromosome 2, within the IL1 gene cluster, the IL1 receptor antagonist gene also has a disease-associated allele. This is a variable number of tandem repeats of an 86bp stretch of DNA in intron 2. Allele 2 of the IL1 receptor antagonist polymorphism is associated with chronic inflammatory diseases of epithelial tissues such as psoriasis, alopecia, scleroderma, ulcerative colitis and autoimmune thyroiditis. Another gene of the IL1 family, IL1 alpha, is associated with the pauciarticular type of juvenile chronic arthritis, and particularly with the complication of inflammatory eye disease (chronic iridocyclitis). A single base transition in the 5' flanking region of IL1β produces an allele that is associated with erosive joint changes in early rheumatoid arthritis.

Thus, cytokine gene polymorphisms are being associated with many chronic inflammatory diseases. Whether these DNA variants are intra-genic markers associated with gene function or chromosomal markers for other linked genes has not yet been established. The picture that is emerging, however, seems to be that cytokine gene polymorphisms are markers for inflammatory disease severity and clinical outcome rather than susceptibility to disease. Whether similar observations will be made in infectious diseases remains to be seen.

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Regulation of Cytokine Receptor Expression and Activity

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Physiological responses to infection and injury include recruitment of a variety of cells of the lymphomyeloid system from the systemic circulation to local sites of injury and their activation at those sites by signals provided both by foreign antigens and by a variety of endogenous regulators. Resolution of the local response involves killing of pathogens and of damaged or pathogen infected cells, and degradation of existing extracellular matrix. Subsequently, proliferation of connective tissue elements such as fibroblasts, deposition of new extracellular matrix and angiogenesis regenerates the damaged tissues. Concurrently, a number of systemic responses occur. These may include increases in body temperature (fever), release of acute phase proteins from the liver, changes in leukocyte trafficking patterns and increases in the rates of proliferation and differentiation of bone marrow precursors of mature leukocytes. All aspects of these processes are under the control of a class of polypeptide mediators referred to variously as cytokines, interleukins, colony stimulating factors and certain growth factors. These polypeptide hormones play a central role both in controlling host responses to injury and infection and also in some cases in normal homeostasis.

The biological effects of cytokines and interleukins are exerted through cell surface receptors. In the last five years, many of these receptor molecules have been characterized by molecular cloning. In two instances three dimensional structures have been determined for receptor-hormone complexes. These studies have allowed us to classify the receptors into several well defined families. The largest group of receptors form what has been termed the hemopoetin receptor family, this includes the receptors for, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, GCSF, GMCSF, LIF, oncostatin M, ciliary neurotrophic factor, growth hormone and prolactin, and one receptor like molecule (cMPL) for which a ligand has not yet been identified. Based on sequence alignments, this family is characterized by an element of approximately 200-220 residues composed of two domains of 100 residues each with detectable homology to one another. Within each 100 residue domain there are a number of conserved cysteine residues, and most characteristic of the family the C-terminal domain of the pair, particularly if proximal to the membrane, contains a motif WSXWS. The tumor necrosis receptors define a second family of related molecules characterized by a 40 residue cysteine-rich domain which repeats several times in the extracellular region of the receptors. This family includes TNFRp80, TNFRp60, TNFRp, CD40, CD30, CD27, OX40, 41BB, the low affinity NGFR and Fas. The majority of the ligands for this family of receptors are type II integral membrane proteins, the majority of which express their activities in this form. Indeed the two TNFs, tumor necrosis factor and lymphotoxin are unusual in this system in that the most physiologically relevant forms are soluble trimers. A third group of cytokine receptors share an element common in many growth factor receptors, namely a cytoplasmic region that possesses endogenous protein tyrosine kinase activity, this group includes the receptors for CSF-1 (c-fms) and mast cell growth factor (c-kit). These receptor are closely related to the platelet derived growth factor receptors. A fourth family is defined by the IL-1 receptors, type I and type II, each of which contains an extracellular ligand-binding region composed of a series of three immunoglobulin-like domains, a third receptor-like molecule (ST2) has recently been found which is structurally related to these two, since this molecule does not bind any of the three forms of IL-1, it may interact with other as yet unidentified IL-1-like molecules. In addition alignment of the cytoplasmic regions of IL-1RI,
ST2 and the related Drosophila protein TOLL, shows a significant level of sequence homology suggesting a conserved signaling mechanism between these molecules. Finally, the group of small inflammatory cytokines or chemokines of which IL-8 can be taken as prototypic, all likely act on cells through receptors which are member of the serpentine receptor family, having seven membrane spanning regions and acting as major regulators of heterotrimeric G protein activity.

In the majority of the systems mentioned in the above survey, the receptor chains identified by cloning when expressed in recombinant form do not mimic the natural receptors, instead binding ligands with much lower affinities. This discrepancy arises because most of the natural receptors are hetero-oligomeric structures. Thus the receptor for IL-2 contains three different chains IL-2Rα, IL-2Rβ and IL-2Rγ. It is also becoming clear that these chains are not uniquely assigned to one receptor complex. Thus the IL-2Rγ is also part of the IL7 and IL4 receptor complexes; similarly the IL6 receptor a complex of IL6Rα and two copies of a larger member of the hemopoetin receptor family, gp130, shares this latter chain with the LIF receptor, the CNTF receptor and the IL-11 receptor. In addition gp130 is a low affinity receptor for oncostatin M. Similar types of subunit promiscuity are beginning to be found in the TNF receptor family.

In addition to functioning as cell surface receptors and signal transducers of cytokine action, cytokine receptors can also be found as soluble molecules which variously act as carriers, antagonists or agonists of cytokine action. These soluble receptors can be generated from alternately spliced mRNAs or by proteolytic shedding of the membrane bound forms. The physiological relevance of these soluble forms is underscored by the finding that virally encoded forms of soluble IL-1 and TNF receptors have been described which act as negative regulators of host defenses to viral infection. Recent data strongly suggest that the true biological function of one of the two IL-1 receptors is as an endogenous attenuator of IL-1 responses. In addition soluble IL-6 receptors can complex with IL6 to form a heterodimer that can bind to and activate gp130 on cells lacking any cell surface IL6 receptors.

In conclusion, our understanding of the molecular basis of cytokine receptor action has reached the point where we can see that these processes have become highly complex with receptors existing as multiple subunit complexes, and as soluble forms. These complex patterns of interaction presumably exist to allow a high degree of fine tuning of the location, duration and extent of responses to cytokines during immune and inflammatory responses pointing to the complexity of the regulation of these processes during host defense.

Rearrangement of T cell receptor genes: regulation and mechanism. Scott K. Durum and Kathrin Muegge. National Cancer Institute, Bldg. 560, Rm. 31-45, Frederick MD, 21702-1201.

Rearrangement of genes is a unique process that is restricted to immature lymphocytes. The T cell receptor genes (α, β, γ and δ) are rearranged during T cell development in the thymus. During gene rearrangement, a few hundred different V, D, and J segments can combine into billions of different possible receptors, resulting in a vast repertoire for recognizing foreign antigens. However, the rearrangement process is risky; a common cause of lymphoid neoplasia is probably erroneous rearrangement, for example when an oncogene is translocated into the receptor locus.

The structure of genes before and after rearrangement are well studied, but little is known of the rearrangement process or its regulation, primarily because the cells that are actively rearranging their genes are exceedingly rare in the thymus. To detect these rare cells, we have developed a PCR method to detect their rearranged DNA product. We determined that the rearrangement of the TCRβ gene takes place in the thymus on day 15 of mouse embryogenesis, so we looked for signals from the thymus that triggered this process. Thymocytes from day 14 embryos were cultured in vitro and a number of stimuli were tested for induction of rearrangement of TCRβ in these cells. IL7 was unique among many cytokines and other stimuli in inducing the rearrangement of TCRβ, and later of TCRα. This effect is partly through IL7's induction of RAG1 and RAG2, which are known to be required for the rearrangement process. IL7 is produced by the thymic epithelial cells, and from our findings, supports rearrangement of these genes.

We have also identified an inhibitor of rearrangement. Antibodies that cross-link CD16 on the cell surface completely turn off the rearrangement of TCRβ. CD16 was originally defined as a low affinity Fc receptor, but it may have other functions on these immature thymocytes. CD16 is known to transduce signals on other cell types, and in our experiments is presumably turning off some component of the rearrangement process (although not the RAG genes, which remain switched on). The thymocytes treated with anti-CD16, having been aborted in T cell development, show the surprising response of developing into large granular cells with the NK1 marker (presumably NK cells). Hence, immature thymocytes can develop into either T or NK cells, and anti-CD16 diverts them into the latter.

The machinery that cuts and splices the rearranging genes has not been defined as yet. The RAG genes noted above appear to regulate
the process, but have not been shown to directly interact with the DNA. We sought to identify the direct DNA-binding components of the recombinase machinery. To enrich for the rare cells in the thymus that are actively undergoing rearrangement, we synchronized T cell development by irradiating mice (which kills their thymocytes), reconstituted them with unrearranged stem cells, and at a time when many thymocytes were synchronously rearranging, we extracted their nuclei and looked for proteins that could bind to TCRβ genes. We identified a protein (termed "RP" for recognition protein) that binds to a motif that flanks all rearranging genes. This motif consists of a heptamer motif and a nonamer motif, separated by 12 or 23 bases of spacer DNA, and RP binding requires both heptamer and nonamer motifs. RP was highly expressed in this population of thymocytes that were highest in rearrangement activity, much lower in more mature thymocytes, and absent in mature T cells. RP may serve as the DNA-binding unit of the recombinase machinery and current efforts are to purify and clone it.

Thus, we have found three facets of the rearrangement process of TCRβ. IL7 induces the rearrangement, CD16 crosslinking blocks rearrangement and diverts cells into an NK pathway, and RP may serve as part of the recombinase machinery.
CYTOKINES AND THE ACUTE PHASE RESPONSE

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As a result of infection or trauma, the body initiates an orchestrated series of responses aimed at stopping tissue destruction, recruiting leukocytes to remove infectious agents and initiating repair mechanisms to return the organism to normal function. The term describing this reaction is acute inflammation and one prominent aspect is the response of the liver, the hepatic acute phase response, resulting in dramatic enhanced gene regulation and expression in hepatocytes with release of increased levels of plasma proteins, acute phase reactants, into the circulation. Three classes of cytokines are known to mediate the hepatic gene regulation through modulation of specific nuclear factor/gene interactions and resulting in enhanced gene transcription. IL-6 and the related family of cytokines, including Leukemia Inhibitory Factor, IL-11, Oncostatin M and Ciliary Neurotrophic Factor, acting through a common gp130 receptor cause major regulation of all acute phase proteins, while IL-1 and TNF cause synergistic and additive regulation of a subset of these genes. Glucocorticosteroid is a third hormone required for maximal gene activation by the other cytokines. Examples from transgenic overexpression and/or knockout animals demonstrate the cytokine dependent regulation of the acute phase
response and the crucial nature of its maintenance and control for species survival.

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Coordination of reproductive events is accomplished by the carefully regulated release and activity of the blood-borne chemical messengers, hormones. While hormones facilitate communication between distant tissues, various growth factors may serve as paracrine regulators of reproductive function. Recently, it has been proposed that cytokines may also serve as local modulators of hormone synthesis and secretion. Bidirectional communication may exist between immune cells and endocrine cells. The effects of interleukin-1 on hypothalamic-pituitary function are well documented (Sapolsky et al., 1987 and others). Interleukin-1, IL-6 and tumor necrosis factor-α (TNF-α) are found in uterine and trophoblastic tissues (Hunt et al., 1992, and others), and may be involved in embryonic-uterine signaling at the time of implantation. A variety of cytokines have been found to have either positive or negative effects on hormone production by gonadal cells. Gonadotropin-stimulated steroidogenesis and formation of gonadotropin receptors are inhibited by IL-1, TNF-α, and interferon-γ (IFN-γ) in both ovarian and testicular cells. This work has been recently reviewed by Adashi (1990), Pate (1994) and Pate and Townson (1994).

Many changes that occur during cyclic ovarian function, such as ovulation and corpus luteum formation and regression, involve extensive tissue remodeling. These events are thought to trigger immune or inflammatory responses. The hypothesis to be presented in this talk is that cytokines may be involved in regulation of corpus luteum function, especially at the time of luteal regression.

