The links between the neuroendocrine and the immune systems: Views of an immunologist

G. Aleph Prieto Moreno and Yvonne Rosenstein
Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Mexico

Abstract
The immune system was long considered a self-contained system where cells that had been taught to differentiate “self” from “non-self” had the ability to eliminate foreign pathogens. It was thought that the immune system was relatively resistant to environmental pressures, and had little or no interactions with other physiological systems. However, over the last decades this view has dramatically changed. It is now known that the complex machinery of the immune system
modifies and is modified by other systems, particularly the nervous and endocrine systems. Cytokines, hormones and neurotransmitters all function in a similar way: synthesized and released by specific cells, these molecules operate in a paracrine and endocrine fashion through specific receptors expressed in neural cells as well as immune cells, and regulate a variety of immune and neuroendocrine processes. The cross-conversation between systems ultimately fine tunes the homeostasis, providing the organism with exquisitely subtle and sophisticated mechanisms to perceive the internal and external environment in the broadest sense we can think. This review focuses on the influence of the immune system on the neuroendocrine systems. Emphasis was put on those immune mechanisms that alert the organism of danger, and their role in central nervous system-controlled functions such as sickness behavior, sleep regulation, fever onset and mood disorders.

Introduction
The immune system, the nervous system and the endocrine system: A “ménage à trois”

The understanding of immune regulation has focused essentially on molecular and cellular factors within the immune system as primary regulators of immune function. However, many non-immune factors, derived from the nervous and endocrine systems participate in the physiological modulation of immune function. The nervous system monitors the immune system at several levels: through direct interaction between specialized immune cells and neural-released factors or through direct nerve fiber connections with immune tissues. The Hypothalamus-Pituitary Axis modulates immune function stimulating the secretion of glucocorticoids, prolactin, and growth, thyroid and sex hormones [1].

The brain and the immune systems are hardwired through the autonomic nervous system: the sympathetic and parasympathetic pathways innervate lymphoid tissues including the bone marrow, thymus, lymph nodes and vasculature as well as spleen and gut-associated lymphoid tissue [1-3]. Moreover, peripheral sensory nerves may be retrogradely activated to release neuropeptides into tissues, where these molecules also affect lymphoid cells migration, activation and mediator release. The principal end products of the sympathetic and parasympathetic pathways are norepinephrine and epinephrine (members of the catecholamine compounds group), and acetylcholine, respectively. These circuits are essential for regulating the inflammatory response: vagal nerve stimulation and acetylcholine release inhibit secretion of IL-1β, TNF-α, IL-6 and IL-18, thus modulating septic shock [4], while catecholamines inhibit inflammatory but stimulate anti-inflammatory cytokine secretion [1]. In addition, immune cells display receptors for a plethora of
neurotransmitters: acetylcholine [5], dopamine [6, 7], adrenergic receptors (α and β) [3], vasoactive intestinal peptide [8], pituitary adenylate-cyclase polypeptide [9], substance P [10], serotonin, histamine [11], leptin [12] ... All these neurotransmitters are sensed by lymphoid cells through specific receptors that will generate intracellular signals and ultimately affect immune cell function and immune response in many different ways. These non-immune factors determine the susceptibility to inflammatory, autoimmune, allergic or infectious diseases that are under the control of the immune system.

Conversely, immune factors regulate the nervous system at the molecular, cellular, organ and system levels, thus playing an important role in neuronal cell death and survival, development and activity in normal and pathological conditions. Immune factors participate importantly in the onset of sickness behavior, as well as in the pathogenesis of neurodegenerative diseases, the neuro-regeneration that follows trauma, and brain normal functions as diverse as cognition, memory consolidation and mood. The two systems communicate by means of chemical messengers ranging from small molecules such as nitric oxide to neuroendocrine peptides to large proteins such as cytokines and growth factors and their respective receptors. This review focuses mainly on the influence of the immune system on the neuroendocrine systems. Emphasis was put on those immune mechanisms that alert the brains of danger, and their role in central nervous system-controlled functions such as stress response, fever onset, sleep regulation, mood disorders, and sickness behavior. A deeper comprehension of the neural-immune systems interactions involved in both, pathological and homeostatic mechanisms will reveal a more complete appreciation of physiological responses, as it will provide new avenues for treatment of disease.

