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Conference Perspective
NEUROIMMUNOMODULATION: STRESS AND IMMUNE FUNCTION
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At this meeting, as at a number of recent meetings wherein the latest cellular and molecular techniques have been used to revisit and re-evaluate long-standing biological questions and observations, the past has indeed become prologue. In many cases, as with sections of this meeting, the questions or observations are 25–30 years old, such as the seminal studies by Dr William R Beisel and his colleagues at the US Army Medical Research Institute of Infectious Diseases. This series of studies documented the relatively constant constellation of metabolic, physiological and endocrinological changes which occur during bacterial, viral and rickettsial diseases in humans and other species. The wisdom of the ancient Greeks also was demonstrated at this conference: the virtues of a sound mind in a sound body.

The meeting began with Dr Seymour Reichlin (New England Medical Center and Vail, Arizona) listing both the clinical conditions and preclinical data that attest to the presence and consequences of neuroendocrine–cytokine interactions. As some of the consequences of such interactions he cited: pituitary–adrenal activation; inappropriate ADH secretion; sick euthyroid syndrome; sick hypogonadal syndrome; sick bone syndrome; impaired insulin secretion and insulin resistance; and sick brain syndrome, also known as sickness behaviour. In every case there is growing evidence of endocrine–cytokine interactions. Moreover, these interactions between cytokines and neurohormones may elicit adverse neurological side-effects when cytokines are given therapeutically. For example, in a 1987 study of 44 patients given IL-2 and lymphokine-activated killer cells, 5 patients developed moderate cognitive impairment, 22 displayed delirium and severe cognitive impairment, while 7 exhibited delusions, escape behaviour and picking at their bedclothes (the last a condition which Dr Reichlin noted was described by Galen and is often found in the severely ill). Interferon-α_2 treatment of hepatitis C also is generally accompanied by fever, chills and fatigue, but in 17% of cases evokes serious brain dysfunction to include suicidal tendencies.

Dr Rodney Langman (Salk Institute for Biological Studies) argued that the immune system 'hijacked' existing effector functions to create a scheme whereby the host can distinguish between self and non-self. He suggests that the immune system is unique to vertebrates while 'immune responses' can be found even in single-cell creatures. Dr Langman made an impassioned plea to begin collecting the data on cytokine and neuroendocrine effects and interactions in a central location so the

information can be processed and integrated into a 'consensus model of neuroendocrine immunology'. He volunteered to be the contact person to begin collecting data on the ligands, the receptors and the cell types, as well as on the biological actions of, and physiological responses to, hormones and cytokines. Some in the audience thought that this would be an insurmountable task unlikely to yield much information about a complex system; however as meta-analysis has shown, computer systems can often find patterns in multiple sets of data that may not be apparent from individual studies.

Dr Edward Bernton (Walter Reed Army Medical Center, Washington, DC) reminded the audience that despite the bloody nature of battle, it was infections not injuries that have caused more casualties in war, even as recently as the Korean, Vietnam and Gulf wars. In addition to being hazardous, war and the training for war is stressful. To assess the effects of such stresses on the endocrine–immune system(s) 300 Ranger trainees were studied over a period of 8 weeks during the desert, mountain, forest and swamp phases of their training. On average these healthy individuals lost 9–10% of their body weight, virtually all as fat, had 3–4 hours of sleep per day, operated at a high level of activity, and were subjected to a wide variety of stresses such as might occur in battle. By the end of the 4th week, at the completion of the mountain phase of training which was the most strenuous phase, serum cortisol levels increased while those of testosterone and IGF-1 decreased. At this time, the skin test to an array of antigens was 40% of normal while 20% of the trainees were anergic. The skin test response correlated with testosterone levels. While T cell function was decreased, IgE levels were increased. White cell secretion of IL-1α, IL-6 and TNFα in response to endotoxin was decreased. Attempts to eliminate these alterations in the neuroimmune responses by increasing food intake yielded equivocal results.

While exhausting exercise coupled with sleep deprivation and a variety of additional stresses clearly is immunosuppressive, according to Dr Laurie Hoffman-Goetz (University of Waterloo, Ontario, Canada) acute maximal exercise by itself appears to increase the number of natural killer (NK) cells and increase NK and lymphokine activated killer (LAK) cell activities. Whether these increases in natural immune functions have benefit to the host may depend upon the nature of the disease, for example, the dissemination of mammary adenocarcinoma tumours appears to be unaffected by NK or LAK cell activity.

In regard to stress, it appears to make no difference whether one is man or mouse. Dr John Sheridan (Ohio State University) showed that restraint stress increased plasma corticosterone levels and tissue responses to norepinephrine, but reduced the cellular immune responses of C57 Black 6 mice to respiratory influenza A infection. The elevated plasma corticosterone levels correlated with reduced lymphocyte trafficking to the lungs and to the draining lymph nodes of the infected mice. While cell accumulation at the inflammatory site and lymphadenopathy were restored by treating the animals with a glucocorticoid receptor antagonist, restoration of T cell activation only occurred when a β-adrenergic receptor antagonist was given, suggesting a role for catecholamine in the regulation of T cell activation.

According to Dr Bruce McEwan (Rockefeller University), glucocorticoids can modulate immune cell trafficking as a function of the glucocorticoid receptors on such cells, as well as of tissue factors such as corticosteroid binding globulin and steroid catabolizing enzymes. Diurnal and stress-induced increases in glucocorticoid elicit

increases in neutrophil and eosinophil counts and decreases in B and T cells. The decrease in B cells appears to be totally glucocorticoid dependent, whereas the effects on other immune cells appear to involve factors in addition to glucocorticoid.

Given that neuro-endocrine–cytokine interactions exist, one might expect that cytokines would need to cross the blood–brain barrier to have an effect. Dr William Banks (Veterans Affairs Medical Center, New Orleans) demonstrated that IL-1 does cross the blood–brain barrier by means of a saturable transport system and that IL-1 receptor antagonist (IL-1ra) inhibits transport of IL-1α at 50–60-fold lower concentrations of IL-1ra than is needed to produce an equivalent inhibition of IL-1β transport. The physiological and immunological consequences of this selective inhibition are as yet unknown, but these data should be kept in mind if IL-1ra is used to treat acute or chronic inflammation.

Evidence that minute (femtomolar) amounts of IL-1β injected into the brain are sufficient to suppress a variety of peripheral immune cell responses was provided by Dr Jay Weiss (Emory University, Atlanta, GA). CNS IL-1 suppression of peripheral immune response requires corticotrophin releasing factor (CRF) as an intermediate in that antiserum to CRF blocked the IL-1-induced immunosuppression. In addition, Dr Weiss has recently found that the HIV envelope protein gp120 injected into rat brain causes a release of IL-1 and suppression of peripheral cellular immune responses. Dr Jean Merrill (University of California, Los Angeles) showed that a full-length HIV envelope protein containing gp120 and gp41 induced the production of both IL-1 and TNFα in rodent glial cell cultures. Detailed analysis of selected protein fragments showed that IL-1 and TNFα were induced by different epitopes.

Diminished intellectual function and motor impairment is also common in people with AIDS. Dr Anna da Cunha (National Institute of Mental Health, NIH, Bethesda, MD) found that there is an increase in the mRNA encoding the peptide neurotransmitter preprosomatostatin (SRIF) in layer IV of the frontal cortex in HIV-seropositive children and SIV-infected juvenile rhesus macaques concomitant with cognitive impairment. IL-1 is known to induce SRIF, but whether IL-1 or other cytokines are actually responsible for both the cognitive and motor dysfunction of AIDS dementia cannot be determined from neuro-anatomical studies alone.

Dr Robert Dantzer (INSERM, Bordeaux, France) provided evidence for a scheme by which cytokines released peripherally can induce the centrally (CNS) mediated sickness behaviour described by Dr Reichlin. This scheme involves activation of primary nerve endings by a prostaglandin-dependent mechanism; conduction of this signal via the vagus nerve (cutting the vagus nerve blocks the sickness behavioural effects of peripherally administered IL-1); induction of cytokine gene expression in the brain; the action of these CNS-produced cytokines on neurons and/or glial cells (centrally administered IL-1ra blocks the behavioural effects of peripherally administered IL-1); and the induction by these CNS cytokines of hormones (e.g. glucocorticoids) and neuropeptides (e.g. vasopressin) which act to moderate the behavioural effects of the cytokines.

One particular aspect of sickness behaviour is disruption of sleep patterns, generally characterized by an initial period of enhanced slow-wave sleep (SWS) followed by a period of reduced sleep. Dr James Krueger (University of Tennessee, Memphis, TN) described what is known about cytokines and the regulation of sleep. IL-1α and β, TNFα and β, interferon-α, and acidic fibroblast growth factor all enhance sleep whether given intravenously or intracerebroventricularly. The effect of

these cytokines appears not to be limited to illness-associated sleep changes. Antibodies to IL-1β or to TNFα, as well as a soluble TNF receptor and IL-1ra reduce normal sleep. The mechanisms by which cytokines elicit sleep do not appear to involve prostaglandins, opioids or insulin, but may be mediated by growth-hormone-releasing hormone (GHRH). Growth hormone release is linked to SWS and GHRH is somnogenic in rats, rabbits and man. Antibodies to GHRH block IL-1-induced growth hormone release and IL-1-induced sleep responses. Nitric oxide (NO) is also somehow involved; GHRH and IL-1 enhance NO production, while inhibition of NO production blocks IL-1-induced sleep.

Another aspect of sickness behaviour is fever. Dr Matthew Kluger (The Lovelace Institutes, Albuquerque, NM) presented evidence that IL-1β acts in the anterior hypothalamus to produce fever and that IL-6 appears to be involved. Intrahypothalamic injection of neutralizing antibody to IL-1β attenuates fever and suppresses the rise in hypothalamic IL-6. According to Dr Bryan Spangelo (University of Nevada, Las Vegas), IL-1β also induces IL-6 release in the anterior pituitary via lysophosphatidylcholine activation of protein kinase C. IL-6 stimulates the release of prolactin, growth hormone and luteinizing hormone from male rat anterior pituitary cells in vitro. Antibody to IL-6 reduced, but did not eliminate prolactin release.

