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*J Clin Invest.* 1997;100(12):2941-2947. <https://doi.org/10.1172/JCI119846>.

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# Perspectives Series: Cytokines and the Brain

## Pathways and Mechanisms for Cytokine Signaling of the Central Nervous System

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Early in the field of study of neural-immune interactions there was significant doubt that the peripheral immune system could signal the brain. The assumption was that large molecules such as cytokines (15–20-kD proteins) could not cross the blood-brain barrier (BBB,<sup>1</sup> an anatomical and functional separation between brain parenchyma and peripheral tissues consisting of the vascular endothelium, basement membrane, neuroglial membrane, and glial perivascular feet, see Fig. 1). It was initially thought that cytokines could not easily enter the central nervous system (CNS) to affect their target sites. However, recent work has demonstrated several routes by which peripheral cytokines can either directly cross the BBB or indirectly signal the brain through other informational substances.

There are three known routes by which peripheral cytokines can exert their effects on the brain: (a) peripheral tissues, which are innervated by the peripheral and autonomic nervous systems, can send direct signals to the brain via peripheral nerves; (b) brain vasculature can convey signals through secondary messengers, such as nitric oxide (NO) or prostanooids, produced in response to cytokines; and (c) cytokines can directly act at the level of the brain parenchyma after crossing the BBB or after entering brain areas that lack a BBB. Cytokine action at these sites, which are not mutually exclusive, may depend on the location and route of exposure to inflammatory stimuli and the disease state of the organism. This *Perspective* considers recent advances in our understanding of cytokine action at these sites.

Peripheral tissues, including immune organs, are innervated by the autonomic and peripheral nervous system. Peripheral autonomic nerves can send an immune signal to the brain. It has been shown that subdiaphragmatic, but not hepatic vagotomy, blocks intraperitoneal IL-1 $\beta$ -induced hypothalamic norepinephrine depletion and attenuates intraperitoneal IL-1 $\beta$ -induced increases in serum corticosterone (1). Subdiaphragmatic vagal transection attenuates the acquisition and facilitation

tates the extinction of conditioned taste aversions induced by intraperitoneal IL-1 $\beta$  or TNF- $\alpha$  administration (2). Vagotomy also attenuates the depression in social exploration induced by intraperitoneal, but not intracerebroventricular, administration of IL-1 $\beta$ , indicating that vagal afferent nerves are involved in the transmission of an immune message from the periphery to the brain, but that vagotomy does not impair the direct sensitivity of the brain itself to immune signals (3). In these studies, initial questions were raised regarding the mechanism by which cytokines could activate the vagus nerve. Recent studies indicate that IL-1 receptors, as indicated by binding studies, exist in whole vagus (abdominal, laryngeal, and thoracic) and in the hepatic segment of the vagus (4). These receptors appear to be located on dendritic-like cells interdigitating in the nerve parenchyma, as well as in cells within the paranganglia around the vagus. The similarity of these para- and intraneuronal collections of immune cells to the well-described components of the mucosal immune system, the “GALT” (gastrointestinal-associated lymphoid tissue) and “BALT” (bronchial-associated lymphoid tissue), have led Maier and Watkins (4) to suggest that this vagal-related immune tissue might be termed “NALT” for neural-associated lymphoid tissue. Whether this intriguing concept of an interface tissue between the immune and nervous system turns out to be representative of a larger, more generalizable neural-related peripheral immune tissue remains to be proven.

The vagal route by which cytokines within the peritoneal cavity can signal the brain may not apply to circulating cytokines. An important mechanism by which circulating cytokines may affect CNS functioning without crossing the BBB is by acting at the level of brain vasculature (Fig. 1). Cytokine activation of blood vessels in the brain results in the production of informational substances, such as prostaglandins or NO, that can mediate the effects of immune molecules on brain function. In this regard, Hashimoto et al. (5) showed that biologically active, gold-labeled IL-1 binds to the vascular wall, in the region of the anteroventral third ventricle. Those authors concluded that the binding of circulating IL-1 on the endothelium of that region acts as the initial step to fever induction.

