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

Holiday Inn at Yale:

Saturday, June 25	12:00 Noon–6:00 pm

Yale Univ., Sterling Hall of Medicine

Saturday, June 25	6:30 pm–8:30 pm
Sunday, June 26	8:00 am–5:30 pm
Monday, June 27	8:30 am–5:30 pm
Tuesday, June 28	8:30 am–1:30 pm

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.

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

6:15 pm	Hotel to Calhoun to Harkness
9:45 pm	Harkness to Hotel
9:45 pm	Harkness to Calhoun

Sunday

7:15 am	Hotel to Commons
8:45 am	Commons to Harkness
5:00 pm	Harkness to Commons
7:15 pm	Commons to Harkness
10:15 pm	Harkness to Calhoun to Hotel

Monday

7:15 am	Hotel to Commons
8:45 am	Commons to Harkness
5:45 pm	Harkness to Calhoun to Hotel
6:45 pm	Hotel to Commons

Tuesday

7:15 am	Hotel to Commons
8:45 am	Commons to Harkness
5:00 pm	Harkness to Calhoun to Hotel
6:15 pm	Hotel to Calhoun
10:00 pm	Calhoun to Hotel

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.
11:00 Cytokines and Vascular Smooth Muscle. **Debbie Beasley.** New England Med. Ctr., Boston.
11:40 The Effects of Cytokines on Sleep Patterns. **James Krueger**. Univ. Tennessee.

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

1:30 Identification of Novel Signal Transduction Proteins of Macrophage Origin. **Bruce Beutler**. Howard Hughes Med. Inst., Dallas.
2:10 Cytokine Genetics. **Gordon Duff**. The Hallam Royal Hosp., Sheffield, UK.
2:50 Break.
3:20 Regulation of Cytokine Receptor Expression and Activity. **Steven Dower**. Immunex R&D Corp., Seattle.
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.
2 8:30 **5.2** Tumor necrosis factor-α modulates the transepithelial resistance of LLC-PK_1 epithelial cell *sheets.* ***C.W. Marano, K.V. Laughlin, L.M. Russo, A. Peralta Soler, and J.M. Mullin***. Lankenau Med. Red. Ctr., Wynnewood, PA.
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.

4 8:30 **5.4** Cytokines signal protein tyrosine kinase/protein phosphatase concept in breast tumor proliferation and aggressiveness. **A.A. Hakim**. Cell. & Molec. Biol., Kankakee, IL.

5 7:30 **5.5** Potential interaction of IL-4 with endogenous cytokine *in vivo*. **L. Sullivan, L. Bober, M. Grace, S. Braun, H. Macosko, F. Payvandi, C. Pugliese-Sivo, and S. Narula**. Schering-Plough Res. Inst.

6 8:30 **5.6** Endogenous tumor necrosis factor is not required for endotoxin mediated gene expression for inducible nitric oxide synthase II in alveolar macrophages and neutrophils. **S.S. Greenberg, J. Xie, M. Li, J. Kolls, S. Nelson, G. Bagby, and W. Summer.** LSU Med. Ctr.

7 7:30 **5.7** Purification and characterization of the cytokine macrophage migration inhibitory factor. **J. Bernhagen, T. Calandra, R.A. Mitchell, A. Cerami, and R. Bucala**. The Picower Inst. for Med. Res., Manhasset, NY.

8 8:30 **5.8** Excretion of cytokines and related factors in the urine by astronauts during and after spaceflight. **T.P. Stein, M.F. Schluter, and L.L. Moldawer.** UMDNJ, Stratford, NJ and Univ. Florida.

9 7:30 **5.9** Lymphokine expression in rat lungs after antigen challenge. **P.M. Renzi, J.P. Yang, J. Martin, and O. Hamid.** Royal Victoria Hosp., McGill Univ., and St-Luc Hosp., Montreal, Quebec.

10 8:30 **5.10** Ozone exposure, exercise, and the systemic acute phase response in humans. **M.W. Frampton, F. Malek, J.P. Fogarty, R.J. Looney, P.E. Morrow, and M.J. Utell.** Univ. Rochester.

11 7:30 **5.11** Platelet enhanced IL-1α production by monocytic cells. **B.D. Clark, E.C. Donaldson, K. Aiura, R.G. Tompkins, C.A. Dinarello, J.F. Burke, and J.A. Gelfand.** New England Med. Ctr., Tufts Univ., Mass. Gen. Hosp., and Harvard Med. Sch.

6.0 Cytokines and the Cardiovascular, Respiratory and Renal Systems

7:30 PM—Harkness Lounge

Board #

12 8:30 **6.1** Interferon-γ, IFNγ, down-regulates cystic fibrosis transmembrane regulator, CFTR, gene expression. **A. Edelman, G. Przewlocki, I. Baro, A.S. Honge, D. Escande, and F. Besançon**. INSERM, Paris and CNRS, Orsay, France.

13 7:30 **6.2** Detection of endothelin peptide and receptor gene expression in bovine corneal epithelium. **W. Tao, G. Liou, and P. Reinach**. Med. Col. Georgia.

14 8:30 **6.3** Inflammatory cytokines lower erythropoietin gene expression. **W. Jelkman, S. Frede, and J. Fandrey.** Univ. Bonn, Germany.

15 7:30 **6.4** Adult mammalian cardiac myocytes express both TNF receptors. **D.L. Mann, G. Torre-Amione, S. Kapadia, and R. Levobitz**. Baylor Col. Med.

16 8:30 **6.5** Transforming growth factor-β_1 differentially regulates fibroblast growth factor-induced DNA synthesis in cardiac and skin fibroblasts. **A. Sigel and M. Eghbali**. Yale Univ.

7.0 Cytokine Inhibitors

7:30 PM—Harkness Lounge

Board #

17 7:30 **7.1** Inhibition of endotoxin-induced microvascular changes by blockade of the IL-1 receptor. **J. Norman, C. Baker, and E.T. Sutton**. Univ. South Florida.

18 8:30 **7.2** The effects of pentoxifylline on IL-2 toxicity. **A.B. Lentsch, J.A. Anderson, F.N. Miller, and M.J. Edwards**. Univ. Louisville.

19 7:30 **7.3** Correlation of serum levels of TNF and IL-6 with pathophysiology in a baboon model of septic shock. **T. Emerson, Jr., A. Chang, G. Peer, M. Fournel, M. Duerr, and L. Hinshaw**. Miles Inc. and Oklahoma Med. Res. Fndn.

Monday, June 27

Breakfast

7:30 AM—Commons Dining Hall

Symposium

8.0 Cytokines in Stress, Trauma and Disease

Chair: **Alan J. Lewis**. Wyeth-Ayerst Res.

9:00 AM—Harkness Auditorium

9:00 Cytokines and the Acute Phase Response. **Jack Gauldie**. McMaster Univ., Hamilton, Ontario.

9:40 Cytokines and Reproduction. **Joy L. Pate**. Ohio State Univ.

10:20 Break.

11:00 Possible Involvement of IL-1α and IL-1β in Arthritis —Studies in Animals. **Ivan G. Otterness**. Pfizer Inc.

11:40 Dysregulation Between Cytokine and Cytokine Inhibitor Production in Infection. **L.L. Moldower**. Cornell Univ. Med. Col.

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 #

20 1:30 **9.1** Effects of central IL-1 on peripheral metabolism in the rat. **R.D. Stith and T. Templer**. Univ. Oklahoma Hlth. Sci. Ctr.

21 2:30 **9.2** IL-1α stimulates dopamine release by increasing protein kinase A activity in PC-12 cells. **A.R. Gwosdow, A. Mendrinos, N.A. O'Connell, M.S.A. Kumar, A. Mohan, R.K. Agarwal, and A.B. Abou-Samra**. Mass. Gen. Hosp. and Shriners Burns Inst., Boston.

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.

23 2:30 **9.4** Effect of tumor necrosis factor on glucose and lipid metabolism. **Y. Sakurai, X-J. Zhang, and R.R. Wolfe**. Shriners Burns Inst. and Univ. Texas Med. Branch, Galveston.

24 1:30 **9.5** Serotonin-induced IL-1α is required for the production of collagenase in uterine smooth muscle cells. **J.A. Dumin, B.D. Wilcox, and J.J. Jeffrey.** Albany Med. Col.

25 2:30 **9.6** The role of epinephrine on TNF and IL-6 production in isolated perfused rat liver. **J.F. Liao, J.A. Keiser, W.E. Scales, and M.J. Kluger.** The Lovelace Insts., Univ. Michigan, and Warner-Lambert Co.

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.

27 2:30 **9.8** Modulation of production of IL-6 by progesterone in human gingival fibroblasts challenged with IL1-β. **C.A. Lapp and M.E. Thomas.** Med. Col. Georgia.

10.0 Cytokines and the Central Nervous System

7:30 PM—Harkness Lounge

Board #

28 1:30 **10.1** The central location of glucocorticoid negative feedback during psychological stress-induced fever. **J.L. McClellan, L.E. Morrow, J.J. Klir, and M.J. Kluger.** Univ. Michigan.

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.

30 1:30 **10.3** Systemic but not central administration of tumor necrosis factor α attenuates lipopolysaccharide-induced fever in rats. **J.J. Klir, J.L. McClellan, W. Kozak, Z. Szelényi, and M.J. Kluger**. The Lovelace Insts.

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.

