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Conceptual development of the immune system as a sixth sense

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Abstract

Understanding how and why the immune and nervous systems communicate in a bidirectional pathway has been fundamental to the development of the psychoneuroimmunology (PNI) field. This review will discuss some of the pivotal results that found the nervous and immune systems use a common chemical language for intra and inter-system communication. Specifically the nervous and immune systems produce a common set of peptide and nonpeptide neurotransmitters and cytokines that provides a common repertoire of receptors and ligands between the two systems. These studies led to the concept that through the sharing of ligands and receptors the immune system could serve as a sixth sense to detect things the body cannot otherwise hear, see, smell, taste or touch. Pathogens, tumors, and allergens are detected with great sensitivity and specificity by the immune system. As a sixth sense the immune system is a means to signal and mobilize the body to respond to these types of challenges. The paper will also review in a chronological manner some of the PNI-related studies important to validating the sixth sense concept. Finally, the review will suggest ways to apply the new found knowledge of the sixth sense to understanding a placebo effect and developing new therapeutic approaches for treatment of human diseases.

Keywords: Psychoneuroimmunology; Neuropeptides; Neurotransmitters; Cytokines; IL-10; Interferon; CNS; Leukocytes; Vagus

1. Introduction

In 1984 Blalock proposed that the immune system also serves a sensory role, a "sixth sense" to detect things the body cannot otherwise hear, see, smell, taste or touch (Blalock, 1984). The proposition was that immune system has evolved to detect foreign entities such as pathogens, tumors, allergens with great sensitivity and specificity. Consequently, as a sensory organ it would be a means to signal and mobilize the body to respond to these challenges. But individual leukocytes are not physically connected to the nervous system, so how could such signaling work? This of course was precisely the type of question that one faced when first presenting this concept to a skeptical scientific community. Due to the format, this cannot be a fully comprehensive review so the goal is to introduce the development of the "sixth sense" concept in a historical manner and put this into perspective through selected examples of its progression and implications for the future.

1.1. Cytokine hormonal activity

The foundations for this concept began nearly a decade before with studies of the first of the yet to be named cytokines, interferon (IFN). Receptors for IFNs are ubiquitous on all cells and specialized/differentiated cells treated with IFN displayed endocrine-like stimulation (Blalock, 1989). For example, murine myocardial cells' beat frequency was enhanced by murine IFNα/β treatment just as noradrenaline would elicit. With little known about intracellular signaling pathways at the time, we assumed that the IFN receptor and adrenergic receptors shared intracellular messengers such as cAMP. It was found that IFN could mimic the actions of several factors such as β-adrenergic agonists, neurotransmitters, and neuropeptide hormones

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(corticotrophin, a.k.a. ACTH) by increasing the beat frequency of myocardial cells, enhancing the firing rate of neurons and inducing steroidogenesis in adrenal cells, respectively (Blalock, 1989). IFN treatment of Y-1 adrenal tumor cells caused them to change morphologically to a rounded shape and to enhance their corticosteroid production. Human IFN-α demonstrated opioid-like analgesic properties when injected i.c.v. into mice (Blalock and Smith, 1980, 1981). These mice had reduced sensitivity to thermal pain and would become transiently catatonic. Both effects were preventable and reversible with naloxone. IFN-α also bound to opioid receptors in murine, brain membrane preparations. This led to the prediction that IFN and maybe other cytokines would have many hormonal-like activities and result in a multitude of physiological changes. Recent studies have shown that IFN-γ and IFN-α receptors activate different components of the Jak-Stat pathway which affect many genes and could account for many of IFN's activities.

Coincidently, within this same time frame another cytokine, the first "interleukin" (IL-1) was recognized to have diverse activities including hormonal-like systemic effects. In fact one of IL-1's original names was the "endogenous pyrogen" due to its fever inducing characteristic. Treatment of AtT-20 pituitary tumor cells with IL-1 showed that it stimulated the release of ACTH on an equimolar basis as corticotrophin releasing hormone (CRH) (Woloski et al., 1985). Several studies by Berkenbosch and colleagues, Sapolsky and colleagues, plus others quickly followed further exploring IL-1's activities on hypothalamic and pituitary function (Turnbull and Rivier, 1999). Another cytokine tested in those studies, termed hepatocyte stimulating factor and later renamed IL-6 was an even more potent stimulator of ACTH production.

These "hormonal"-like effects of the first cytokines led us to speculate that similar or other central nervous system (CNS) effects would manifest themselves when high doses were used clinically. In fact, this turned out to be true and has been a major limiting factor for clinical use of cytokines.

1.2. Leukocyte production of proopiomelanocortin (POMC)

The initial studies of IFN-α's hormonal activity were complicated by the limited technology of the period. The IFN sequence at the amino acid or nucleic acid level was unknown and homogenous, recombinant DNA-based IFN was not available either. Therefore, when IFN-α was found to have ACTH-like immunoreactivity and biological activity, one possibility was that the IFN-α molecule contained an ACTH sequence (Blalock and Smith, 1980). Another possibility was that ACTH was co-produced with the IFN and present in the preparation. Biochemical approaches were used to distinguish between these possibilities (Smith and Blalock, 1981; Blalock and Smith, 1980). Bona fide ACTH is resistant to proteolysis by pepsin unlike IFN. The ACTH-like activity remained and the antiviral activity was destroyed by pepsin digestion. Thus it was likely a true ACTH peptide involved, but this experiment did not clarify the structural relationship between IFN and ACTH. Human IFN-α was cloned this same year and analysis of the DNA showed that there were no ACTH or β-endorphin sequences. Affinity purification and separation on SDS–polyacrylamide gels later showed that a 22-Kd biosynthetic intermediate of pro-opiomelanocortin (POMC) had been co-purified with the 23-Kd form of IFN-α (Smith and Blalock, 1981).

Subsequent studies established that the leukocyte ACTH and endorphin were biologically active and antigenically identical to the pituitary peptides. Thus, at the basis of the sixth sense concept these were the first signal molecules discovered to be produced, in common to the immune and neuroendocrine systems.

1.3. Discovery of neuropeptide and neurotransmitter receptors in immune functioning

During the same period of the early observations of neuropeptide production by leukocytes were reports that neuropeptide and neurotransmitter receptors were expressed on the same cells. Initially, the studies were based on functional assays and later with radioligand binding studies. One of the better characterized class of neuropeptide receptors on leukocytes are the opioid receptors (Carr et al., 1996). Wybran and colleagues in 1979 found that morphine and methionine enkephalin would inhibit rosette formation by human T-lymphocytes. T-lymphocyte frequency and antibody production could also be altered with opioids, especially natural killer cell activity. The clinical implications for functional opioid receptors on leukocytes is tremendous, particularly for understanding aspects of drug abuse or anesthesia on immune function.