Using primary cultures of luteal cells, it has been possible to determine both acute and chronic effects of cytokines on luteal cell function and viability. Gonadotropin-stimulated progesterone production is inhibited by either IL-1β, TNF-α, or IFN-γ, but the later two cytokines are much more effective than IL-1 in this regard. In contrast, all three cytokines are potent stimulators of prostaglandin production by these cells. The mechanism by which prostaglandin synthesis is enhanced may differ slightly for each cytokine. In luteal cells, TNF-α appears to act primarily through stimulation of phospholipase A₂, whereas IL-1β may activate phospholipase C and PGH synthase in addition to phospholipase A₂. The mechanism of action of IFN-γ has not yet been determined. In addition to the observed functional effects, cytokines may also directly promote cell death during
luteal regression. Although the three cytokines mentioned have little or no effect on viability of cultured luteal cells when administered alone, combined treatment with TNF-α and IFN-γ results in a substantial decrease in number of viable cells. Inhibition of cytokine-stimulated prostaglandin production does not alter the cytotoxic effect of these cytokines. Expression of Class I major histocompatibility (MHC) molecules on luteal cells is enhanced, and Class II MHC molecules are induced, by exposure to IFN-γ. This is especially intriguing, since Class II MHC expression increases prior to luteal regression in vivo.

In conclusion, evidence is accumulating which supports the hypothesis that cytokines, and perhaps other immune response mechanisms, are involved in regression of the corpus luteum. This is only one example of a number of putative roles for cytokines in reproduction. Cytokines may play a role in follicular development and/or atresia, testicular function, and hypothalamic-pituitary regulation, as well as maternal recognition of pregnancy and embryo development.

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POSSIBLE INVOLVEMENT OF IL-1α and IL-1β IN ARTHRITIS - STUDIES IN ANIMALS.

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Introduction: ProIL-1α and proIL-1β are synthesized as 32 kDa proteins from related genes. They both lack a signal sequence and are synthesized on free ribosomes without passing though the Golgi apparatus. Both IL-1's are processed to a mature, proteolysis resistant 17 kDa protein and released by an incompletely defined secretory pathway. There is a common signaling receptor (type 1) for both IL-1's. This commonality of structure and receptor signaling raises questions as to why there should be two distinct forms of IL-1.

Hypothesis: Both IL-1α and IL-1β play a significant role in arthritis.

Results: We have used hamsters, a species that will run 10-14 km/night on a wheel, to explore the changes that occur after induction of an arthritis¹, ². Intra-articular (i.a.) IL-1 leads to an inhibition of wheel running activity, a rapid loss of proteoglycan (PG) from non-calcified articular cartilage, inhibition of new proteoglycan synthesis, and infiltration of inflammatory cells into the synovial fluid and lining. We estimate a maximal decrease in rate of new PG synthesis of circa 40% and a doubling of the catabolic rate of PGs. Although PG remains depleted and the synovial lining infiltrated by cells, running is restored within 12 hrs after IL-1. After 48 hrs, if a 2nd i.a. IL-1 injection is given, inhibition of running is much more profound, and whereas the effects of the first i.a. IL-1 injection could be completely inhibited by prostaglandin biosynthesis inhibitors, after the 2nd i.a. IL-1 injection only a 20-30% restoration of normal running could be obtained. In these and other instances, the effects of IL-1 in an already inflamed site differ significantly from those in a naive site. In these experiments, we cannot distinguish IL-1α and IL-1β: their effects are equivalent and additive; they either synergize nor antagonize each other.

Examination of the distribution of cellular staining with IL-1α or IL-1β specific antibodies demonstrates differential expression of the two IL-1 molecules. For example, after oral challenge with Y. enterocolitica,
Peyer's patches, which were previously negative for IL-1α and β, now show a distinct cellular distribution for IL-1α and IL-1β stained cells. Only a minority of the cells are double staining. In the spleen, where cells do not have direct contact with the antigenic challenge, IL-1α and IL-1β mRNA is induced without expression of IL-1 protein. Again the pattern of cells expressing IL-1α and IL-1β mRNA is spatially distinct. After i.v. LPS, the IL-1α and IL-1β proteins are induced and the cellular distribution of protein staining recapitulates the selective pattern of mRNA staining.

These results suggest that different cells may be programmed to produce IL-1α and IL-1β, and this difference in expression is consistent with known differences in transcriptional regulation and maturation pathways of IL-1α and IL-1β.

These differences can be reflected in in vivo utilization of IL-1. Using specific antibodies to block the activity of IL-1α or IL-1β, the cytokine dependency of physiological pathways can be determined. For example, the weight loss due to turpentine can be completely reversed by anti-IL-1β, but not anti-IL-1α or anti-IL-6 antibodies. Using such antibodies, we explored the role of IL-1α and IL-1β in a murine model of antigen-induced arthritis. Combined anti-IL-1α and anti-IL-1β antibodies prevented the inhibition of PG synthesis, but not the acceleration of PG catabolic rate. Overall there was a significant decrease in edema, cell infiltration, and proteoglycan loss in anti-IL-1 treated animals. Neither anti-IL-1α nor anti-IL-1β antibody alone was effective in this arthritis; both antibodies were required for a good therapeutic effect. The failure to inhibit PG catabolism and completely resolve the arthritis indicates that IL-1 independent factors also make a significant contribution to this disease.

Dysregulation Between Cytokine and Cytokine Inhibitor Production During Infection

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The importance of TNF\(\alpha\) and IL-1 in mediating the acute detrimental host responses to lethal endotoxemia and overwhelming gram-negative bacterial infections is no longer a matter of general dispute. However, it has only been recently recognized that the integrated cytokine response to infection and injury is complex, and that, ultimately, tissue responses depend not only upon the absolute concentrations of IL-1 and TNF\(\alpha\), but also upon the presence of cytokine inhibitors, anti-inflammatory cytokines, and the number of cellular receptors. A pivotal advance in the past two years has been the identification of at least two new classes of specific IL-1 and TNF inhibitors which prevent ligand binding to their respective receptors. These include IL-1 receptor antagonist and the soluble receptors for IL-1 and TNF.

The integrated cytokine response to an acute, nonlethal, inflammatory insult (as represented by mild endotoxemia in volunteers) encompasses the early synthesis and release of at least two proinflammatory cytokines (IL-1 and TNF) whose concentrations at the local site of tissue production are greater than those in the plasma (1). This early release of proinflammatory cytokines into the local tissue milieu acts to initiate and orchestrate many of the beneficial responses aimed at improving antimicrobial function and reducing tissue damage.

However, this initial release of proinflammatory cytokines is ultimately short-lived. IL-1 activity is mitigated by the synthesis and release of IL-1ra which interferes with IL-1 binding to its cellular receptors [2]. Similarly, TNF\(\alpha\) activity is modulated by the subsequent shedding of the extracellular domain of its two cellular receptors which complexes with TNF and prevent its binding to the cellular TNF receptors (3). Following a mild endotoxemia, sTNFR I and II concentrations peak one to two hours after TNF\(\alpha\) and remain elevated for longer periods. The loss of cellular TNF\(\alpha\) receptors from target tissues serves two purposes: the transient desensitization of cells to repeated exposure to TNF\(\alpha\), and the formation of receptor-ligand complexes which attenuate peak free TNF\(\alpha\) concentrations, and may act as a buffer to deliver low levels of cytokine over extended periods.

A second mechanism by which the host regulates proinflammatory cytokine production and activity is the subsequent release of mediators which suppress IL-1 and TNF\(\alpha\) production, and increase IL-1ra release. For example, increased production of prostanoids and the counter-regulatory endocrine response (e.g. cortisol, CRF, and aMSH) all down-regulate IL-1 and TNF\(\alpha\) production and activity. More recent evidence has established that the synthesis and release of the anti-inflammatory cytokines, IL-4 and IL-10, also serves to limit the release of proinflammatory cytokine production.

The catastrophic host responses to overwhelming bacterial infections, and propagation of the systemic inflammatory response syndrome with multisystem organ
dysfunction in ongoing inflammatory processes represent dysregulation of this normal homeostatic process. For example, in acute septic shock due to gram-negative bacteremia or endotoxemia, the magnitude of proinflammatory cytokine response (TNFα and IL-1) is excessive. The quantities of TNFα and IL-1 produced are greater than can be mitigated by the release of IL-1ra (2) and TNF soluble receptors (4). Studies by ourselves and by Dayer have revealed that in patients expiring from infections, the balance between TNF and its soluble receptors favors the proinflammatory cytokine (3,4), a finding strikingly different than seen in patients with recoverable infections. Furthermore, the timing of the release of these cytokine inhibitors is sufficiently delayed in septic shock such that excess proinflammatory cytokines are produced initially in the reticuloendothelial system. They are also produced in the blood by circulating monocytes and vascular endothelial cells where their effects on endothelial cells lead to hemodynamic collapse.

Similarly, in ongoing inflammatory processes, such as those which occur in hospitalized patients with systemic inflammatory response syndrome (SIRS) or sepsis syndrome, the mechanisms which ultimately down-regulate proinflammatory cytokine release are ineffective (5). In both septic shock and SIRS, the beneficial aspects of proinflammatory cytokine production (including stimulation of nonspecific host immunity, increased antigen specific T-cell proliferation, macrophage and NK-cell bactericidal capacity) are offset by the adverse consequences of continued exposure to elevated TNFα and IL-1 concentrations.

In conclusion, the studies suggest that the production and action of the proinflammatory cytokines, TNF and IL-1, are tightly regulated. During a mild inflammatory process, there is the initial release of TNF and IL-1 which initiates and orchestrates the proinflammatory response. In patients with septic shock or SIRS, this initial release of TNF and IL-1 is either excessive or continuous in nature, and endogenous mediators such as IL-1ra, soluble receptors for TNF or IL-1, and anti-inflammatory cytokines are unable to modulate the exaggerated proinflammatory response. In such cases, the adverse effects of exaggerated or continuous production of the proinflammatory cytokines are manifested.


Measuring Cytokines: The Jurassic Park Syndrome

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The application of molecular biology methods to leukocyte biology through the 1980s has led to a phenomenal growth of information regarding intracellular signaling. Recombinant cytokines and/or antibodies are now available for 13 interleukins, the interferons, tumor necrosis factors, several colony stimulating factors and many growth factors. However, the power of molecular biology techniques has led to a false sense of security: since cytokines can be harvested in high yield after expression in E. coli, investigators did not need to confront issues relating to binding proteins, proteolytic degradation and other sources of interference in biological fluids in order to obtain reagents. Now most investigators simply buy them. In contrast, isolation of classical protein hormones in an earlier era required extraction and purification from natural sources. Thus, endocrinologists were aware of potential sources of interference when it came time to develop hormone assays.

At present, numerous biotechnology companies offer immunoassay kits for various cytokines. However, as reported in a recent World Health Organization (WHO) study, measurements made in a set of control and patient sera with 9 TNFα kits, 8 IL-6 kits and 6 IL-2 kits showed that major discrepancies in results are obtained from one kit to the next [1]. The WHO investigators found that the various kits had differing susceptibilities to interference by soluble cytokine receptors. Although this WHO study has highlighted one source of interference, acute phase proteins, heterophilic antibodies and many other factors need to be taken into account as well [2]. The fact that the cellular sources of cytokines are collected in a blood sample means that collection procedures are critical. Fetal bovine serum routinely used in cell cultures can have cytokine inhibitors or cytokines themselves as contaminants. Thus, culture supplements need to be carefully considered.

The contrast between previous studies in endocrinology and many current investigations of circulating cytokines can be summed up in observations made by Dr. Ian Malcolm, a fictitious scientist in Michael Crichton's novel Jurassic Park [3]. The work of endocrinologists of a previous era "required a substantial sacrifice ... an apprenticeship, a discipline lasting many years." The current state of the cytokine assay literature can be likened to the mind-set of the creators of the dinosaurs running amok in Jurassic Park, as characterized by Dr. Malcolm: "There is no discipline lasting many decades. There is no mastery: old scientists are
ignored ... The buyer [of a cytokine assay kit] simply purchases the power, like any commodity. The buyer doesn't even conceive that any discipline might be necessary." [3]

The aim of my talk during the workshop on Measurement of Cytokines in Tissues and Fluids will be to review historical efforts to measure circulating interleukin-1 in humans, beginning with Greisman and Hornick's attempts to measure "endogenous pyrogen" using fever assays [5]. The talk will progress through successive modes of in vivo and in vitro bioassays and various immunoassay formats. Problems and discrepancies in these early efforts will be portrayed as foreshadowings of recent discoveries of soluble receptors, receptor antagonists and other factors. Also to be addressed is the concept that for any cytokine, there is not one assay that will satisfy all needs: assays with different characteristics will answer different biological questions [4]. It is hoped that this presentation can stimulate a discussion of what disciplines need to be engendered in those purchasing cytokine assay kits or attempting to measure circulating cytokines.