32 1:30 **10.5** IL-4 inhibits the production of nitric oxide in stimulated glial cells. **N. Goldreich and C. Brodie.** Bar-Ilan Univ., Ramat-Gan, Israel.

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. Tirosh, and A. Weinreb**. Bar-Ilan Univ, Ramat-Gan, Israel.

34 1:30 **10.7** Role of TNF in the fever mechanisms induced by intravenous perfluorocarbon emulsion in rats. **J.D. Bradley, S. Otto, D. Smith, G. Neslund, and S.F. Flaim**. Alliance Pharm. Corp.

35 2:30 **10.8** HIV-1 glycoprotein 120 alters rat sleep. **M.R. Opp, T.K. Hughes, Jr., and E.M. Smith.** Univ. Texas Med. Branch, Galveston.

36 1:30 **10.9** L-NAME, a nitric oxide synthase inhibitor, does not alter the febrile response to lipopolysacharide. **N.C. Long and S.A. Shore.** Harvard Sch. Publ. Hlth.

37 2:30 **10.10** Colocalization of Fos-like immunoreactivity and nitric oxide synthase activity following immunological stimulation. **J.K. Elmqvist, T.E. Scammell, C.D. Jacobson, and C.B. Saper.** Harvard Med. Sch., Beth Israel Hosp., Boston, and Iowa State Univ.

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.

39 2:30 **11.2** Development of a human IL-1β precursor elisa. **L.S. Casey, L. Esposito, C. Alley, B. Lytwyn, and R.S. Dondero.** Cistron Biotechnol., Inc., Pine Brook, NJ.

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

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

Physiology of the Activity and Release of Cytokines

June 25–28, 1994
Yale University, New Haven, CT

Sessions with Contributed Abstracts

Invited Speaker Abstracts

Poster Abstracts

Cytokine and Hormone Interactions

Adriana del Rey
Institute of Physiology, Philipps 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 estimulate 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.

1. Besedovsky, H.O. and del Rey, A. In: *Frontiers in Neuroendocrinology*, W.F.. Ganong and L. Martini, Eds., Raven Press, 13: 61-94 (1992).

2. del Rey, A. and Besedovsky, H.: *Eur.J.Clin.Invest.*, 22, 10-15 (1992).

CYTOKINES AND BEHAVIOR. Robert Dantzer. Integrative Neurobiology, INSERM U394, Rue Camille Saint-Saens, 33077 Bordeaux Cedex, France

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 quantitites 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 such 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 imune system to the brain is represented by primary afferent 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

1. Bluthé RM, Dantzer R, Kelley KW, Brain Res 1992, 573, 318-320.
2. Bluthé RM, Walter V, Parnet P, Layé S, Lestage J, Verrier D, Poole S, Stenning BE, Kelley KW, Dantzer R, submitted.
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4. Dantzer R, Bluthé RM, Kent S, Kelley KW, in de Souza E. (ed) Neurobiology of Cytokines, Academic Press, 1993, vol. 17, pp. 130-150
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CYTOKINES AND VASCULAR SMOOTH MUSCLE

Debbie Beasley, Ph.D., New England Medical Center, Boston, MA

Vasodilatation is a major feature of infection and inflammation, occuring 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 PGI_2 and PGE_2 (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 artherosclerotic 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 radioimmunnoassays 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.

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Effects of Cytokines on Sleep Patterns

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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-1β SWS will occupy about 65% of this time. In contrast to SWS, rapid eye 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:

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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

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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 [1 2 3]. In two instances three dimensional structures have been determined for receptor-hormone complexes [4 5]. 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, GÇSF, 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 he receptors. This family includes TNFRp80, TNFRp60, TNFRrp, 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 from 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 [678]. 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 process during host defense.

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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 rearrenged DNA product. We determined that the rearrangement of the TCRβ gene takes place in the thymus on day 15 of mouse embrogenesis, 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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CYTOKINES IN REPRODUCTION

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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-1ß, 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 A_2, whereas IL-1ß may activate phospholipase C and PGH synthase in addition to phospholipase A_2. 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 sythesized 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 distict 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 arthritis[1, 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. entercolitica,

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[3]. 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[4]. Combined anti-IL-1α and anti-IL-1β antibodies prevented the inhibition of PG synthesis, but not the acceleration of PG catabolic rate[5]. 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.

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Dysregulation Between Cytokine and Cytokine Inhibitor Production During Infection

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The importance of TNFα 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 TNFα, 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, TNFα 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 TNFα and remain elevated for longer periods. The loss of cellular TNFα receptors from target tissues serves two purposes: the transient desensitization of cells to repeated exposure to TNFα, and the formation of receptor-ligand complexes which attenuate peak free TNFα 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 TNFα production, and increase IL-1ra release. For example, increased production of prostanoids and the counter-regulatory endocrine response (*e.g.* cortisol, CRF, and αMSH) all down-regulate IL-1 and TNFα 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.

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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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Cytokines and the Pathogenesis of Fever. Matthew J. Kluger, Institute for Basic and Applied Medical Research, The Lovelace Institutes, Albuquerque, New Mexico 87108.

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 it 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 ^{125}I 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-1ß (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-1ß 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 defence responses (eg fever, neuroendocrine responses behavioural changes), and influence neuronal degeneration and repair and glial activation[1-3]. 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, TNFα, MIP-1 and IFNγ 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-1ß and IL-6 as endogenous pyrogens in the brain derives from observations that central administration of neutralising 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 behavioural changes, fever and hypermetabolism. Pyrogenic and thermogenic responses to IL-1ß, IL-6 and IL-8 in the rat are inhibited by central pretreatment with either a CRF receptor antagonist or neutralising antibody, whereas responses to IL-1α and TNFα are unaffected by this treatment. Several pieces of data now indicate that central effects of IL-1α and IL-1ß on fever and thermogenesis involve different mechanisms, and that responses to IL-1ß 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-1ß and IL-6, but not IL-1α or TNFα. 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 (ie IL-1ß 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.

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CYTOKINES/HYPOTHALAMIC-PITUITARY-ADRENAL(HPA) AXIS INTERACTIONS

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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. Intracerebraoventricular (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)

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INTERLEUKIN-1 RECEPTOR ANTAGONIST. Robert C. Thompson, Ph.D.
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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.

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ANTISENSE OLIGONUCLEOTIDE DEVELOPMENT AS THERAPEUTIC AGENTS.

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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 constituitive 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 concommitant 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 ballon 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. Schoool 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.

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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, eg., 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 occurence 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].

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EFFECTS OF SMALL MOLECULES ON CYTOKINE PRODUCTION AND RESPONSES. Katherine L.Molnar-Kimber, Inflammatory Diseases Div., Wyeth-Ayerst Res., Princeton, NJ 08852.

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 (TNFα), 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 dibutryl cyclic AMP suppressed the production of TNFα 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 TNFα production. Combination studies indicate that the effects are additive. These data suggest opposing roles for cGMP and cAMP levels in TNFα 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 FKBPs, 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 $p70^{S6}$ 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 4^{th} and 5^{th} 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.

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5.1

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

IL-1 receptor antagonist (IL-1ra) is stimulated by immobilized IgG-induced monocyte Fcγ 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 ($1x10^6$/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-FcγRI or FcγRIII antibodies did not (adherent cells: 1.4±0.3; anti-FcγRII:8.8±2.4 ng/ml; anti-FcγRI:2.0±0.7 ng/ml; and anti-FcγRIII: 4.7±1.2 ng/ml, respectively) (p=0.011 for FcγRII antibody versus no antibody). In an attempt to block FcγR-induced IL-1ra, monocytes were preincubated with soluble Fab anti-FcγR antibodies for 1 hour (1 μg/ml) before an 18 hour incubation on immobilized IgG (Sandoglobulin). Soluble anti-FcγRII (n=5) significantly suppressed IL-1ra (p=0.001), but anti-FcγRI or FcγRIII did not. Lastly, to confirm the role of FcγRII in IL-1ra regulation, subjects homozygous for the FcγRII arginine$_{131}$ polymorphism ("high responder") were compared to homozygous histidine$_{131}$ individuals ("low responder") for IL-1ra induction by immobilized monoclonal anti-FcγRII antibody 41H16 (which only recognizes the arginine polymorphism). In high responder FcγRII monocytes (n=2), immobilized 41H16 (1 μg/ml) induced IL-1ra to 140% of IgG-stimulated IL-1ra (control), compared to 66% of control for low responders. In contrast, immobilized mIgG$_3$ (1 μg/ml), which binds to human FcγRI and FcγRIII, induced monocyte FcγR crosslinking as assessed by H_2O_2 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 α (TNF) MODULATES THE TRANSEPITHELIAL RESISTANCE OF LLC-PK$_1$ EPITHELIAL CELL SHEETS. Colleen W. Marano*, Kathleen V. Laughlin, Linda M. Russo, Alejandro Peralta Soler and James M. Mullin*. Lankenau Medical Research Center, Wynnewood, PA 19096

The transepithelial resistance (TER), an inverse measure of the tight junctional (TJ) permeability across renal epithelial LLC-PK$_1$ cell sheets, displays a multiphasic and reversible response to the presence of the cytokine, Tumor Necrosis Factor α (TNF). Following a 90 minute delay after TNF administration, TER abruptly decreases and then rapidly recovers. The flux of [^{3}H]-mannitol across the epithelium, a direct measure of tight junctional permeability, increases as the TER decreases, as does the paracellular penetration of the electron dense dye ruthenium red. The effect of TNF on TER can be prevented by antibodies directed toward TNF as well as by a monoclonal antibody to the p55 TNF receptor. TNF can exert its effects on the TER when presented to the apical or basolateral surfaces alone, but the decrease in resistance is greatest when TNF is applied to both surfaces. The inhibitory action of genistein on the TNF-induced TER response supports a role for tyrosine kinase in mediating the effect. Inhibiton of PKC (staurosporine, calphostin-C, or H-7) or of its associated protein phosphatase by okadaic acid has no measurable effect on the TNF-induced TER response. On the other hand, activation of cAMP-dependent protein kinase A, subsequent to pharmacologic elevation of cAMP, inhibits the TER response to TNF. Preventing the TER response to TNF by inhibition of lipoxygenase (NDGA or ETYA) and potentiation of the TER response in the presence of epoxygenase inhibitors (ketoconazole and SKF-525A) supports a role for arachidonic acid metabolism 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 also that elevations of serum TNF might underly such pathophysiological conditions as inflammation, edema, cachexia, and multiorgan failure in part due to TNF's ability to render leaky the epithelial linings of major organs. (Supported by 2R01CA48121 and a JDF postdoctoral fellowship).