Melanocortin (MC-R) receptors, particularly MC2-R, the ACTH receptor was another neuropeptide receptor found during this early period to be present and functional on leukocytes (Blalock, 1989). Again, early evidence was functional, such as ACTH stimulated B-cell growth and antibody synthesis at low concentrations. However, at high concentrations ACTH inhibited cytokine synthesis and antibody synthesis. With an "experiment of nature," it was firmly established that the prototypical adrenal gland, ACTH receptor is the same as that on leukocytes (Smith et al., 1987). Individuals who are congenitally insensitive to ACTH-mediated steroidogenesis (ACTH insensitivity syndrome) that is due to an aberrant adrenal ACTH receptor, also lack high affinity ACTH binding sites on their peripheral blood leukocytes as compared to normal individuals.

The discoveries of a great many neuropeptide, neurotransmitter and neuroendocrine hormone receptors on leukocytes have now been reported and characterized to various extents (Table 1) (Blalock, 1989). Such a diversity of receptors on leukocytes suggests that a wide range of regulatory responses could be mediated by neuropeptides and neurotransmitters in communication with the sixth sense.

Table 1
Peptide hormones and neurotransmitters produced in the immune system

ACTH[a]
Arginine vasopressin[a]
Atrial naturetic peptide
Chorionic gonadotropin
CRH[a]
Endorphins[a]
Follicle stimulating hormone
Growth hormone[a]
Insulin-like growth factor 1[a]
Luteinizing hormone
Luteinizing-hormone-releasing hormone
[Met]enkephalin[a]
Oxytocin
Prolactin[a]
Parathyroid-hormone-related protein
Substance P[a]
Thyroid-stimulating hormone
Vasoactive intestinal peptide[a]

[a] Receptors for these ligands have been characterized on leukocytes.

2. Bidirectional communication between the immune and nervous systems

These observations listed above, among others led the field to recognize that the nervous system and immune system speak a common biochemical language and communicate via a bidirectional circuit. Shared ligands such as neurotransmitters, neuroendocrine hormones, cytokines and the respective receptors regulate this circuit. The discovery of neuropeptide and neurotransmitter receptors on leukocytes and likewise receptors for cytokines on nervous and neuroendocrine cells began to explain the early, puzzling observations in the field of psychoneuroimmunology (PNI). In particular, this connection can account in part for the age-old anecdotical based notion that the mind can influence the bodies' susceptibility and resistance to disease as well as aspects of the placebo effect.

One of the most dramatic examples of the mind–immune system connection was the demonstration that an immune response could be ''conditioned'' much in the same manner as the classic experiments by Pavlov (Ader and Cohen, 1975). By pairing administration of an immunoregulatory substance as an unconditioned stimulus with an external event which is the conditioned stimulus (i.e., in the case of Pavlov, a ringing bell), the conditioned stimulus alone would alter the immune response. This was first shown by Metal'nikov and Chorine in 1926, then Ader and Cohen (1975) independently rediscovered the phenomena which in turn led to the modern phase of PNI (Ader and Cohen, 1975). Other strong evidence that supported the mind/immune system concept was the many effects of stress on immune function and the finding that behavioral characteristics can predict susceptibility to autoimmunity in an animal model of multiple sclerosis (Kavelaars et al., 1999). Taken together, these observations left no doubt that the mind could influence the immune system and suggested that the earlier anecdotal observations were real, but the mechanisms for this communication were still unknown.

That stimulation or ablation of various regions of the brain could, depending upon the region, inhibit or enhance immune responses strongly suggested that the immune system could influence the CNS or even that immunoregulatory entities resided in the CNS.

The question was how are these entities operating? Two observations serve as the groundwork for answering this question. One, it was observed that peripheral immune responses, such as antibody production could alter the firing rate of neurons in the CNS (Besedovsky et al., 1977). Secondly, the innervation of immune tissues and organs provided a conduit for such information to reach the CNS (Stevens-Felten and Bellinger, 1997). The question then becomes what is the nature and source of the information, and how and by what means is it received? This becomes clearer as previously discussed, leukocytes can produce neuropeptides such as β-endorphin and other neurotransmitters and neurons can make cytokines such as IL-1. In addition, leukocytes and the CNS have receptors for cytokines as well as neuropeptides and neurotransmitters. Therefore neurotransmitters, neuropeptides and cytokines represent the signaling molecules relaying chemical information and depending on the stimulus either neurons or immune cells can be the initial source. The chemical information in turn can be received by both neurons and leukocytes since they share receptor repertoires.

Based on these observations that the immune and nervous/neuroendocrine systems share common signal molecules and receptors, it has long been our contention that this allows the immune system to function as a sensory organ (Blalock, 1984; Blalock and Smith, 1985). A sixth sense, that completes our ability to be cognizant of agents and responses such as infections, tumors, or allergens which cannot normally be seen, touched, heard, smelled, or tasted. Recognition of these ''noncognitive stimuli'' could then be signaled to the CNS and/or neuroendocrine systems through the aforementioned common signal molecules and receptors (Fig. 1). The outcome of this recognition would be a physiological response that is beneficial to the host by optimizing the homeostatic conditions or immune response to the challenge, hence a reciprocal arm in the bidirectional communication pathway (Fig. 2). Our bias in this review has been towards IFN, CRH, and POMC pathways, on which we have done research. But it should be pointed out that in the overall picture other factors including glucocorticoid, serotonin, epinephrine/norepinephrine pathways that are not discussed would also be involved.

3. 1987–1996: molecular biology approaches support the sixth sense

3.1. Neuropeptides and neurotransmitter production by the immune system

As PNI was emerging as a field and the journal *Brain Behavior and Immunity* was launched, the newly developed molecular biology approaches were revolutionizing