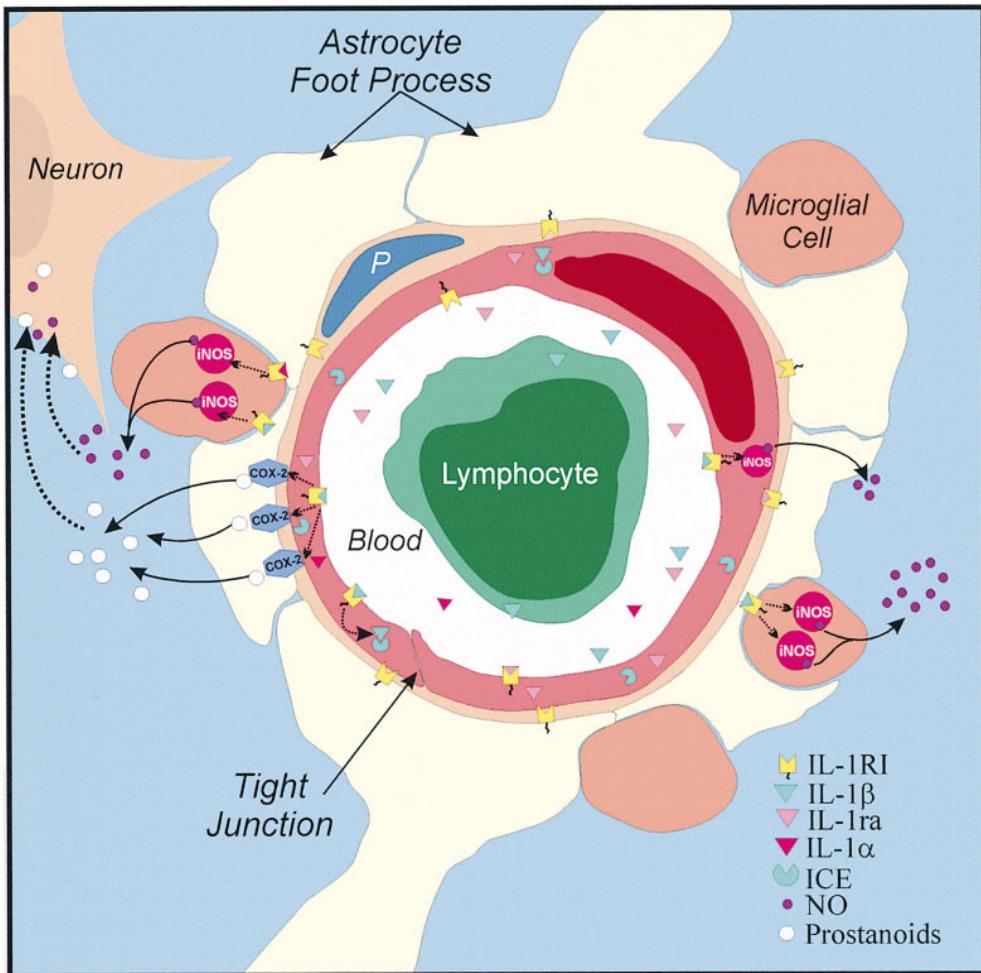
IL-1 action is regulated by a complex network of molecules that include multiple ligands (IL-1 $\alpha$ , IL-1 $\beta$ , and IL-1 receptor antagonist [IL-1ra]), several binding sites (IL-1 receptor type I [IL-1RI], IL-1RII, IL-1 accessory protein, soluble receptors, and autoantibodies), and a key regulatory enzyme (IL-1 $\beta$  converting enzyme [ICE], also known as caspase 1). We have found that the genes encoding IL-1 $\alpha$ , IL-1 $\beta$ , ICE, IL-1ra, and IL-1RI are constitutively expressed in blood vessels (6, 7), and that after peripheral LPS injection, there is induction of IL-1 $\beta$  mRNA within brain vasculature associated with high levels of vascular and perivascular inducible NO synthase (iNOS) mRNA expression (8). Based on these findings, we have concluded that the vascular and perivascular induction of iNOS