5.3

INTERACTIONS OF NUCLEAR FACTOR IL-6 WITH THE LONG TERMINAL REPEAT OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1. Valerie M. Tesmer and Minou Bina. 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. 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 three distinct regions of the long terminal repeat bind bacterially expressed NF-IL6 in DNaseI footprinting assays. In one of these regions, known as NRE1, the NF-IL6 recognition motif overlaps an E-box (CANNTG) motif which provides a binding site for a group of transcription factors known as basic helix-loop-helix proteins. Insertional mutagenesis was performed to differentiate the protein binding properties of these two sequence motifs in NRE1. Analysis of mutated 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 TYROSINE KINASE(PTK)/PROTEIN PHOSPHATASE(PP) CONCEPT IN BREAST TUMOR PROLIFERATION AND AGGRESSIVENESS. Anwar A. Hakim. Cellular & Molecular Biology. P.O.Box984 Kankakee. Illinois 60901.

Human breast carcinoma (BCa) cells synthesize and release a large variety of biologically active compunds including EGF, TGF, PDGF, proteases and plasminogen activators(Hakim Experien tia 32:1057-1059,1976, CBB 5:63-74,1980) and cytines (tumor necrosis facto TNF) whic modulate BCa cell proliferation & aggressiveness by blocking the host defense mechanisms. These substances combined with hormonal estrogens (Hakim Ann.d'Inst. d'Immunol. 131C:155-170,1980 &ibid CII 8:133-142,1980) to modulate macrophage functions (Hakim CBB: 3:85-92,1979).Cytokines are the critical regulators of proliferation & differentiation for hematopoietic cells. BCa-associated membrane glycoprotein signal peripheral blood lymphocytes(Hakim Immun. Commun. 4:251 276,1975), to release MIF, IL-2 (Hakim Am.Fed.Clin.Res.23:491, 1975) & other cytokines. Sera from animals bearing spontaneous mammary AdCa enhanced tumor growth(Hakim Surg.Forum 26:175-177, 1975;J.Surg.Oncol. 8:383-390,1976), with simultaneous release of Immunosuppressing factor(s) which correlated with tumorigene sis(Hakim Europ J Cancer14:1249-1260,1975, & release of trypsin inhibitor (Hakim Klin.Wochenschrifts 56:767-777,1978).TNF is a monokine that inhibits the proliferation of a variety of tumor cells in vitro & induces the hemorrhagic necrosis of solid tumors in vivo.The present study examined the relationship between estrogen receptors(ER) & TNF-alpha expression in BCa cells from estblished cell lines & fresh tissue biopsies.

5.5

POTENTIAL INTERACTION OF IL-4 WITH ENDOGENOUS CYTOKINE IN VIVO. Lee Sullivan, Loretta Bober, Michael Grace, Sera Braun, Heather Macosko, Faribourz Payvandi, Catherine Pugliese-Sivo and Satwant Narula. Schering-Plough Research Institute, Kenilworth, NJ 07033.

We delivered alginate 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-IL3 mAb, albeit to a lesser extent. The effect of anti-IL5 mAb was of a lesser magnitude than either GM-CSF or IL-3 mAbs, 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.

5.6

ENDOGENOUS TUMOR NECROSIS FACTOR IS NOT REQUIRED FOR ENDOTOXIN MEDIATED GENE EXPRESSION FOR INDUCIBLE NITRIC OXIDE SYNTHASE II IN ALVEOLAR MACROPHAGES AND NEUTROPHILS. Stan S. Greenberg, Jianming Xie*, Mina Li*, Jay Kolls*, Steve Nelson*, Greg Bagby and Warren Summer. Depts of Medicine and Physiology, LSU Medical Center, New Orleans, LA 70112

Intratracheal (i.t.) administration of endotoxin (0.2 mg) to rats stimulates alveolar macrophage (AM) production of tumor necrosis factor$_\alpha$ (TNF) and up-regulates nitric oxide synthase II (iNOS) and NO production by AM and neutrophils (PMN). TNF$_\alpha$ is believed to be the mediator of gene expression for iNOS in response to LPS. We tested this concept by pretreating rats with a polyclonal anti-TNF$_\alpha$ antibody (ATab) or non-immune IgG (IgG) (7.3 mg/rat, im) 2 hr. before or pentoxyfylline (PTF, 25 mg/kg i.p.) 0.5 hr. before i.t. LPS to male Sprague Dawley rats. AM and PMN were obtained from bronchoalveolar lavage fluid (BALf) from lungs of rats killed at 2 and 4 hrs post LPS. AM and PMN were assyed for mRNA for NOS II with competitor equalized RT-PCR. BALf and plasma were analyzed for TNF$_\alpha$ and NO. ATab and PTF abolished LPS-induced increases in TNF$_\alpha$ but did not suppress the NO content of the BALf or gene expression for iNOS by AM or PMN. We conclude that LPS-induced upregulation of gene expression for TNF$_\alpha$ and iNOS in AM and PMN are temporally related events. However, TNF$_\alpha$ is not required for induction of iNOS. mRNA for iNOS II is resistant to suppression by PTF.

5.7

PURIFICATION AND CHARACTERIZATION OF THE CYTOKINE MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF). J. Bernhagen, T. Calandra, R. A. Mitchell, A. Cerami, and R. Bucala. The Picower Institute for Medical Research, Manhasset, NY 11030.

MIF, previously described as a T-cell cytokine, has been re-discovered recently as a pituitary and hormone that plays an important role as a mediator of endotoxic shock (Bernhagen *et al.*, 1993, *Nature 365*, 756-759). Despite the molecular cloning of a human MIF in 1989, characterization of the structural and biological properties of MIF 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 milligram quantities of protein 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_r of native MIF was identical with that of recombinant MIF as assessed by SDS-PAGE/Western blotting (12.5 kD monomer) and mass spectrometric analysis. Both recombinant and native MIF exhibited comparable biological activities. MIF (1 μg/ml) induced the secretion of TNF-α (1.2-6.9 ng/ml) and stimulated nitric oxide (NO) production from murine macrophages. However, significant NO generation (5.7-20.4 μM) was observed only in cells primed with IFN-γ. MIF activity in a monocyte migration assay was dose-dependent and peaked at 0.1 μg/ml. Circular dichroism spectroscopy revealed that both human and murine MIF belong to the α/β-class of proteins. Both MIF species displayed high percentages of β-sheet (murine: 39%, human: 51%) and α-helix (murine: 18%, human: 31%) conformation. In guanidinium hydrochloride (GdnHCl)-induced unfolding studies, the midpoint of unfolding was determined to be at 1.8 M GdnHCl for muMIF, and the free energy change of unfolding was found to be at 11.75 kJ/mol. These studies establish the identity of the native and cloned, recombinant protein and provide a first insight into the conformational and structural stability properties of this critical inflammatory mediator.

5.8

EXCRETION OF CYTOKINES AND RELATED FACTORS IN THE URINE BY ASTRONAUTS DURING AND AFTER SPACEFLIGHT. T.P. Stein, M.F. Schluter and L.L. Moldawer. Depts. of Surgery, UMDNJ-SOM, Stratford, NJ 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, sTNFR-II 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-1 (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) sTNFR-II was only increased after landing. (iii) The pattern of IL-6 excretion post flight was different from that of sTNFR-II and IL-1ra.

5.9

LYMPHOKINE EXPRESSION IN RAT LUNGS AFTER ANTIGEN CHALLENGE. PM Renzi, JP Yang, J Martin, Q Hamid. Meakins-Christie Labs, Royal Victoria Hospital, McGill University, André Viallet Research Institute, Pulmonary Unit, St-Luc Hospital, Mtl, 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) rats, 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 for the measurement of interleukin (IL)-2 and 4 production by in-situ hybridization 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 JT Costello Memorial Research Fund, the MRC of Canada and RHNCE).