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Fever is one of the most common host defense responses of an infected organism (1). The rise in body temperature associated with fever is a highly regulated process, involving numerous sophisticated physiological and behavioral responses. As in many regulatory processes there appear to be multiple factors that influence the regulated body temperature during fever -- some raise the thermoregulatory set-point and others prevent the set-point from rising too high. The former are called "endogenous pyrogens," and the latter "endogenous antipyretics" or "endogenous cryogens." Endogenous pyrogens include such cytokines as IL-1β, IL-6 and perhaps others. Endogenous antipyretics or cryogens include arginine vasopressin, α-melanocyte stimulating hormone, glucocorticoids, and in some cases TNFα(TNF).

Hypotheses:
1. IL-1β is an endogenous pyrogen acting at the level of the anterior hypothalamus in the rat.
2. IL-1β exerts its pyrogenic action via the local release of IL-6 within the anterior hypothalamus.
3. Endogenously produced TNF may act as an endogenous antipyretic or cryogen.
4. Glucocorticoids modulate the rise in body temperature via receptors located in the anterior hypothalamus.

Summary:
Injection of neutralizing antibody to IL-1β into the anterior hypothalamus (AH) of rats blocks much of the rise in body temperature caused by intraperitoneal (ip) injection of lipopolysaccharide (LPS) (2). There are many in vitro data showing that IL-1 induces the production of IL-6. In the above study, it was also found that the AH administration of antibody to IL-1β also blocked the rise in AH IL-6. In an earlier study, we had shown using push-pull perfusion, that the AH concentration of IL-6 rises during fevers caused by ip injection of LPS (3). Furthermore, when IL-6 was infused into the AH of rats, this caused fevers of similar magnitude to that which occurred following injection of LPS. These data support the hypothesis that IL-6 is critically important in the fever pathway.

In many studies from our laboratory, we have shown that the addition of neutralizing polyclonal antibody to TNF results in larger fevers in response to ip injection of LPS (e.g. 4). We have interpreted these data as supporting the hypothesis that endogenously-produced TNF may act as an endogenous antipyretic or cryogen. Although some studies have shown that the addition of monoclonal antibodies to TNF results in smaller fevers, in those studies either no data were shown demonstrating that the antibody neutralized the TNF bioactivity, or data were actually shown indicating that the circulating concentrations of TNF were actually enhanced by the addition of the antibody.
In data that will be presented in this meeting, we have shown that the antipyretic actions of TNF probably occur outside the CNS, and thus a higher circulating level of TNF might actually (as may occur when non-neutralizing antibody to TNF is injected into an organism), be the result of the higher circulating concentration of this cytokine.

Glucocorticoids rise in the circulation during most infections that result in fever. We have recently shown that the rise in corticosterone results in the attenuation of LPS-induced and psychological stress-induced fevers (5). Based on studies using the progesterone and glucocorticoid antagonist, RU38486, we have found that the site of negative feedback of glucocorticoids on LPS-induced fever is the anterior hypothalamus. Preliminary data support the hypothesis that the site of negative feedback of glucocorticoids on fevers due to psychological stress is the hippocampus.

Conclusions:
1. Fever is a complex regulatory process that involves numerous pyrogenic and cryogenic cytokines and hormones.
2. IL-1β appears to be an endogenous pyrogen, which acts at the level of the anterior hypothalamus.
3. Much of the pyrogenic action of IL-1β may be via an increase in IL-6 within the anterior hypothalamus.
4. Although TNF may be a pyrogen when injected into an organism, there are data that support the hypothesis that endogenously produced TNF is an endogenous antipyretic.
5. Glucocorticoids exert an antipyretic action, most likely at the level of the anterior hypothalamus.

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Transport of Cytokines Across the Blood-Brain Barrier

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Blood-borne cytokines can affect various functions under the control of the central nervous system (CNS) such as sleep, appetite, and body temperature. Included in the various mechanisms by which circulants can affect the brain is direct penetration of the blood-brain barrier (BBB). The BBB, comprised of the capillary bed of the brain and the ependymal lining of the choroid plexus, separates the blood from the cerebrospinal and interstitial fluids of the CNS. The BBB can act as a near absolute barrier to blood-borne peptides and proteins, as in the case of albumin, or can regulate their passage, as exemplified by the enkephalins (1). Passage of cytokines across the BBB could provide a mechanism by which peripherally administered or secreted cytokines could directly interact with neurons deep within the CNS. We will review some of the results that demonstrate that interleukin 1α (IL-1α) and other cytokines can cross the BBB.

Several methods have been used to determine the degree to which the BBB is permeable to 125I labeled IL-1α. Multiple-time regression analysis has shown that the BBB is about 40 times more permeable to IL-1α than to serum albumin (3). This relatively high permeability is due to the presence of a saturable system (2) that also transports IL-1β (5). About 0.05-0.08% of an iv injected dose is taken up by each gram of brain. This compares favorably with the uptake rates of other centrally active substances such as morphine (0.01-0.02%) and L-DOPA (0.1%) and suggests that sufficient IL-1α may be crossing the BBB to affect brain function. IL-1α reached every region of the brain examined, including cerebral cortex, hippocampus, striatum, cerebellum, and hypothalamus. Capillary depletion, a method that separates the brain parenchyma from the capillary bed of the brain that comprises much of the BBB, and sampling of cerebrospinal fluid demonstrate that IL-1α reaches areas deep within the CNS and well behind the BBB (4). Samples of radioactivity recovered from cerebrospinal and brain interstitial fluids and submitted to analysis by HPLC showed that IL-1α penetrated the BBB in intact form. Under the conditions of these experiments, no disruption of the BBB to doses of IL-1α as high as 50 µg/kg could be demonstrated.

The transport system for IL-1α and IL-1β was not affected by and did not transport IL-2, IL-6, or tumor necrosis factor-α (TNF-α). Dexamethasone, indomethacin, α-MSH, and morphine also did not effect transport. Studies performed with a series of antibodies indicated that the portion of the IL-1α molecule that binds to the BBB transport system is very
similar to that which binds to the Type I receptor of the T lymphocyte. However, the BBB transporter itself appears to be related to, but immunologically distinguishable from, the Type I receptor.

Other cytokines that have been examined for BBB penetration include IL-2 and TNF-α. IL-2 penetrates the BBB to a low degree by a non-saturable mechanism. By contrast, TNF-α crosses the BBB by a saturable transport system distinct from that described for IL-1α. After intravenous injection, TNF-α was recovered from both cerebrospinal and brain interstitial fluids; integrity of the molecule was confirmed with HPLC. Up to 0.1% of a dose of TNF-α injected iv entered each gram of brain. Passage across the BBB in neonates was particularly rapid and was due to the presence of the saturable transporter at this age.

The results indicate that some cytokines are able to penetrate the BBB to a degree that may be sufficient to affect some functions of the CNS.

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Interactions between cytokines and neuropeptides in the brain.

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Several cytokines have been demonstrated to exert potent actions on the brain where they mediate systemic host defense responses (e.g., fever, neuroendocrine responses, behavioral changes), and influence neuronal degeneration and repair and glial activation\(^1\). The most extensively studied cytokine in the brain, interleukin-1 (IL-1) has been established as an important neuromodulator which exhibits diverse biological actions\(^4\) and interacts with several neuropeptides.

Cytokines and/or their receptors are expressed in brain and can be induced by local or systemic tissue injury, infection, and inflammation. IL-1, IL-6, IL-8, TNF\(\alpha\), MIP-1 and IFN\(\gamma\) all elicit marked increases in body temperature (fever) and hypometabolism (thermogenesis) when injected into the brains of experimental animals. Evidence to support the role of IL-1\(\beta\) and IL-6 as endogenous pyrogens in the brain derives from observations that central administration of neutralizing antibodies to these cytokines attenuates fever and thermogenic responses to endotoxin in the rat. The pyrogenic actions of many cytokines are dependent on prostaglandin synthesis, with the exception of the chemokines IL-8 and MIP-1. The neuropeptide corticotrophin releasing factor (CRF) mediates some actions of cytokines in the brain including pituitary adrenal activation, suppression of peripheral immune function, certain behavioral changes, fever, and hypermetabolism. Pyrogenic and thermogenic responses to IL-1\(\beta\), IL-6 and IL-8 in the rat are inhibited by central pretreatment with either a CRF receptor antagonist or neutralizing antibody, whereas responses to IL-1\(\alpha\) and TNF\(\alpha\) are unaffected by this treatment. Several pieces of data now indicate that central effects of IL-1\(\alpha\) and IL-1\(\beta\) on fever and thermogenesis involve different mechanisms, and that responses to IL-1\(\beta\) are not dependent on Type I (80k Da) IL-1 receptors. The site and mechanism of action of CRF on fever and thermogenesis is unknown, but probably involves efferent brain pathways rather than modification of the set point for body temperature.

Genetically obese rodents with defective CRF regulation show impaired responses to IL-1\(\beta\) and IL-6, but not IL-1\(\alpha\) or TNF\(\alpha\). Lipocortin-1 (annexin-1) is a potent endogenous inhibitor of cytokine action in the brain, and mediates the antipyretic effects of glucocorticoids. Lipocortin-1 inhibits febrile and thermogenic responses to cytokines which depend on CRF (i.e., IL-1\(\beta\) and IL-6), and apparently acts by suppressing hypothalamic CRF release.
Similar mechanisms may be involved in cytokine actions on neurodegeneration. Blocking IL-1 action in brain by central injection of IL-1ra markedly inhibits ischaemic, traumatic and excitotoxic brain damage in the rat. Although these forms of neurodegeneration appear to be independent of prostaglandins, ischaemic and excitotoxic damage are inhibited by injection of a CRF receptor antagonist or lipocortin-1. The effects of other peptides which inhibit pyrogenic actions of cytokines such as αMSH and vasopressin may also modify neurodegeneration.

Although some common mechanisms mediate the diverse actions of cytokines in the brain, important differences have also been demonstrated and multiple pathways are involved in specific actions or effects of different cytokines in the CNS.

References


Presence of bi-directional interactions between the neuroendocrine and immune systems has been firmly supported by evidence found in numerous experiments (Blalock, 1987; Maclean and Reichlin, 1981; Reichlin, 1993). This paper discusses two topics of cytokines/HPA axis interactions: (1) the route and mechanism by which immune signals are transmitted to the brain and activate HPA axis; (2) non-inflammatory stress also activates immune system in terms of IL-6 production, but stress-induced HPA activation is not directly related to IL-6 response to stress.