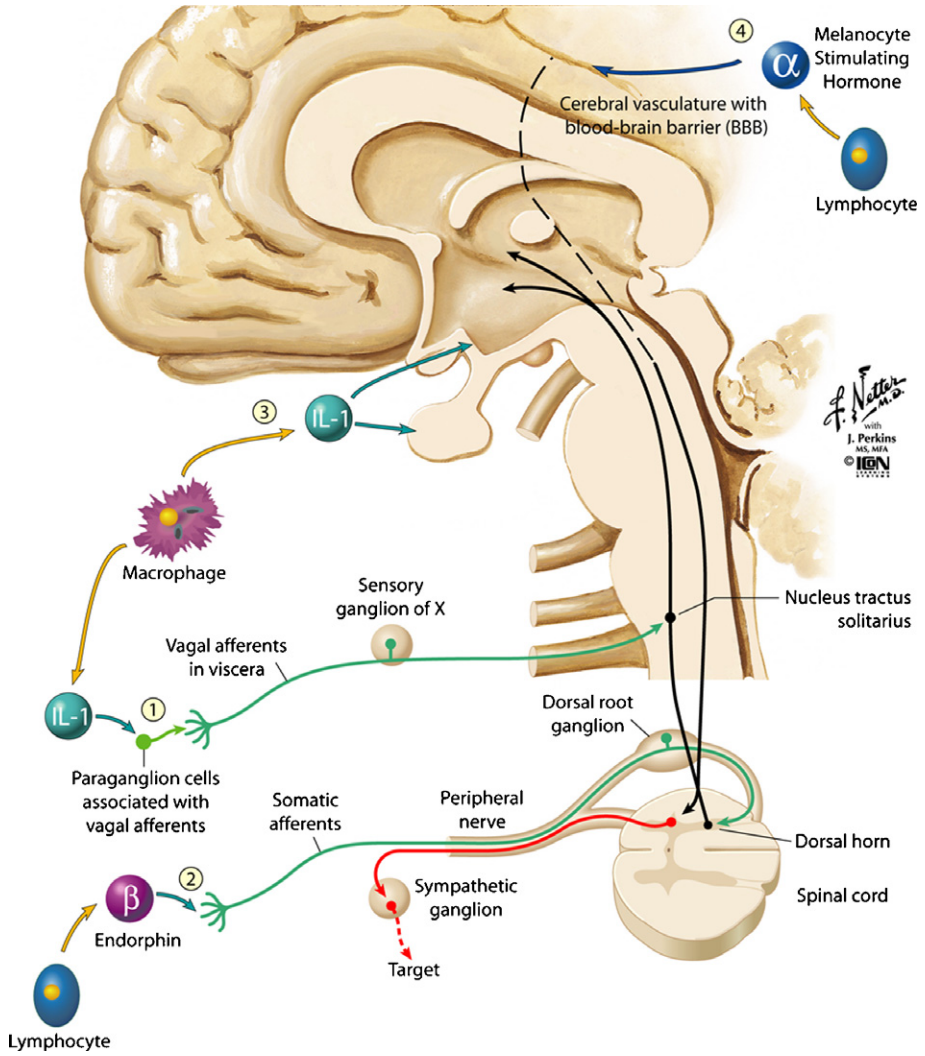

Fig. 1. Examples of afferent nerve pathways used for immunosensing. (1) Cytokines like IL-1 act on the vagus nerve to cause behavioral changes and illness symptoms. (2) Lymphocyte-derived neuropeptides such as β-endorphin modulate pain sensations by acting on peripheral sensory nerves. (3) IL-1 can act on the hypothalamus and pituitary to produce CRH and ACTH, respectively. (4) Leukocyte-derived hormones like α-melanocyte stimulating hormone (MSH) cross the BBB and affect signaling on the sympathetic nervous system. Reproduced with permission by Blackwell Publishing from reference (Blalock, 2005).

biomedical research. This had dramatic implications for determining the fidelity and specificity of neuroendocrine structures and functions in the immune system. As with many new fundamental and unexpected discoveries there had been much controversy over the finding that leukocytes produced neuropeptide hormones. At the start of this period Cobi Heijnen, Annemieke Kavelaars and their colleagues reproduced and extended many of the initial findings (Kavelaars et al., 1989). Then utilizing the burgeoning molecular biology technology Keith Kelley, Holley Westley, and colleagues together with John Funder's group (Lolait et al., 1986) plus others provided a definitive molecular foundation for the earlier observations and predictions by demonstrating POMC mRNA expression in leukocytes. Despite even this evidence the authenticity of the leukocyte neuropeptides continued to be questioned. The identity between pituitary and leukocyte POMC peptides was eventually unequivocally established by demonstrating the expression of full length POMC mRNA in leukocytes and that the amino acid and nucleotide sequences of splenic and pituitary ACTH and POMC are identical (Smith et al., 1990; Galin et al., 1991).

Another major advance in the sixth sense concept during this period was in the diversity of neuropeptides and neurotransmitters found to be produced by the immune system. This meant there were potentially many more signals and nervous or neuroendocrine system responses that could be impacted by the immune system. Many of these, such as growth hormone will be covered in depth in other articles of this series, and are only listed in Table 1. One, CRH is particularly important to the sixth sense story in regards to the leukocyte POMC production. CRH treatment of human and mouse lymphocytes was found to induce the production of POMC and ultimately its cleavage products ACTH and endorphin (Smith et al., 1986). In addition a synthetic corticosteroid, dexamethasone