5.10

OZONE EXPOSURE, EXERCISE, AND THE SYSTEMIC ACUTE PHASE RESPONSE IN HUMANS. Mark W. Frampton*, Fadi Malek*, John P. Fogarty*, R. John Looney*, Paul E. Morrow*, Mark J. Utell*. University of Rochester, NY 14642

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 0.22 ppm ozone and 1 exposure to air, each for 4 hours with exercise, separated by at least 3 weeks. Phlebotomy 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 immunoreactive 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 WBC, LDH, AST, 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-1α PRODUCTION BY MONOCYTIC CELLS. Burton D. Clark, Ellen C. Donaldson, Koichi Ajiura, Ronald G. Tompkins, Charles A. Dinarello, John F. Burke, and Jeffrey A. Gelfand. 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 affirmed 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 monocytic cell line, MonoMac 6. In our studies, thrombin-activated platelets (P) were incubated with MonoMac 6 cells (M) at ratios of 0, 16, 80, 160, and 800 platelets/MonoMac with or without endotoxin (LPS) or heat-killed *S. epidermidis* (SE). IL-1α was quantitated by a specific and sensitive radioimmunoassay subsequent to 4h and 8h incubations. The incubation of activated platelets with monocytic cells (16:1 ratio) for 4h in the presence of LPS (M+P+LPS) at 50,100 and 500 pg/ml resulted in 362%, 479%, 489% increases in IL-1α, respectively, compared to monocytes exposed to LPS without platelets (baseline, M+LPS). After 8h incubation, the overall percentage change from baseline decrease by approximately one-half, reflected by a corresponding decrease in baseline (M+LPS) cytokine production. Monocytes plus platelets incubated with *S. epidermidis*for 4 h (M+P+SE) showed a 350% increase over baseline (M+SE), and a 200% increase at 8h. There was a dose response for cytokine production with increased ratios of P:M. Overall, these results suggest that activated platelets may magnify the cytokine response observed during both Gram-positive and Gram-negative infection.

6.1

INTERFERON-γ, IFNγ, DOWN-REGULATES CYSTIC FIBROSIS TRANSMEMBRANE REGULATOR, CFTR, GENE EXPRESSION. A. Edelman[1*], G. Przewlocki[1*], I. Baro[3*], A.S. Honge[3*], D. Escande[3*], F. Besançon[2*], [1]INSERM U.323, [2]INSERM U.245, [3]CNRS URA 1121,75015 Paris and Orsay, France

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 IFNs which are produced in response to infections during CF, the effects of IFNα, β and γ on CFTR mRNA levels were tested in HT29 and T84 cell lines. Treatment of these cells with IFNγ, but not with IFNα and IFNβ, strikingly reduced the amount of CFTR mRNA in a concentration and time-dependent manner. The effect was detectable at a concentration as low as 0.1 IU/ml. Short-term treatment by 100IU/ml IFNγ (< 1h) was inefficient, the inhibition was maximal at 12 h. IFNγ, α and β (100IU/ml) did not affect β actin mRNA content and increased the steady state level of 2'-5' oligodenylate synthetase mRNA, one of IFN inducible genes. We also investigated IFNγ effects on the rate of CFTR gene transcription, and compared the CFTR mRNA half-life in control and IFNγ-treated cells, respectively. IFNγ did not change the transcription rate of CFTR gene suggesting a postranscriptional regulation. Accordingly, CFTR mRNA half-life was strongly reduced in the IFNγ-treated cells compared to the controls (6 and 14 hrs, respectively) suggesting that the down-regulation of the CFTR mRNA occurs through destabilization of the transcripts. Western blot analysis using anti-CFTR antibodies indicated that CFTR protein content was diminished in the cells treated by IFNγ. To establish whether the decrease in CFTR mRNA was reflected at the functional level, we assayed CFTR function by comparing the cyclic AMP(cAMP)-stimulated $^{36}Cl^-$ efflux and whole-cell currents in control and IFNγ-treated cells. Both assays indicated that cAMP-stimulated fluxes were decreased in the IFNγ-treated cells indicating inhibition of CFTR function. These data suggest that production of IFNγ in response to infections might modulate Cl secretion.

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6.2

DETECTION OF ENDOTHELIN PEPTIDE AND RECEPTOR GENE EXPRESSION IN BOVINE CORNEAL EPITHELIUM (BCE). Wenhong Tao, Gregory Liou* and Peter Reinach. Medical College of Georgia, Augusta, GA 30912.

The endothelins are a group of three different isopeptides. One of them (ET-1) when applied as eyedrops to rabbits stimulates corneal epithelialization in a wound healing model. This stimulation involves possible interactions with two different receptor subtypes (ET_A and ET_B). We sought evidence for the endogenous gene expression of ET-1, ET-2 and ET-3 as well as the two ET receptor subtypes in the BCE. Hybridization riboprobes were prepared by PCR and DNA cloning and their identity was verified by DNA sequencing. Their gene expression was detected by RNase protection analysis (RPA). Deoxigenin labeled riboprobes were prepared for the detection of the pattern of distribution of ET_A and ET_B gene expression in the intact BCE. The riboprobes were selective based on Southern blot analysis and they also were not cross reactive. RPA revealed that there is gene expression of ET-1, ET-2, ET_A and ET_B. The ET-1 expression level was 3-fold higher than that of ET-2. The ET_B expression level was also 3-fold higher than that of ET_A. In situ hybridization indicated that there is an appreciably higher level of ET_A gene expression in the proliferating basal cells than in the differentiated suprabasal cells. With regards to ET_B, its pattern of distribution was the reverse of the one seen for ET_A. These results show that there is constitutive gene expression of ET-1, ET-2, ET_A and ET_B in BCE. This level of expression suggests that ET-1 and ET-2 act as endogenous mitogens to stimulate basal cell proliferation. Accordingly, the multilayered corneal epithelium can continuously be renewed permitting it to protect the cornea against physical trauma and pathogenic invasion.

6.3

INFLAMMATORY CYTOKINES LOWER ERYTHROPOIETIN GENE EXPRESSION. Wolfgang Jelkmann, Stilla Frede* and Joachim Fandrey* Institute of Physiology I, University of Bonn, D-53115 Bonn, Germany

In patients with the anemia of chronic disorders the plasma level of the hormone erythropoietin (Epo) is often low in relation to the blood hemoglobin concentration. We have carried out in vivo and in vitro studies to find out whether inflammatory cytokines impair hypoxia-induced Epo production. In rats exposed to hypoxia (8% O_2 in the inspiratory gas) for 6 h, single injections of bacterial lipopolysaccharides (LPS, 0.1 mg/kg, i.p.) caused lowered serum Epo protein and renal Epo mRNA levels, as measured by radioimmunoassay and competitive polymerase chain reaction, respectively. 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 and that they act through lowering Epo gene expression.

6.4

ADULT MAMMALIAN CARDIAC MYOCYTES EXPRESS BOTH TNF RECEPTORS. DL Mann*, G Torre-Amione*, S Kapadia*, and R Levobitz*, 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-hTNF-α for myocytes was $9X10^{-9}$. 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 myocardial 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.

6.5

TRANSFORMING GROWTH FACTOR-β_1 DIFFERENTIALLY REGULATES FIBROBLAST GROWTH FACTOR-INDUCED DNA SYNTHESIS IN CARDIAC AND SKIN FIBROBLASTS. Andreas Sigel, Mahoubeh Eghbali, New Haven, Connecticut 06510

Previously, we demonstrated that transforming growth factor-β_1 (TGF-β_1) specifically modulates phenotypic characteristics of cardiac fibroblasts in culture and that skin fibroblasts, under identical conditions, do not exhibit molecular or morphological signs of phenotypic modulation. Further studies proved that exogenous TGF-β_1 inhibits fibroblast growth factor (bFGF)-induced stimulation of DNA synthesis in cardiac fibroblasts and that blockade of autocrine TGF-β_1 leads to increased mitogenic activity of bFGF in cardiac fibroblasts. In this study we demonstrate that mitogenic effect of bFGF in skin fibroblasts, as measured by [^{3}H]-thymidine incorporation into DNA, was not affected by exogenous TGF-β_1. Furthermore, stimulation of DNA synthesis by bFGF decreased significantly in skin fibroblasts that were treated with anti-sense oligonucleotides complementary to the translation initiation site of TGF-β_1 mRNA. This effect was not observed in sense-treated cells. Together, these data indicate that autocrine TGF-β_1 is necessary for mitogenic effect of bFGF in skin fibroblasts. They further point to inherent or tissue-specific predispositions of skin and cardiac fibroblasts that contribute to their differential response to TGF-β_1.

7.1

INHIBITION OF ENDOTOXIN-INDUCED MICROVASCULAR CHANGES BY BLOCKADE OF THE INTERLEUKIN - 1 RECEPTOR. James Norman, Carleton Baker, E.Truitt Sutton. Depts of Surgery and Physiology, Univ. of So. FL, Tampa. 33612.