The road to the CNS: The blood brain barrier

The brain is protected from exposure to blood cells and blood products by the blood brain barrier (BBB), an endothelial barrier made of functionally and anatomically distinct brain capillary endothelial cells connected by tight junctions and with discrete vesicular activity. Under normal resting physiological conditions, this structure excludes most molecules, except small lipophilic ones; hydrophilic molecules and essential nutrients are mostly taken by active transport across the BBB. It was long thought that the BBB provided a hermetic wall that isolated the central nervous system (CNS), and that immune surveillance was not available. It is now clear that immune cells can traffic through the CNS under routine immunosurveillance and pathological conditions and that systemic cytokines cross the BBB at very specific places.

Interestingly, most immune cells can be found in the CNS. During viral infections, ischemia or inflammatory diseases such as multiple sclerosis, leukocytes cross the BBB and accumulate within enlarged perivascular spaces;
following that, cells will migrate to different regions of the CNS parenchyma. In experimental models of multiple sclerosis (Experimental autoimmune encephalitis; EAE) [13, 14] and in bacterial infections of the meningeal spaces [15] neutrophils and macrophages are found in addition to T lymphocytes. To date it is not clear whether the molecular mechanisms to recruit those cells to the CNS and to further activate them are the same in the different brain regions [16].

Immune-cell derived cytokines and interleukins cross the BBB at the circumventricular organs located at various sites along the walls of the cerebral ventricles (median eminence, organum vasculosum of the lamina terminalis, subfomical organ, choroid plexus, neural lobe of the pituitary). These areas, usually rich in blood vessels, lack tight junctions, making it possible for large molecules to cross from the blood to the brain tissue; moreover, some of them (median eminence) are located between the hypothalamus and the pituitary gland, two areas that secrete numerous neurohormones. There is also evidence for active transport of cytokines such as IL-1β, IL-1R, GM-CSF, IL-6, TNF-α and IFN-γ, allowing these key molecules to cross the BBB at different places. In addition, the afferent fibers of the vagal nerve may convey pyrogenic cytokines to the thermoregulatory centers of the hypothalamus. The vagus innervates visceral structures such as the lung and the gastrointestinal tract, where there may be frequent contact with pathogens. It has been proposed that IL-1β is a key component of vagally-mediated immune-to-brain communication, by inducing expression of the activation marker c-fos in vagal primary afferent neurons [17].

**Cytokines**

Cytokines constitute an extremely elaborated network of peptide signaling molecules that are synthesized when immune cells recognize specific pathogen associated molecular patterns (PAMPS) through an evolutionary conserved family of receptors, the Toll-like receptors (TLR) [18]. Lipopolysaccharide (LPS) on gram-negative bacteria is recognized by TLR4, lipopeptides on gram-positive bacteria by TLR2, TL1 and TLR6, bacterial flagellae by TLR5 and TLR11, unmethylated bacterial and viral DNA by TLR9, guanosine-uridine rich viral RNA by TLR 7 and TLR8, double-stranded RNA by TLR3. Circulating cells of the innate immune system, such as neutrophils and monocytes, natural killer cells and γδ T lymphocytes as well as resident cells such as dendritic cells (professional antigen-presentation cells) and macrophages express TLRs, through which they recognize pathogens. Tissue mast cells express also TLRs and play a role in innate immunity by releasing vasoactive peptides such as histamine and serotonin, which can directly activate sensory neural fibers. When they are exposed to microbes or stimulated by cytokines or other mediators, all these cells release cytokines.
Once released by activated cells, cytokines constitute an alarm system whereby neighboring cells are alerted that the host is under attack. In the periphery, chemotactic cytokines will attract inflammatory cells such as neutrophils to provide a higher level of defense. This in turn will increase the local concentration of cytokines that will flow to the circulatory system and eventually reach the brain, thus acting like hormones, on organs distal to their site of production. Cytokines affect directly and indirectly the neuroendocrine and the autonomic nervous systems by modulating neural cells that express receptors for those cytokines or the chemistry of the brain (cytokine-cytokine, cytokine-hormone or cytokine-neurotransmitter interactions) [19]. In addition, recently it has been shown that peripheral cytokines can induce the synthesis of cytokines centrally, without need to cross the BBB. For instance, TNF-α injection in the periphery is associated with the production of IL-1 in certain brain areas.