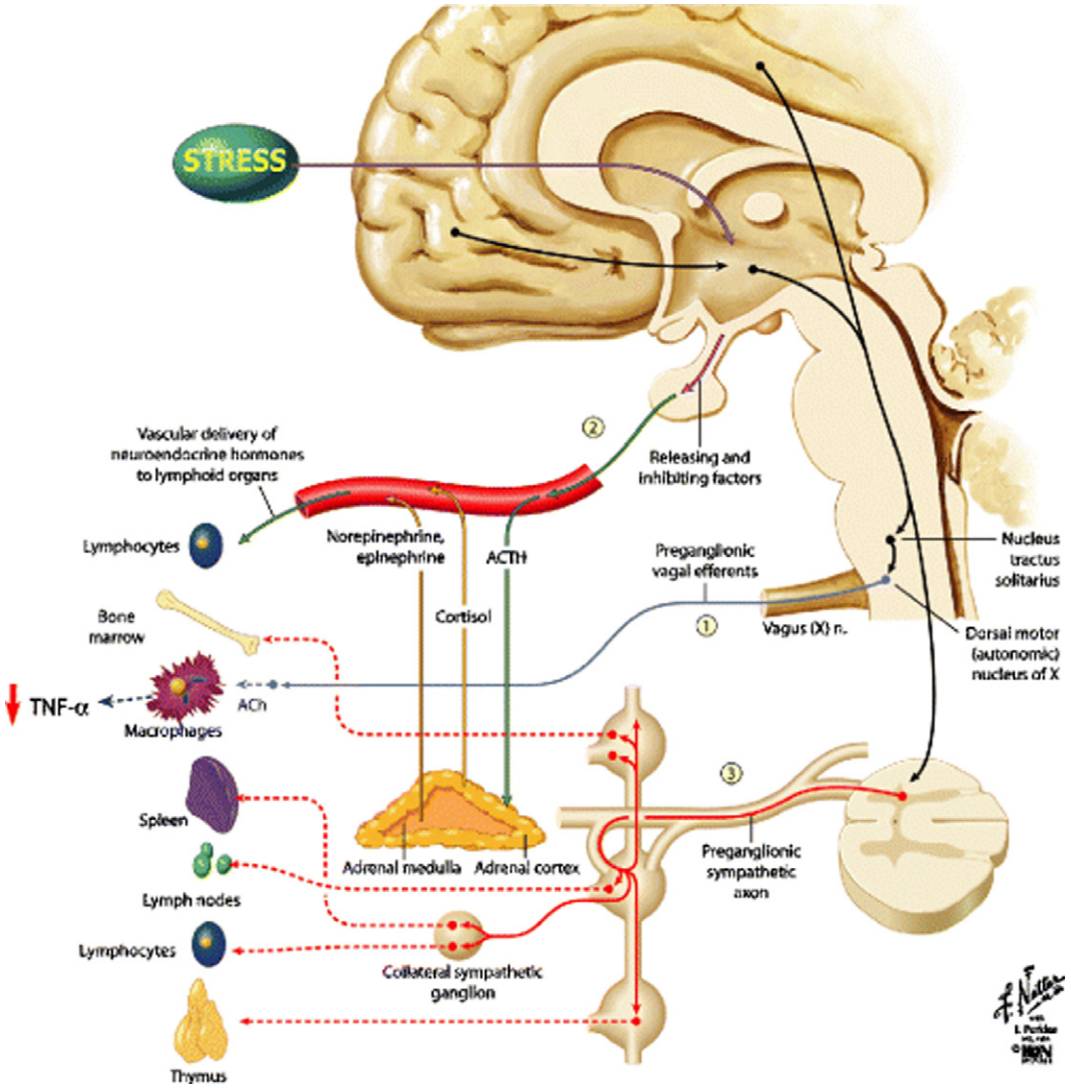

Fig. 2. Examples of efferent nerve pathways that modulate immune function. (1) Vagal acetylcholine acts on macrophages to blunt proinflammatory cytokine synthesis. (2) Hormones from the hypothalamic–pituitary–adrenal axis modulate lymphocyte function. (3) Sympathetic outflow can regulate the function of immune tissues and their cells. Reproduced with permission by Blackwell Publishing from reference (Blalock, 2005).

inhibited POMC production. Thus, leukocyte POMC regulation was similar to that in the pituitary and an example of how the neuroendocrine system could regulate the sixth sense. In 1990, Stephanou et al. reported the expression of CRH in immune tissue (Stephanou et al., 1990). Therefore, leukocytes could produce a hypothalamic releasing factor which possibly could act on the pituitary gland or other leukocytes to produce POMC and thus another shared neuropeptide demonstrating a common mode of action for both the immune and the neuroendocrine systems.

3.2. Opioid receptors

Interestingly, the finding of neuropeptide and neurotransmitter receptors on leukocytes was much better accepted than neuropeptide production by leukocytes. Perhaps it was due to the ability to measure immune responses altered by the neuropeptide hormones and neurotransmitters, which was generally easier than measuring low concentrations of neuropeptides by immunoassays. Molecular biology techniques, particularly cloning of receptors began to facilitate PNI research during this decade, but maybe even more so was the development of new and highly specific receptor antagonists. Initial studies showed functional opioid receptors on leukocytes (Carr et al., 1996). Because there are several opioid receptor types with different affinities for the same ligands, it was difficult to prove which type opioid receptor was present and functioning on leukocytes. The development of new, highly specific ligands and antagonists proved to be a major advance for the characterization of the opioid receptors on leukocytes. As an example, Carr et al. used a delta class-selective ligand ([^{3}H]*cis*-(+)-3-methylfentanyl-isothiocyanate), to specifically label a binding site with a molecular weight of 58 kDa on murine lymphocytes and P388d1 macrophage cells (Carr et al., 1989). A lymphocyte membrane protein exhibiting mu-class selectivity, also

of a molecular weight of 58 kDa could be labeled with a mu-selective, site-directed acylating agent ([^{3}H]2-Δ-ethoxybenzyl-1-[*N*,*N*-diethylamino]ethyl-5-isothiocyanato-benzimidazole). In addition to the mu and delta-opioid receptors, the kappa-selective, site-directed acylating agent [3H](1s,2s)-(−)-*trans*-2-isothiocyanato-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide, labeled a 38–42 kDa protein on lymphocytes that exhibited kappa-class selectivity.

Once the brain delta and mu opioid receptors were cloned, it provided probes to detect opioid receptor gene expression in leukocytes (Carr et al., 1996). When the amplified cDNA was sequenced, the open reading frame proved to be 98–99% identical to the brain opioid receptor sequences for both delta and mu opioid receptors. The T-lymphocyte cell line, R1.1 which had previously been shown to have a kappa receptor linked to a guanine nucleotide binding protein was found to express kappa opioid receptor mRNA. Thymocytes have also been shown to express a full length delta-opioid receptor mRNA, which has been sequenced to confirm its identity. Interestingly, using the same procedures has identified a new, ''orphan'' non-opioid receptor with a 60% sequence homology to the classic opioid receptors in murine lymphocytes and a related one in human lymphocytes. Experiments using antisense RNA determined that this orphan receptor is up regulated following cell activation and has functional significance in lymphocyte proliferation and antibody production. Thus, a major advance was made during this period using the new molecular biology techniques and high specificity reagents to confirm the earlier findings of opioid receptors on leukocytes and to accurately distinguish between the subtypes that are expressed, to establish the diversity of opioid receptors acting in the sixth sense.

3.3. Cytokines

This period was a time of great advances in understanding the role of cytokines in the sixth sense. Several trends facilitated this progress. First, this was also a time of rapid advance in identifying new cytokines, their receptors, and mechanisms of action as immune system regulators. Secondly, recombinant DNA methodology allowed production of concentrated and highly purified cytokines to test for action in the CNS and neuroendocrine system. Also, the new molecular biology techniques such as RT-PCR, in situ hybridization made it possible to detect and localize the cytokine and receptor expression in complex tissues like the CNS.

The focus of the previous decade on pro-inflammatory cytokines and the hypothalamic pituitary axis (HPA) continued, particularly in regards to IL-1, tumor necrosis factor α (TNFα), and IL-6 (Turnbull and Rivier, 1999). Radioligand binding, in situ immunohistochemical staining, and RT-PCR studies showed IL-1 receptor expression in the hippocampus and hypothalamus, although there was some controversy on which receptor subtype was expressed most and where. Also, high levels of IL-1 receptor I and II expression were demonstrated in the pituitary. Much less work focused on the presence of IL-6 or TNF receptors in neuroendocrine tissues. Receptor expression for these cytokines could be detected in the pituitary and in the brain for IL-6, but like IL-1 there was some controversy about the expression in the brain for TNF receptors. Nonetheless the studies provided anatomical substrates for the signals of the sixth sense to act on HPA and CNS activity.