Dr Kluger also showed that physiological levels of corticosterone increased TNF and IL-6 release from isolated perfused rat livers. Epinephrine via a β-mediated pathway also led to an increase in IL-6 secretion from liver. It appears that these stress hormones exhibit a degree of duality; they can increase the circulating levels of some cytokines as well as moderate the effects of these self same cytokines.

According to Dr Adrian Dunn (Louisiana State University Medical Center, Shreveport, LA) many of the behavioural responses associated with sickness, while initiated by cytokines such as IL-1, are mediated by corticotrophin-releasing factor (CRF). Intracerebroventricular (icv) injections of CRF elicit sickness behaviour and icv injections of a CRF antagonist block these behavioural responses.

It appears that not only are many forms of stress immunosuppressive, but so too are substances we often use when under stress – tobacco and alcohol. Dr Mohan Sopori (The Lovelace Institutes, Albuquerque, NM) showed in both in-vivo and in-vitro studies that nicotine activates lymphocytes making them refractory to subsequent antigen-mediated activation. The effect of nicotine may be related to the presence of nicotine acetylcholine receptors on lymphocytes. Chronic alcohol consumption also is immunosuppressive; this effect of alcohol appears to be a dominant genetic trait. Chronic ethanol ingestion suppresses the antibody plaque-forming cell response in LEW rats and LEW × F344 progeny, but not in F344 rats.

Dr J Edwin Blalock (University of Alabama, Birmingham, AL) suggested that the immune system acts as a sensory organ for non-cognitive stimuli, such as bacteria, viruses and tumours. He indicated that the neuroendocrine and the immune systems share many of the same ligands and receptors. For example, lymphocytes have been shown to produce ACTH, prolactin, TSH, endorphins and growth hormone; in fact growth hormone appears to act as autocrine regulator in lymphocytes. Conversely, the pituitary has a receptor for IL-1 and IL-1 can act directly to induce CRF which stimulates ACTH release. CRF also sensitizes the pituitary so that subsequent exposure to IL-1 causes a persistent ACTH release. Such sensitization might allow a series of mild inflammatory stresses to produce a disproportionate glucocorticoid

response. Dr Blalock also briefly discussed a pituitary-derived factor called 'suppressin' which appears to block the blastogenic response and, in preliminary studies, to inhibit lymphoid tumour growth.

Evidence for a role of the sympathetic nervous system in modulating immune system responses was provided by Dr Suzanne Felten (University of Rochester School of Medicine). Treatment of adult mice with 6-hydroxydopamine (6-OHDA) depletes peripheral organs, including lymphoid organs, but not the CNS, of 90–95% of norepinephrine content within 24 hours. This chemical sympathectomy increases background proliferation in inguinal and axillary lymph nodes, spleen and bone marrow in non-immune mice. Upon stimulation lymph node T cell response was reduced and B cell response increased, but with a reduction in IgM and an increase in IgG. Splenic B cell responses differed from those of lymph nodes; LPS-induced proliferation was decreased with no changes in IgM or IgG concentrations.

Much of the focus of stress research in general and this meeting has been on the factors of neuro, endocrine or cytokine origin which propagate stress. As an antidote, Dr James Lipton (University of Texas SW Medical Center, Dallas, TX) discussed α-MSH which appears to be following IL-1 into the pantheon of virtually uniquitous endogenous mediators, which may be appropriate considering the range of its potent anti-inflammatory activities. α-MSH is found in many areas throughout the brain and body, with large amounts found in the skin and gut. α-MSH has been shown to moderate acute inflammation induced by irritants and cytokines; the delayed hypersensitivity reaction; chronic inflammation (mycobacterium arthritis); and systemic inflammation associated with endotoxaemia, sepsis, peritonitis and adult respiratory distress (ARDS). In a caecal ligation model of peritonitis which is uniformly fatal, α-MSH increases survival to 40% and acts additively with gentamicin to attain 70% survival. In ARDS, α-MSH inhibits neutrophil migration and accumulation of neutrophils in the bronchi. α-MSH may exert its anti-inflammatory actions by its ability to inhibit nitric oxide production by macrophages. Dr Lipton proposed an interesting inflammo-modulatory cycle based on the fact that macrophages both produce and have receptors for α-MSH while neutrophils appear to have only receptors for α-MSH thus, in macrophages α-MSH could act as an autocrine. Since macrophages succeed neutrophils at wound sites one wonders if this difference between macrophages and neutrophils allows for a non-specific inflammatory response, which could be destructive if unchecked, to be converted into a controlled healing response.

If cytokines are active in the CNS, one might expect that repeated or chronic administration of pro-inflammatory cytokines might induce CNS dysfunction or disease. Dr Iain Campbell (The Scripps Research Institute, La Jolla, CA) described the effects of chronic expression of cytokines in the CNS of transgenic animals. A GFAP expression vector was used to target astrocytes in the CNS for the expression of IL-6, IL-3 or IFN-α. Cerebral expression of each of these cytokines at high levels led to an early death. Expression at low levels was associated with a progressive neuropathy, but allowed time for development of breeding lines. All cytokines caused neurodegeneration and astrocytosis. IL-6 alone caused demyelination, angiogenesis, diffuse blood–brain barrier leakage and upregulation of acute-phase protein synthesis as well as increased deposition of these proteins. IL-3 alone caused menigoencephalitis; both IL-3 and IL-6 caused perivascular cuffing and microgliosis. IFN alone caused induction of MHC class I and 2,5-oligoadenylate synthase gene

expression. Whether or not such transgenic animals can be developed into models for a variety of neurodegenerative diseases remains to be determined, but it is clear that chronic expression of cytokines in the CNS produces many of the changes associated with such diseases.

Neurocytokine interactions appear to occur even in the womb. Evidence that cytokines might play a role in brain function and perhaps in brain development at the fetal stage was adduced by Dr G Miller Jonakait (Rutgers University, Newark, NJ). Murine interferon-γ (IFN-γ) markedly increases choline acetyltransferase (ChAT) activity in cultured rat embryonic septal nuclei with adjacent basal forebrain. The data indicate that IFN-γ increases the amount of mRNA coding for ChAT and suggests that IFN-γ encourages the differentiation of cholinergic neurons from undifferentiated precursors. A microglial-derived intermediate appears to be involved.

In the event that there remained any doubt that a powerful link exists between brain function and immune function, Dr Nicholas Cohen's (University of Rochester Medical Center, Rochester, NY) presentation of the effects of a gustatory conditioned stimulus on antibody production provided food for thought. In short, after conditioning, a booster dose of antigen which by itself was unable to elicit a significant antibody response was found to be as effective as a 100-fold greater dose of antigen in inducing a secondary antibody response. Could these data indicate that the potency of drugs and vaccines might be enhanced by carefully chosen psychological stimuli? Could the mechanism of the placebo effect be so explained?

The meeting closed with a masterful summary by Dr David Felten (University of Rochester Medical Center) who listed a series of key themes that emerged during the meeting. One theme involved the accumulation of evidence that there were functional receptors for hormones and neurotransmitters on tissues and cells of the immune system, the thymus, the spleen, T and B lymphocytes and macrophages. A second theme had to do with the growing evidence that neurohormones are produced, and are active in, the immune system and conversely that cytokines are produced and active in what has classically been considered the neuroendocrine system. It should be emphasized that though there are many instances of neuroimmune interactions, not every stimulus that generates an immune response also elicits a neuroendocrine response. For example, while LPS stimulates both immune and neuroendocrine responses, KLH evokes only an antibody response without any glucocorticoid response.

The third theme emphasized that there was a wide range of cytokine interactions with the nervous system and conversely, that there is a diversity of neurotransmitter interactions with primary (thymus and bone marrow) and secondary (spleen, lymph nodes) lymphoid tissues, as well as with target organs such as liver. It has long been known that neurotransmitters interact with one another to produce synergistic or antagonistic effects and a parallel situation has been observed to exist among the cytokines. But only recently has it become clear that these two systems crosstalk extensively. While this crosstalk probably works to the advantage of the host during sickness, following injury, and in health, it does not make the elucidation and/or manipulation of these interactions easier for the preclinical and clinical investigators.

Dr Felten ended by reminding the audience that most of the studies reported at this meeting were done in young healthy animals or humans subjected to an acute or limited-duration physiological or inflammatory stress or induced disease. These studies leave unanswered the effects of a chronic stress or disease and the alterations

and adaptations associated with ageing. An additional factor which was not discussed was the perception and effect of pain on neuroimmune interactions. Amelioration of pain has been shown to facilitate healing after surgery.

CONCLUDING COMMENTS

As this conference amply demonstrated, molecular biology provides the tool to examine in exquisite detail the individual components of complex biological systems. However, molecular biology, in and of itself, gives little or no insight as to how these components are arrayed and interact to form a functional system. It is only by the concomitant study of molecular, cellular, intercellular (local), tissue, organ and organism (systemic) changes that a clinically relevant corpus of information will be derived. The present and foreseeable scarcity of research funds relative to the plethora of proposed projects suggests that more such integrative studies should be designed to use the funds that are available most fruitfully. Meetings such as this, in which multiple facets, systemic as well as local, of neuroimmune interactions in sickness and in health are discussed, gives some indication of the value of this approach.