Endotoxic shock induces vascular changes resulting in hypotension, arteriolar constriction, decreased vasoconstrictor sensitivity to norepinephrine (NE) (Circ. Shock 12:165; 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 cytokine cascade at the level of the interleukin-1 (IL-1) receptor has been shown to attenuate endotoxin-associated hypotension, the arteriolar and aortic effects of IL-1 receptor antagonist (IL-1ra) were studied. *Methods:* Cremaster muscle A_1, A_2, and A_3 arterioles of 16 rats were studied by videomicroscopy. Following baseline measures of mean arterial pressure (MAP) and arteriolar diameter, dose-response curves to topical NE (10^{-8}-10^{-3}M) and ACh (10^{-6}-10^{-3}M) were obtained. Prior to the administration of *E. coli* endotoxin (6mg/kg iv, LD_{100}) one half of the animals were randomly assigned to receive recombinant IL-1ra (0.2 mg/kg/min iv for 5 hours). All animals were followed with hourly measures of arteriolar diameters and NE/ACh thresholds for five hours post-endotoxin or until death. Aortic endothelium was examined by electron microscopy (EM). *Results:* All control animals, but no IL-1ra animals died within 6 hours ($p<0.01$). IL-1ra administration significantly attenuated endotoxin-induced vasoconstriction of A_1, A_2, and A_3 arterioles ($p<0.05$ compared to untreated control). This was associated with maintenance of MAP and baseline NE vasoconstrictor sensitivity (both no change from 0 hour, significantly greater than control, $p<0.01$). Arteriolar dilation in response to ACh was maintained at baseline levels in all animals, however, EM demonstrated the maintainance of aortic endothelium 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 endotoxic shock which is associated with maintainence of MAP, reactivity of arterioles to NE and ACh, and the integrity of the aortic endothelium. (Supported by Am. Heart Assn., FL Affiliate)

7.2

THE EFFECTS OF PENTOXIFYLLINE ON INTERLEUKIN-2 TOXICITY. Alex B. Lentsch*, Jim A. Anderson*, Frederick N. Miller and Michael J. Edwards*. Departments of Physiology and Surgery, and the Center for Applied Microcirculatory Research, University of Louisville, Louisville, KY 40292

Interleukin-2 (IL-2) causes the regression of metastatic cancer and several toxicities. These effects are thought to be mediated by tumor necrosis factor-α (TNF), possibly through lymphocytic activation. In this study we determined if pentoxifylline (PTXF), a drug which may inhibit TNF production, could also inhibit IL-2 induced upregulation of lymphocyte adhesion molecules and IL-2 induced toxicity. 40 female C57BL/6 mice were divided into four groups and treated ip for 6 days: control, PTXF (150mg/kg/day), IL-2 (10^6U/day), and IL-2 and PTXF. Spleen cell suspensions were tagged with fluorescent antibodies to the adhesion molecules CD11a and CD49d and analyzed by flow cytometry for fluorescent intensity per cell (FI/c). Indices of toxicity and blood chemistry were evaluated. Data in the table (mean ± SEM) show flow cytometry data and two indices of toxicity, SGPT (serum glutamate pyruvate transaminase) and BILI (bilirubin). $P \leq 0.05$: (*) compared to control and PTXF, and (#) compared to all other groups.

	CD11a (FI/c)	CD49d (FI/c)	SGPT (IU/L)	BILI (mg/dL)
Control	386 ± 10	36 ± 1	56 ± 21	.3 ± .05
PTXF	431 ± 40	35 ± 1	41 ± 2	.7 ± .36
IL-2	1020 ± 32*	50 ± 2*	228 ± 27*	2.4 ± .87#
IL-2 & PTXF	987 ± 35*	50 ± 5*	238 ± 8*	.7 ± .12

IL-2 induced increases in SGPT and the expression of CD11a and CD49d were not attenuated by PTXF. PTXF did decrease bilirubin levels toward control values. These results indicate that this dose of PTXF had a limited effect on the responses to chronic 6-day IL-2 treatment.

7.3

CORRELATION OF SERUM LEVELS OF TNF AND IL-6 WITH PATHOPHYSIOLOGY IN A BABOON MODEL OF SEPTIC SHOCK. T. Emerson, Jr., A. Chang*, G. Peer*, M. Fournel, M. Duerr* and L. Hinshaw. Miles, Inc., West Haven, Ct 06516 and OMRF, Oklahoma City, OK 73104.

A number of cytokines are thought to be involved in inflammatory disease states. This report targets the possible roles of TNF and IL-6 in the pathophysiology of septic shock. Baboons were infused i.v. with lethal amounts of live E. coli bacteria and treated i.v. with 15, 7.5, 5.0, or 1.5 mg/kg anti-TNF monoclonal antibody (TNF MAb). The 15 mg/kg study has been published (Circ. Shock, 30:279, 1990). Survival in these groups was 100%, 57%, 9% & 0%, respectively. TNF MAb at 15 mg/kg prevented death and no pathology was evident in lungs, kidneys, adrenals, spleen or liver. However, as the dose of TNF MAb was decreased, survival time decreased, permanent survival decreased and organ damage increased progressively, including vascular congestion, hemorrhage, edema and necrosis. No serum TNF was detectible at any dose of TNF MAb. However, there was a direct relationship between serum IL-6 levels and death as well as multiple organ pathology. These data suggest that serum IL-6 may play a major role in the pathophysiology of septic shock in the baboon, and hence may be of prognostic value.

9.1

EFFECTS OF CENTRAL INTERLEUKIN-1 ON PERIPHERAL METABOLISM IN THE RAT. R.D. Stith and L. Templer. Univ. Okla. Hlth. Sci. Ctr., Oklahoma City, OK 73190.

Many investigations have focused on the peripheral actions of interleukin-1beta (IL1), but little is known about the efftects of centrally acting IL1 on peripheral metabolism. We studied dose-responses to IL1 icv in fasted, male Sprague-Dawley rats over 6 hours. Peak core body temperature (39.6°, 39.3°, and 38.2°; control=37.6°) and plasma (pl) corticosterone (685, 666, and 682 ng/ml; control=188 ng/ml) were measured after 15, 25, and 50 ng IL1, respectively. Pl glucose increase was to 125% of control at all doses, but the duration of hyperglycemia was dose-dependent. Hepatic glycogen content was diminished to 50%, 40%, and 70% of control after 15, 25, and 50 ng. Pl insulin levels after 15 ng seemed to parallel pl glucose, but were blunted after 25 and 50 ng. No changes in pl glucagon were observed. Activity of liver phosphoenolpyruvate carboxykinase (PEPCK) was reduced to 60%, 66%, and 81% of control. The same doses of IL1 injected iv did not affect any parameter measured. Since IL1 stimulates pituitary IL6 release, we investigated the effects of peripheral IL6. When 1 ug of IL6 was injected iv, pl glucse, corticosterone, insulin and glucagon increased, while liver glycogen and PEPCK were decreased. We conclude that central IL1 induced changes in peripheral metabolism could be partially, but not wholly, explained by peripheral actions of IL6.

9.2

INTERLEUKIN-1α STIMULATES DOPAMINE RELEASE BY INCREASING PROTEIN KINASE A ACTIVITY IN PC-12 CELLS. Andrea R. Gwosdow, Alexandra Mendrinos,* Nancy A. O'Connell*, M.S. A. Kumar,*A. Mohan,*R.K. Agarwal* and Abdul B. Abou-Samra.* Massaschusetts General Hospital and Shriners Burns Institute, Boston, MA. 02114.

A recent study from this laboratory (Am. J. Physiol., 263:E461-E466, 1992) has shown that the inflammatory mediator, interleukin-1α (IL-1α), stimulates catecholamine release from primary cultures of rat adrenal cells. The present studies were conducted to determine a) if IL-1α stimulates catecholamine (dopamine) release from PC-12 cells and b) if protein kinase A (PKA) is involved in IL-1α–induced dopamine release from PC-12 cells. The results indicate that IL-1α significantly ($P<0.05$) elevated dopamine release after a 24 hr incubation period. IL-1α did not stimulate cAMP accumulation at any time period between 5 min and 24 hr. In contrast, forskolin elevated ($P<0.05$) intracellular cAMP levels and increased PKA activity and dopamine release. Because IL-1α did not affect cAMP accumulation, the effect of IL-1α on PKA was investigated. IL-1α increased ($P<0.05$) PKA activity at 15 and 30 min and returned to control levels by 1 hr. Inhibition of PKA with the PKA inhibitor, H-8, blocked activation of PKA and dopamine secretion by both IL-1α and forskolin in PC-12 cells. These observations demonstrate that a) IL-1α stimulated dopamine release from PC-12 cells, b) the mechanism of IL-1α activation of PKA is independent of cAMP and c) PKA is an intracellular mediator used by IL-1α to stimulate dopamine secretion from PC-12 cells.

9.3

PRODUCTION OF INTERLEUKIN-1β AND TNFα BY PBMC DOES NOT DECLINE WITH AGE. R Roubenoff, TB Harris, JG Cannon, L Abad, PWF Wilson, CA Dinarello. USDA HNRC/Tufts, Boston, MA 02111; NIA, NIH, Bethesda, MD 20892; Framingham Heart Study, Framingham, MA 01701.

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 monocytic arm of the immune system. We examined the production of the inflammatory cytokines IL-1β and TNFα in 420 elderly Framingham Heart Study participants (Group E; mean [± SD] age 78 ± 5 y), 20 healthy younger Framingham controls (Group FC; age 40 ± 10 y), and 30 laboratory controls (Group LC; age 40 ± 12 y). Samples were shipped from Framingham to Boston on ice within 1h of drawing, and mononuclear cells were separated by Ficoll-Hypaque centrifugation and cultured for 24h in pyrogen-free RPMI alone or 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 to stimulation were similar in all groups. Unstimulated production of both cytokines was 2-3-fold higher in FC than LC ($p < 0.01$), suggesting an effect of transport, which disappeared with stimulation (p = NS). IL-1β production by E and FC was within 10% at each level of stimulation (including RPMI only), but TNF production was 30-120% higher in E at all levels of stimulation ($p < 0.01$ except 1 ng/ml LPS, $p < 0.1$). We conclude that there is no senescence of TNFα or IL-1β production in humans, and there may be a pro-inflammatory tendency with higher TNF production in E. Analysis of disease and risk-factor associations with IL-1 and TNF production are now underway. Supported by: Interagency Agreement Y01-AG-2-0195 and NIH Grant DK02120.