Cytokines can be broadly classified in pro-inflammatory or anti-inflammatory cytokines, and they include chemotactic cytokines, immunomodulatory interleukins, as well as endocrine hormones such as prolactin and growth hormone. The pattern of cytokines depends on the nature of the antigenic stimulus, and the cell source that is being stimulated. Since a given pathogen has the ability to activate a specific combination of TLRs, the molecular signature of a pathogen is different from that of another pathogen and this is translated at the level of the cytokine mixture that will be released. Under pathologic conditions, the specific composition of the cytokine milieu can overactivate the immune response, leading to inflammatory disease or oversuppress it, increasing the susceptibility to infectious disease or autoimmunity. Cytokines turn out to be particularly important for the evolution of infectious diseases of the brain (neuro-aids and toxoplasmosis), and can contribute to neuro-degeneration and dementia. Thus the receptors for those cytokines are good targets for developing therapeutic approaches for reduction of brain injury. However, cytokines and immune factors are not always toxic, and the presence of specific activated T cells at critical times after nerve trauma are important for neuro-regeneration and can prevent paralysis in a model of rodents with spinal cord injury [20].

**Effect of cytokines on CNS functions**

Over the last decades it has become clear that the CNS, long time considered as an immune privileged region, is regulated by the immune system (IS) through cytokines. The effects of cytokines are mediated through specific receptors. Interestingly, and probably reflecting the expression of those receptors on different sets of cells of different areas of the CNS, cytokines can mediate a wide range of responses. For example, IL-2 and IFN-γ stimulate neuronal activity in the cortex and hippocampus [21]; IL-1 and IL-6 inhibit the
neurons of the anterior hypothalamus, but IL-1 stimulates the neurons of the supraoptic nucleus of the hypothalamus [22].

Moreover, not all the cytokines affecting the CNS functions are produced in the periphery. Cytokines are also produced within the CNS. Astrocytes, the cells that provide mechanical and metabolic support for neurons by regulating the environment in which neurons function, and microglia, cells derived from the macrophage lineage, produce several cytokines [23]. In addition, certain neurons can produce cytokines such as IL-1 [24]. Cytokines (and their receptors) such as IL-1, IL-3, IL-6, IL-8, IL-12 and IFN-γ, have been found to be constitutively expressed in the CNS (reviewed in [24]), while expression of TNF-α in the CNS is still under debate. The precise localization of the cells that produce those cytokines is not yet available. In addition, it is now known that T lymphocytes trafficking to the CNS will contribute to modify the balance of cytokines, regulating inflammation [16]. CNS-derived cytokines are considered to act more like growth factors and exert paracrine/autocrine effects on hormone secretion and cell proliferation. Depending on the pattern, cytokines expressed within the CNS participate in cell death [25, 26] or survival [27]. In addition, brain-produced cytokines such as IL-1β, can cause a systemic anti-inflammatory response (higher levels of IL-10) in response to LPS, through the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system [28]. Central and peripheral cytokine networks are thought to be independent and different levels of cytokines are found in the brain and the periphery.

When acting on the brain, cytokines can induce fever, sleep and sickness behavior; they can also modify the mood, the memory consolidation and cognition as well as regulate the neuroendocrine stress response. Early studies were done with complex infectious and inflammatory stimuli, suggesting that these effects were mediated by cytokines. With the availability of large enough quantities of recombinant cytokines, and knockout mice it has been possible to show that these diverse effects were directly mediated by the cytokines themselves and not by non-specific effects of the disease model.

**Activation of neuroendocrine stress response**

A stressor can be defined as a stimulus that activates the hypothalamic–pituitary–adrenal (HPA) axis and/or the sympathetic nervous system to help an organism to adapt physiologically to deal with a threat. More broadly, psychological stress develops when events or environmental demands exceed an individual’s perceived ability to cope with the circumstances [29]. Activation of the immune system is frequently accompanied by activation of the neuroendocrine system: during inflammation, immune cell-derived cytokines stimulate hypothalamic release of corticotrophin releasing hormone (CRH) into the portal blood system and hence to the pituitary
gland; the net effect is a rapid increase in plasma adrenocorticotrophic hormone (ACTH) with subsequent release of glucocorticoids (GC) from the adrenal gland.

In turn, ACTH itself has immunoregulatory functions, both on humoral and cell-mediated processes [1]. Additionally, GC, together with the ability to raise levels of blood glucose, has potent anti-inflammatory effects and dampens humoral and cellular immune responses. By interfering with the activity of the NFkB transcription factor and with the enzymes involved in the synthesis of inflammatory mediators such as prostaglandins and nitric oxide, naturally occurring GCs, such as corticosterone in rodents and cortisol in humans, and pharmacologic GC preparations such as dexamethasone suppress antibody production, delayed-type hypersensitivity, and alter immune cell trafficking, differentiation, maturation and activation. In general, there are evidences that insufficient GC response are associated with predisposition to inflammatory or autoimmune disease, and excess GC production, as occur in chronic stress, predisposes to infection diseases and other sequelae of immunosuppression [30]. In the first case, hypo-HPA axis responsiveness has been shown in animal models and in humans affected with systemic lupus erythematosus (SLE), arthritis and multiple sclerosis. This is consistent with the immediate therapeutic effects of GC on rheumatic diseases [31].