ABSTRACTS OF PAPERS PRESENTED AT THE SYMPOSIUM ON NEUROIMMUNOMODULATION: STRESS AND IMMUNE FUNCTION AT THE LOVELACE INSTITUTES, ALBUQUERQUE, NEW MEXICO, 7–9 OCTOBER, 1994

Transport of IL-1 receptor antagonist across the blood–brain barrier: comparisons to the transport of IL-1α and IL-1β

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IL-1α and IL-1β have been shown to cross the blood–brain barrier (BBB), suggesting a mechanism by which blood-borne IL-1 can affect the central nervous system (CNS). Il-1 receptor antagonist (RA) is a naturally occurring cytokine with structural similarities to IL-1α and IL-1β. The only described function has been the ability to block receptors in both the periphery and the CNS. No information is available in the literature about RA/BBB interactions. Three key questions were addressed by experiments involving the iv injection into mice of recombinant human cytokines.

1. Does RA block the BBB transport of IL-1α and IL-1β?

The entry of IL-1α and IL-1β radioactively labelled with ^{125}I were both blocked by RA to a statistically significant extent. However, entry of IL-1α, which is normally faster than that of IL-1β, was much more easily inhibited than the entry of IL-1β, with the dose of RA needed to achieve 50% inhibition of entry being 5.8 μg/kg for IL-1α and 3907 μg/kg for IL-1β. This suggests that high blood levels of RA may be able to convert effectively a preference by the BBB for 1α transport to a preference for 1β.

2. Does RA cross the BBB?

^{125}IRA crossed the BBB with a unidirectional influx constant of 5.19(10^{-4}) ml g^{-1} min^{-1}, a rate of entry similar to that for IL-1α and IL-1β. HPLC of the radioactivity extracted from brain tissue confirmed that intact cytokine had crossed the BBB; radioactivity that was acid precipitable was also recovered from cerebrospinal fluid. Capillary depletion showed that the majority of cytokine taken up by brain completely penetrated the capillary bed that comprises the BBB to reach the brain's parenchyma and interstitial fluid. The percentage of the iv injection that entered the brain peaked at about 0.33%/g of brain 30 min after injection. This is about 4–5 times higher than the percentage of an iv injection accumulating in the brain for IL-1α. This suggests that, in experimental designs or therapeutic manoeuvres that give IL-1α and RA simultaneously, the RA/IL-1α ratio would be higher behind the BBB than in the periphery and CVOs, leading to a relatively greater blockade of central receptors.

3. Is the transporter for RA related to that for IL-1α or IL-1β?

A dose of 0.47 μg/kg of IL-1β inhibited entry of ^{125}IRA by 50%, but IL-1α did not consistently inhibit entry. This pattern of cross-inhibition suggests that while these three cytokines probably share transporters, it is likely that transporter subtypes exist. Although previous studies have found IL-1α and IL-1β to be effective self- and cross-inhibitors at about the 10–20 μg/kg dosage range, the current data suggests that the relationship with RA is quite different. The ability to inhibit RA even better than itself suggests that IL-1β at high blood levels would tend to decrease the RA/IL-1β ratio in the CNS relative to the peripheral ratio. In contrast, increasing blood levels of IL-1α, even at levels that would be inhibiting IL-1α entry, would have little effect on RA entry; this would tend towards higher RA/IL-1α ratios.

Conclusions

These data show that blood-borne RA is able to cross the BBB and perhaps, therefore, exert an influence on brain function. In addition, the ability of these molecules to differentially inhibit their own and each other's entry into the CNS suggests that relative alterations in CNS ratios may also contribute to effects on brain function.

Endocrinological and immunological adaptations to chronic stress in students at the US Army Ranger School

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The relationship between stress and susceptibility to infectious diseases has been the subject of increasing discussion and research, although animal studies in this area date to the time of Louis Pasteur. The Army Ranger School is a rigorous infantry leadership course designed to mimic the psychological and physical stressors inherent in infantry combat missions. Outbreaks of infectious diseases are recurrent problems in stressed populations such as military trainees, military combatants, and refugees. The Ranger School was used as a model for such stressors, and this study examined sleep, neuroendocrine responses, delayed-type hypersensitivity (DTH) skin-test responses, and antibody responses to polysaccharide and protein recall antigens. The study utilized a repeated measures design, obtaining data on ranger trainees prior to the course, and after 4, 6, and 8 weeks. Data was collected on two cohorts, totalling 112 subjects, in 1991 and 1992. During the first study trainees were immunized with diphtheria-conjugated *Haemophilus influenza* B (HIB) vaccine at the course mid-point, and their responses to both antigens compared with those of a control group. DTH testing utilized a standard 7-antigen multiple tine-test device. Sleep and activity were documented continually by wrist-worn activity monitors. Saliva and 0700 blood samples were collected for neuroendocrine and cytokine measurement.

AM cortisols were significantly elevated throughout the course, compared to baseline. Testosterone levels were significantly suppressed. Dehydroepiandro- sterone sulphate (DHEA-S) increased concomitantly with cortisol, but the DHEA-S/cortisol ratio decreased with stress. Free T4 and T3 levels decreased and TSH increased, all

significantly. LH and PRL levels changed insignificantly. DTH responses to the 7 antigens decreased significantly ($p<0.001$) during the period of stress with 18% of students developing anergy and 40% decreasing to 1 or less antigen (hypoergic). In contrast, students immunized with HIB at the 4 weeks, when DTH was maximally depressed, increased their titres to both antigens comparably to simultaneously immunized age-matched ranger instructor controls. A direct correlation existed between depressed serum testosterone levels and loss of DTH reactivity. DTH responses showed no correlation with percent body weight loss. Serum IL-1α and IL-6 were maintained with remarkable constancy across all time-points. However, the ability of blood mononuclear cells to secrete TNF and IL-1 when incubated ex vivo with lipopolysaccharide was suppressed during the stress period.

This study demonstrated that suppression of DTH responses occurs in healthy individuals during a period of chronic stress, and suggests that the high wartime prevalence of infectious diseases in combatants and refugees, often attributed to deficiencies in personal hygiene and public health, may also reflect stress-associated alterations in immune host defences.

Endocrine regulation of immune cells

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Today there is little doubt that there is bidirectional communication between the immune system and the central nervous system (CNS). A commonality of ligands and receptors would seem to represent the molecular mechanism of such cross-talk. Thus, the immune system is now recognized as a source of neuropeptides and their receptors, while cytokines and their receptors are found within the CNS. Furthermore, regardless of the system, the same secondary messenger molecules can be generated as a result of a particular ligand/receptor interaction. Therefore, discrimination between functional responses would seem in part to reside in how a certain cell type is genetically programmed to respond. In addition, subtle differences in transcriptional regulation and post-translational modification of the neuropeptides and cytokines may contribute to differential responsivity of the immune and neuroendocrine systems. Physiologically, these findings suggest that the immune system functions as a sensory organ for stimuli not recognized by the CNS. Indeed, immune system initiation of stress responses as well as local antinociception through lymphocyte-derived endorphins support this view. On the other hand, the association of certain animal models of autoimmune disease with hypothalamic disorders suggests an immunoregulatory capacity for the CNS. A complete understanding of this circuitry holds great promise for understanding and treating human neuroimmunological disorders.

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Calcitonin gene related peptide (CGRP) induced apoptosis in vitro in murine thymocytes via receptor insensitive to the antagonist $CGRP_{8-37}$ with a potency similar to that induced by glucocorticoid

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The nervous and neuroendocrine sytems have been shown to play an important role in the modulation of immunity. CGRP is a molecule that serves as both a hormone and a neuropeptide, and has been identified by immunocytochemistry in cell bodies and nerve fibres of the primary gland of the immune system, the thymus. Receptors for CGRP have been characterized on thymocytes and T cells, and their activation by physiological levels of CGRP was found to suppress Con A-stimulated proliferation of mature virgin CD 4 thymocytes. This suppression is blocked by the antagonist for CGRP ($CGRP_{8-37}$). CGRP also inhibits the proliferation of Con A- and antigen-stimulated splenic T cells but to a lesser degree than that observed with thymocytes.

Given the magnitude of difference between the effects of CGRP on thymocytes vs. splenocytes it was important to determine if some or all of the CGRP-induced suppression of thymocyte proliferation was due to apoptosis. We further wanted to determine whether, if CGRP does, in fact, induce apoptosis, is it physiologically similar to the glucocorticoid-induced apoptosis in thymocytes. Alternatively, CGRP might antagonize glucocorticoid-induced apoptosis in a manner similar to that with which J. Ashwall has shown that T cell receptor-activated apoptosis inhibited gluco-corticoid-induced apoptosis.

For the mitogen proliferation assay, thymocytes were plated out with the appropriate reagent and evaluated for tritiated thymidine incorporation after 72 h. Propidium iodide and a FASC were used to distinguish apoptotic cells.

At 8 and 24 h, Con A did not significantly induce apoptosis in thymocytes, whereas CGRP alone and in the presence of Con A induces a two-fold increase in apoptotic cells ($p<0.05$). The antagonist, $CGRP_{(8-37)}$, did not cause apoptosis alone or in the presence of Con A, nor did it block programmed cell death caused by Con A or CGRP. This data suggests that apoptosis in thymocytes is mediated by a CGRP receptor not sensitive to the antagonist. However, since Con A-induced proliferation of thymocytes is inhibited by the antagonist, $CGRP_{(8-37)}$ this peptide appears to mediate at least two separate functions on subpopulations of thymocytes via two different CGRP receptors. At 8 h, corticosterone (10^{-7} mol/L) induces rapid apoptosis in thymocytes but had no effect on reversing apoptosis or suppression of cell proliferation induced by CGRP (10^{-8} mol/L) induces rapid apoptosis in thymocytes but had no effect on reversing apoptosis or suppression of cell proliferation induced by CGRP (10^{-8} mol/L) either in the presence or absence of Con A. At 24 and 48 h, the amount of apoptosis induce by either CGRP or corticosterone were similar suggesting that the same population of cells (the CD4/CD8 double positive) are affected by these naturally occurring molecules. The necessity for these two means of apoptotic induction to coexist in the thymus remains unclear. However, a better understanding of stress, circadian rhythms and peptide release mechanisms may shed light on apoptotic function of these molecules.