9.4

EFFECT OF TUMOR NECROSIS FACTOR ON GLUCOSE AND LIPID METABOLISM

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We have previously reported that, in conscious dogs, TNF caused an increase in plasma glucagon concentration associated with the increase in endogenous glucose production and the decrease in FFA flux. In this study, we have tested the hypothesis that the changes in glucose and lipid metabolism during TNF infusion was due to the increase in plasma glucagon concentration. We employed a pancreatic clamp technique to keep plasma insulin and glucagon concentration at constant levels during TNF infusion. After 2 h base line period (Period 1), TNF was infused into a group of animals ($n=7$) as a primed-constant infusion (Prime, 2.5 $\mu g \cdot kg^{-1}$; constant, 62.5 $ng \cdot kg^{-1} \cdot min^{-1}$) for 2 h (Period 2). The control group ($n=6$) received saline instead of TNF. Glucose and lipid kinetics were measured by means of the primed-constant infusion of [6,6-d_2]glucose and [d_2]palmitic acid.

	[Glucose] *	Glucose Ra ‡ §	Glucose clearance ¶	FFA Ra **	[FFA]*
Basal	8.06±0.51	17.8±1.1	2.18±0.18	13.9±2.0	0.54±0.07
TNF-treated	6.81±0.89‡‡	16.5±0.7	3.26±0.49‡‡	9.1±1.2‡‡	0.25±0.04‡‡

* μmol/ml; ‡ Ra, the rate of appearance; § μmol/kg·min; ¶ ml/kg·min; ** FFA, free fatty acids. ‡‡ Different from basal value with paired t-test, $P<0.05$.

We conclude that whereas TNF did not increase endogenous glucose production when plasma glucagon was clamped, the significant increase in glucose utilization and the decrease in FFA flux during TNF infusion was observed independent of changes in plasma glucagon and insulin.

9.5

SEROTONIN-INDUCED INTERLEUKIN-1α IS REQUIRED FOR THE PRODUCTION OF COLLAGENASE IN UTERINE SMOOTH MUSCLE CELLS.

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The involvement of interstitial collagenase in the regulation of the connective tissue of the uterus is crucial for the return of this organ to post-partum reproductive competence. Serotonin, (5HT) has been shown to be an obligate, cell-specific inducer of collagenase in uterine smooth muscle cells (SMC). 5HT activates the collagenase gene through the $5HT_2$ receptor subtype. Recent studies have shown that bacterial lipopolysaccharide (LPS) acts co-operatively with 5HT to induce collagenase prior to its induction in cells treated with 5HT alone. Studies by others have shown that SMCs respond to LPS by inducing IL-1 mRNAs and that IL-1 can induce itself by an autocrine loop. Accordingly, SMCs were examined for LPS-mediated induction of IL-1. Treatment of cells with 5HT and LPS resulted in the induction of IL-1α mRNA which closely paralleled the production of collagenase. Removal of 5HT from the culture medium or treatment with selective antagonists of the $5HT_2$ receptor prevented the induction of both IL-1α and collagenase mRNA. Conversely, phorbol ester or selective agonists of the $5HT_2$ receptor fully substituted for 5HT in inducing IL-1α and collagenase mRNA. Induction of IL-1α mRNA by serotonin was completely insensitive to cycloheximide, but was blocked by progesterone. Nuclear run-on indicated that 5HT-induced increases in IL-1α mRNA were accompanied by an increase in IL-1α transcription. To determine if IL-1 is necessary for collagenase production, the IL-1 receptor antagonist (IL-1ra) was utilized. SMCs cultured in the presence of 5HT and IL-1ra failed to produce IL-1α or collagenase mRNAs. Removal of IL-1ra resulted in the rapid induction of IL-1α and collagenase mRNA. This suggests that 5HT-induced IL-1α is required for the production of collagenase and may act via an autocrine loop.

9.6

THE ROLE OF EPINEPHRINE ON TNF AND IL-6 PRODUCTION IN ISOLATED PERFUSED RAT LIVER (IPRL)

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A bidirectional communication exists between the nervous system and the immune system. Evidence has accumulated suggesting that cytokines/immune peptides influence sympathetic neuronal survival, and that cytokines can promote the secretion of catecholamines. However, it is unclear if catecholamines influence the production/secretion of cytokines. Using an IPRL preparation, we have shown that the liver is an important source of circulating cytokines in response to LPS, and that corticosterone dose-dependently influenced LPS induced production of TNF and IL-6. In this study, we investigated the effect of epinephrine on hepatic TNF and IL-6 production. We demonstrated that epinephrine (1 μm/ml) alone significantly increased release of IL-6 bioactivity from IPRL, but did not induce TNF. LPS-induced increase in TNF release were inhibited by epinephrine. Both responses were totally inhibited by the β blocker propranolol (10 μm/ml). Anisomycin, a protein synthesis inhibitor, completely blocked of the rise in TNF and IL-6 concentration in the IPRL effluent, supporting the hypothesis that synthesis (or release) of these cytokines was dependent on protein synthesis. In isolated Kupffer cells and hepatocytes, epinephrine alone had no effect on TNF or IL-6 production. However, epinephrine treatment significantly decreased LPS-induced release of TNF bioactivity, and increased LPS-induced release of IL-6 bioactivity from Kupffer cells. Our data support the hypothesis that epinephrine can promote IL-6 secretion from IPRL, but not from isolated cells. Epinephrine decreased LPS induced TNF production, but increased LPS induced IL-6 production in both IPRL and isolated Kupffer cells. These effects of epinephrine on TNF and IL-6 production in liver are β-receptor mediated processes. Research supported by NIH Grants AI27556 and MH48609.

9.7

REGULATION OF INSULIN-LIKE GROWTH FACTOR (IGF)-I CONTENT AND BINDING PROTEINS BY IL-1ß.

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The enhanced muscle catabolism observed during infection and trauma is not fully understood, but various lines of evidence support the role of proinflammatory cytokines as putative mediators. *In vivo* studies indicate that IL-1 can influence protein balance. Experiments have also shown that IGF-I alters protein metabolism and that circulating levels of this peptide are decreased by trauma and infection. Thus, it is possible that stress-induced changes in IL-1 may regulate the IGF system thereby influencing the catabolic response. The present study characterized the changes in circulating and tissue concentrations of IGF-I and selected IGF-binding proteins (BPs) in fasted rats after IV injection of IL-1ß (6 μg/rat). Circulating levels of IGF-I were reduced 40-60% as early as 3 h and remained depressed for at least 9 h. At 6 h, IL-1ß also decreased the IGF-I content in liver (25%), heart (46%), and a variety of skeletal muscles with different fiber type compositions (40-60%). In contrast, IL-1ß almost doubled the IGF-I levels in kidney. Western ligand blots and immunoprecipitation of plasma indicated that IL-1ß increased BP-1 (3-fold) and a BP with a *Mr* of 28,000 (i.e., BP-28K; 50%). These changes in the IGF system were associated with increases in body temperature, and plasma glucagon and corticosterone concentrations. These data indicate that the IGF system is sensitive to IL-1ß, and provides support for the role of IGF-I and IGF-BPs in the enhanced tissue catabolism of disease states associated with elevations in IL-1. (Supported by NIH grant GM 38032).

9.8

MODULATION OF PRODUCTION OF INTERLEUKIN-6 BY PROGESTERONE IN HUMAN GINGIVAL FIBROBLASTS CHALLENGED WITH INTERLEUKIN 1-β.

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During pregnancy, the occurrence of gingivitis is common, producing pain, bleeding, edema, and erythema of the gingiva. It has long been assumed the that the "pregnancy hormones" - eg. progesterone (P) and 17β-estradiol (E2) - are the causative agents in this condition, but the mechanism by which these hormones might effect such changes has not been established. The hypothesis being tested was that some of the hormones of pregnancy might modulate the production of interleukin-6 (IL-6), which is secreted by gingival fibroblasts in response to an inflammatory challenge. Although these cells also release IL-6 after stimulation with lipopolysaccharides as well as other inflammatory mediators, the system we used here depended on interleukin-1β to provide this stimulation. We developed a serum-free medium in which to test the effects of these hormones. Fibroblasts from clinically healthy patients were grown from explants in standard DMEM with 10% fetal bovine serum, and used between passages 3-10. Steroid experiments were conducted in phenol red-free DMEM containing 15mM HEPES, non-essential amino acids, insulin, transferrin, selenium, bovine serum albumin and oleic acid. Under these conditions, we found that P dose-dependently and significantly ($P<0.05$) inhibited the secretion of IL-6. These results were seen with cells from males and females. IL-6 secretion at 10^{-6}M P was 40% of control, and the estimated EC_{50} was 2.7×10^{-7}M, a level found in later pregnancy. This study was funded in part by the Medical College of Georgia Research Institute.

10.1

THE CENTRAL LOCATION OF GLUCOCORTICOID NEGATIVE FEEDBACK DURING PSYCHOLOGICAL STRESS-INDUCED FEVER. J.L. McClellan, L. E. Morrow, J. J. Klir, M. J. Kluger. Univ. of Michigan, Ann Arbor, MI 48109.