Blood-borne immune signals carried by cytokines, particularly by IL-1, elicit rapid activation of HPA as indicated by an acute increase in plasma ACTH and corticosterone levels after iv injection of IL-1 in conscious rats. The rapid ACTH response is mediated by increased release of corticotrophin releasing factor (CRF) into the hypophysial portal vessels, not by direct action of the cytokine on the pituitary gland. This was supported by the finding that iv administration of CRF antiserum completely prevented IL-1-induced ACTH response, and that addition of IL-1 into the primary culture of rat pituitary cells did not stimulate release of ACTH into the media, although different laboratories reported different results. Blood-borne IL-1 appears to enter the brain through the fenestrated endothelium of circumventricular organs, in particular organum vasculosum laminae terminalis (OVLT) in rats, bind to its receptors on astrocytes tightly surrounding OVLT and stimulate production and release of PGE2 from astrocytes. Microinjection of IL-1 receptor antagonist into OVLT almost completely suppressed IL-1-induced ACTH response in rats. The critical role of astrocytes in the transmission of blood-borne immune signal carried by IL-1 through PGE2 production was supported by a series of experiments. In cultured rat astrocytes, addition of IL-1 increased release of PGE2 into the culture media dose-dependently. Moreover, rapid increase in extracellular PGE2 concentrations in OVLT after iv injection of IL-1 was confirmed by microdialysis. Furthermore, PGE2 response of astrocytes to IL-1 was suppressed by co-incubation with CRF or somatostatin, and augmented with angiotensin, suggesting the presence of regulatory interactions between various neuropeptides and IL-1 in production of PGE2 by astrocytes. The essential role of astrocytes in transmitting the blood-borne immune signal to the brain has also been supported by the experiments with lesion in OVLT in rats. Placement of lesion in OVLT by radiofrequency or kainic acid which permanently destroys neurons, but not astroglia, did not only suppress ACTH release induced by IL-1 iv, but enhanced the ACTH response. PGE2 released into the extracellular space in OVLT appears to diffuse or be transported humorally to nearby preoptic area (POA). The importance of POA in the transmission of the signal of blood-borne IL-1 to HPA axis was supported by the finding that lesion of POA by radiofrequency or kainic acid significantly decreased ACTH response of IL-1 iv, being contrast to the finding of lesion in OVLT. Moreover, microinjection of PGE2, not PGD2, in POA induced a rapid increase in plasma ACTH concentration. The activated neurons in POA appear to transmit their signal to CRF neurons in PVN. However, the transmission of the signal from POA to PVN appears to be mediated by a catecholaminergic system, since microinjection of 6-hydroxydopamine (6-OHDA) into PVN suppressed IL-1-induced ACTH response.
On the other hand, catecholaminergic system is activated by central CRF and appears to play a key role in rapid increase of IL-6 in circulation during non-inflammatory or non-infectious stress, such as immobilization, hemorrhage/reinfusion and foot shock in rats. Magnitudes of stress-induced increase in plasma IL-6 concentration are generally inversely related to pre-stress plasma corticosterone levels in rats. However, maintenance of normal or slightly higher than normal levels of plasma corticosterone levels in adrenalectomized rats by implanting corticosterone pellet resulted in a greater IL-6 response to stress than normal animals, suggesting biphasic effect of corticosterone in stress-induced IL-6 response. Although host-defense responses including ACTH, corticosterone and IL-6 responses to LPS were reduced 24 hours after hemorrhage stress, the post-stress reduction of IL-6 response was not only prevented, but also reversed by maintaining plasma corticosterone concentrations at upper normal range in adrenalectomized rats.

Although non-inflammatory stress induces a rapid increase in plasma IL-6 concentration, the response does not appear to be directly related with activation of HPA axis by the stress. Intracerebroventricular (icv) injection of CRF indeed increased circulating IL-6 levels, but microinjection of CRF antagonist icv failed to suppress stress-induced IL-6. The CRF antagonist injected icv significantly decreased ACTH response to the stress. On the other hand, either central and systemic administration of 6-OHDA suppressed stress-induced IL-6 response. This indicates that the mechanisms for stress-induced IL-6 and ACTH release are independent. Furthermore, systemic administration of CRF antiserum significantly reduced stress-induced IL-6 response, suggesting a possible involvement of tissue CRF in the IL-6 response.

In conclusion, immune signals carried by blood-borne IL-1 are transmitted to the brain and activates HPA axis through increased synthesis and release of PGE2 by astrocytes. OVLT, POA and PVN are critical sites for the transmission of these signals in rats and the catecholaminergic system appears to mediate activation of CRF neurons in PVN. Catecholaminergic system also plays a critical role in IL-6 response to non-inflammatory stress, but the stress-induced IL-6 response is not directly related with HPA activation induced by the stress. Involvement of tissue CRF in stress-induced IL-6 response was also suggested. (These studies were supported in part by a grant from the Office of Naval Research)


INTERLEUKIN-1 RECEPTOR ANTAGONIST.  

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The discovery of a human protein that is a highly specific interleukin-1 receptor antagonist (IL-1ra) (Ref. 1) has significantly advanced our understanding of the physiological actions of IL-1. It has shown how IL-1 acts as a pro-inflammatory rather than as an immunostimulatory in vivo, how its actions are controlled and it has provided an opportunity to treat a range of diseases that are thought to result from excessive IL-1 activity.

Using IL-1ra, we and other workers have shown that the pro-inflammatory actions of IL-1 are a significant factor in a range of diseases. IL-1ra ameliorates outcome in animal models of diseases as varied as sepsis, rheumatoid arthritis, inflammatory bowel disease, GVHD and stroke (Ref. 2). The reduction of disease severity is generally associated with an inhibition of leukocyte recruitment, coagulation disorders, catabolic activity and fibrosis at sites of injury and inflammation. Most of these activities are easily understood in terms of the in vitro and in vivo properties of the cytokine. In contrast, IL-1ra has very little effect on the specific immune response, even though IL-1 has been demonstrated to have significant immunostimulatory effects in vivo and in vitro. It appears that the immunostimulatory effects of IL-1 are duplicated by other cytokines.

IL-1ra is a part of the natural regulatory mechanism of IL-1 action as suggested by its increased synthesis under a number of inflammatory conditions. Recent evidence from Cominelli’s group showing that antibodies to IL-1ra greatly exacerbate the severity of an immune complex mediated model of bowel inflammation in the rabbit has confirmed this possibility (Ref. 3). Furthermore, the high levels of IL-1ra detected in the normal and the diseased rabbit colon have raised important questions concerning the natural state of the IL-1 receptor that must be answered before we can truly understand the role of IL-1 and IL-1ra in inflammation.

The properties of IL-1ra as a natural anti-inflammatory agent have led to clinical trials of its effect on sepsis syndrome and on rheumatoid arthritis. IL-1ra showed a reduction in sepsis mortality from 34% to 29% in an 893 patient Phase III trial, a result that was not statistically significant (Ref. 4). However, a retrospective analysis of the approximately two-thirds most severely ill patients defined by the presence of ARDS, DIC, renal dysfunction or clinically significant shock showed that IL-1ra reduced 28 day mortality from 43% to
28%. The increased survival time in these patients is significant at the level \( p = 0.0003 \). An ongoing 1,400 patient trial will test the hypothesis that IL-1 is a potent mediator of pathology and mortality in sepsis induced organ dysfunction and shock, and that IL-1ra can ameliorate these conditions in a clinical setting.

When viewed in a broader context recent work on IL-1ra is one instance of the way in which studies of natural regulatory mechanisms can help the physiologist, pharmacologist and the clinician understand the role of cytokines in the response to injury and infection.

References


Antisense nucleic acids as therapeutics exploits the high degree of specificity offered by Watson-Crick base recognition of RNA target sequences. Oligonucleotides have great potential for the development of gene specific therapeutic agents (1-3). A number of obstacles that affect the usefulness of oligonucleotides as therapeutic agents includes (i) the need to be stable in the body, (ii) the requirement for efficacious cellular uptake, and (iii) the need that activity be sequence specific. Genta has focused on the development of two nucleic acid backbone analogues that are nuclease resistant: phosphorothioate (increased stability) and methylphosphonate (stable) oligonucleotides. In both cases, the natural phosphodiester backbone has been modified at the non-bridging oxygen of the phosphate and replaced with a sulfur or a methyl group, respectively. Progress toward increasing the affinity of the oligomers to RNA target sequences has also been made by making the oligomer chirally enriched or chirally pure. This has led to increases in affinity for RNA on the order of 10^3 greater than racemic methylphosphonate oligomers. To have a basis for comparing the consequences of the alterations, Genta is using as a target reporter gene, the bacterial gene chloramphenicol acetyl transferase (CAT). Using this system, modified forms of the CAT gene which incorporate splicing and poly A signals allow their evaluation in mammalian cells. Biological evaluation of expression has been done in cells by microinjection of various oligonucleotides and allow comparisons that are independent of cellular uptake. Enhancement of oligonucleotide delivery has also been accomplished using liposome reagents, such as cationic lipids.

The ability to inhibit IL1 beta expression in keratinocytes was evaluated as a potential target in psoriasis has been carried out in collaboration with Drs. K. Cooper and C. Hammerberg, U. Mich. Medical Center. IL1 beta protein levels are elevated in psoriatic lesions and recede prior to resolution of psoriatic lesions which suggests a potential intracrine role in epidermal hyperproliferation. Treatment of a keratinocyte cell line with antisense phosphorothioate oligomer (0.25 uM with Lipofectin®), directed to the initiation codon of IL1 beta led to reduction (~65%) of either constitutive or TNF alpha induced levels of IL1 beta. The inhibition of protein synthesis was sequence specific since control oligomers with 4 mismatches to the target sequence showed no inhibition. A concomittant effect of antisense IL1 beta oligomer on keratinocyte proliferation was seen but its exact mechanism is less clear since non-sequence specific inhibition by phosphorothioate oligomers has also been observed.
Genta is also developing phosphorothioate oligomers for selected therapies and one disease state under evaluation is restenosis associated with balloon angioplasty. Studies are carried out with a localized single treatment with two antisense oligomers directed against the cell cycle genes, cdc2 and PCNA. Genta's collaborator, Dr. V. Dzau, Stanford Univ. School of Medicine, has demonstrated a significant and long-term inhibition of stenosis in injured rat arteries (4) and these findings are being extended to studies in other animal species.

TNF RECEPTORS AND ANTAGONISTS. PERSPECTIVES OF NEW THERAPEUTIC STRATEGIES

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The many different activities of TNF on cells include cytotoxic as well as growth factor activities, and the induction or upregulation of a variety of genes encoding, e.g., adhesion and histocompatibility antigen molecules. The integrated activities of TNF at the level of tissues, organs or the whole organism may be understood as those of a pleiotropic mediator in immunologic, inflammatory and metabolic host defense, but in some disease states endogenous mediators such as TNF and other cytokines produced in excess contribute to and maintain pathologic conditions and thus develop highly toxic activities. Two distinct cellular TNF receptors, p55TNFR and p75TNFR, have been identified. Both receptors bind the related factors TNFα and TNFβ, jointly referred to as TNF. In general both receptors are expressed simultaneously on cells, but they have been found to mediate distinct TNF responses in various cells. The distinct cellular responses to p55TNFR and p75TNFR triggering may open the possibility to dissect the spectrum of TNF activities using agonists which in contrast to wildtype TNF selectively bind to p55TNFR or to p75TNFR. For example, both p55- and p75TNFR mediate proliferative signals in lymphoid cells, but cell adhesion to endothel appears under dominant p55TNFR control [1, 2]. Prototypes of such agonists have been identified by introducing point mutations into the human TNF sequence [3], and TNFR type selective TNF mutants have been investigated in human endothel cell culture and cytotoxicity, as well as in vivo studies.

Soluble TNF binding proteins naturally occur in serum. They were identified as fragments of the extracellular regions of both TNF receptors having retained TNF binding activity; they are variably thought to act as TNF inhibitors, or slow TNF release reservoir. In an attempt to develop TNF antagonists for clinical use, recombinant soluble TNF receptors and chimaeric TNF receptor-immunoglobulin fusion constructs have been expressed in eucaryotic cells. The soluble receptor proteins were found to bind TNF with an affinity comparable to that of the membrane-bound receptors. The TNF receptor-immunoglobulin chimaeras (TNFR-IgG) are expressed as dimeric molecules which initially were constructed to obtain longer in vivo halflifes than the soluble receptors; they were, however, found also to have a higher binding avidity which may be understood from the structure of a TNFR / TNF complex which has been solved by protein X-ray diffraction analysis [4], and from which it can be envisioned that the two receptor moieties of one TNFR-IgG molecule simultaneously bind to two receptor binding sites of a TNF trimer. The studies of the binding properties of the two TNF receptors
revealed that p55- and p75TNFR-IgG had similar equilibrium binding affinities. However, a strongly pronounced difference in binding kinetics was discovered, p55TNFR forming the kinetically more stable complex with TNF [5]. This difference in binding kinetics carries over to the respective chimaeric fusion constructs, and p55TNFR-IgG therefore was chosen as TNF antagonist candidate.