Administration of LPS directly into the bloodstream of laboratory animals, or subcutaneous or intramuscular injection of the irritant turpentine result in enhanced plasma levels of TNF-α, IL-1-β and IL-6 [32, 33]. Although the main site of action of IL-1 is the hypothalamus, where it stimulates the production of CRH, this cytokine was also shown to affect ACTH release during long-term cultures of normal pituitary cells [34]. Administration of high doses of IL-6, TNF-α, and IFN-γ also stimulates the pituitary-adrenal axis, although to a lesser extent than IL-1. Even so, during local inflammation, IL-6 levels remain elevated to a greater extent and for greater periods than is IL-1 [24]. Similarly, in humans, intravenous or subcutaneous administration of IL-1α, IL-1β, IL-2, IL-6, TNF-α, IFN-α, IFN-β and IFN-γ elevates plasma ACTH and/or cortisol concentrations within 1-4 h. Both in cancer patients and normal healthy males, it was observed that HPA axis is remarkably responsive to IL-6 [35]. Apart from cytokines, other potential immunological messengers capable of influencing neuroendocrine process are histamine and serotonin, which are released by mast cells as part of the innate immune response [36].

Interestingly, physical or psychological stress have also been associated with IL-1β and IL-6 production. Acute psychological stress induces the mobilization of lymphocytes into the blood [29], a process known as lymphocytosis. Mobilization of the immune cells into the tissues readies the organism to deal with physical trauma and a potential infection that may result. In the stress-induced redistribution of lymphocytes, catecholamines and
cortisol play a critical role. In a wider context, sympathetic leucocytosis can be thought as a less-recognized facet of the ‘fight-or-flight’ response [2].

A recent report described a new pathway by which C3a, a complement-derived molecule that mediates numerous pro-inflammatory activities, directly stimulates anterior pituitary hormone release. C3a signals in pituitary hormone secreting and non-hormone secreting cells through C3a receptors and activates the hypothalamic-pituitary-adrenal axis, a reflex central to the stress response and to the control of inflammation, resulting in the release of prolactin, growth hormone, and adrenocorticotropin and a net increase in the corticosterone serum levels [37]. In addition, results derived from in vitro work have shown that IL-1, IL-2 and IL-6 can increase the release of GCs by acting directly on the adrenal gland [38]. By contrast, IL-4 and low doses of IFN-γ inhibit the HPA axis in vivo [39, 40].

Overall, these data show that changes in neuroendocrine functions observed during certain pathologies may be mediated by factors released when the immune system is activated. Reciprocally, the effector molecules in the HPA axis could act as a feedback loop during immune activation. Determining which immune-derived factors are affecting neuroendocrine processes during a given disease, and whether they are ameliorating or aggravating its course, is of clinical relevance.

**Fever**

Fever is a common response to infection, inflammation and trauma. It is a complex physiological response that results of the communication between the immune system and the brain. Following contact with a pathogen or an inflammatory stimulus, immune cells in the periphery release cytokines. As mentioned above, cytokines will cross the BBB or interact with peripheral neural components such as the vagus [41], and will signal the temperature sensitive cells of the preoptic area of the hypothalamus to increase the thermal set point. Fever is stimulated by cytokines such as IL-1, IL-6, IL-8, MIP1-β (macrophage-inflammatory protein 1β) and IFN-γ. On the other hand, antipyretic cytokines (IL-10) and molecules such as AVP (arginine vasopressin), α-melanocyte stimulating hormone (α-MSH) and glucocorticoids limit the magnitude and duration of fever. TNF-α seems to exert pyrogenic as well as anti-pyretic effects, depending on the experimental conditions. The sum of the pyrogenic and antipyretic elements will ultimately favor the onset of behavioral and physiological mechanisms that increase heat production and decrease heat loss, determining the amplitude of the temperature elevation; that is fever.

Most of our knowledge regarding the role of cytokines in the regulation of fever has been achieved with traditional pharmacological techniques: injection of cytokines in different compartments of the periphery or of the brain, usage
of neutralizing antibodies to antagonize the effects of endogenously produced cytokines, administration of pathogen-derived pro-inflammatory products (the most frequently used is LPS, a cell wall component of gram-negative bacteria). These techniques have facilitated the identification of specific anatomic regions implicated in fever regulation and a role for the injected substances in fever onset. However, in those experimental models it is difficult to ascertain that a cytokine has been neutralized in all areas of the body, opening the possibility that it still may be regulating the response. The recent availability of mice that lack functional genes for cytokines or cytokines receptors in all tissues of the body has provided a tool to study the role of a given cytokine as well as the effect of the absence of that cytokine on the production and regulation of other cytokines in fever control.