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Cytokine–CNS interactions: lessons from transgenic models

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Cytokines are potent biological response modifiers that exhibit a spectrum of cellular actions. These factors have been implicated as important mediators of physiological and possibly pathophysiological processes within the CNS. Consistent with the notion that cytokines may contribute to tissue injury in CNS disease, altered neural expression of various cytokine genes has been observed in a variety of neurodegenerative diseases including MS, HIV encephalopathy, Alzheimer's disease and spongiform encephalopathy. We wished to evaluate the neurological consequences of the prolonged CNS expression of cytokines. To accomplish this, a GFAP expression vector was employed that targeted the expression of the cytokines IL-6, IL-3 or IFN-α to astrocytes in the CNS of transgenic mice. Cerebral expression of each of these cytokines at high levels produced mice with severe clinical features (e.g. runting, reduced activity, ataxia and seizures) and was invariably associated with early death. On the other hand, expression at low levels was associated with the onset at a later age of progressive neuropathy and allowed the development of breeding lines for further evaluation. In these animals diverse and extensive cellular and molecular neuropathologic changes were observed and included neurodegeneration (all), demyelination (IL-6), perivascular cuffing (IL-6 and IL-3), meningoencephalitis (IL-3), astrocytosis (IL-6, IL-3 and IFN), microgliosis (IL-6 and IL-3), angiogenesis (IL-6), diffuse blood–brain barrier leakage (IL-6), upregulated acute-phase protein synthesis and deposition (IL-6) and induction of MHC class I and 2,5-oligoadenylate synthase gene expression (IFN). We conclude that: (1) transgenic expression of the cytokines IL-6, IL-3 and IFN-α in the CNS results in the development of acute (high expression) or chronic progressive (low expression) CNS disease associated with a spectrum of clinical, physiological and pathological manifestations, (2) although the clinical, cellular and molecular phenotype produced by the cerebral expression of the various cytokines showed some overlap, the differences were more prominent, reflecting the unique actions of each cytokine, (3) these transgenic models recapitulate many of the structural and functional impairments seen in human neurodegenerative diseases. Therefore, our studies indicate that cytokines, which normally function as primary regulators of the host response, also have the potential

to mediate significant injury in the CNS, (4) these transgenic models offer not only a valuable tool for further understanding the CNS pathobiology of cytokines but also provides a unique resource for the development and testing of therapies aimed at abrogating the toxic actions of these important mediators.

Behaviour and immunity

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For many years, immunologists considered an immune response to be a direct consequence of, and solely determined by, the administration of antigen to an autonomous immune system. In the past decade, however, we have witnessed a paradigm shift, and it is now generally accepted that the immune system is subject to regulation by neural and endocrine signals, evoked, in many instances, as part of a behavioural response to environmental stimuli. To exemplify this point, recent data from our laboratory will be presented revealing that: (1) in a conditioning protocol in which immunization was associated with a novel taste, re-exposure to the novel taste, in conjunction with a minimally antigenic stimulus, effected enhanced serum antibody titres compared with relevant controls, and (2) that differential housing, or pheromones from footshocked mice, differentially affected cytokine production by TH1 and TH2 lymphocytes and the immune responses they regulate.

Conditioning studies: Male BALB/c mice received an intraperitoneal injection of 50 ng keyhole limpet haemocyanin (KLH) paired with a gustatory conditoned stimulus (CS) on each of 5 conditioning trials given at 3-week intervals. Three weeks after the mice received their last conditioning trial, they were divided into several subgroups. One subgroup of conditioned mice was re-exposed to the CS; a second subgroup was exposed to a booster injection of an amount of KLH (0.5 ng) that was, by itself, too small to elicit a significant antibody response: a third group received the CS plus 0.5 ng KLH; and a fourth subgroup received an immunogenic concentration of 50 ng KLH as a positive control. The 0.5 ng KLH boost ensured that a stimulus salient for the immune system was available to trigger the immune system since it seemed highly unlikely that a CS should stimulate antibody production by itself. Mice that received the CS together with the booster immunization made significantly more IgG anti-KLH antibody than the groups of conditioned animals that received either CS alone or the booster injection in the absence of the CS. Indeed, the antibody response of the animals that received the 0.5 ng KLH boost plus the CS was virtually identical to that of conditioned animals that had received 100-fold more antigen (i.e. the positive control group). Critical documentation that the antibody response in the subgroup that received the 0.5 ng KLH boost plus the CS was indeed a conditioned response, was provided by the observation that antibody titres in an additional group of mice that had been pre-exposed to the CS prior to the onset of the conditioning trials, did not differ from conditioned animals that received a booster injection in the absence of the CS.

Stress–immune system interactions: Exposure of BALB/c mice to HPA axis-activating 'pheromones' from footshocked male BALB/c mice results in suppression of mitogen-induced proliferation, NK cell activity, and Con A-induced IL-2 production on the one hand and enhanced IgM and IgG anti-KLH antibody titres and IL-4 production on the other. The enhanced IL-4 production could be blocked by implanting pellets containing the glucocorticoid receptor antagonist, RU486, prior to stress-odour exposure. These observations suggest that in the BALB/c mouse, the neuroendocrine response to stress pheromones may eventuate in differential activation of T-helper cell subsets.

We also addressed the issue of psychosocial factors and TH1- and TH2-derived cytokine dominance in a differential housing model in which the neurochemical milieu is stably altered over an extended period of time (rather than transiently altered, as is likely to be the case following stress-odour exposure). Following a tertiary in-vivo immunization with KLH, antigen-stimulated splenocytes from individually housed C57BL/6 mice (a 'TH1 dominant' strain) produced more IL-2 that KLH-treated splenocytes from group-housed (four/cage) animals, whereas splenocytes from individually housed BALB/c mice ('TH2 dominant) produced more IL-4 than did lymphocytes from group-housed BALB/c animals. We are currently evaluating whether these strain differences result from differential neurochemical responses to the housing conditions and/or differential immune system sensitivity to the neurochemical signals.

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Neuroanatomical and neurochemical correlates of HIV/SIV-mediated cognitive and motor impairment

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Diminished intellectual function and motor impairment (HIV-associated dementia) is common in individuals with AIDS. The cause of CNS dysfunction appears to be the immunodeficiency virus itself, rather than other opportunistic pathogens. The exact mechanism(s) by which the virus affects brain function is not clear. The affected brain does not always express high levels of viral antigens or viral RNA, or show virus-associated neuropathology, suggesting that the cause of CNS dysfunction may be an indirect rather than a direct effect of HIV. Indirect effects of HIV could include cytokine release in the brain and subsequent alteration of neuronal function.

Our postmortem studies show that the mRNA encoding a peptide neurotransmitter, preprosomatostatin (SRIF) is up-regulated in layer IV of the frontal cortex in HIV-seropositive children with but not without HIV-associated dementia. Neurons in subcortical areas known to contain the highest amounts of viral antigen project to and synapse selectively on neurons within layer IV. IL-1 and TNFα are potential products of viral-infected cells which alter neuronal activity and alterations in neuronal activity increase SRIF mRNA. Interestingly, two children with, as opposed to those without, viral-infected cells in the brain had the highest levels of SRIF mRNA in layer IV. Layers IV in adults with AIDS contains increased numbers of astrocytes, which correlates with HIV-associated dementia. Astrocytes have IL-1

immunoreactivity. IL-1 induces SRIF mRNA. Thus, an increase in SRIF mRNA could also be due to astrocytosis in layer IV.

To determine which of these alterations may be significant early in the course of HIV infection, juvenile rhesus macaques were infected with SIV and euthanized at the time of onset of motor or cognitive impairment. An up-regulation of SRIF mRNA was seen exclusively in layer IV in animals with predominantly cognitive (rather than motor) impairment. There was no ongoing astrocytosis in layer IV. Hence, this increase in SRIF mRNA is probably due to alterations in neuronal activity produced by IL-1/TNFα originating in subcortical regions of the brain, rather than local astrocytosis.

Animals with predominantly motor impairment had an up-regulation in SRIF mRNA in layers V, VI and subcortical white matter (SWM) in addition to layer IV. Also, there appeared to be ongoing astrocytosis in these layers and SWM. Thus, this increase in SRIF mRNA may be due to local elevations in IL-1 and TNFα in astrocytes in these cortical laminae and SWM. Ongoing astrocytosis may be associated with neurodegeneration on subcortical regions with large amounts of viral antigens, to which these cortical laminae and SWM connect.

These data indicate that an up-regulation in SRIF mRNA expression appears early, is laminar-specific and underlies neuronal dysfunction in HIV/SIV disease. Cognitive impairments are probably caused by cytokines acting at distant sites, whereas motor impairment may be caused by cytokines acting locally on somatostatinergic neurons.

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Cytokines and sickness behaviour

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Non-specific symptoms of infection include fever and profound alterations in mood and behaviour. Sick individuals experience fatigue, malaise, listlessness and inability to concentrate. They also show hypersomnia, anorexia, depressed activity and loss of interest in their environment. These non-specific changes are collectively termed 'sickness behaviour'. They can be induced in experimental animals and in human subjects by systemic administration of lipopolysaccharide (LPS), the active fragments of endotoxin, and recombinant preparations of the proinflammatory cytokines that are released by activated accessory immune cells during infection (mainly interleukin-1, interleukin-6, tumour necrosis factor-α, α-interferon).

Using pharmacological tools in the form of recombinant cytokines and their antagonists, we have confirmed that TNFα and the two molecular forms of interleukin-1, IL-1α and IL-1β, have potent suppressing effects on social exploration and food motivated behaviour in laboratory rodents. These effects are not the result

of physical debilitation since cytokine-treated animals can still adjust their behaviour in accordance with their terminal needs, e.g. they increase their response rate when needed.