The efficacy of p55TNFR-IgG to protect from mortality in endotoxin or bacterial challenge was investigated in animal models. It was found to fully protect mice sensitised with D-galactosamine from endotoxin challenge as well as from lethal intravenous E. coli challenge. Baboons were also protected from lethal E. coli challenge, and various haemodynamic, haematologic, coagulation, metabolic and secondary cytokine disorders in these animals were significantly attenuated by the TNF antagonist treatment. However, in mice with sepsis developing from generalised peritonitis induced by intraperitoneal administration of various gram(+) and gram(-) organisms the protective efficacy was lower. The occurrence of death was postponed, but the treatment only marginally improved the ultimate rate of survival in these peritonitis models. The conclusion that TNF is an important, but not the only toxic mediator in septic disease models is also supported by the finding that mice whose p55TNFR was deleted by gene targeting are insensitive to LPS after D-GalN sensitisation, but equally succumb to the lethal activity of LPS as wildtype mice in the absence of sensitisation [6].


Cytokines play a pivotal role in intercellular interactions. The signal transduction pathways resulting in cytokine production and subsequently in cytokine responses offer fertile ground for intervention by small molecules. These small molecules can be used to probe the signal transduction pathways as well as investigate the role of various cytokines and their actions in immune disorders. Regulation of intracellular concentrations of cyclic nucleotides, calcium ion fluxes (Ca++), phosphorylation and dephosphorylation plays differential roles in the production and responses to cytokines. Small molecules have aided in deciphering the signal transduction pathways of IL-1, tumor necrosis factor alpha (TNF\(\alpha\)), IL-2, IL-3, IL-4, IL-10, IFNg, EGF and PDGF (1-4).

Phosphodiesterases metabolize the intracellular cyclic nucleotides (cNMP) and thus play a major role in modulating their intracellular concentrations. The five distinct families differ in their cyclic nucleotide substrate specificities and tissue distribution. Compounds which elevate cAMP such as nonselective PDE inhibitors, PDE IV inhibitors (rolipram, nitraquazone, WAY-PDA-641), and dibutyl cyclic AMP suppressed the production of TNF\(\alpha\) from endotoxin-stimulated monocytes. The nonselective PDE inhibitors and dbcAMP also suppressed the levels of steady state mRNA. Delayed addition experiments and mRNA studies suggest that cAMP plays a role in modulating translation and mRNA stability. In contrast, agents which elevate cGMP such as sodium nitroprusside, exogenous cGMP, and zaprinast, stimulate TNF\(\alpha\) production. Combination studies indicate that the effects are additive. These data suggest opposing roles for cGMP and cAMP levels in TNF\(\alpha\) secretion. However, the various effects of agents which modulate cNMP levels on IL-1 production suggest that the role of cAMP and cGMP can be influenced by experimental conditions.

The macrolides, FK506, FK520, rapamycin and cyclosporine (CsA), are potent immunosuppressive agents. They must bind to a member of the immunophilin protein class, that is, of the FK506 binding protein or cyclophilin families, respectively (3,4) in order to mediate their immunosuppressive effects as shown by the ability to block the activities of these compounds with high molar excess of structurally related analogs (FK506BD, WAY-124466, WAY-129765, CsAme6). Although FK506, FK520, and rapamycin bind to FKBP5, their activities and mechanisms of action are distinct. FK506, FK520, and cyclosporine inhibit IL-2, IL-4 production induced by Ca++ dependent stimuli and under certain experimental conditions IL-1. FK506, FK520, or cyclosporine-immunophilin complexes bind to calcineurin and inhibit its phosphatase activity, a critical step in activation by Ca++ dependent stimuli. Thus, the cytoplasmic NF-AT subunit remains phosphorylated and cannot migrate to the nucleus where it would bind to the nuclear subunit of NF-AT and
stimulate IL-2 mRNA transcription (4,5). These molecules also block apoptosis, which involves the calcineurin pathway. Although FK506, FK520, and cyclosporine inhibit responses to IL-1 + PHA or TCR stimulation, such as IL-2 production and T-cell proliferation, they do not inhibit responses to IL-2, IL-3 and IL-4. In contrast, rapamycin which exhibits minimal inhibition on IL-2, IL-1, IL-3, and IL-4 production, readily suppresses many cytokine-induced responses such as IL-2, IL-3, IL-4, EGF, and PDGF induced protein and DNA synthesis, antibody and IFNg production. The effects on protein synthesis and proliferation are mediated at least in part by rapamycin's effects on activation of p70S6 kinase and the cdk2/cyclinE kinase complex pathways (3,4,6).

Although rapamycin's mechanism remains elusive, second derivative circular dichroism studies of rapamycin, FKBP12 and rapamycin-FKBP complexes indicate that the triene region of rapamycin adopts a more rigid, planar conformation in the complex. X-ray crystallography studies indicate that the conformation of FKBP's loop between the 4th and 5th beta pleated sheets assumes a distinct conformation upon binding to rapamycin in contrast to native FKBP or FKBP complexed with FK506 (7). Since conformational changes are observed in both FKBP and rapamycin in the complex, the target of rapamycin (TOR) protein(s) can interact with FKBP, rapamycin, or more likely, both FKBP and RAPA in the complex, as observed for CsA-CyP and FK506-FKBP binding of calcineurin (8). Two TOR proteins have been identified in yeast and the mammalian TOR proteins are being feverishly pursued.

Small molecules which modulate cytokine production and responses are potential therapies for asthma, transplantation and autoimmune diseases. PDE-IV inhibitors are being pursued primarily for asthma indications. FK506, FK520, and rapamycin as well as CsA are potent immunosuppressive agents in vivo in both transplantation and autoimmune models and may be useful therapeutic agents in man. Combination studies indicate that co-administration of rapamycin with CsA, or FK506 can yield synergistic immunosuppressive effects.

5.1 HUMAN Fc RECEPTOR II (FcγRII) REGULATES IL-1 RECEPTOR ANTAGONIST PRODUCTION (IL-1Ra) PRODUCTION: Clay B. Marsh, Clark L. Anderson, Mark D. Wewers. The Ohio State University, Columbus, OH 43210.

IL-1 receptor antagonism (IL-1Ra) is stimulated by immobilized IgG-induced monocytic Fc receptor crosslinking. This study was designed to determine which monocyte Fcγ receptor regulates IL-1Ra production. Using immobilized mouse Fab anti-human FcγR antibodies (1 µg/ml) (Medarex), containing <10 pg/ml of endotoxin, we stimulated IL-1Ra release from enriched normal human monocytes (10^6/ml). After 18 hours, supernatants were assayed by IL-1Ra ELISA. Compared to adherent cells, immobilized anti-FcγRII (n=6) significantly induced IL-1Ra, but equivalent concentrations of anti-FcγRIII antibodies did not ( adhere): n=4, 1±0.3 anti-FcγRIII B.B2.2.4 µg/ml, anti-FcγRIII 2.0±0.7 µg/ml; and anti-FcγRII: 4.7±1.2 µg/ml, respectively) (p<0.01 for FcγRII antibodies versus no antibody). In an attempt to block Fcγ-induced IL-1Ra, monocytes were preincubated with soluble anti-Fcγ antibodies for 1 hour (1 µg/ml) before an 18 hour incubation on immobilized IgG (Sandoglobulin). Soluble anti-FcγRI (n=5) significantly suppressed IL-1Ra (p=0.001), but anti-FcγRII or FcγRIII did not. Lastly, to confirm the role of FcγRII in IL-1Ra regulation, subjects homozygous for the FcγRII arginine polymorphism (“high responder”) were compared to homozygous histidine responders (“low responder”) for IL-1Ra induction by immobilized immunochemical anti-FcγR antibody 41H10 (which only recognizes the arginine polymorphism). In high responder FcγRII monocytes (n=2), immobilized 41H10 (1 µg/ml) induced IL-1Ra up to 140% of IgG-stimulated IL-1Ra (control), compared to 66% of IL-1Ra for low responders. In contrast, immobilized IgGl (1 µg/ml), which binds to human FcγRI and FcγRII, induced monocyte FcγR crosslinking as assessed by FcγRII production, but did not induce monocyte IL-1Ra release over background. These data suggest that monocyte FcγRII primarily regulates IL-1Ra release.

5.2 TUMOR NECROSIS FACTOR (TNF) MODULATES THE TRANSEPITHELIAL RESISTANCE OF LLC-PK1 EPITHELIAL CELL SHEETS. Colleen W. Marcus, Kathleen Y. Lechlin, Linda M. Russo, Alejandro Peralta Soler and James M. Mullin. Lankenau Medical Research Institute, Wynnewood, PA 19096.

The transepithelial resistance (TER), an inverse measure of the tight junctional (TJ) permeability across renal epithelial LLC-PK1 cell sheets, displays a multiphasic and reversible response to the presence of the cytokine, Tumor Necrosis Factor (TNF). Following a 90 minute delay after TNF administration, TER abruptly decreases and then rapidly increases. This finding, together with an understanding of the changes in TER during TNF administration, suggests that the mechanism of tight junctional permeability, increases as the TER decreases, as does the peritubular penetration of the electron dense dyes ruthenium red. The effect of TNF on TER appears to be blocked by antibodies directed to the TNF receptor. The use of monoclonal antibodies directed to the p55 TNF receptor. TNF can exert its effects on the TER present to the apical or basolateral surfaces alone, but the decrease in resistance is greatest when TNF is applied to both surfaces. The inhibition of production of TNF-induced TER responses supports a role for tyrosine kinase in mediating the effect. Inhibition of PKC (preincubation with calphostin C, or H-7), or the expression of an adenosine receptor, via adenosine receptor antagonist, reduces the TNF-induced TER responses. On the other hand, activation of a-cAMP-dependent protein kinase A, subsequent to pharmacological elevation of cAMP, inhibits the BTN response to TNF. Preventing the TER response to TNF by inhibition of lipoprotein (LDL or ETYA) and potentiation of the TER responses in the presence of eicosanoids inhibitors (indomethacin and BES) aborbs the problem in the mediation of the effect. These results confirm that TNF mediated regulation of TJ permeability is a complex response to numerous positive and negative intracellular signaling pathways. This research suggests that elevations of serum TNF might underly such pathophysiological conditions as inflammation, edema, cachexia, and multigland failure inuri to TNF’s ability to regulate locally the epithelial lining of single organs. (Supported by SBO1506181 and a JDF postdoctoral fellowship).

5.3 INTERACTIONS OF NUCLEAR FACTOR IL-6 WITH THE LONG TERM TERMINAL REPEAT OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1. Valerie M. Teasler and Minou Rina. Department of Chemistry, Purdue University, West Lafayette, IN 47907-1393.

In response to IL-6 induction, nuclear factor IL-6 (NF-IL6) has been shown to activate the production of several inflammatory proteins. IL-6 has also been implicated in playing a central role in the activation of the human immunodeficiency virus type 1 (HIV-1). Transient expression analysis revealed that NF-IL6 could upregulate transcription mediated by the HIV-1 long terminal repeat. This activation might be controlled by multiple DNA sequence elements, since an analysis of the terminal repeat of the enhancer revealed the existence of basic helix-loop-helix proteins. Insertional mutagenesis was performed to differentiate the protein binding properties of these two sequence motifs in NF-IL6. Analysis of mutagenic probes using nuclear extracts revealed increased levels of NF-IL6 binding to its cognate sequence in NRE-1 during macrophage differentiation of U937 cells.

5.4 CYTOKINES SIGNAL PROTEIN VEGF RECEPTOR (V/FR) PHOSPHORYLATION (PDGF) CONCEPT IN ENHANCED TUMOR PROLIFERATION AND AGGRESSIVENESS: Anwar A. Hakim. Cellular & Molecular Biology, P.O.Box 984 Ramey APO, 05056-0984, Colorado, 9S 80515.

Human breast carcinomas (HCA) cells synthesize and release a large variety of biologically active compounds including EGF, PDGF, FGF, prolactins and plasminogen activator. (1, 2) and some cytokines (IFN, TNF) that play a role in carcinogenesis (3). Binding proteins for VEGF, EGF, PDGF, TGF, are present in cancerous tissues and tumors. The role of VEGF in tumor growth and angiogenesis has been extensively studied (4). The role of VEGF in breast cancer is still under investigation. We used breast cancer tumor samples from established cell lines & fresh tissue biopsies, to release VEGF, EGF, PDGF, TGF, TNF, and other cytokines. Overexpression of cytokines is correlated with tumor growth (5). In a variety of cancers, VEGF, EGF, PDGF, TNF, and other cytokines are expressed by tumor cells, breast cancer cells, cancerous tissues, and normal cells. This research suggests that elevations of serum TNF and other cytokines in cancer patients may be a direct measure of tumor size and location. In this study, we investigated the role of cytokines in promoting tumor growth and their effect on tumor angiogenesis.