The IL-1 family members are IL-1α, IL-1β and IL-1rn (IL-1 receptor antagonist). IL-1β is thought to be the primary secreted form, while IL-1α is mostly a membrane–associated molecule, and IL-1rn acts as an antagonist of IL-1 by binding to IL-1 receptors without inducing intracellular signals. Two receptors for IL-1 have been cloned: type I receptor is considered as the signaling receptor and type II receptor is thought to function as a negative regulator of IL-1 functions [42]. Studies that have examined the fever response to peripheral injection of LPS in IL-1β knockout mice have found that IL-1β is not essential for fever production in response to LPS [43, 44]. Interestingly, although it is thought that IL-1β induces fever through IL-6, the LPS-induced levels of IL-6 was similar in the IL-β knockout mice and in the wild-type mice, raising thus the possibility that IL-1α may compensate for the lack of IL-1β, and bind to the IL-1r1 to induce fever. When similar experiments were performed with the IL-1r1 knockout mice, hence lacking the ability to respond to either form of IL-1, identical fevers were observed in those animals and wild-type mice in response to low (100ug/kg ip) or high (5 mg/kg ip) levels of LPS [45]. In contrast, the injection of turpentine to induce local inflammation and a strong acute phase response (fever, cachexia, acute phase protein production and anorexia) in the IL-1β and IL-1r1 knockout mice revealed that fever and the sickness behavior associated with turpentine were dependent on the expression of IL-1β or IL-1r1 [46-48].

IL-6 is also an important mediator of fever. Sepsis, the peripheral injection of LPS or turpentine induce a rise in plasma and brain IL-6 concentrations [49-51]. IL-6 knockout mice are resistant to fever in response to low doses of LPS [52], but, when given high doses of LPS, they develop fevers similar to wild type mice [43], suggesting that other cytokines can compensate for the lack of IL-6. Those cytokines could be, as IL-6, members of the gp130 family of cytokines (cytokines that signal through the gp130 receptor). It was recently shown that although IL-11, a gp130 cytokine, is not pyrogenic when administered into the brain ventricles, central application of hrIL-11 altered
body temperature in conditions of pyrogenic stimulation [53]. Alternatively, other cytokines that do not use the gp130 receptor may produce fever in the IL-6 knockout mice. Data obtained with the IL-6 knockout mice show that IL-6 is indispensable for the fever induced in response to turpentine [43, 54].

TNF-α has been considered as a pro-inflammatory cytokine; when injected into several species, it can induce fever [55]. However, data obtained with mice lacking both the TNFp55 and p75 receptors (TNFR) show that endogenous TNF has little role in LPS-induced fever and functions more like an antipyretic. These animals develop very high fevers in response to septic-like doses of LPS, supporting a role for TNF-α in hypothermia. The enhanced fever of TNFR knockout mice correlated with lower levels of IL-10, suggesting that the anti-pyretic role of TNF-α depends on IL-10. Interestingly, TNFR knockout mice develop the same fever as wild-type animals in response to turpentine [56].

IL-10 is an anti-inflammatory TH2 cytokine. It inhibits the LPS-induced production of IL-1β, IL-6 and TNF-α [57]. Data obtained with the IL-10 knockout mice support these findings. As for TNF-α, turpentine-induced fever is not under the control of IL-10 [58].

Despite some discrepancies between all these studies, the overall conclusion is that IL-1 and IL-6 are not essential for fever onset in response to LPS and other microbial derived molecules yet they are essential mediators for fever induced by turpentine and molecules that induce a non-microbial inflammatory response. Moreover, the production of IL-6 in response to turpentine is dependent on IL-1β production, whereas that dependent of LPS is not. In addition, TNF-α seems to function more like an IL-10 dependent anti-pyretic molecule in response to high doses of LPS, while it does not play a significant role in turpentine-induced fever.