The mechanisms which mediate the induction of sickness behaviour by peripherally released cytokines involve several steps: (1) peripheral immune stimuli activate primary nerve endings by a prostaglandin-dependent mechanism, as evidenced by the induction of sensory neuropeptides in the spinal cord in response to peripherally injected LPS; (2) this neural message is transmitted to the brain to exert its effects since intraperitoneal administration of LPS induces the expression of *c-fos* protein at the level of the brain projection areas of the vagus nerve, the main afferent nerve from the abdominal cavity to the brain, and section of the vagus nerve blocks the behaviourally depressing effects of peripherally administered LPS and IL-1; (3) the neural message that is transmitted to the brain via primary afferents is transduced back into an immune message, since the induction of cytokine gene expression in the brain in response to peripherally released cytokines is blocked by section of the vagus nerve; (4) the cytokines which are synthesized in the brain in response to peripheral immune stimuli are released as evidenced by in-vitro studies; (5) centrally released cytokines act on neurons and/or glial cells which have membrane receptors for these cytokines, resembling those characterized at the periphery; furthermore, in the case of IL-1, central administration of a specific antagonist of IL-1 receptors, IL-1ra, blocks the behaviourally depressing effects of peripherally administered IL-1; (6) centrally released cytokines activate a variety of hormonal systems (e.g. glucocorticoids) and neuropeptides (e.g. vasopressin) which limit the extent of behavioural changes induced by cytokines and promote recovery.

Although there are still many unanswered questions concerning, in particular, the nature of the mechanisms which code the transmission of the immune message in peripheral afferent nerves and the way this message is decoded by the brain, the existence of a functional cytokine compartment in the brain opens fascinating perspectives for the elucidation of a variety of psychopathological disorders, from the chronic fatigue syndrome to the neural manifestations of bacterial and viral infections, including AIDS.

Immune challenge as a CNS stressor

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Stress is associated with an activation of catecholaminergic systems and the hypothalamo–pituitary–adrenocortical axis (HPAA). The catecholaminergic activation involves not only the sympathetic nervous system and the adrenal medulla, but also noradrenergic (and possibly also dopaminergic and adrenergic) systems in the brain. Activation of the HPAA involves the release of corticotropin-releasing factor (CRF) from the hypothalamus, which in turn stimulates the secretion of pituitary ACTH and β-endorphin, and glucocorticoids from the adrenals. Some evidence also indicates an activation of CRF-containing neurons not associated with the HPAA.

Most people would regard infections as stressful, and consistent with this there is considerable evidence that infections and other immune challenges activate both

central and peripheral catecholaminergic systems, and the HPAA. For example, infection of mice with influenza virus increases circulating concentrations of ACTH and corticosterone as well as brain noradrenergic (NE) systems, and there is evidence for sympathetic activation also. Endotoxin and interleukin-1 (IL-1) have similar effects. These effects appear to involve increased synaptic release as indicated by in-vivo microdialysis studies. Interestingly, whereas physical and psychological stressors tend to activate cerebral dopaminergic systems, influenza virus infection and IL-1 have very little effect, although endotoxin has a minor effect on dopamine metabolism. Moreover, the regional pattern of the NE activation is somewhat different; the effects of influenza virus and IL-1 are selective for the hypothalamus, whereas most stressors have regionally non-specific effects on NE metabolism. The effect of endotoxin on dopamine metabolism is also regionally non-specific, whereas physical stressors selectively affect the prefrontal cortex.

Physical and behavioural stressors also affect cerebral serotonin metabolism, and the same is true for 'immune stressors'. In all cases the effects of serotonin appear to be mediated by an increase in brain-free tryptophan which is in turn caused by peripheral sympathetic activation. The use of endotoxin has permitted the separation of the cerebral serotonergic and catecholaminergic responses in stress, because endotoxin-resistant mice fail to show a NE (and an HPAA) activation, but normal tryptophan and serotonergic responses, whereas NO synthesis inhibitors prevent the latter without altering the former.

Infections and immune stimuli also share behavioural responses with physical and behavioural stressors. Sickness is associated with anorexia, hypomotility, increased sleep time, and a reduction in exploration, sexual libido, etc. Such behavioural changes are mimicked by IL-1 and by endotoxin. Some of them are also associated with other stressors, and can be mimicked by intracerebroventricular (icv) application of CRF. Indeed the behavioural responses to IL-1 can be antagonized by icv application of a CRF antagonist.

In sum, infections and immune challenges can elicit the same physiological responses as physical stressors, and some of the same behavioural ones. It seems likely that the same messengers and pathways are used for both kinds of stressors.

Interactions between the sympathetic nervous system and the immune system

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Noradrenergic (NA) nerve fibres have been identified along the vasculature and among the parenchymal elements of both primary and secondary lymphoid organs using both histochemistry for norepinephrine (NE) and immunocytochemistry (ICC) from the catecholamine synthetic enzyme tyrosine hydroxylase (TH). The close proximity of nerve terminals, identified at the ultrastructural level using TH ICC, to lymphocytes and macrophages, combined with data demonstrating transmitter release and the presence of adrenergic receptors on lymphocytes and macrophages, suggests a direct functional role for NE in the modulation of immune function.

Although functional alterations have been reported by α and β receptor stimulation, both in-vitro and in-vivo, these laboratories have chosen to characterize

the relationship between NA sympathetic nerves and lymphoid cells by using chemical sympathectomy in adult rodents. Treatment of adult (3-month-old) mice with 6-hydroxydopamine (6-OHDA) depletes lymphoid organs (and other peripheral organs, but not central nervous system) of 90–95% of the NE content by 24 h after treatment. Controls for non-neuronal toxicity include pretreatment with desmethylimipramine to prevent neuronal uptake of 6-OHDA, thereby preventing sympathetic nerve damage, and various α and β receptor antagonists to control for transient effects of release from damaged nerves.

Treatment with 6-OHDA increases background (non-stimulated) proliferation in inguinal and axillary lymph nodes, spleen and bone marrow in non-immune mice. Migration of lymphocytes taken from lymph nodes (LN) into LN of recipient mice was altered as well, with lymphocytes from 6-OHDA-treated mice showing decreased accumulation in control LN at 1 h after injection when compared with lymphocytes from control mice. Lymphocytes from control LN showed increased accumulation in LN of 6-OHDA-pretreated mice.

Proliferation in-vivo in 6-OHDA-treated mice is increased; T-cell numbers in LN are decreased, while sIgM cell numbers are increased. Treatment with 6-OHDA decreases Con A-induced proliferation in LN and splenic lymphocytes treated ex-vivo. This decrease in proliferation was not associated with a change in IL-2/4. LPS-induced B cell proliferation was enhanced. Isotype analysis revealed a reduction in IgM and an increase in IgG, suggesting an isotype switch. This was accompanied by increased INF-γ production, pointing to a possible cell cytokine cause for this isotype switch. Splenic B cell responses were opposite to those found in LN; LPS-induced proliferation was decreased, with no changes in IgM^+ cells, IgM or IgG concentrations.

These and a growing number of other studies continue to point to the sympathetic nervous system as an important contributing factor in immune responsiveness.

Exercise and the immune response

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Millions of adult North Americans participate in regular exercise. One reason for this is the common belief by the public that regular exercise improves one's resistance to prevalent chronic diseases. Except for heart disease and diabetes mellitus, the validity of this assumption has not been systematically tested to any extent. However, a limited number of epidemiological studies suggest that regular exercise may be protective against certain site-specific cancers, including cancers of the colon, breast and lung. The biological basis for this has been hypothesized to involve exercise-induced changes in natural immune functions; changes in the activity of natural killer (NK) cells, lymphokine activated killer (LAK) cells, macrophages and cytokines have been the primary focus of this research.

Acute maximal or submaximal exercise in humans results in an immediate increase in NK (CD16+ and CD56+ lymphocytes) numbers and a corresponding increase in NK and LAK cell cytolytic activities. This is followed by transient decreases, probably due to numerical shifts and modulation by prostaglandins from circulating monocytes. Maximal NK serine esterase (granzyme) activity, involved in apoptotic killing, increases after acute exercise. Changes in the expression of the NK

cellular adhesion molecule, LFA-1, on NK cells also influence cytolytic activity after acute exercise. However, the clinical significance of these transient changes in natural immune function needs to be determined.

Much less is known about the impact of training on natural immunity. In elite athletes, levels of NK and LAK-cell activity and NK cell numbers obtained from subjects at rest are elevated compared with untrained subjects. Training-associated elevations in basal NK and LAK cell number and function may play a role in the control of haematogenous metastasis of some experimental tumours. Rodents trained by treadmill or voluntary wheel running had fewer NK-sensitive H-ras-transformed 10T½ fibrosarcoma tumour cells in the lungs compared with untrained animals. This effect was partially eliminated when the animals were pretreated with antibodies that block NK cells. In contrast, for experimental mammary adenocarcinoma tumours that are not NK or LAK sensitive, despite a small increase in splenic NK and LAK activity associated with training, the number of lung tumours was not influenced by prior exercise training of the host. These results point to the need for caution in interpreting the clinical relevance of exercise-associated changes in the natural immune system, especially for complex multifactorial diseases such as cancer.

Interferon-γ promotes cholinergic differentiation of embryonic septal nuclei and adjacent basal forebrain

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In cultured rat embryonic septal nuclei with adjacent basal forebrain, murine interferon-γ (IFNγ) produces a striking increase in choline acetyltransferase (ChAT) activity. The effect of IFNγ on cholinergic differentiation is age dependent; IFN is far more potent in E14 cultures than in older cultures. The effect of IFN is somewhat specific for this area of brain. Non-cholinergic populations (hippocampus and cortex) do not become cholinergic and striatum responds only poorly. In searching for a mechanism by which IFN elevates ChAT levels, we found that IFN does not cause a change in the affinity of ChAT for choline, but rather raises mRNA coding for the ChAT enzyme. [^{3}H]-Thymidine incorporation studies reveal that IFN, a well-known antimitotic agent, does not affect cell proliferation in the cultures. While IFN doubles the number of neurons, cholinergic cell number increases more than 7-fold keeping pace with ChAT activity.