5.5 POTENTIAL INHIBITION OF IL-4 WITH ENDOGENOUS CYTOKINES IN VIVO. Lee Sullivan, Loretta Boher, Michael Grace, Sara Braun, Heshch Madooh, Fariboud Payandei, Catherine Puglisse-Sive and Satvyat Narula. Schering-Plough Research Institute, Kenilworth, NJ 07033.

We delivered aliginate entrapped cells secreting recombinant IL-4 to study the effect of this cytokine on the hematopoietic cells of normal mice. Cells in the peripheral blood, peritoneal cavity and spleen were monitored over a two-week period. The most dramatic effects were an increase in neutrophils in all populations and an increase in the peritoneal phagocytic cell population, correlating with an increase in eosinophils and monocytes. These increases could be specifically inhibited by monoclonal antibodies (mAbs) to IL-4. Anti-GM-CSF mAb also strongly inhibited all IL-4 mediated changes in the cell populations tested, as did anti-IL-3 mAb albeit to a lesser extent than either anti GM-CSF or IL 3 mAb, except that it dramatically inhibited the expansion of eosinophils in the peritoneal cavity. These results strongly suggest that the continuous administration of IL-4 in vivo can induce or interact with endogenous GM-CSF, IL-3 and IL-5 which in turn can alter hematopoietic cell number and function.


Intratracheal (i.t.) administration of endotoxin (0.2 µg) to rats stimulates alveolar macrophage (AM) production of tumor necrosis factor, (TNF) and up-regulates nitric oxide synthesis by AM and production of oxygen radicals (O2). TNF is believed to be the mediator of gene expression for NO in response to LPS. We tested this concept by treating rats with an intravenous anti-TNF antibody (Atab) or non-immune IgG (IgG) (1.3 mg/rat, iv) 9 hr before or pentoxifylline (PTF, 25 mg/kg, i.p.) 0.5 hr before i.t. LPS to male Sprague Dawley rats. AM and PMW were obtained from bronchoalveolar lavage fluid (BALF) from lungs of rats killed at 2 and 4 hrs post LPS. AM and PMW were assayed for NO for NO release with paper chromatography with BALF. BALF and plasma were analyzed for TNF, NO, Atab and PTF abolished TNF-induced increases in TNF, but did not suppress the NO content of the BALF or gene expression for iNOS by AM or PMW. We conclude that PTF-induced upregulation of gene expression for TNF, and iNOS in AM and PMW are temporally related events. However, TNF is not required for induction of iNOS. mRNA for iNOS II is resistant to suppression by PTF.
5.7


MIF, previously described as a T-cell cytokine, has been re-discovered recently as a mitogen and a cytokine that plays an important role as a mediator of endotoxin shock (Bersohnen et al., 1993, Nature 365, 756-759). Despite the molecular cloning of a human MIF and the characterization of its signal transduction and biological properties, its precise function and location has remained incomplete in large part because significant quantities of pure, bioactive MIF protein have been unavailable. We cloned and expressed both murine and human MIF and purified significant quantities of both, for biological and structural characterization. For comparison purposes, native MIF was purified to homogeneity from mouse liver using sequential ion exchange and reverse-phase chromatography. The M, of native MIF was identical with that of recombinant MIF as assessed by SDS-PAGE/Western blotting (125 kDa monomer) and mass spectrometry analysis. Both recombinant and native MIF exhibited comparable biological activities. MIF (1 ng/ml) inhibited IL-1 beta-induced NO production from murine macrophages. However, significant NO generation (5.7-20.4 pM) was observed only in cells primed with IFN-gamma. MIF activity in a monococyte migration assay was dose-dependent and peaked at 0.1 pg/ml. Circular dichroism spectroscopy revealed that both human and murine MIF belong to the Cu/Zn class of proteins. Both MIF species displayed high percentages of p-sheet (murine: 39%, human: 51%) and a-helix (murine: 18%, human: 31%) conformation.

5.8

EXCRETION OF CYTOKINES AND RELATED FACTORS IN THE URINE BY ASTRONAUTS DURING AND AFTER SPACEFLIGHT. J.P. Stein, M.P. Schuler and L.L. Moldawer, Dept. of Surgery, UMDNJ-SOM, Stanford, NY and Univ. of Florida, Gainesville, FL.

Ascent to and living under the microgravity conditions found during spaceflight is an unfamiliar environment for mankind. The adaptation to the space environment may elicit a stress/inflammatory response by the body as a stress. The objectives of this study were to determine whether spaceflight is associated with increased cytokine activity. The mean daily urinary IL-6, IL-1ra, and sTNF-RII and cortisol excretion rate were measured on 24 hr urine pools collected from the four payload crew members from eleven days before launch to seven days after landing for a total of twenty seven days. The experiment was conducted before, during and after the 1991 9.5 day SLS-I (Columbia) Space shuttle mission. Dietary intake and urine output were monitored continuously for the 27 day period for the four payload crew. Results: (i) Urinary IL-6, IL-1ra and cortisol excretion were increased on the first day of spaceflight suggesting an acute phase response. (ii) sTNF-RII was only increased after landing. (iii) The pattern of IL-6 excretion post-flight was different from that of sTNF-RII and IL-1ra.

5.9

LYMPHOKINE EXPRESSION IN RAT LUNGS AFTER ANTIGEN CHALLENGE. BM Kuroo, JP Yang, JM Martin, GM Hazel, MCH Medive, The Christie Lab, Royal Victoria Hospital, McGill University, Andre Viallet Research Institute, Pulmonary Unit, St. Luc Hospital, Mil, PQ, Canada.

Lymphokines are believed to participate in the inflammatory response which follows allergen challenge (AC). To assess whether certain lymphokines are involved in the physiological responses after AC we evaluated lymphokine mRNA expression in the lungs of 2 strains of rats. Brown Norway (BN) are high IgE producers which develop late airway responses (LR) and increased airway responsiveness after AC and Sprague Dawley (SD) rats, low IgE producers which develop little LR and no increased airway responsiveness after AC. Rats were sensitized with ovalbumin and challenged 14 days later. Eight hours after AC the rats were killed, the vasculature washed, the right lung fixed in paraformaldehyde (PFA) and the left lung frozen for total RNA determination, reverse transcription and PCR for interleukin (IL)-2, 4, 5, 10 and interferon gamma (IFN). PCR was standardised on lung RNA for each cytokine. Results from PCR were also confirmed by Southern hybridization. All BN lungs expressed IL-4 and 10 but no lungs expressed IL-2 or IFN eight hours after AC. All SD lungs expressed IL-2, 10 and IFN. IL-5 was expressed in some lungs of BN and SD rats. IL-4 was also expressed in the lungs of some SD rats. In situ hybridization confirmed the results for IL-2 and showed that IL-4 was expressed by more cells in BN rats. The differences in cytokine expression after AC may be involved in the physiological changes which occur after AC in BN rats. (Supported by the MRC of Canada and RHNGC).

5.10


Exposure to ozone with exercise results in airway inflammation, with increases in PMN and inflammatory mediators in bronchoalveolar lavage (BAL) fluid, including interleukin-6 (IL-6). We sought to determine whether ozone exposure increases circulating levels of IL-6 or induces a systemic acute phase response. Eighteen physically fit nonsmokers underwent 2 exposures to 0.99 ppm ozone and 1 exposure to air, each for 4 hours with exercise, separated by at least 3 weeks. Phalbution and BAL were performed either immediately or 18 hours after exposure. Exercise in air resulted in an acute phase response, with a 4-fold increase in circulating immunoactive IL-6, as well as increases in circulating WBC, lactate dehydrogenase (LDH), and aspartate amino transferase (AST), immediately after exercise. C-reactive protein (CRP) increased 3-fold 18 hours after exposure. Ozone exposure with exercise was associated with a marked additional increase in IL-6 in 4 of 8 subjects, but did not enhance the WRC. IL-6, CRP, or subsequent CRP increases. Exercise induces an acute phase response in physically fit subjects; IL-6, but not CRP, is further increased by exercise in ozone.

5.11

PLATELET ENHANCED IL-1alpha PRODUCTION BY MONOCYTIC CELLS. Burton D. Clark, Ellen C. Donaldson, Rekha A. And, Ronald G. Toccalino, Charles A. Cingolani, John F. Burke, and Jeffrey A. Galland. Dept of Medicine, New England Medical Center, Tufts Univ. School of Medicine, Surgical Services, Massachusetts General Hospital, and Dept. of Surgery, Harvard Medical School, Boston, MA 02111.

It is widely alleged that platelets are involved in maintaining hemostasis yet it is less widely appreciated that platelets have other physiological roles not normally associated with thrombosis. We have analyzed the potential role of platelets in the inflammatory response. More specifically, we have investigated the role of platelets with monocytes and their enhancement of cytokine production by the monocyte cell line, MonoMac 6. In our studies, through-activated platelets (P) were incubated with MonoMac 6 cells. Results: the IL-1 production was found to be dose-dependent with platelets (P+) yielding 3.5, 8.0, 8.9 and 9.2 fold increases in the IL-1 production at platelet to cell ratios of 0, 16, 32, 160, and 800 platelets/MonoMac with or without endotoxin (LPS) or heat-killed S. epidermidis (SE). IL-1 was quantitated by a radioligand and sensitive radioassay subsequent to 4h and 8h incubations. The incubation of activated platelets with monocyte cells (16:1 ratio) for 4h in the presence of LPS (M+P+LPS) at 5.000 and 1000 pM/LPS resulted in 36%, 47%, 489%, increases in IL-1alpha, respectively, compared to monocytes exposed to LPS without platelets (baseline, M+LPS). After 8h incubation, the overall percentage change from baseline decreases by approximately one-half, reflecting by a corresponding decrease in baseline (M+LPS). Overall, these results indicate that activated platelets may magnify the cytokine response observed during both Cram positive and Cram-negative infection.

Cystic fibrosis (CF) is caused by mutations in the 250 kb gene encoding for a CFTR protein. To assess a possible regulation of CFTR gene expression by IFN-γ, which is produced in response to infections during CF, the effects of IFN-γ, on CFTR mRNA levels were tested in H292 and 184B cell lines. Treatment of these cells with IFN-γ, not with IFN-γ, and IFN-γ, resulted in a reduced amount of CFTR mRNA in a concentration-dependent manner. This effect was detectable at a concentration as low as 0.1 IU/ml. Short-term treatment by 100IU/ml IFN-γ did not affect the protein, nor the mRNA levels of β-actin and β-2 microglobulin (β 2 M). Thus, IFN-γ did not affect β-actin mRNA content and increased the steady state level of Z-2-oligodeoxynucleotide synthetase mRNA, one of IFN inducible genes. We also investigated IFN-γ effects on the expression of CFTR mRNA levels in the H292 cells compared to the controls (0 and 14 hrs, respectively) suggesting that the down-regulation of the CFTR mRNA occurs through desensitization of the transcripts. Western blot analysis using anti-CFTR antibodies indicated that IFN-γ protein content was diminished in the cells treated by IFN-γ. To establish whether the decrease in CFTR mRNA was reflected at the functional level, we assayed CFTR function by measuring the cyclic AMP/cAMP-stimulated fluxes in contra1 and IFN-γ-treated cells. Both assays indicated that AMP-stimulated fluxes were decreased in the IFN-γ-treated cells indicating inhibitory function. These data suggest that production of IFN-γ in response to infections might modulate CFTR secretion.

Supported by INSERM, Ministère de la Recherche et de l’Espace and AFLM, France.

6.3 INFILTRATORY CYTOKINES LOWER ERYTHPOIETIN GENE EXPRESSION. Wolfgang Felmann, Stella Frede and Joachim Pandrau, Institute of Physiology I, University of Bonn, D-53115 Germany.