Cytokines such as circulating IL-1 can then interact with IL-1 receptors on endothelial cells of the vasculature, and stimulate signaling molecules such as nitric oxide synthase or prostaglandins through a COX-2 dependent pathway [59]. Prostaglandin, E2 and NO can cross the BBB, and stimulate temperature increase. It was recently shown that PGT and MRP4 likely play a role in transporting prostaglandins through the blood-brain and blood-cerebrospinal fluid barriers and may be involved in the maintenance of prostaglandin homeostasis in the brain and in the initiation of fever response. Interestingly, LPS treatment increases the expression of PGT in the supraoptic and paraventricular nuclei of the hypothalamus, but not that of MRP4, suggesting that the dichotomy between the infection-induced fever and the non-infection induced fever follow different signaling pathways [60]. In addition, the signaling leading to fever occurs through NFkB, p38, and Jun [61, 62]. Treatment with non-steroidal anti-inflammatory agents blocks these routes and prevent fever onset.
Sleep

Deprivation of sleep is considered to make individuals more susceptible to illness, particularly to viral infections such as cold and influenza. On the other hand, illness is often associated with somnolence. These observations strongly suggest that the status of the immune system at a given time can alter sleep and conversely, that sleep has an important role in restoring the immune system. Some epidemiological studies suggest that sleep duration might influence mortality, as increased mortality has been found to be associated with sleep duration of less than or greater than seven hours [63, 64], pointing that in addition to the length of sleep and the repairing processes coupled to it, other factors are involved. The exact mechanisms by which sleep and immunity regulate each other are far from being elucidated and often data obtained with different species are different, possibly revealing variations in the immune and neuroendocrine responses between species [65].

Sleep is divided into two distinct states: non-rapid-eye-movement (NREM and rapid-eye-movement (REM) sleep. On an overall, clinical and experimental data suggest that acute infection with nonneurotropic pathogens or administration of components such as muramyl peptides or LPS derived from these microorganisms [66-68], induce stereotypic changes in NREM sleep, particularly slow-wave sleep time is increased, and often there is a reduction of REM sleep time.

The study of spontaneous infections in humans has provided interesting insights. Human African trypanosomiasis, known as sleeping sickness, constitutes a unique model to investigate the relation between infection and sleep. Rather than increased sleep duration, these patients have disturbances in the circadian regulation of the sleep/wake cycle. In these patients, the level of disturbance is proportional to the severity of the illness [69] and trypanosomicide treatment reverses the sleep/wake cycle disorders [70]. Sleep-structure distortion is one of the earliest and most consistent signs of HIV infection, with patients having an increased duration of small wave sleep during the latter half of the night [71]. On the other hand, rabies, a disease that directly involves the CNS, induces marked changes in the sleep architecture, with increased wakefulness times and decreased REM sleep [72].

Infection-associated sleep and spontaneous sleep are regulated, in part, by cytokines and their specific receptors. Cytokines such as TNF-α, interleukins and interferons are produced during the acute phase response that follows infection, as well as under normal conditions in the CNS [73]. Augmenting the brain or plasma levels of either TNF-α or IL-1β increase the duration of small-wave sleep [74-76]; conversely, decreasing their levels diminishes small-wave sleep [77-79]. Interestingly, each of these cytokines influences the effect of each other on sleep, and mice defective in either IL-1R1 or TNF receptor have significantly less baseline sleep than control mice [80, 81].
The role of other cytokines on sleep is more ambiguous. IL-2, IL-15, IL-18, IFN-α and IFN-γ have been associated with enhanced duration of sleep [82, 83], while IL-4, IL-10 and IL-13 have rather been shown to inhibit spontaneous sleep [84]. Whether these cytokines are acting directly on sleep or indirectly, through other cytokines, particularly TNF-α and IL-1β is presently not known. Alternatively, the variations in sleep pattern that follows infections with different microorganisms is a consequence of different cytokines combinations in response to the different microorganisms. Overall, it seems that pro-inflammatory cytokines tend to be somnogenic, while anti-inflammatory ones are not.

From a molecular point of view, TNF-α- and IL-1β-mediated signaling activate NFκB and enhance sleep, and IL-4 and IL-10 and stimuli that activate IκB inhibit sleep [84]. In turn, NFκB activation constitutes a positive signal towards the production of TNF-α and IL-1β, as well as of IL-2, which is also considered as a somnogenic factor [82].