Retroviral studies in which neuronal precursors were labelled in culture showed that IFN increased the number of cholinergic neurons that developed from these precursors, suggesting that IFN (or its intermediate) encourages the differentiation of cholinergic neurons from undifferentiated precursors.

We sought to determine whether this was a direct action of IFN on neurons or whether it was mediated by a non-neuronally derived soluble intermediate. IFN signal transduction involves the phosphorylation and translocation to the nucleus of a specific cytoplasmic protein known as p91. Using an antibody directed against this molecule, we found that ameboid microglia constitute the responding cell type in the cultures.

Having identified a non-neuronal responder, we sought to determine the identity of the intermediate. While NGF effects are additive with those of IFN, antibodies to NGF do not block the action of IFN, suggesting that NGF is not the intermediate.

Similarly, while bFGF significantly elevates ChAT activity, antibodies to FGF do not block the action of IFN. While neither NGF nor FGF are the intermediates, there does exist a microglial-derived intermediate that increases cholinergic differentiation. The identity of this molecule is still under investigation, but it is not one of the neurotrophins, nor is it aFGF, TGFβ, TNF, IGF, GM-CSF, EGF or VIP.

Cytokines and the production of fever

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Fever is one of the most common host defence responses of an infected organism. It is clear from dozens of studies that the rise in body temperature associated with fever is a highly regulated process, involving numerous sophisticated physiological and behavioural responses. As for 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'. In addition to infection-induced fever, the phenomenon of 'stress-induced hyperthermia' in many ways has the characteristics of true fevers (i.e. an elevated thermoregulatory set-point).

Numerous cytokines and hormones are considered putative endogenous pyrogens or endogenous antipyretics or cryogens. The demonstration that a substance (e.g. cytokine X) causes a fever (or anapyrexia) when injected into an animal is not sufficient evidence to conclude that that substance is an endogenous pyrogen (or endogenous cryogen). Evidence will be presented that supports the hypothesis that IL-1β is involved in LPS-induced fever, and that the site of action of this cytokine is the anterior hypothalamus. IL-1β probably induces fever via the release of the cytokine IL-6 within this same area of CNS (Klir et al., 1994). Data will also be presented that indicate that endogenously produced TNFα is an endogenous antipyretic, rather than a pyrogen, under many conditions (Kluger, 1991).

We believe that there are sufficient data to support the renaming of 'stress-induced hyperthermia' to 'stress-induced fever'. The precise pathways between contact with a pathogen or exposure to a psychologically stressful situation are not identical. However, there is considerable evidence indicating that stress-induced rises in body temperature are regulated, thus fulfilling the necessary criterion for this phenomenon to be called a fever (Kluger, 1991). In addition, data will be presented that indicate that the stress hormones, corticosterone and epinephrine, stimulate the release of the cytokines from the liver. Infusion of physiological levels of corticosterone into an isolated perfused rat liver resulted in increases in TNF and IL-6 in the effluent solution. Infusion of epinephrine led to a rise in IL-6 from the IPRL (Liao et al., 1994).

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Cytokines and the regulation of sleep

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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 of man with viruses result in complex sleep responses dependent upon the infectious agent and route of administration. The general response is characterized by an initial period of enhanced sleep; this is followed 6–48 h later by a period of reduced sleep. Bacterial products probably responsible for sleep and fever 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, tumour necrosis factor (TNF) α and β, interferon-α (IFN-α) and acidic fibroblast growth factor (aFGF). Other cytokines, basic FGF, IL-2, IL-6 and IFN-β do not affect sleep although IL-6 is pyrogenic. The major effect of cytokines is to increase 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 h depending upon dose and route of administration, e.g. rabbits normally sleep about 45% of the time between 9.00 am and 3.00 pm; 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 remain 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 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 probably 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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Immunology: evolutionary implications

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The evolutionary forces that shaped the immune system are probably linked with the ability of terrestrial vertebrates to 'walk' across a rapidly changing landscape of infectious viruses and bacteria. Instead of parasites and their hosts slowly evolving in isolated 'ponds', the highly mobile vertebrates were exposed to many different infectious environments during a single life time. Thus, instead of the usual slow evolution of germline-encoded recognition of bacteria and viruses, vertebrates developed a means of distinguishing self from non-self on a somatic time scale. This radically new mechanism of recognition of parasites allowed the evolution of even more destructive levels of effector function as this destruction could be specifically and locally delivered. The need to make an effective self–non-self discrimination essentially forces the separation of recognitive events and the destructive effector events needed to protect against lethal infection.

For the purpose of analysing neuroendocrine reactions in the immune system, it is logical to clearly separate the antigen-specific events from the antigen-non-specific components, which are essentially common to all multicellular animals. In principle, neuroendocrine intervention could take place at the antigen-specific level or the antigen-non-specific level, although, at the antigen-specific level, intervention would have to be functionally non-specific. Thus, given that antigen-specific immune events can result in a wide variety of effector reactions, it seems inevitable that neuroendocrine intervention will be restricted to particular effector pathways. Here neuroendocrine effects could range from influencing the type of effector function the immune response will promote, to modulating the intensity of a class of effector functions, especially those functions that are common to non-specific effector functions.

The theme of this symposium on neuroimmunology is modulation, stress and immune function. From an immunological and evolutionary perspective, it seems useful to class infection as a stressor and examine on the one hand the interplay between different stressors, and on the other hand to evaluate the effects of various stressors on different components of the immune effector reactions.

Finally, having seen the value of computer models in integrating our understanding of the immune system, it seems timely to ask if the neuroendocrinology community thinks that it is ready to start construction of a neuro–, endocrine–immunomodel that aims to take the overview without necessarily remaining correct in every detail. To make a cellular automata type of model it is important to characterize ligands and receptors in terms of molar concentrations and binding constants, and to think of switches that are on/off or are like volume controls. It will also be important to consider the effects of combinatorial sets of ligand receptor interactions in the

control of the cellular responses that are so diverse when the ligand/receptor pairs available to regulate these responses are relatively few.

Neuroimmunomodulation of inflammation by α-MSH

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α-MSH is a tridecapeptide derived from pro-opiomelanocortin. It occurs in widespread regions of the brain and body; barriers to the external environment (skin, gut) are known to contain α-MSH. Specific receptors for this molecule, melanocortin receptors, have recently been identified and cloned. In addition to its antipyretic effect, α-MSH has potent anti-inflammatory influences. This peptide is effective in all major forms of experimental inflammation: acute inflammation induced by irritants or by cytokines and other inflammatory mediators; delayed hypersensitivity reaction; chronic inflammation; systemic inflammation (endotoxaemia, peritonitis, sepsis, adult respiratory distress syndrome). Results obtained with animal models further suggest that the peptide exerts its anti-inflammatory effect via actions both within the CNS and in the periphery. Observations on patients indicate that the peptide has a physiological role in modulation of inflammatory reactions: there are clear differences in α-MSH in certain AIDS patients, in patients with arthritis, and in patients with cardiac ischaemia undergoing thrombolysis. It appears that the peptide becomes available to modulate local and systemic inflammatory reactions in humans. The mechanism of such actions of the peptide is likely to involve inhibition of production/action of proinflammatory cytokines and/or inhibition of another mediator that is common to all forms of inflammation. These possibilities are being explored using in-vitro tests of stimulated cultured peripheral cells and molecular biology techniques. The results to date are consistent with modulation by the peptide of responses of peripheral host cells via its action on specific peptide receptors.

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Adrenal steroids are multifunctional modulators of immune function: studies of receptor distribution and immune cell trafficking

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Adrenal steroids exert both positive and negative effects on the immune system. Their main effects include the regulation of apoptosis of immature thymus lymphocytes as part of the T cell selection process, the switching of class of an immune response by enhancing humoral immunity and suppressing cellular immunity, inhibition of inflammatory and autoimmune responses, and modulation of immune cell movement between the blood and various tissue compartments ('trafficking'). This presentation will deal primarily with the distribution of adrenal steroid receptor types and the role

of adrenal steroids in immune cell 'trafficking'.

In order to accomplish these various roles, immune cell types express different amounts of adrenal steroid receptors. These exist as two types, mineralocorticoid or type I, and glucocorticoid or type II receptors. Cells of the thymus gland express the highest levels of type II receptors compared with mature T and B cells, whereas neutrophils express very low levels of type II receptors. Type I receptors are expressed by cells of the rat spleen but are not evident in rat thymus. The presence of type I and type II receptors in the same cells of the spleen is indicated by the ability of agonists for the type I receptor to negatively regulate the level of type II receptors.

The presence of adrenal steroid receptors in a tissue is only one aspect of its sensitivity to circulating adrenal steroids. Access to the target tissue is regulated by tissue factors that include corticosteroid binding globulin (CBG) and steroid catabolizing enzymes such as 11β-hydroxysteroid dehydrogenase (11-HSD). Synthetic steroids such as dexamethasone bypass these protective mechanisms and gain ready access to spleen and thymus type II receptors, whereas the natural glucocorticoid, corticosterone, is limited in its access to these tissues. As a result, patterns of type I and type II receptor occupancy differ as a function of the time of day (diurnal variation of corticosterone) and presence or absence of a stress response. These findings help to explain functional immune data indicating that the spleen is a protected site with regard to access by endogenous glucocorticoids but is not protected with regard to access by dexamethasone. Therefore, caution must be exercised in not generalizing data from synthetic glucocorticoid effects on immune function.