Previous work has shown that glucocorticoids attenuate both lipopolysaccharide (LPS) and psychological stress-induced fevers within the central nervous system (CNS). There is evidence that glucocorticoids (as well as other modulators of fever) act in the anterior hypothalamus (AH) during LPS fevers, and those fevers can be exacerbated by microinjection of RU38486 (a type II glucocorticoid receptor antagonist) into the AH. While intracerebroventricular microinjection of RU38486 will attenuate stress-induced fevers, AH microinjection will not. Thus, the central location of glucocorticoid negative feedback during psychological stress-induced fevers has not been determined. The hippocampus contains a large number of glucocorticoid receptors, and is involved in glucocorticoid negative feedback of several physiological events. To determine if glucocorticoid modulation during psychological stress-induced fever occurs in the hippocampus, the fornices of rats were transected to block hippocampal communication with the AH. This resulted in significantly larger psychological stress-induced fevers (1.19 ± 0.11 vs 0.83 ± 0.13 °C, P = 0.02) in animals with transected fornices compared to animals with sham surgeries. There were no differences between these groups for LPS-induced fevers (P = 0.92). To determine where in the hippocampus glucocorticoids might exert their negative feedback during psychological stress, rats were microinjected with either 1 ng RU38486 or vehicle into the dentate gyrus region prior to a 30 minute exposure to an open field (a psychological stressor). There was no difference between the psychological stress-induced fevers of the RU38486 and vehicle-injected groups (P = 0.21), indicating these fevers are modulated elsewhere in the hippocampus. These data support the hypothesis that glucocorticoids modulate psychological stress-induced (but not LPS-induced) fevers in the hippocampus, although their precise site of action is still not known. **Research supported by AI-27556 and MH-48609**

10.2

CENTRAL INJECTION OF IL-1α INCREASES BOTH HEPATIC GLUCOSE PRODUCTION AND PERIPHERAL GLUCOSE UPTAKE. PE Molina, NN Abumrad* and CH Lang. Department of Surgery, SUNY at Stony Brook, Stony Brook, NY 11794-8191.

IL-1 is produced endogenously within the brain following systemic injection of endotoxin and thus may be an important component of the central regulation of glucose homeostasis. The present study examined whether central administration of IL-1, at a dose that is without affect when injected peripherally, is capable of regulating glucose flux. Intracerebroventricular (ICV) injection of IL-1α (100 ng) into fasted rats produced a hyperglycemia that resulted from an increased rate of hepatic glucose production (HGP; 110%) that exceeded the rise in glucose uptake (GU; 82%) by peripheral tissues. This enhanced flux was associated with increased plasma levels of catecholamines, glucagon, corticosterone and insulin. IV infusion of α- and ß-adrenergic antagonists prior to IL-1 injection prevented the IL-1α induced increase in HGP. Using the *in vivo* injection of [^{14}C]-2-deoxyglucose, IL-1α was shown to increase GU in skeletal muscle (117%), diaphragm (50%) and heart (110%). GU by other tissues (liver, spleen, lung, skin, gut and whole brain) was not different from control values. When somatostatin was infused to prevent the IL-1 induced hyperinsulinemia, the increased GU by muscle was blocked or greatly attenuated. These results indicate that IL-1α acts centrally to stimulate whole body glucose flux. Furthermore, the enhanced HGP appears to be mediated by the increased plasma catecholamines and the increased GU by peripheral tissues mediated by the early hyperinsulinemia. (Supported by NIH GM 38032).

10.3

SYSTEMIC BUT NOT CENTRAL ADMINISTRATION OF TUMOR NECROSIS FACTOR α ATTENUATES LPS-INDUCED FEVER IN RATS. John J. Klir, Jennifer L. McClellan, Wieslaw Kozak, Zoltán Szelényi and Matthew J. Kluger. Institute for Basic and Applied Medical Research, The Lovelace Institutes, Albuquerque, NM 87108.

The purpose of this study was to test the hypothesis that tumor necrosis factor α (TNFα) limits fever induced by lipopolysaccharide (LPS) in rats, and to determine whether such antipyretic action of this cytokine is systemic or in the central nervous system (CNS). The CNS effects on LPS-induced fever were tested by injecting a subpyrogenic amount (0.20 µg) of human recombinant TNFα (hrTNF) intracerebroventricularly (icv), or by a slow infusion into the anterior hypothalamus of an amount previously measured in this brain region during LPS fever (0.24 U in 0.13 µl of artificial cerebrospinal fluid/min) for 3 hours starting 1 hour after an ip injection of LPS (50 µg/kg). The peripheral effects of this cytokine on LPS fever were tested by injecting 1 µg/kg of hrTNF ip, or by ip administration of 300 µg/kg of human TNF soluble receptor, p80 (TNFsr). The core temperature (measured by biotelemetry) during LPS fever was not significantly affected by central nervous administrations of hrTNF (ANOVA, P = 0.920 for the icv administration; and unpaired t test, P = 0.260 for the intrahypothalamic infusion). The febrile response to LPS was abolished by an ip injection of hrTNF such that the mean temperature over the time period between 2 and 8 h after the injections (37.28 ± 0.12°C) was not significantly different from the mean temperature during 1 h before the injections, designated as a baseline temperature (37.01 ± 0.12°C) (paired t test, P = 0.301). When rats were injected ip with TNFsr, the febrile response to LPS was enhanced (unpaired t test, P = 0.013). These results support the hypothesis that TNFα acts to limit the magnitude of LPS-induced fever, and that this action occurs outside the CNS. Research supported by NIH AI27556.

10.4

SLEEP PATTERNS IN HEALTHY AND INFLUENZA-INFECTED MICE ARE CORRELATED WITH ALLELES OF THE *If-1* GENE. Linda A. Toth. St. Jude Children's Research Hospital, Memphis, TN 38105.

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 intranasal inoculation with influenza virus. Differences in alleles of the *If-1* gene in these strains permit C57BL/6 mice to produce more IFN during some viral infections than do BALB/c mice.

C57BL/6 and BALB/c mice housed at 22 ± 1°C on a 12:12 h light:dark cycle demonstrated different baseline patterns of sleep, locomotor activity and temperature. C57BL/6 mice (n=12) exhibited marked circadian variations in these parameters, whereas circadian variations in BALB/c mice (n=7) were much less pronounced. After influenza infection, both strains developed decreases in temperature, locomotor activity and delta-wave amplitude during sleep. These effects were similar in magnitude and time course in both strains, began within 24 h after viral inoculation, and persisted for at least 4 days. However, infected C57BL/6 mice also showed robust increases in the amount of sleep, whereas infected BALB/c mice did not. Sleep enhancement in C57BL/6 mice began within 24 h after inoculation, persisted for at least 4 days, and was most prominent during the "lights-off" period, which was associated with a relatively low amount of sleep during the baseline recording period.

Thus, sleep enhancement after influenza infection is correlated with the *If-1* allele for high IFN production in mice. Because IFN is somnogenic, this suggests a mechanism for the difference in sleep propensity observed after influenza infection in C57BL/6 and BALB/c mice.

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10.5

IL-4 INHIBITS THE PRODUCTION OF NITRIC OXIDE IN STIMULATED GLIAL CELLS. Nurit Goldreich 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. Recently, 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 glycoprotein 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-4 did not change the basal levels of NO production but inhibited that induced by INF-γ. The inhibitory effect of IL-4 was more pronounced in cells pre-treated for 24 hr before stimulation with INF-γ. This effect of IL-4 was not mediated through a decrease in the level 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-4. 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 T CELL FUNCTION IN PATIENTS WITH GLIOBLASTOMAS: A SELECTIVE IMPAIRMENT OF THE IL-2 SYSTEM. Chaya Brodie*, Alex Tsukerman*, Ely Ashkenazi**, Motti Deutsch, Reuven Tirosh and Arye Weinreb. *Department of Life Sciences, **Department of Neurosurgery, Hadassah, Ein Karem, The Jerome Schottenstein Cellscan Center, Department of Physics, Bar-Ilan University, Ramat-Gan 52900, 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 glioblatomas has been associated with broad impairment of immunocompetence as demonstrated by a variety of *in vivo* and *in vitro* parameters. Glioblastoma patients express deficiencies in cell-mediated immunity, such as cutaneous anergy reduced number of circulating T cells and decreased lymphocyte stimulation by mitogens. In this study we have analyzed the mechanisms responsible for diminished lymphocyte reactivity by examining the function of T lymphocytes in patents with malignant glial tumors. We found that the proliferative response of lymphocytes stimulated with PHA or anti-CD3 was significantly reduced in patients with malignant glial tumors as compared to patients with meningiomas, oligodendrogliomas and healthy individuals. Stimulated T cells appear to express lower levels of the α-subunit (p55) of the IL-2R, whereas, no significant differences were observed in the level of the β-subunit (p75) as determined by flow cytometry. In parallel we found increased levels of sIL-2R in the supernatants of stimulated PBMC. No changes were observed in either secretion of IL-4 or the level of IL-4R. These results indicate that T cells obtained from patients with glioblastomas exhibit a selective defect in the production of IL-2 and in the expression of functional IL-2R.

10.7

ROLE OF TNF IN THE FEVER MECHANISM INDUCED BY INTRAVENOUS PERFLUOROCARBON EMULSION IN RATS. J.D. Bradley, S. Otto, D. Smith, G. Neslund, and S.F. Flaim. Alliance Pharmaceutical Corp., San Diego, CA 92121.