In addition to the combination of cytokines, infection-related and normal cytokines-induced sleep patterns depend on the circadian rhythms of immunity as well as on the responsiveness of an individual to neuroendocrine stress and corticotropin releasing factor (CRH). CRH, produced by the hypothalamus, has been found to antagonize the IL-1β-induced increase in small wave sleep and is a potent inducer of waking [65]. On the contrary, GHRH (growth hormone releasing hormone) promotes slow-wave sleep [85]. GHRH is also thought to mediate some of the sleep-promoting activity of IL-1β, whereas cortisol blocks the production of IL-1β and other cytokines [86]. There is also a reciprocal effect of immune components on hormones. For example, IL-6 [87] and TNF-α [88] also stimulate ACTH secretion. IL-1 induces the expression of the gene for CRH [89], and that of ACTH from the anterior pituitary [90]. IL-2 [91] and IL-1 [92] stimulate AVP (arginin vasopressin) secretion from the hypothalamus.

Sleep depravation also affects the immune system. Chronic sleep loss is more detrimental than acute sleep loss, which instead might boost the immune system. Sleep can influence the immune system through the action of centrally produced cytokines that are regulated during sleep; thus generating a regulatory loop where immune functions and sleep are closely interconnected [65]. Although little information is available, primary sleep disorders seem to be associated with changes in immune competence, with chronic insomnia being associated with a decreased number of T lymphocytes and NK cells [93]. In addition, a link between insomnia unrelated to medical disorders and a shift in the Th1/Th2 balance toward Th2 dominance was recently described [94].

The relation between immunity and sleep are not only important from the physiological point of view where homeostasis and well-being are the main
goal, but also from an economic point of view. Sleep loss and sleep disruption are key economic and public health issues. With pressure to work longer hours and around-the-clock shifts, the population sleeps less, affecting circadian rhythms and immunity, increasing the risk for accidents, and illness as well. A deeper understanding of the relations between sleep and immunity might lead to novel interventions and therapies.

**Mood**

Cytokines also alter mood. Treatment of cancer or AIDS patients with IL-2 or IFN-γ has been shown to profoundly affect mood, including serious depressive symptoms, as well as suicide [30]. Recent findings have shown that the development and severity of depressive symptoms positively correlated with the magnitude of the decrease in tryptophan concentration during treatment of patients, suggesting that nutritional or pharmacological interventions to enhance serotonin availability could participate in the prevention of the cytokine induced depressive symptoms. Interestingly, the administration of the selective serotonin reuptake inhibitor, paroxetine, is associated with a significant reduced incidence of depressive disorders in patients undergoing IFN-α therapy [95]. In humans, depression has also been associated with variations in the plasma levels of IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-2, soluble IL-2 receptors, IL-6 and soluble IL-6 receptors [96], while animals treated with LPS, IL-1β or TNF-α usually appear withdrawn from the environment [97].

**Food intake**

Acute and chronic pathological processes are accompanied by food intake suppression. In animals, systemic administrations of IL-1 and TNF-α suppress feeding and drinking. This effect has been observed using various measurements of food and water intake in ad libitum as well as deprived conditions [98, 99]. In general, anorexia induced by cytokines is proposed to involve the modulation of hypothalamic-feeding associated sites, prostaglandin-dependent mechanisms, modifications of neurotransmitter systems, gastrointestinal, metabolic, and endocrine factors [99]. In the case of IL-1β, the hypothalamus may serve as a central site of action for the depressive effects on food intake [98]. Electrophysiological studies have shown that IL-1 and TNFα can inhibit glucose sensitive neurons in the lateral hypothalamus area, considered a “hunger center” [100]. Consistent with this, IL-1β was shown to reduce both meal size and meal duration, whereas higher doses also decreased meal frequency [99]. In addition to their individual effects, IL-1β and TNF-α, synergistically disrupt the consumption of a highly palatable food source (chocolate milk) in male rats [101].
The decreased food intake that accompanies fever appears to be inconsistent with the enhanced energy requirements of thermogenesis, making the anorexic effect of IL-1 and TNF-α difficult to reconcile with the pyrogenic activity of these molecules. To resolve this paradox, it has been proposed that cytokine-induced anorexia spares energy required for foraging and prevents a weakened organism to run into the risk of being exposed to a predator during the search of food. From this perspective, it can be predicted that cytokines should be more effective to suppress the foraging than the consummatory components of food intake [97]. This appears to be the case since LPS- or IL-1-treated animals stopped pressing a lever for food but still ate the food pellets that were delivered independently of their behavior [102]. Also, it has been proposed that temporary anorexia during acute disease may be beneficial to an organism since a restriction in the intake of micro- and macro-nutrients will inhibit bacterial growth [99].

Together with IL-1, IL-6 and TNF-α, leptin acts as an acute-phase reactant during inflammation. Under conditions of food deprivation, leptin levels fall, as this hormone is produced in proportion to the body fat mass. Lowered leptin levels lead to reduced metabolic expenditure, freeing energy required for supporting the functions of vital organs [103]. Regulation in the leptin levels during the course of sickness could represent a feedback mechanism for the modulation of the immune response [104].