The early studies of systemic administration of IFN-α and other cytokines found that it led to various side effects and these were often the limiting factor for the dosage that could be utilized. Many of these side effects were often predictable based on previous in vitro and animal studies, for example the opioid-like effects of IFN-α that were discussed above (Blalock and Smith, 1981). During the decade following 1987 there were many advances in identifying new activities of systemically and centrally administered cytokines as well as possible mechanisms for their action. In general, proinflammatory cytokines, IL-1, TNFα, and IL-6 plus IFN-α were shown to increase slow wave sleep, induce fever, decrease activity, and decrease food intake, among other effects. Hart had collectively termed these types of behaviors as ''sickness behavior'' and investigations proceeded to explore the behaviors and mechanisms causing them in response to peripheral or central cytokines (Kelley et al., 2003). A major question was how did large molecules like cytokines cross the blood–brain barrier (BBB), which was thought to be very exclusive, to affect the CNS? Although this will be covered in depth in other articles of this series, some transport of cytokines across the BBB appears to occur. However, the discovery of a vagal nerve pathway that is involved in transducing this signal from the periphery (and sixth sense) to the CNS to mediate this sickness behavior eliminates the absolute need for cytokines to cross the BBB (Fig. 1). A subdiaphragmatic vagotomy blocked pain, fever, and other sickness behaviors in response to intraperitoneal endotoxin or IL-1 which indicates afferent vagal fibers are carrying signals to the brain (Maier, 2003). IL-1 binding sites have been located on paraganglia near vagal fibers and IL-1 receptor mRNA has been detected in afferent vagal fibers. In addition, the peripheral IL-1 or electrical stimulation of the vagus induces the induction of IL-1 in the hypothalamus and hippocampus (Kelley et al., 2003; Maier, 2003). Thus, these studies indicate that nervous system is expressing in common with the immune system, cytokine receptors and cytokines which seem to transduce signals as part of the sixth sense.

4. 1997–2006: the sixth sense in action

The decade starting in 1997 has brought many more studies that supported and extended the concept of the immune system as a sixth sense. Perhaps an oversimplification, but in regards to the sixth sense concept this period seemed largely to bring more diversified and sophisticated examples rather than many new fundamental mechanisms.

Therefore this section will focus on a few representative but not exclusive examples to support the concept.

4.1. Neuropeptides and neuropeptide receptors

While other neuropeptides and neurotransmitters such as endomorphins were shown to be produced by leukocytes during this period, CRH is arguably the most important one for the sixth sense concept. Even though CRH production by leukocytes was discovered previously, it was during this period that an exciting physiologic role for it has been discovered (Rittner et al., 2005). During inflammation when tissue is destroyed or invaded by leukocytes, proalgesic mediators such as proinflammatory cytokines are released and contribute to the pain sensation. These same conditions stimulate CRH production by leukocytes among other sources and factors at the site of inflammation. CRH's first reported effect on the immune system was the induction of ACTH and endorphin production by lymphocytes (Kavelaars et al., 1989; Smith et al., 1986). Initial questions raised about leukocyte production of ACTH/POMC concerned the amount produced and whether it was sufficient for activating the adrenal glands or other function. In vivo production of CRH by leukocytes during inflammation seems to address these questions. Opioid receptors are present on the peripheral endings of sensory neurons and binding of leukocyte endorphins or other opioids can lead to a clinically relevant inhibition of pain at the site of inflammation (Rittner et al., 2005). This has been confirmed by using opioid receptor antagonists or antisera and anti-sense RNA to block CRH production. Therefore at a site in the periphery there is an immune to nervous system communication involving ligands and receptors common to both systems. This also appears to be an example of how the sixth sense can act in a beneficial manner for the host, a self-contained, localized analgesic response to counter inflammatory pain.

4.2. Cytokines

As IFN was at the foundation of the sixth sense concept, it continues to the present as an important interest as a mediator of sixth sense signaling on the CNS. There is a growing incidence of hepatitis C virus (HCV) infections worldwide. It is a particularly insidious infection that is acquired through sexual activity, contaminated needles, and decreasingly so by transfusions and transplanted tissues. The infection can remain undetected on the order of 20 years and then present as a chronic infection, liver disease and/or hepatocellular carcinoma. The current, most common treatment for chronic infection with HCV is a combination of IFN-α and the antiviral drug, ribavirin. Although an effective therapy, IFN-α is associated with the development of depression in 20–50% of patients, depending on the dose and duration of treatment (Raison et al., 2005). Similar incident rates of depression are seen in cancer patients undergoing treatment with IFN-α (Capuron et al., 2004). IFN-α therapy for HCV infection serves as an excellent, representative paradigm for the involvement of the sixth sense. First, the studies with IFN-α administered for clinical therapy and during clinical trials show a direct association with depression (Capuron et al., 2004; Raison et al., 2005). This is further validation and in humans, of IFN-α's opioid and CNS effects (Blalock and Smith, 1981; Carr et al., 1996; Wang et al., 2000). Secondly, studies have demonstrated that depressive symptoms can be associated with decreased immune function and to influence the course and outcome of a wide range of disorders, such as viral infections (Raison et al., 2005). In a preliminary study, Raison et al. found that patients who experienced significant increases in depressive symptoms during IFN-α therapy were less likely to clear the HCV (Raison et al., 2005). Therefore, this paradigm suggests the sixth sense does indeed function. The cytokine is acting on the CNS, resulting in depressive symptoms, and the CNS is feeding back to regulate the immune response. If the sixth sense is beneficial, one wonders why it works against the host (failure to clear virus), but it may be a function of super physiologic levels of IFN-α that can be administered exogenously. Alternatively and maybe more likely is that we do not understand what is or should be the beneficial effect in this situation.

Another trend during this period, as with the previous decade has been the ongoing discovery of new cytokines. Many of these are potential mediators of immune and nervous system communication and further highlight the potential diversity of the sixth sense signaling. In particular are the chemokines, a group of structurally related cytokines that mediate leukocyte trafficking especially as part of an innate immune response (Rossi and Zlotnik, 2000). The fact that many of these are expressed in the brain, a chemokine receptor is a co-receptor for the human immunodeficiency virus (HIV) and a likely factor in neuro-AIDS does emphasize the importance of this family of cytokines for neuro–immune interactions.

Another cytokine, interleukin 10 (IL-10), is synthesized by T-helper 2 (Th2) lymphocytes and among many anti-inflammatory functions, it inhibits synthesis of Th1 cytokines like IFN-γ and proinflammatory cytokines like IL-1 and TNF α (Smith et al., 2006). At the end of the previous period the production and action of IL-10 was described in pituitary cells and hypothalamus tissue (Smith et al., 2006). Here was another cytokine shared between the immune and neuroendocrine systems and another signal molecule for the sixth sense. As an anti-inflammatory cytokine that inhibits production of IL-1 and TNFα, IL-10 could serve a negative feedback role to counter proinflammatory cytokines in the CNS. In support of this idea, when administered intracerebroventricularly, IL-10 has the opposite effects as IL-1 and TNFα. The proinflammatory cytokines enhance slow wave sleep whereas IL-10 decreases it, resulting in more time awake (Smith et al., 2006). Administration of the HIV envelope protein, gp120

intracerebroventricularly initially enhances and then decreases slow wave sleep. The kinetics correspond with IL-1 mRNA expression, followed by IL-10 mRNA (Smith et al., 2006). One of the major symptoms associated with HIV infection, even prior to developing AIDS is fatigue and a disrupted sleep pattern. The gp120 studies suggest that this may be in part due to cytokine expression in the CNS. While IFN, HCV, and HIV have been our focus (or bias) in this review, other cytokines and viruses could also have been discussed in this context and will be represented in other articles of this series.