In patients with the anemia of chronic disorders the plasma level of the hormone erythropoietin (Epo) is often found to be low in relation to the blood hemoglobin concentration. We have carried out our study in vivo and in vitro studies to find out whether infiltratory cytokines impinge hypoxia-induced Epo production. In rats exposed to hypoxia (8% O2 in the inspiratory gas) for 6 h, single injections of bacterial lipopolysaccharides (LPS, 0.1 mg/kg, i.p.) caused serum Epo protein and renal Epo mRNA levels, as measured by radioimmunoassay and competitive polymerase chain reaction, respectively, to increase in relation to the blood hemoglobin concentration. We have carried out our study in vivo and in vitro studies to find out whether infiltratory cytokines impinge hypoxia-induced Epo production. In rats exposed to hypoxia (8% O2 in the inspiratory gas) for 6 h, single injections of bacterial lipopolysaccharides (LPS, 0.1 mg/kg, i.p.) caused serum Epo protein and renal Epo mRNA levels, as measured by radioimmunoassay and competitive polymerase chain reaction, respectively, to increase in relation to the blood hemoglobin concentration. In an attempt to identify the inflammatory mediators of the impaired synthesis of Epo, effects of various recombinant cytokines were studied in an Epo-producing human hepatoma cell line (HepG2). Interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) proved to be the most potent cytokines in suppressing the Epo mRNA level. We propose that IL-1, TNF-α, and possibly other cytokines are responsible for the defective Epo production in renal and nonrenal immune responses to disease. The Epo gene expression is regulated by cytokines, and thus provide an experimental basis for understanding the cardiovascular effects of TNF-α.

6.4 ADULT MAMMALIAN CARDIAC MYOCYTES EXPRESS BOTH TNF-RECEPTORS. RL Mann, G. Torres-Amiouve, C. Respaud and R. Levollet, Baylor Coll Med, Houston, TX.

We have previously demonstrated that tumor necrosis factor alpha (TNF-α) exerts significant negative inotropic effects in adult feline cardiac myocytes. TNF-α binds to two specific receptors: TNF-R1 and TNF-R2. While most cell types express both TNF receptors, their presence in cardiac myocytes has not been shown. Therefore, we sought to determine whether adult cardiac myocytes express TNF-R1 or TNF-R2. Saturation binding isotherms using human (h) 125-I-TNF-α in isolated adult feline cardiac myocytes displayed saturable binding, indicating the presence of specific TNF receptors. The affinity of binding of 125-I-TNF-α for myocytes was 9X10^9 M^-1. To determine whether TNF-R1 or TNF-R2 were also expressed in the human heart as well, tissue sections of normal human myocardium were stained by immunoperoxidase using monoclonal antibodies that specifically recognize TNF-R1 or TNF-R2. All specimens tested showed specific extracellular staining of myocaridal fibers with anti-TNF-R1 and anti-TNF-R2 antibodies. These data demonstrate that adult cardiac myocytes express TNF-R1 and TNF-R2, and thus provide an experimental basis for understanding the cardiovascular effects of TNF-α.
INHIBITION OF ENDOTOXIN INDUCED MICROVASCULAR CHANGES BY BLOCKADE OF THE INTERLEUKIN-1 RECEPTOR.  


Department of Surgery and Physiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235.  

Endotoxin induces vascular changes ranging from hypotension, arteriolar constriction, decreased vasocoractor sensitivity to norepinephrine (NE) (Circ. Shock 12:163, 1984) and loss of endothelium in large arteries (Circ. Shock 41:71, 1993). Endothelium-dependent arteriolar vasodilation in response to acetylcholine (ACH), however, is maintained (Am. J. Physiol. 264:H1118, 1993). Since blockade of the cyclooxygenase cascade at the level of the interleukin-1 (IL-1) receptor has been shown to attenuate endotoxin-associated hypotension, hypothermia, and acute lung injury, we studied antagonism of the IL-1 receptor antagonist (IL-1ra) in vivo in rats. Methods: Cremaster muscle arterioles, A2, and A3 arterioles of 16 rats were studied by video microscopy. Following baseline measures of mean arterial pressure (MAP) and arteriolar diameters, dose-response curves to topical NE (10^-8 to 10^-4M) and ACh (10^-8 to 10^-4M) were obtained. Prior to the administration of IL-1 ra (0.5 mg/kg iv, 10 mg/kg one half of the animals were randomly assigned to receive recombinant IL-1ra (0.2 mg/kg/mice iv for 5 hours). All animals were followed by hourly measures of arterial diameters and NE sensitivity for two hours. Results and Discussion: Administration of IL-1ra treatment prevented death in all IL-1ra treated animals but no untreated animals (p<0.01). It is concluded that antagonism of the IL-1 receptor produces a significant increase in survival during endotoxemia which is associated with maintenance of MAP, reactivity of arterioles to NE and ACh, and the integrity of the endothelium. (Supported by Am. Heart Assn., FL Affiliate).  

SUNDAY  
CYTOKINE INHIBITORS  
A-47  

INHIBITION OF ENDOTOXIN-INDUCED AKINESIA AND ACHE inhibition BY INHIBITION OF TNF.  


Dep. of Physiol. and Pharmacol., Oklahoma State University, Stillwater, OK 74078.  

The use of endotoxin as a model for the study of endotoxemia is limited by the high mortality rate. In an attempt to study endotoxemia with a lower mortality rate, we examined the axotomy and the acetylcholinesterase (AChE) inhibition by a monoclonal antibody (mAb) against TNF. Methods: Animals (100 g) were injected ip with LPS (2 mg/kg) and divided into 5 groups 1-5: (1) control, (2) 15 mg/kg of TNF mAb, (3) 50 mg/kg, (4) 250 mg/kg, and (5) 1000 mg/kg. Each group contained 10 animals. Neuronal axotomy was evaluated by determining the percentage of sciatic nerve fibers with axons growing into the nerve graft. AChE inhibition was assessed by measuring the rate of hydrolysis of acetylcholine in homogenates of sciatic nerves. Results: Axotomy was significantly increased in 15 mg/kg TNF mAb group compared to control. AChE activity was decreased in the 15 mg/kg TNF mAb group compared to control and increased in the 50 mg/kg TNF mAb group compared to control.  

METHODOLOGY IN A BABOON MODEL OF SEPTIC SHOCK.  


Departments of Surgery, Physiology, and Pathology, University of Oklahoma Health Science Center, Oklahoma City, OK 73104.  

Many investigations have focused on the peripheral actions of TNF and IL-6 in the pathophysiology of septic shock. This report targets the possible roles of TNF and IL-6 in the pathophysiology of septic shock.  

EFFECTS OF CENTRAL INTERLEUKIN-1 ON PERIPHERAL METABOLISM IN THE SEPTIC BABOON.  

SUNDAY CYTOKINE INHIBITORS A-48  

MONDAY  
CYTOKINES AND METABOLIC/ENDOCRINE INTERACTIONS INCLUDING REPRODUCTION  

9.1  
EFFECTS OF CENTRAL INTERLEUKIN-1 ON PERIPHERAL METABOLISM IN THE RAT.  

R. D. Stith and L. Templer.  


Many investigations have focused on the peripheral actions of interleukin-1b (IL-1b) but little is known about the effects of centrally acting IL-1 on peripheral metabolism. We studied dose responses to IL-1b in fasted, male Sprague-Dawley rats over 6 hours. Peak core body temperature (39.6, 666, and 682 rig/ml; control=188 rig/ml) were measured after 15, 25, and 50 ng IL-1b, respectively. Plasma corticosterone (pl) increases 50 (200 mug/kg/day), IL-2 (106U/day), and IL-2 and PTXF. Spleen cell suspensions were tagged with fluorescent antibodies to the adhesion molecules CD1 la and CD49d and analyzed by flow cytometry for fluorescent intensity per cell (FIc). Indices of toxicity and blood chemistry were evaluated. Data in the table (mean ± SEM) show flow cytometry data and two indices of toxicity, SOPT (serum glucose pyruvate transaminase) and BILI (bilirubin). F1c=0.05: (*) compared to control and PTXF, and (b) compared to all other groups.  

<table>
<thead>
<tr>
<th></th>
<th>CD1a</th>
<th>CD49d</th>
<th>SOPT (IU/L)</th>
<th>BILI (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>355 ± 10</td>
<td>15 ± 1</td>
<td>5 ± 2</td>
<td>0.5 ± 0.05</td>
</tr>
<tr>
<td>PTXF</td>
<td>411 ± 40</td>
<td>35 ± 1</td>
<td>41 ± 2</td>
<td>0.7 ± 0.05</td>
</tr>
<tr>
<td>IL-2</td>
<td>1500 ± 32*</td>
<td>50 ± 3*</td>
<td>276 ± 27*</td>
<td>3.4 ± 0.78*</td>
</tr>
<tr>
<td>IL-2 &amp; PTXF</td>
<td>297 ± 35*</td>
<td>50 ± 3*</td>
<td>228 ± 4*</td>
<td>0.7 ± 0.12*</td>
</tr>
</tbody>
</table>

IL-2 induced increases in SOPT and the expression of CD1la and CD49d were not attenuated by PTXF. PTXF did decrease bilirubin levels toward control values. These results indicate that these dose of PTXF had a limited effect on the responses to chronic 6-day IL-2 treatment.

There is evidence that T-cell mediated immunity and IL-2 production decline with age in mice and humans. However, it is not clear if there is similar senescence of the monocyte arm of the immune system. We examined the production of the inflammatory cytokines IL-1β and TNFα by 420 elderly Framingham Study participants (Group E; mean ± SD: age 78 ± 5 y, 20 healthy younger Framingham controls (Group F; age: 54 ± 10 y). IL-1β was measured for 54h in sera of the PBMC and with LPS 1 or 100 ng/ml or heat-killed S. epidermidis as stimulants. Mature cytokines were assayed using a sensitive and specific RIA. Dose-responses of the stimulated proliferation of T-cells, and the IL-1β production was tested in all groups in this study. We have observed that IL-1β can influence protein balance. Experiments have also shown that IL-1β can influence the catabolic response. The present study characterized the role of IL-1β and TNFα on the production of collagenase.

* pmol/ml; r Ra, the rate of appearance; ; n ml/kg/min; ** FFA, free fatty acids. # Different from basal value with paired r-test, P<0.05.
10.1 THE CENTRAL LOCATION OF GLUCOCORTICOID NEGATIVE FEEDBACK DURING PSYCHOLOGICAL STRESS-INDUCED FEVER. J.L. McCullan, L.E. Morrow, J.J. Keil, M.J. Kluger, University of Michigan, Ann Arbor, MI 48109.

Previous work has shown that glucocorticoids (GCs) administered directly to the CNS can inhibit fever, but the site of action remains to be elucidated. To determine whether such antipyretic action of this cytokine is systemic or in the central nervous system (CNS), we examined the effect of intracerebroventricular (icv) or by a slow infusion into the anterior hypothalamus (AH) of an amount previously measured in this brain region during LPS fever (0.24 U in 0.5 μl of saline) on fever induced by lipopolysaccharide (LPS) in rats. fever was measured by means of baseline temperature (37.01 ± 0.12°C) (paired t test, P = 0.301). When rats were injected ip with TNF, the febrile response to LPS was abolished or greatly attenuated. These results indicate that IL-1β acts centrally to stimulate whole body glucose flux. Furthermore, the enhanced HGP is mediated by the increased glucocorticoids. Enhanced HGP can be blocked by glucocorticoid receptor antagonists and inhibited by glucocorticoid receptor antagonists. These data suggest that the hypothalamus modulates the hypothalamic temperature set point (37.0°C) by a mechanism that is independent of circulating glucocorticoids. These data also suggest that the hypothalamus modulates the hypothalamic temperature set point (37.0°C) by a mechanism that is independent of circulating glucocorticoids. These data also suggest that the hypothalamus modulates the hypothalamic temperature set point (37.0°C) by a mechanism that is independent of circulating glucocorticoids.

10.3 SYSTEMIC BUT NOT CENTRAL ADMINISTRATION OF TUMOR NECROSIS FACTOR α ATTENUATES LPS-INDUCED FEVER IN MICE. John L. Tompsett, L.E. Mottram, W.J. Keil, M.J. Kluger, Institute for Basic and Applied Medical Research, The Lovelace Institute, Albuquerque, NM 87117.