**Sickness behavior**

The typical symptoms of sickness include weakness, inability to concentrate, decreased appetite and locomotion, depressed mood and increased somnolence. Until recently, these symptoms were considered as non-specific symptoms, offering little information for diagnostic. Sickness behavior is now considered a normal response, rather than a debilitating state, that allows an organism to cope with infection in a regulated fashion [97].

Most of the symptoms associated with sickness behavior have been observed after the administration of LPS, thus supporting the role of infection as a generator of these warning signs. Not surprisingly, cytokines released when the organism senses a pathogen play an important role in sickness behavior. Along the text, we reviewed how LPS activates the immune system and induces the release of cytokines such as IL-1, IL-6 and TNF-α, which in turn can reach the brain. Experimentally, it has been shown that local injection of IL-1, IL-2, IL-6 and TNF-α into the peritoneum, the vein or the ventricles reproduces the symptoms of sickness behavior (reviewed in [105]). The wide range of symptoms associated with sickness behavior suggests that cytokines could alter the signaling pathways of different neurotransmitters, particularly that activated through G-protein signaling. Supporting this notion, it has been shown that TNF-α stabilizes RGS7, a regulator of G-protein signaling [106].
Interestingly, sickness behavior has been found in all organisms studied to date. From an evolutionary point of view, sickness behavior is thought to be protective, in part, as a mechanism to allow the organism to conserve energy to cover for the cost of immune responsiveness during infection. Weakness, lethargy, depression or pain (induced by IL-1β), will favor immobility, thus saving energy. Dissection of the interface connecting the neuro-endocrine and immune systems with metabolism is a field that has gained wide interest recently, and it is clear that a better knowledge of the intricate network of interactions between these three systems will lead to identify valuable targets to develop novel therapies.

**Concluding remarks**

The nervous, endocrine and immune systems constitute a system of communicating flasks. Changes on one system will promote changes in the other two. To balance the influence of each system over the other two, the organism has developed elaborated and intricate networks of anatomical and molecular interactions. A thorough understanding of the mechanisms that rule these networks will certainly provide new and exciting insights into the regulation of these systems.

**Acknowledgments**

We thank our colleagues for their comments and discussions, and Juan Manuel Hurtado Ramirez and Shirley Ainsworth for technical help. Work in our laboratory is partially funded by grants from the Dirección General de Personal Académico from the Universidad Nacional Autónoma de México and from the Consejo Nacional de Ciencia y Tecnología, (CONACyT), Mexico. Aleph Prieto Moreno is the recipient of a scholarship from CONACyT.

**References**

Tand B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow
7. Kipnis, J., Cardon, M., Avidan, H., Lewitus, G.M., Mordechay, S., Rolls, A.,
Shani, Y., Schwartz, M. 2004, Dopamine, through the extracellular signalregulated
kinase pathway, downregulates CD4+CD25+ regulatory T-cell activity:
peptide acts as a potent suppressor of inflammation in vivo by trans-deactivating
Henche, N., Brabet, P., Leceta, J., Gomariz, R.P. 2002, Anti-inflammatory role in
septic shock of pituitary adenylate cyclase-activating polypeptide receptor. Proc
10. Brain, S.D., Williams, T.J. 1988, Substance P regulates the vasodilator activity of
11. Jutel, M., Watanabe, T., Klunker, S., Akdis, M., Thomet, O.A., Malolepszy, J.,
Zak-Nejmark, T., Koga, R., Kobayashi, T., Blaser, K., Akdis, C.A. 2001,
Histamine regulates T-cell and antibody responses by differential expression of H1
leptin in the induction and progression of autoimmune encephalomyelitis. J
Immunol., 166, 5909-16.
polymorphonuclear cell interactions during autoimmune demyelination. Am J
Pathol., 139, 1401-9.
oligodendrocyte glycoproteins induce experimental autoimmune encephalomyelitis
138, 195-201.
trafficking to the CNS: anatomical sites and molecular mechanisms. Trends
Immunol., 26, 485-95.
Watkins, L.R. 1999, Interleukin-Ibeta in immune cells of the abdominal vagus
1999, Autoimmune T cells protect neurons from secondary degeneration after


37. Francis, K., Lewis, B.M., Akatsu, H., Monk, P.N., Cain, S.A., Scanlon, M.F., Morgan, B.P., Ham, J., Gasque, P. 2003, Complement C3a receptors in the