IL-10 also appears to play a role in the homeostatic functioning of the HPA axis. In vitro, IL-10 has contrasting effects on HPA functions (Smith et al., 2006). It will induce the production of CRH from hypothalamic median eminence tissue, stimulate ACTH production by the pituitary, yet it inhibits corticosteroid production by adrenal cells. The latter effect appears to be through inhibition of enzymes in the biosynthetic pathway upstream from corticosterone. In the whole animal, the inhibition on the adrenal steroidogenesis may be the major effect since IL-10 deficient mice produce higher levels of corticosterone in response to stressors.

IL-10 also plays a role in modulating another sensory function, the ability to touch or more specifically feel pain (Smith et al., 2006). Proinflammatory cytokines are known to increase nociception, the ability to sense painful stimuli. So it is not surprising that administration of exogenous IL-10 into a site of spinal cord inflammation decreased pain sensitivity (Smith et al., 2006; Ledeboer et al., 2006). Presumably this is through IL-10's anti-inflammatory effects. Conversely, IL-10 deficient mice have decreased sensitivity to both thermal and capsaicin stimuli (Smith et al., 2006). Antisera to IL-10 produced the same result of decreased nociception when injected into wild type mice. These data suggest that endogenous IL-10 increases nociception, which is in contrast to the decreased nociception observed with exogenous administration of IL-10. Clearly, the role of IL-10 in pain perception is complicated and the mechanism as to how IL-10 regulates nociception in response to inflammatory and non-inflammatory stimuli is in need of further investigation.

Taken together, the effects of immune-derived opioids and IL-10 on pain control is an example of the sixth sense integrating with one of our classic sensory systems. Perhaps that is the major contribution of this decade to the concept of the immune system as the sixth sense; that it really does belong and function with the other "classical" senses.

5. 2007 and beyond: the sixth sense in the future

What will the future bring to our understanding of the immune system as a sixth sense and may this knowledge be utilized clinically? Now that there is definitive data showing that there is diverse, powerful, and bidirectional communication between the immune and nervous systems it should be possible to take advantage of it for purposes such as resistance to disease or enhanced anti-tumor therapy.

A common theme seems to be rapidly emerging as the sixth sense is better understood. An understanding of the physiology and pathophysiology that results from this bidirectional communication will have a revolutionary impact on the understanding and practice of medicine that is not unlike what has resulted from deeper insight into other components of the nervous system. That understanding led to circuit specific drugs like those now used for Parkinson's disease, depression, etc. Furthermore it may reveal many of the deep secrets of our everyday experiences, anecdotal experiences, and mechanisms of Eastern or other alternative medicines that have defied explanation. A case in point concerns a new interpretation of an earlier observation that CRH induced pituitary corticotrophs to be sensitive to IL-1 (Payne et al., 1994). Ensuing with the original report of IL-1 induced ACTH release from a pituitary cell line (Woloski et al., 1985) and subsequent confirmations with primary pituitary cell cultures, a controversy raged over whether in vivo IL-1 mediated ACTH increase originated at the level of the hypothalamus or pituitary gland (Turnbull and Rivier, 1999). The consensus of studies concluded that ACTH release occurs as a result of IL-1 stimulating CRH release from the hypothalamus and that CRH is entirely responsible for pituitary ACTH secretion (Turnbull and Rivier, 1999). It was difficult to reconcile this view with the abundance of IL-1 receptors in the pituitary gland as compared to the hypothalamus and the observation that CRH up regulated IL-1 receptors on AtT20 cells suggested a solution to this paradox (Webster et al., 1991). Perhaps any direct response of the pituitary to IL-1 is CRH-dependent, possibly through up regulation of IL-1 receptors. This could explain many of the inconsistencies such as complete inhibition of ACTH release when antiserum against CRH or a CRH receptor antagonist is administered with IL-1 (Turnbull and Rivier, 1999). Something that blocked CRH would also in turn block IL-1 direct effects by preventing the receptor upregulation. In the study by Payne et al. (1994) it was shown that administration of the phosphate buffered saline (PBS) vehicle control mediated an IL-1 induced ACTH similar to that of a low dose of CRH. Experimental controls suggested it was CRH stimulated by the PBS administration. Therefore, this was essentially a placebo effect, but one now with a molecular explanation (Fig. 3) (Blalock, 2005).

But beyond the academic knowledge, can understanding the communication between the immune and nervous systems have practical or clinical applications? There seems to be a wealth of possibilities, more so than can be covered in a limited review. A few examples come immediately to mind. The first, relates to the observation of IFN-α's opioid-activity (Blalock and Smith, 1981), and the identification of distinct sites within the IFN-α molecule that bestow different properties upon the cytokine (Wang et al., 2000). Mutation of a single amino acid residue from Tyr to Ser knocks out almost entirely the antiviral/immunoregulatory activity

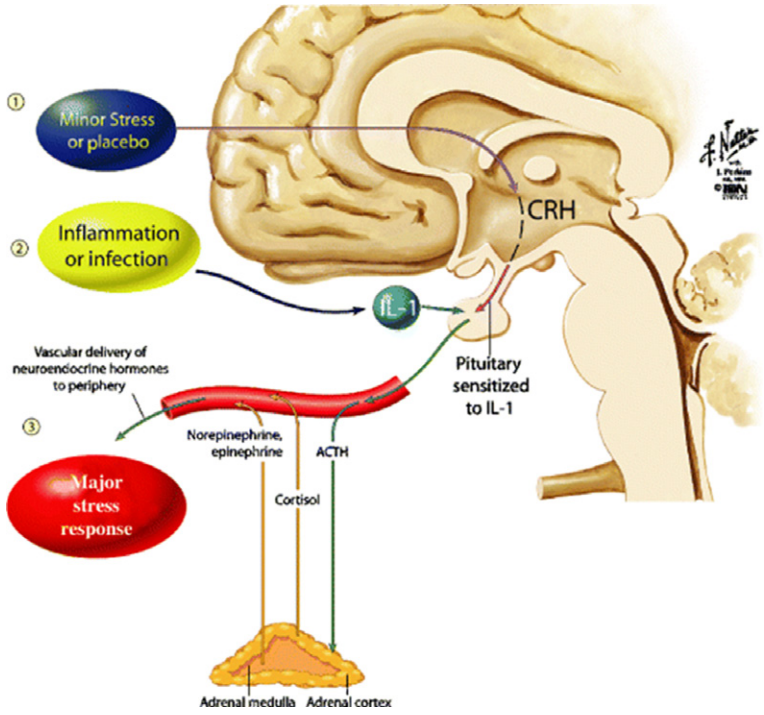

Fig. 3. Crossroads of the Classic Senses and the Sixth Sense. (1) Mild, perhaps imperceptible, stress, or a placebo cause the hypothalamus to release CRH in quantities too low to evoke pituitary ACTH release but sufficient to upregulate pituitary IL-1 receptors. (2) Coincident or subsequent inflammation or infections elicits IL-1 which acts directly on the pituitary to cause ACTH release and (3) a stress response that is above and beyond what would be expected for the level of inflammatory stress. Reproduced with permission by Blackwell Publishing from reference (Blalock, 2005).

but not the analgesic activity. Conversely, mutation of a different residue, the 122nd Tyr was changed to Ser, eliminated the analgesic activity but not the antiviral activity. This sets the stage for "designer cytokines." For example, an IFN-α without its principal side effects (i.e. fever, fatigue, and often flu-like symptoms) but with antiviral activity. Higher doses of this designer IFN-α might then be used for more effective treatment of tumors and hepatitis C. Or an IFN-α without the immunoregulatory activity that could still bind opioid receptors as a possible new analgesic. A related development is a form of erythropoietin that has lost hematopoietic activity but retains its potent ability to confer neuroprotection in many pathologies (Leist et al., 2004). Such an erythropoietin might be effective in the treatment of stroke and other neurological disorders.