Intravenous (i.v.) infusion of perfluorochemical emulsions (PFC) 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 chronically fitted with intraperitoneal body temperature telemeters and a jugular vein catheter and allowed to recover overnight. Animals were then infused with saline (3 mL/kg, n=4), lipopolysaccharide (0.1 µg LPS/rat, n=4), or a 90% w/v PFC emulsion (3 mL/kg, n=4) and core body temperature was monitored by telemetry for 12 hrs. A fourth group of animals was pretreated with dexamethasone (Dex, 0.2 mg/kg) at both 12 and 2 hrs prior to PFC infusion (n=4). Rats were unrestrained 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 (pg/mL) and mean area under the curve (AUC) of the change in body temperature. Data are Means ± SEM.

GROUP	AUC	Baseline	1 hr	2 hrs	3 hrs	6 hrs
Saline	13.7	0 ± 0	0 ± 0	3.8 ± 1.4	1.4 ± 0.9	0.3 ± 0.3
LPS	15.4	1.4 ± 1.4	3179 ± 799	235 ± 65	25 ± 7	3.5 ± 3.5
PFC	27.9	0 ± 0	110 ± 36	197 ± 60	162 ± 37	35 ± 9
Dex + PFC	10.9	1.2 ± 1.2	0 ± 0	1.3 ± 0.9	0.6 ± 0.6	0 ± 0

There were no significant changes in TNF or body temperature in saline control animals. After LPS, TNF levels increased sharply by 1 hr. Despite high TNF levels, this dose of LPS did not induce fever (while higher LPS doses induce fevers 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 ~1.25°C above baseline at 5 hrs. Dex ablated both the increase in TNF and the fever following PFC. These data suggest that serum TNF levels do not always correlate with fever. However, the timecourse or duration of TNF release may play a role in the production of fever.

10.8

HIV-1 glycoprotein120 ALTERS RAT SLEEP. MR Opp, TK Hughes, Jr.,* and EM Smith*. Depts of Psych & Behav Sci and Microbiol. Univ of Texas Med Branch, Galveston, TX 77555

Excessive daytime fatigue and sleepiness are prominent and persistent symptoms associated with HIV infection. Concentrations of cytokines increase during the course of HIV infection, and some cytokines, particularly IL-1, are somnogenic. gp120, the major envelope protein of HIV-1, has many neurological actions and induces IL-1 secretion in brain. As such, gp120-induced IL-1 secretion within the CNS is one potential mechanism whereby sleep may be altered during HIV infection. Alternatively, gp120 may exert direct actions on "sleep centers" within the CNS.

Rats were surgically prepared with instrumentation to allow determination of sleep/wake activity. The rats were injected ICV with one of two doses of recombinant gp120 (ABT, Inc., Cambridge, MA: 100- or 500 ng) or with pyrogen-free saline (vehicle). Rats responded with increases in the amount of time spent in non-rapid-eye-movements sleep (NREMS) relative to values obtained after vehicle injection, regardless of the dose of gp120 injected. This period of enhanced NREMS lasted 4 - 8 h depending on dose, and was followed by a reduction in sleep relative to values obtained after vehicle administration. We speculate this period of reduced sleep may be due to gp120- and/or IL-1-induced elevations in endogenous cytokine inhibitors, possibly the IL-1 receptor antagonist, IL-10, or corticotropin-releasing hormone. These substances all reduce sleep in experimental animals.

10.9

L-NAME, A NITRIC OXIDE SYNTHASE INHIBITOR, DOES NOT ALTER THE FEBRILE RESPONSE TO LPS N.C. Long and S.A. Shore, Physiology Program, Harvard Sch. of Public Health, Boston, MA 02115.

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 cryogen, limiting the magnitude of fever (Long et al., *Am. J. Physiol*, R332, 1990). One potential mechanism for this response is that TNF moderates fever by inducing the release of nitric oxide (NO), a potent vasodilator, which could lead to increased heat loss. NO could also act centrally to alter the febrile response. To test the hypothesis that NO is involved in moderating fever, we compared the febrile response of rats that had been treated with the NO inhibitor L-NAME, to that seen in rats treated with its inactive enantiomer, D-NAME. L-NAME and D-NAME were administered continuously in the rats' drinking water (70 mg/100 ml) starting 8 days before the study began. The L-NAME-treated rats had a mean blood pressure of 151 mmHg, while the D-NAME animals had a mean blood pressure of 127 mmHg (p=0.007), confirming the efficacy of the L-NAME treatment. Body temperature (Tb) of the rats was monitored by implanted biotelemetry devices. We found no difference in the baseline Tb of the rats (L-NAME: 36.4±0.2°C vs D-NAME: 36.3±0.3°C; p=0.71). The injection of 10 µg/kg of LPS (i.p.) into the L-NAME-treated rats induced a mean change in Tb of 1.11±0.18°C between 2 and 8 h post-injection. This did not differ from the fever seen in the D-NAME rats during the same time interval (1.09±0.12, p=0.92). These data do not support the hypothesis that the vasodilatory effect of NO mediates the cryogenic effect of TNF during LPS fever. It is possible, however, that NO acts centrally, and that the L-NAME did not reach the region of the brain responsible for this response. Supported by HL19170.

10.10

COLOCALIZATION OF FOS-LIKE IMMUNOREACTIVITY AND NITRIC OXIDE SYNTHASE ACTIVITY FOLLOWING IMMUNOLOGICAL STIMULATION. J.K. Elmquist*, T.E. Scammell, C.D. Jacobson, and C.B. Saper. Dept. of Neurology, Harvard Medical School/Beth Israel Hospital, Boston, MA 02115, and Dept. of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

Nitric oxide (NO) is a biologically active molecule implicated in numerous physiological functions including participation in the acute phase response (APR). Recent studies suggest that NO is involved in regulating neuroendocrine function following immune system activation including alteration of ACTH, vasopressin, and oxytocin secretion. Additionally, inhibition of NO production following LPS challenge has detrimental effects (including increased mortality rates) 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 i.v. LPS as a model of sepsis and have mapped the resultant Fos-IR. In addition, we have examined subsets of cells in the brain that contain both Fos-IR and NADPH-diaphorase staining (NO synthase activity) following LPS challenge. Cells that contained both NO staining and Fos-IR were observed in the periventricular, supraoptic, and paraventricular hypothalamic nuclei, and in the medial preoptic area. Double staining was also seen in circumventricular organs including the organum vasculosum of the lamina terminalis (OVLT) and in the subfornical organ. The results of this study provide anatomical evidence for the role of NO in regulating neuroendocrine responses following immune activation. These studies further suggest that neuronal NO is involved in coordination of the complex physiological responses of the APR.

METHODOLOGICAL ISSUES IN CYTOKINE MEASUREMENT

11.1

PRO-IL-1β IS RELEASED FROM MONOCYTES IN VITRO IN A FORM THAT IS RESISTANT TO PROCESSING BY IL-1β CONVERTING ENZYME. Mark D. Wewers* and Heidi A. Pope*. The Ohio State University, Columbus, OH. 43210

The processing and release of 31 kDa proIL-1β 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 mononuclear phagocytes in response to endotoxin stimulation in vitro (J.Immunol.149:3052,1992). Since the site of processing of the 31 kDa proIL-1β is not known, we hypothesized that the released proIL-1β may represent IL-1β in a pre-processing phase or IL-1β that has been modified to prevent processing. To study released proIL-1β, we measured supernatant proIL-1β from endotoxin stimulated monocytes by immunoprecipitation of ^{35}S-methionine labeled protein, by Western blots, and by our recently developed enzyme linked immunoassay (ELISA) specific for proIL-1β (J.Immunol. Meth. 165:269,1993). Although supernatant proIL-1β represented 20-40% of the total released IL-1β as measured by SDS-PAGE with densitometry, this proIL-1β was not detected by our proIL-1β specific ELISA. The ELISA's inability to detect proIL-1β was not due to inadequate sensitivity or subsequent degradation in the ELISA. Importantly, the 31 kDa protein was confirmed to be proIL-1β since its immunoprecipitation was specifically blocked by the immunogenic peptide used to generate the proIL-1β specific antibody. Finally, since supernatant proIL-1β can be immunoprecipitated, we asked whether affinity purified supernatant proIL-1β can be processed to mature IL-1β when incubated with recombinant IL-1β converting enzyme (ICE) (Merck). In two separate purifications, immunoaffinity purified cytosolic proIL-1β was processed by ICE but identically purified supernatant proIL-1β was not. These findings imply that proIL-1β can be released from monocytes in a unique form that may reveal important clues to monocyte regulation of proIL-1β processing and release.

11.2

DEVELOPMENT OF A HUMAN INTERLEUKIN-1 BETA PRECURSOR ELISA. Leslie S. Casey, Lisa Esposito, Cynthia Alley, Beverly Lytwyn, and Richard S. Dondero. Cistron Biotechnology, Inc., Pine Brook, NJ 07058.

A highly-sensitive, rapid, and specific ELISA was developed for measurement of the 33kD human interleukin-1β (IL-1β) precursor protein. The monoclonal antibody-based ELISA reproducibly detects as little as 10pg/ml of precursor. Proteolytic cleavage of IL-1β precursor by IL-1 converting enzyme (ICE) or other enzymes leads to release of the active, 17.5kD form of IL-1β, which is known to be a key mediator of inflammation in normal immunity and in disease. Cleavage of purified IL-1β precursor protein in vitro resulted in markedly reduced signals in the IL-1β precursor ELISA, with concomitant increased signals in assays 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.