The movement of immune cells between the blood and various tissue compartments is a visible, but poorly understood, aspect of glucocorticoid action on the immune system. Both diurnal increases and stress-induced increases in corticosterone secretion produce reversible changes in immune cell concentration in blood. These include increases of neutrophil and eosinophil concentrations and decreases of B and T cell as well as NK cell and macrophage concentrations. The decrease of B cells levels is particularly striking and appears to be almost completely dependent on circulating corticosterone, whereas there appear to be extra-adrenal influences on the trafficking of other immune cell types in addition to the effect of corticosterone. Acute administration of corticosterone to adrenalectomized animals results in a close replication of the stress-induced changes observed in adrenal intact animals. Acute administration of the type II receptor agonist, Ru28362, replicates the stress-induced changes in blood leukocyte numbers, whereas the type I receptor agonist, aldosterone, reverses a stress-induced increase in leukocyte numbers observed in adrenalectomized animals.

Although the changes in blood levels of immune cells is relatively small compared with the total immune cell number in the body, these changes appear to reflect redistribution of cells to tissue compartments that may become especially important under immune system challenge. Current experiments are underway to assess whether 'trafficking' represents a means of providing immune cells to parts of the body undergoing challenge by various antigens including cancer cells and viral or bacterial pathogens.

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The mapping of HIV-1 GP160 epitopes required for IL-1 and TNFα production in glial cells

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Nested, deletion mutants from the gp160 amino terminus representing folded, glycosylated, serially truncated recombinant envelope proteins were expressed in eukaryotic cells via a vaccinia virus vector (vPE). These proteins and linear gp120/gp41 peptides as well as polyclonal and monoclonal antibodies reactive to defined regions of gp120/gp41 were used to map the epitopes involved in production of interleukin-1 (IL-1) and tumour necrosis factor alpha (TNFα) in rodent glial cell cultures. A full-length env protein containing both gp120 and gp41 (vPE16) induced both IL-1 and TNFα. vPE16 induced IL-1 in-vitro for a longer period of time and at lower concentrations than were required to induce TNFα. Both the V4 loop to C5 region of the carboxy terminus of gp120 as well as the gp41 protein were critical in TNFα production. V1–3 and C1–3 of gp120 were not important in TNFα production. The V3 loop of gp120 appeared to be critical for IL-1 while V1, V2, V4, V5, and C1–5 domains of gp120 and gp41 were not important. Synthetic peptides representing linear epitopes in the V3 loop and C5 domain of gp120, and the ectodomain of gp41 were all strong inducers of TNFα, although a protein representing almost the entire gp41 was the strongest inducer of TNFα. Linear peptides in the region from the V4 loop to the C4 domain were strong inducers of IL-1. IL-1 and TNFα production were induced by different epitopes in gp160. The strength of the induction varied depending on

whether the epitopes were conformational or linear determinates. Cytokine induction by env protein occurred at the messenger RNA level as determined by Northern blot analysis. Virus infection, therefore, is not required for the induction of these two cytokines and gp160 epitopes other than the classical CD4 binding domain are involved. It is expected that cell surface receptors other than CD4 interact with gp160 epitopes to produce IL-1 and TNFα from the glial cells. These data suggest envelope proteins in intact as well as degraded HIV-1 virions could induce proinflammatory cytokines which are possibly important in CNS AIDS.

Neuroendocrine consequences of inflammatory disease: adaptive significance
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Systemic infection, and inflammation as encountered clinically or induced in experimental animals, brings about dramatic changes in metabolic state, in part mediated by altered endocrine function. In addition to direct effects of circulating and intrinsic inflammatory cytokines on endocrine glands, many of the interleukins act in the central nervous system altering neuropsychological and hypothalamic–pituitary function. In addition to causing fever, cytokines produced within the brain, including IL-1, IL-2, IL-6, TNFα, and IFN, cause cognitive dysfunction, disorientation, and delirium. Peripheral or central administration of bacterial toxin, interleukins 1, 2, 6, and TNFα activate central CR neuronal systems, and alter the secretion of CNS-neurotransmitters. Among the neurotransmitters which are stimulated are catecholamines, NO, GHRH, CCK, and somatostatin. Release and/or synthesis of acetylcholine, GNRH and TRH are inhibited. Synthesis of the neurotoxin substance, Alzheimer precursor peptide, is also enhanced. Central changes in neuroendocrine function caused by inflammation lead to activation of the pituitary–adrenal axis, inhibition of pituitary–thyroid function ('sick euthyroid syndrome'), inhibition of pituitary–gonad function ('sick hypogonadal syndrome') and release of vasopressin (syndrome of inappropriate ADH secretion, SIADH). In each of these disorders, altered hypothalamic activity causes either excess secretion, or inhibits appropriate feedback responses. Though not specifically neuroendocrine, inflammatory cytokines adversely affect bone synthesis and inhibit pancreatic islet function.

Given this array of responses, many of which are apparently harmful, and the finding that blockade of IL-1 and TNF receptors increases survival in sepsis, it is reasonable to question why inflammatory cytokine responses, appearing early in eukaryote evolution, have been conserved in higher forms. The pituitary–adrenal response is modulatory to overexuberant cytokine activity in sepsis and autoimmunity, but still uncertain is the teleological value of suppressed thyroid function, hypogonadism, increased ADH release and altered carbohydrate and bone metabolism. Fever has been shown to have positive survival value in infection, but anorexia, cognitive defects and delerium are consequences of the neural response which leads to altered temperature 'set-point'.

Cytokine-induced activation of the hypothalamic–pituitary–adrenal axis: mechanisms and influence of alcohol

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Administration of cytokines stimulates the activity of the hypothalamic–pituitary–adrenal (HPA) axis of the rodent, an effect that represents one arm of the bilateral communication taking place between the immune and the neuroendocrine systems. The stimulatory influence of cytokines on corticosteroid secretion is important in making available energy resources necessary to the increased metabolism of organisms exposed to pathogens, and in maintaining cytokine production within acceptable limits. In addition, ACTH exerts direct effects on immune cells, a phenomenon that is probably important during exposure to immune signals. It follows that circumstances that disrupt the cross-talk between the two systems mentioned above can lead to abnormal immune and/or endocrine responses. For example drugs such as alcohol (EtOH) are known to increase the incidence of immune-related diseases, and may do so in part by altering the normal interaction between the immune system and the HPA axis. Our studies were therefore designed, first to understand the physiological role played by immune proteins in mediating the response of the HPA axis to infection or inflammation, and second to investigate the influence of EtOH on cytokine-induced ACTH and corticosterone secretion.

As cytokines present both in the periphery and the central nervous system (CNS) are likely to play a role during immune stimulation, it appears important to determine whether the mechanisms involved in modulating ACTH secretion in response to the systemic and central administration of immune signals are comparable. Specifically, we investigated the role played by corticotropin-releasing factor (CRF), vasopressin (VP) and nitric oxide (NO) in mediating the release of ACTH by cytokines such as interleukin-1β (IL-1β), and studied their role during IL-induced activation of the HPA axis in rats exposed to EtOH. CRF represents a crucial modulator of ACTH released by both IL-1β and EtOH, as indicated by the decrease in plasma ACTH levels in the absence of endogenous CRF. The acute influence of systemic IL-1β on the HPA axis is probably mediated at the level of nerve terminals rather than within the paraventricular nucleus (PVN) of the hypothalamus, whereas this latter structure plays an important role during prolonged activation of the HPA axis by blood-borne cytokines as well as following the intracerebroventricular (icv) delivery of IL-1. VP is released by IL-1β, and plasma levels of this peptide are elevated shortly after EtOH exposure. Peripheral IL-1 administration does not increase PVN VP gene expression, while icv delivery does. A role for endogenous VP in the HPA axis response to the central, but not systemic injection of IL-1 is further provided by the finding that VP immunoneutralization blunts ACTH secretion in response to the icv but not iv injection of the cytokine. VP also participates in the response of the HPA axis to EtOH. Indeed a single EtOH injection increases VP mRNA levels in the PVN, and ACTH levels of rats administered the drug are decreased in the presence of VP antibodies. Finally, we investigated the role played by NO. Blocking the activity of the enzyme responsible for NO formation, NO synthase (NOS) with arginine derivatives, such as L-N$_{\omega}$nitro-L-arginine-methylester (L-NAME), augments the ACTH response to the iv, but not icv, injection of IL-1β, and does not significantly alter the influence of EtOH. Interestingly, only the icv administration of IL-1β, but neither its systemic

injection nor exposure to EtOH augments NOS gene expression in the hypothalamus. These results suggest that NO exerts a restraining influence on ACTH released by systemic administration of cytokines, but that its appearance in the hypothalamus is not crucial during stimulation of this brain area by IL-1β.

Prior exposure to CRF-dependent events often results in an increased responsiveness of the HPA axis. However, the acute administration of moderate doses of EtOH does not alter the response of this axis to systemic injection of IL-1β, while significantly decreasing the effect of the icv administration of the protein. As this phenomenon is accompanied by a blunting of hypothalamic c-*fos* gene expression normally caused by the cytokine, it appears to reflect an inhibitory influence of EtOH on the CNS pathways impinging on the hypothalamus. These data suggest that despite their common reliance on endogenous CRF to release ACTH, in acute experiments, EtOH does not facilitate the stimulation of the HPA axis induced by blood-borne IL-1β; furthermore, the net influence of alcohol becomes inhibitory following central injection of the cytokine. Different results are obtained during long-term exposure to EtOH, a situation under which the drug interferes with the systemic effect of IL-1β on pituitary activity. Hypotheses related to the role of pituitary responsiveness to CRF and VP, and to a possible influence of NO (which is reportedly altered by EtOH), were tested. Feeding rats an alcohol diet for 7–10 days decreases ACTH released by the iv injection of IL-1β and VP, but not CRF. Blockade of NOS activity significantly reverses the effect of the drug on VP- but not IL-1-induced ACTH secretion. We still do not know whether EtOH differentially alters NO formation in the pituitary (which is the target for blood-borne VP) and the median eminence (which is the primary target for blood-borne IL-1β), or whether other mechanisms are involved.