The purpose of this study was to test the hypothesis that tumor necrosis factor α (TNF-α) limits fever induced by lipopolysaccharide (LPS) in rats, and to determine whether such antipyretic action of TNF-α is systemic or in the central nervous system (CNS). The CNS effects on LPS-induced fever were tested by injecting a subhypothalamic amount (0.10 μg) of human recombinant TNF-α (hrTNF) intracerebroventricularly (icv), or by a slow infusion into the anterior hypothalamus (AH) of an amount previously measured in this brain region during LPS fever (0.24 U in 0.5 μl of saline) on fever induced by lipopolysaccharide (LPS) in rats. Fever was measured by means of baseline temperature (37.01 ± 0.12°C) (paired t test, P = 0.301). When rats were injected ip with TNF, the febrile response to LPS was abolished or greatly attenuated. These results indicate that IL-1β acts centrally to stimulate whole body glucose flux. Furthermore, the enhanced HGP is mediated by the increased glucocorticoids. Enhanced HGP can be blocked by glucocorticoid receptor antagonists and inhibited by glucocorticoid receptor antagonists. These data suggest that the hypothalamus modulates the hypothalamic temperature set point (37.0°C) by a mechanism that is independent of circulating glucocorticoids. These data also suggest that the hypothalamus modulates the hypothalamic temperature set point (37.0°C) by a mechanism that is independent of circulating glucocorticoids. These data also suggest that the hypothalamus modulates the hypothalamic temperature set point (37.0°C) by a mechanism that is independent of circulating glucocorticoids.

10.4 SLEEP PATTERNS IN HEPATITIS B AND INFECTED INFANTS ARE CORRELATED WITH ALLELES OF THE IF-1 GENE. Linda A. Thot, Judith D. Phillips, Hosp. for Sick Chld., Toronto, ON, Canada.

To evaluate the role of interferon (IFN) in sleep enhancement during viral infections, we monitored sleep in C57BL/6 and BALB/c mice before and after intraperitoneal inoculation with influenza virus. Differences in alleles of the Tlr-2 gene in humans and mice suggest that IFN-α may induce IFN-γ production in influenza infected animals. Influenza infected C57BL/6 mice (n = 7) exhibited markedly higher variation in sleep parameters, whereas circadian variations in BALB/c mice (n = 7) were much less pronounced (paired t test). Infected animals also showed reduced levels of sleep and locomotor activity as well as reduced sleep latency and duration. These data indicate that sleep enhancement in influenza infected C57BL/6 mice and sleep enhancement in BALB/c mice were mediated by IFN-γ production, which is consistent with the hypothesis that IFN-γ production is required for sleep enhancement following influenza infection. These data also indicate that the role of IFN-γ in sleep enhancement following influenza infection is correlated with the IF-1 allele for high IFN production in mice.

10.5 IL-4 INHIBITS THE PRODUCTION OF NITRIC OXIDE IN STIMULATED GLIAL CELLS. Moshe Goldstein and Chaya Brodie. Department of Life Sciences, Bar-Ilan University, Ramat Gan-52900, Israel.

There is growing evidence that glial cells can be stimulated to act as immunocompetent cells in the CNS. Recent studies have shown that glial cells have been reported to express an inducible nitric oxide synthetase which can be activated by LPS and cytokines. In this study we examined the effect of IL-4, a complex cytokine produced by activated T cells, on the production of NO in a number of human and rat glioma cell lines. Treatment of the cells with INF-γ induced a dose-dependent increase in NO production. IL-1β did not change the basal levels of NO production but inhibited that induced by INF-γ. The inhibitory effect of IL-1β was more pronounced in cells pre-treatment for 24 hr before stimulation with INF-γ. This effect of IL-1β was not mediated through a decrease in the amount of INF-γ receptors. Moreover, other effects of INF-γ in these cells, such as the induction of major histocompatibility complex class II was not inhibited by IL-1β. These results suggest a complex cytokine interaction during instances of trauma and inflammation in the CNS and a possible neuroprotective role for IL-4 against activated glial cells.

10.6 INHIBITION OF I CELL FUNCTION IN PATIENTS WITH GLIOBLASTOMAS: A SELECTIVE IMPAIRMENT OF THE IL-2 SYSTEM. Chaya Brodie*, Alex Tsetkerman*, Hy Ashkenazi*, Moto Deutsch, Reuven Tirosh, and Arvyas Weyrauch. *Department of Life Sciences, **Department of Neurosurgery, Hadassah, Ein Karem, The Jerome Schotterstein Cellscan Center, Department of Physics, Bar-Ilan University, Ramat Gan, Israel.

The importance of the nervous system in regulation of the immune system is manifested in various physiological and pathological conditions. Recently, the occurrence of glioblastomas has been associated with broad immunodepression as demonstrated by low in vivo and in vitro immune cell function. Glioblastomas patients express deficiencies in cell-mediated immunity, such as reduced T lymphocyte responses to mitogens and viral antigens. Glioblastomas patients express deficiencies in cell-mediated immunity, such as reduced T lymphocyte responses to mitogens and viral antigens. Glioblastomas patients express deficiencies in cell-mediated immunity, such as reduced T lymphocyte responses to mitogens and viral antigens. Glioblastomas patients express deficiencies in cell-mediated immunity, such as reduced T lymphocyte responses to mitogens and viral antigens. Glioblastomas patients express deficiencies in cell-mediated immunity, such as reduced T lymphocyte responses to mitogens and viral antigens. Glioblastomas patients express deficiencies in cell-mediated immunity, such as reduced T lymphocyte responses to mitogens and viral antigens. Glioblastomas patients express deficiencies in cell-mediated immunity, such as reduced T lymphocyte responses to mitogens and viral antigens.

*Department of Life Sciences, **Department of Neurosurgery, Hadassah, Ein Karem, The Jerome Schotterstein Cellscan Center, Department of Physics, Bar-Ilan University, Ramat Gan, Israel. © 2003 American Society for Neurochemistry. All rights reserved.

Intravenous (i.v.) injection of recombinant human endotoxin (E. coli) produces transient, delayed fevers in animals and humans. Our objective was to determine whether increases in plasma TNF levels correlated with the fever profile following PFC infusion. Rats were acclimated with saline (3 mL/kg, n=6), lipopolysaccharide (0.1 μg LPS/rat, n=6), or a 0.5% w/v TNF emulsion (0.1 μg TNF/rat, n=6) and monitored for 2 h after i.p. administration of 405 mg/kg of sodium salicylate by telemetry for 2 h. A fourth group of animals was pretreated with desamethasone (0.2 mg/kg at both 1 and 2 hrs prior to PFC injection (n=6)). Rats were anesthetized with free access to food and water. Blood was taken at baseline and 1, 2, 3, and 6 hrs post infusion to determine serum TNF levels by ELISA. The table shows TNF levels (ng/mL) and mean area under the curve (AUC) of the change in body temperature. Data are Means ±SE.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Baseline</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>13.7</td>
<td>0.0</td>
<td>0.0</td>
<td>3.8±1.4</td>
<td>14.0±3.0</td>
</tr>
<tr>
<td>LPS</td>
<td>15.4</td>
<td>14.1±4.4</td>
<td>17.9±3.9</td>
<td>23.5±6.5</td>
<td>22.7±5.2</td>
</tr>
<tr>
<td>PFC</td>
<td>127.5±10.6</td>
<td>152.7±10.2</td>
<td>162.5±10.3</td>
<td>173.6±12.5</td>
<td>180.6±14.2</td>
</tr>
<tr>
<td>Dex + PFC</td>
<td>10.9</td>
<td>12.8±1.2</td>
<td>14.6±0.3</td>
<td>13.3±0.9</td>
<td>15.6±0.6</td>
</tr>
</tbody>
</table>

There were significant changes in TNF or body temperature in saline control animals. After PFC administration, TNF levels of both groups increased. Plasma TNF did not induce fever (while higher LPS doses induce fever in this model). A moderate, but sustained increase in TNF was observed after PFC. The sustained increase in TNF is presumably related to the gradual removal of PFC from the circulation by macrophages. After PFC, rats exhibited a transient fever which peaked at ~125°C above baseline at 5 hrs. Sustained increases in TNF and the fever following PFC. These data suggest that serum TNF levels do not always correlate with fever. However, the time course or duration of TNF release may play a role in the production of fever.


Previous studies have shown that injection of antiserum against tumor necrosis factor (TNF) enhances the febrile response to lipopolysaccharide (LPS), suggesting that this cytokine may act as an endogenous pyrogen, limiting the magnitude of fever (Long et al., Am. J. Physiol. 263:R332, 1990). One potential mechanism for this response is that TNF may down-regulate the production of nitric oxide (NO), which, in turn, could inhibit L-NAME, to that seen in rats treated with its inactive enantiomer, L-NAME. L-NAME and L-NAME were administered continuously in the rats' drinking water (70 mg/lOO ml) starting 8 days before the study began. The L-NAME-treated rats had a mean blood pressure of 151 mmHg, while the L-NAME animals had a mean blood pressure of 177 mmHg (p<0.001), confirming that NO is involved in regulation of arterial body temperature. The NO production of the rats was monitored by implanted biotelemetry devices. We found no difference in the baseline Tb of the rats (L-NAME: 36.4±0.1°C vs D-NAME: 36.3±0.2°C), indicating a regulatory role of NO after immunological stimulation. Previous experiments have demonstrated that systemic administration of lipopolysaccharide (LPS) mimics various aspects of the APR. We recently demonstrated that i.p. administration of LPS induces the expression of Fos-like immunoreactivity (Fos-IR) in nuclear groups of the rat brain thought to be involved in regulation of autonomic homeostasis. In the present study, we have used PFC as a model of sepsis and have mapped the resultant Fos-IR addition. We have examined subsets of cells in the brain that contain both Fos-IR and NADPH-diaphorase staining (NO synthase activity) following PFC challenge. Cells that contained both NO staining and Fos-IR were observed in the medullary region. The results of this study provide evidence for a role of NO in regulating neuroendocrine responses following immune activation.

11.1 PRO-IL-B IS RELEASED FROM MONOCYTES IN VITRO IN A FORM THAT IS INACTIVE. PRO-IL-B IS INACTIVATED BY IL-12 AND LPS. JOSEPH D. WENSTER AND A. B. BOYNE. The Ohio State University, Columbus, OH 43210.

The processing and release of 31 kDa pro-IL-β to the mature 17 kDa form of IL-1β is still poorly understood. In this context, we and others have noted that a 31 kDa form of IL-1β is released from monocytes in response to phagocytosis and stimulation in vitro (J. Immunol. 149:3052, 1992). Since the site of processing of the 31 kDa pro-IL-β is not known, we hypothesized that the released pro-IL-β may represent IL-1β in a pro-processing phase or IL-1β that has been modified to prevent processing. To test this hypothesis, we purified the 31 kDa pro-IL-β from the supernatant of LPS-stimulated monocytes by immunoprecipitation of 32S-labeled labeled protein, by Western blots, and by our recently developed enzyme linked immunosassay (ELISA) method. The 31.1 kDa protein was confirmed to be pro-IL-β since its immunoprecipitation was specifically blocked by the immunogenic peptide used to generate the pro-IL-β specific antibody. Finally, since supernatant pro-IL-β can be immunoprecipitated, we asked whether affinity-purified supernatant pro-IL-β could be processed to mature IL-1β when incubated with recombinant IL-1β converting enzyme (ICE/McIlvain). In two separate experiments, a purified cytokine-regulated pro-IL-β-β was processed by ICE/McIlvain, but identically purified supernatant pro-IL-β was not. These findings imply that pro-IL-β can be released from monocytes in a unique form that may reveal important clues to monocyte regulation of pro-IL-1β processing and release.


A highly-sensitive, rapid, and specific ELISA was developed for measurement of the 33KD human IL-1β (hIL-1β) precursor protein. The monoclonal antibody 225-519 ELISA reproductively detects as little as 10pg/ml of protein. Proteolytic cleavage of IL-1β precursor by IL-1 converting enzyme (ICE/McIlvain) results in release of the active, 17.5kD form of IL-1β, which is known to be a key mediator of inflammation in vivo. Assay of purified IL-1β precursor protein in vitro resulted in markedly reduced signals in the IL-1β precursor ELISA, with concomitant increased signals in measuring the active form. Because of the previous lack of availability of a sensitive, specific assay, study of regulated expression of the IL-1β precursor protein has lagged behind that of the active form. Development of this new ELISA for the human IL-1β precursor protein provides an important tool for this unexplored area of cytokine research.