Possible novel uses for known drugs and medical devices also seem likely in light of the immune and nervous system connection. For an example of a novel drug use, an opioid receptor antagonist was shown to be more effective than cyclosporin in prolonging rat renal allograft survival (Arakawa et al., 1992). The discovery that acetylcholine released from the efferent vagus nerve can act on macrophages to block inflammation and shock in an apparent anti-inflammatory pathway (Fig. 2) has identified a new therapeutic approach to treat patients in septic shock (Pavlov and Tracey, 2005). The studies discussed previously on how IL-1 acts through the vagal nerve to mediate sickness behavior and CNS effects, suggests the afferent vagal pathway is also important. Vagus nerve stimulation with a small electronic pacemaker-like device is a safe, effective, and approved therapy for epilepsy and possibly depression. Perhaps this type of device will also prove useful in controlling chronic inflammatory diseases via activation of the cholinergic pathway and to inhibit inflammation or related symptoms. An investigative drug, tetravalent guanylhydrazone (CNI-1493) has been found to cross the blood barrier and it specifically activated this vagal cholinergic pathway and inhibited inflammation (Pavlov and Tracey, 2005). CNI-1493 is currently undergoing Phase II clinical trials for treatment of Crohn's disease and may be useful in many other inflammatory conditions. Supportive evidence of the acetylcholine effect is that the receptor agonist, nicotine also reduces the severity of ulcerative colitis (Pavlov and Tracey, 2005). Similarly, anti-inflammatory effects of α-melanocyte stimulating hormone and salicylates are in part mediated through a sympathetic efferent route which might be further exploited (Ichiyama et al., 1999). The production of neuropeptide hormones and their receptors by leukocytes offers interesting possibilities for diagnosis. Since it is difficult to biopsy nervous and neuroendocrine tissues, the circulating leukocytes represent a surrogate means to access these unobtainable factors. As an example, there was no high affinity ACTH receptor binding on leukocytes of individuals with ACTH insensitivity syndrome which is characterized by defective ACTH receptors (Smith et al., 1987).

Others, including Nobel Laureate immunologists have noted similarities between the immune and nervous system in terms of the number of cells, combinatorial complexity

and memory. In fact, analogies have even been drawn between the immune system, language and grammar (Jerne, 1985). The "immunologic synapse" is an accepted concept and the eminent immunologist Mark M. Davis recently rephrased the now familiar idea with the statement "Lymphocytes and NK cells can be viewed as cell-sized sensory organs, continuously sampling the internal environment for things that do not belong there or for cellular stress or aberrations. Just as rod cells in the eye can detect even a single photon, cytotoxic T-cells can kill on the advice of only three peptide-MHC ligands" (Davis, 2004). The data discussed above describing shared ligands and receptors common to both the immune and nervous systems makes this metaphor very appropriate indeed. What was once viewed as outside the realm of sensory perception is now "seen" otherwise by the sixth sense.

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Due to the limitations of a brief review it was unfortunate that we could not credit all the original papers and had to rely on reviews so that readers may refer to the original studies. The authors wish to thank our many colleagues, Kley Hughes, Ken Bost, Dan Carr, Doug Weigent, Bob LeBoeuf, and Shawn Galin for the crucial role they played in the development of this research area. Thanks also to Diane Weigent for her help with the manuscript preparation. This work was supported in part by grants from the National Institutes of Health (HL077783 and HL68806 to J.E.B. and NS41495 to E.M.S.) and the John Sealy Memorial Endowment Fund of the University of Texas Medical Branch.

References

Ader, R., Cohen, N., 1975. Behaviorally conditioned immunosuppression. Psychosom. Med. 37, 333–340.

Arakawa, K., Akami, T., Okamoto, M., Oka, T., Nagase, H., Matsumoto, S., 1992. The immunosuppressive effect of delta-opioid receptor antagonist on rat renal allograft survival. Transplantation 53 (4), 951–953.

Besedovsky, H., Sorkin, E., Felix, D., Haas, H., 1977. Hypothalamic changes during the immune response. Eur. J. Immunol. 7, 323–325.

Blalock, J.E., 1984. The immune system as a sensory organ. J. Immunol. 132, 1067–1070.

Blalock, J.E., 1989. A molecular basis for bidirectional communication between the immune and neuroendocrine systems. Physiol. Rev. 69, 1–32.

Blalock, J.E., 2005. The immune system as the sixth sense. J. Int. Med. 257, 126–138.

Blalock, J.E., Smith, E.M., 1980. Human leukocyte interferon: structural and biological relatedness to adrenocorticotropic hormone and endorphins. Proc. Natl. Acad. Sci. USA 77, 5972–5974.

Blalock, J.E., Smith, E.M., 1981. Human leukocyte interferon (HuIFN-alpha): Potent endorphin-like opioid activity. Biochem. Biophys. Res. Commun. 101, 472–478.

Blalock, J.E., Smith, E.M., 1985. The immune system: our mobile brain? Immunol. Today 4, 115–117.

Capuron, L., Ravaud, A., Miller, A.H., Dantzer, R., 2004. Baseline mood and psychosocial characteristics of patients developing depressive symptoms during interleukin-2 and/or interferon-alpha cancer therapy. Brain Behav. Immun. 18, 205–213.

Carr, D.J., DeCosta, B.R., Kim, C.H., Jacobson, A.E., Guarcello, V., Rice, K.C., Blalock, J.E., 1989. Opioid receptors on cells of the immune system: evidence for delta- and kappa-classes. J. Endocrinol. 122, 161–168.

Carr, D.J., Rogers, T.J., Weber, R.J., 1996. The relevance of opioids and opioid receptors on immunocompetence and immune homeostasis. Proc. Soc. Exp. Biol. Med. 213, 248–257.

Davis, M.M., 2004. Panning for T-cell gold. The Scientist 18, 28–29.

Galin, F.S., LeBoeuf, R.D., Blalock, J.E., 1991. Corticotropin-releasing factor upregulates expression of two truncated pro-opiomelanocortin transcripts in murine lymphocytes. J. Neuroimmunol. 31, 51–58.