Taken together, our results indicate that vasopressin and nitric oxide, among other secretagogues, participate not only in the response of the HPA axis to cytokines, but also in the ability of alcohol to alter the functional relationship between the immune system and the HPA axis.

Stress and response to influenza in mice

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Recent studies in both humans and animal models have demonstrated that stress is immunomodulatory, and that stress can alter the pathogenesis of microbial infections. During infectious disease, the immunomodulatory nature of stress can be aversive to health. The mammalian response to a stressor is primarily mediated by two neuroendocrine systems: the sympathetic nervous system and the hypothalamic–pituitary–adrenal axis. These neuroendocrine pathways, when activated by exposure to a stressor, release hormones, peptides and neurotransmitters into lymphoid tissues and inflammatory sites. Thus, neuroendocrine factors have the opportunity to interact with specific receptors on antigen-stimulated lymphocytes. Furthermore, neuroendocrine factors can alter the accumulation of mononuclear cells at inflammatory sites by modulating the expression of chemoattractant cytokines and adhesion molecules involved in lymphoid cell trafficking.

A mouse model of an influenza A viral infection has been used, in conjunction with a stressor (physical restraint), to examine the neuroendocrine mechanisms (with emphasis on glucocorticoid and catecholamine responses) which modulate anti-viral

immunity. Restraint stress significantly elevated plasma corticosterone (cort) and tissue catecholamine (norepinephrine) responses and suppressed cell-mediated immunity. IL-2 production (a $CD4^+$Th1 response) in draining lymph nodes and spleen was suppressed in restrained animals, while anti-viral antibody responses (which require $CD4^+$Th2 cytokines) were essentially intact. Elevated levels of plasma cort correlated with reduced lymphocyte trafficking to the lungs and draining lymph nodes of virus-infected mice. Lymphadenopathy and cell accumulation at the inflammatory site were restored by treating restrained animals with a glucocorticoid receptor antagonist (RU486). However, T cell activation in the draining lymph nodes remained suppressed, unless a β-adrenergic receptor antagonist (nadolol) was given. Antagonism of the β-adrenergic receptor suggested a role for catecholamine in the regulation of T cell activation. We hypothesize that stress acts as a co-factor in the progression of infection by enhancing Th2 while diminishing Th1 responses to viral antigen.

Molecular mechanisms for activation of the immune–neuroendocrine axis

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Multiple mechanisms are involved in activation of the immune–neuroendocrine axis. During virus infections, viral products and replicative processes as well as cytokines all can induce lymphocytes to express the pro-opiomelanocortin (POMC) gene. The resulting ACTH and MSH peptides can cause local and systemic effects. Cytokines induced in this process also may have local and systemic effects.

We and others have shown ACTH and MSH to inhibit several immune functions. To further assess the potential of POMC peptides, as autocrine/paracrine cytokines in the immune system, we examined the effects of ACTH and MSH on intracellular transcription factor activity. Human promonocytic U937 and monocytic THP-1 cell lines were treated with MSH or ACTH and assayed for transcription factor binding activity in their nuclear extracts. The human H9 T-cell line was also assayed for changes in transcription factors under similar conditions. In electrophoretic mobility shift assays (EMSA), nuclear extracts from THP-1 or U937 cells demonstrated a transient increase in the CREB transcription factor binding activity which peaked at 15 minutes followed by a reduction over the following two hours. Under similar conditions NF-kB binding activity was reduced at 30 and 60 minutes post-stimulation with ACTH in H9 cells and MSH in U937 cells, following which binding activity returned to pre-stimulation baseline levels. The U937 cells constitutively produce ACTH. Therefore antiserum to ACTH was added to U937 cell cultures and the nuclear extract analysed by EMSA. NF-kB-like binding was increased, which suggests that endogenous ACTH was inhibiting NF-kB activity.

The ability of ACTH and MSH to down-regulate NF-kB suggests a mechanism and a role for lymphoid and circulating POMC products in inhibiting secondary pro-inflammatory response genes. These actions are consistent with these peptides as members of the growing family of anti-inflammatory cytokines and may represent an endogenous mechanism to limit immune system activation. The inhibitory activity of these peptides and their increased production during stress are consistent with

stress-induced immunosuppression and as an important neuro–immune link.

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Chronic exposure to cigarette smoke/nicotine or ethanol affects the antigen-induced activation of lymphocytes

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Tobacco smoke and alcohol are the two most abused drugs in the world and, in the USA alone, the combined death toll resulting from their abuse exceeds 500,000. Both drugs are known to predispose individuals to many diseases, and this susceptibility has been postulated to result from the negative effects of these drugs on the immune system. However, the mechanism through which these drugs affect the immune system is not clearly understood. We have demonstrated that chronic exposure of rats to cigarette smoke inhibits the antibody plaque-forming cell (PFC) response. The immunosuppressive effects of cigarette smoke are associated with the nicotine-containing particulate phase of cigarette smoke, and chronic exposure to nicotine also results in inhibition of the PFC response. Nicotine appears to affect the antigen-induced signal transduction pathway in both B and T lymphocytes. These effects of nicotine may be related to the presence of nicotinic acetylcholine receptors (nAchRs) on the lymphocyte cell surface. In fact, nicotine, both in-vivo and in-vitro, activates lymphocytes leading to enhanced tyrosine kinase and phospholipase C activities. Preactivation of lymphocytes with nicotine makes these cells refractory to the normal antigen-mediated activation signaling. This is the first study to indicate that nAchRs in lymphocytes may be linked to a second messenger system and explain the presence of cholinergic inervations in lymphoid tissues.

We have also demonstrated that, in LEW rats, chronic ethanol comsumption for six weeks inhibits the PFC response. However, under these conditions, F344 animals are totally resistant to the immunosuppressive effects of chronic ethanol intake, suggesting that immunological changes associated with ethanol abuse are genetically regulated. In fact, like the parental LEW, (LEW × F344), rats are sensitive to immunological effects of chronic ethanol treatment. Thus, immunological susceptibility to chronic ethanol intake is a dominant genetic trait, and may be an important component contributing to the variability in published results on the immunological consequences of chronic ethanol consumption

Cytokine regulation of neuroendocrine function

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Anterior pituitary (AP) hormone secretion is subject to multiple levels of regulation. The medial basal hypothalamus secretes releasing hormones (e.g. peptides, biogenic amines) that either inhibit or stimulate AP hormone release. In addition, target tissues elaborate hormones that feedback upon neuroendocrine structures to affect hypothalamic or AP hormone secretion. Finally, recent studies indicate a role for the

neurointermediate pituitary lobe (NIL) in the regulation of AP hormone secretion.

Because prolactin and growth hormone (GH) facilitate certain responses of the immune system, we hypothesized a role for thymic hormones and lymphokines in the regulation of AP hormone secretion. Thus, interleukin-6 (IL-6) stimulated prolactin, GH and luteinizing hormone (LH) release from male rat AP cells in-vitro. We further hypothesized the production of IL-6 within certain subpopulations of AP cells, since circulating levels of this cytokine are not normally significant. Cultured AP cells released IL-6 in response to endotoxin, interleukin-1β (IL-1β) and increased intracellular concentrations of cAMP. These data indicated a possible paracrine role for IL-6 in the facilitation of AP hormone release. Indeed, an anti-rat IL-6 antibody inhibited prolactin release in a time-dependent manner by 30%. The production of IL-6 by its putative cellular source (i.e. the folliculostellate cell) may participate in a paracrine mechanism of enhanced hormone secretion.

We have recently investigated the second messenger pathway activated by IL-1β leading to IL-6 release in AP cells. This monokine enhanced phospholipase A2 activity, generating arachidonic acid (AA) and glycerophosphorylcholine (GroPCho). AA stimulated IL-6 in a 5-lipoxygenase-dependent manner. The hypothetical PLA2 intermediate, lysophosphatidylcholine (LysoPC), also stimulated IL-6 in a concentration-responsive manner. The effect was dependent on the 1-acyl chain length, the presence of the phosphocholine headgroup, and could be blocked by protein kinase C inhibitors (e.g. H7, chelerythrine). Thus, these data indicate that IL-1β induces AP IL-6 release via a novel mechanism involving LysoPC activation of PKC in the absence of increased diacylglycerol or calcium. The resulting increased cytokinergic tone within the AP may result in the facilitation of the secretion of several AP hormones.

Effects of cytokines in the CNS on immune function

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Appropriate stimulating conditions, such as endotoxin, have been found to induce IL-1 throughout much of the brain, resulting in widespread presence of IL-1 in extracellular fluid of the brain. This IL-1 can have a variety of effects. When infused into rat brain, very low doses of recombinant human interleukin-1β (IL-1β; as low as 3.1 fmol) has been found to suppress a variety of peripheral cellular immune responses measured in blood and splenic lymphocytes, including natural killer cell (NK) activity, response of lymphocytes to mitogen, and interleukin-2 (IL-2) production. This suppression occurred very rapidly (within 15 min) and lasted for several hours; rebound enhancement of immune responses was not seen. Infusion of endotoxin (LPS, 10 ng) into the brain to stimulate release of endogenous IL-1 produced similar suppression of these immune responses, and this effect could be blocked by infusion in brain of low doses (e.g. 10 ng) of α-MSH, a known blocker of cytokine action. Thus, exogenous IL-1 or endogenously released IL-1 acting in brain suppresses cellular immune responses. Further studies showed that this suppression of cellular immune responses by IL-1 occurs via activation of both the pituitary–adrenal axis and sympathetic nervous system, and requires corticotrophin

releasing factor (CRF) as an intermediary. Finally, recent studies have shown that infusion of the HIV envelope protein gp120 into the rat brain also stimulates release of IL-1 in brain and thereby suppresses peripheral cellular immune responses. Thus gp120-induced release of IL-1 in brain may be responsible for some characteristics seen in HIV-infected individuals (e.g. elevated circulating cortisol), and may also play a role in the disruption of immune responses seen in HIV-infected individuals.

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