Ichiyama, T., Sakai, T., Catania, A., Barsh, G.S., Furukawa, S., Lipton, J.M., 1999. Inhibition of peripheral NF-[kappa]B activation by central action of [alpha]-melanocyte-stimulating hormone. J. Neuroimmunol. 99, 211–217.

Jerne, N.K., 1985. The generative grammar of the immune system. Science 229, 1057–1059.

Kavelaars, A., Ballieux, R.E., Heijnen, C.J., 1989. The role of IL-1 in the corticotropin-releasing factor and arginine-vasopressin-induced secretion of immunoreactive beta- endorphin by human peripheral blood mononuclear cells. J. Immunol. 142, 2338–2342.

Kavelaars, A., Heijnen, C.J., Tennekes, R., Bruggink, J.E., Koolhaas, J.M., 1999. Individual behavioral characteristics of wild-type rats predict susceptibility to experimental autoimmune encephalomyelitis. Brain Behav. Immun. 13, 279–286.

Kelley, K.W., Bluthe, R.M., Dantzer, R., Zhou, J.H., Shen, W.H., Johnson, R.W., Broussard, S.R., 2003. Cytokine-induced sickness behavior. Brain Behav. Immun. 17 Suppl 1, S112–S118.

Ledeboer, A., Jekich, B.M., Sloane, E.M., Langer, S.J., Milligan, E.D., Mahoney, J.H., Maier, S.F., Leinwand, L.A., Chavez, R.A., Watkins, L.R., Johnson, K.W., 2006. Novel approaches to control paclitaxel-induced neuropathic pain: Efficacy of interleukin-10 gene therapy and the blood-brain barrier permeable glial modulator, AV411. Brain Behav. Immun. 20, 43.

Leist, M., Ghezzi, P., Grasso, G., Bianchi, R., Villa, P., Fratelli, M., Savino, C., Bianchi, M., Nielsen, J., Gerwien, J., Kallunki, P., Larsen, A.K., Helboe, L., Christensen, S., Pedersen, L.O., Nielsen, M., Torup, L., Sager, T., Sfacteria, A., Erbayraktar, S., Erbayraktar, Z., Gokmen, N., Yilmaz, O., Cerami-Hand, C., Xie, Q.w., Coleman, T., Cerami, A., Brines, M., 2004. Derivatives of erythropoietin that are tissue protective but not erythropoietic. Science 305, 239–242.

Lolait, S.J., Clements, J.A., Markwick, A.J., Cheng, C., McNally, M., Smith, A.I., Funder, J.W., 1986. Pro-opiomelanocortin messenger ribonucleic acid and posttranslational processing of beta endorphin in spleen macrophages. J. Clin. Invest. 77, 1776–1779.

Maier, S.F., 2003. Bi-directional immune-brain communication: implications for understanding stress, pain, and cognition. Brain Behav. Immun. 17, 69–85.

Pavlov, V.A., Tracey, K.J., 2005. The cholinergic anti-inflammatory pathway. Brain Behav. Immun. 19, 493–499.

Payne, L.C., Weigent, D.A., Blalock, J.E., 1994. Induction of pituitary sensitivity to interleukin-1: a new function for corticotropin-releasing hormone. Biochem. Biophys. Res. Commun. 198, 480–484.

Raison, C.L., Broadwell, S.D., Borisov, A.S., Manatunga, A.K., Capuron, L., Woolwine, B.J., Jacobson, I.M., Nemeroff, C.B., Miller, A.H., 2005. Depressive symptoms and viral clearance in patients receiving interferon-[alpha] and ribavirin for hepatitis C. Brain Behav. Immun. 19, 23–27.

Rittner, H.L., Machelska, H., Stein, C., 2005. Leukocytes in the regulation of pain and analgesia. J. Leukoc. Biol. 78, 1215–1222.

Rossi, D., Zlotnik, A., 2000. The biology of chemokines and their receptors. Annu. Rev. Immunol. 18, 217–242.

Smith, E.M., Blalock, J.E., 1981. Human lymphocyte production of corticotropin and endorphin-like substances: association with leukocyte interferon. Proc. Natl. Acad. Sci. USA 78, 7530–7534.

Smith, E.M., Brosnan, P., Meyer, W.J., Blalock, J.E., 1987. An ACTH receptor on human mononuclear leukocytes. Relation to adrenal ACTH-receptor activity.. N. Engl. J. Med. 317, 1266–1269.

Smith, E.M., Galin, F.S., LeBoeuf, R.D., Coppenhaver, D.H., Harbour, D.V., Blalock, J.E., 1990. Nucleotide and amino acid sequence of lymphocyte-derived corticotropin: endotoxin induction of a truncated peptide. Proc. Natl. Acad. Sci. USA 87, 1057–1060.

Smith, E.M., Morrill, A.C., Meyer III, W.J., Blalock, J.E., 1986. Corticotropin releasing factor induction of leukocyte-derived immunoreactive ACTH and endorphins. Nature 322, 881–882.

Smith, E.M., Tu, H., Hughes, T.K., 2006. Interleukin-10 and the hypothalamic–pituitary–adrenal axis. In: Plotnikoff, N.P., Faith, R.E., Murgo, A.J. (Eds.), Cytokines Stress and Immunity, second ed. CRC Press, Boca Raton, pp. 167–190.

Stephanou, A., Jessop, D.S., Knight, R.A., Lightman, S.L., 1990. Corticotrophin-releasing factor-like immunoreactivity and mRNA in human leukocytes. Brain Behav. Immun. 4, 67–73.

Stevens-Felten, S.Y., Bellinger, D.L., 1997. Noradrenergic and peptidergic innervation of lymphoid organs. Chem. Immunol. 69, 99–131.

Turnbull, A.V., Rivier, C., 1999. Regulation of the hypothalamic–pituitary–adrenal axis by cytokines: actions and mechanisms of action. Physiol. Rev. 79, 1–71.

Wang, Y.X., Jiang, C.L., Lu, C.L., Song, L.X., You, Z.D., Shao, X.Y., Cui, R.Y., Liu, X.Y., 2000. Distinct domains of IFN[alpha] mediate immune and analgesic effects respectively. J. Neuroimmunol. 108, 64–67.

Webster, E., Tracey, D., De Souza, E.B., 1991. Upregulation of interleukin-1 receptors in mouse AtT-20 pituitary tumor cells following treatment with corticotropin- releasing factor. Endocrinology 129, 2796–2798.

Westly, H.J., Kleiss, A.J., Kelley, K.W., Wong, P.K., Yuen, P.H., 1986. Newcastle disease virus-infected splenocytes express the proopiomelanocortin gene. J. Exp. Med. 163, 1589–1594.

Woloski, B.M., Smith, E.M., Meyer III, W.J., Fuller, G.M., Blalock, J.E., 1985. Corticotropin-releasing activity of monokines. Science 230, 1035–1037.