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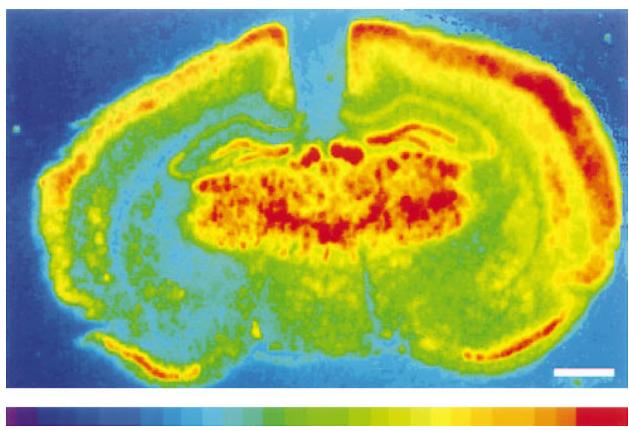
Received for publication 18 November 1997.

1. Abbreviations used in this paper: BBB, blood-brain barrier; CNS, central nervous system; COX-2, cyclooxygenase 2; ICE, IL-1 $\beta$  converting enzyme; IL-1ra, IL-1 receptor antagonist; IL-1RI, IL-1 receptor type I; iNOS, inducible NO synthase; NO, nitric oxide; PVN, paraventricular nucleus; SCF, stem cell factor.

The Journal of Clinical Investigation  
Volume 100, Number 12, December 1997, 2941–2947  
<http://www.jci.org>



**Figure 1.** Transduction of cytokine-mediated signals from the blood to neurons. This diagram shows that circulating or endothelial cytokines can transduce a signal to neurons through informational substances such as NO or prostanoids that are synthesized by enzymes (iNOS and COX-2) whose transcription is induced by IL-1. Note that circulating IL-1 $\beta$  can activate the endothelium to synthesize additional IL-1 $\beta$ , leading to signal amplification. Thus, an IL-1-mediated signal can be conveyed from the blood stream into neurons, without the need for IL-1 $\beta$  to cross the BBB. *P*, Pericyte. Illustration by Naba Bora, Medical College of Georgia.



**Figure 2.** Constitutive localization of stem cell factor (SCF) mRNA in adult rat brain. SCF, which promotes the growth of multilineage hematopoietic cells, is a product of the *steel* (*Sl*) locus of the mouse, and it is a ligand for the *c-kit* protooncogene receptor. Using *in situ* hybridization histochemistry, we have found that the gene encoding SCF is actively expressed in neuron-like cells in the thalamus, cerebral cortex, cerebellum, and hippocampus, particularly in the dentate gyrus, but also in CA<sub>1</sub>, CA<sub>2</sub>, and CA<sub>3</sub>; dark blue denotes areas of lowest hybridization, and red shows areas with the highest levels of SCF mRNA (15). Dyskeratosis congenita is a rare human disease, which has been attributed to dysregulation of SCF function, causing anemia, abnormal calcifications, skin alterations, and mental retardation; dys-

mRNA by IL-1 $\beta$  might represent a mechanism for the modulation of the CNS effects of circulating inflammatory mediators (Fig. 1). IL-1 has been shown to induce NOS mRNA and NO production in vascular cells in vitro and to induce NOS in vivo. The effects of IL-1 on iNOS mRNA in vascular cells are stimulated by tyrosine kinase, potentiated by eicosapentaenoic acid, and inhibited by angiotensin II, actinomycin D, cycloheximide, TGF- $\beta$ 1, and IGF-1. Moreover, IL-1 induces the expression of cyclooxygenase-2 (COX-2) mRNA, prolongs the half-life of that mRNA, and increases the functional activity of COX-2, the rate-limiting enzyme in the conversion of arachidonic acid to prostanoids (Fig. 1). The effects of IL-1 on COX are mediated via NO, and are inhibited by glucocorticoids (for review see reference 7).

Actions of cytokines at the vascular level in the brain may be of pathophysiological relevance, as there is increasing evidence that the IL-1 system may activate cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM), E-selectin, and complement regulatory proteins, and may also cause platelet adhe-

function of SCF in the brain may be related to the biological basis of the mental retardation associated with dyskeratosis congenita. Bar, 0.14 cm.

sion, possibly leading to vascular occlusion and stroke. Platelet adhesion to human vascular endothelium is modulated by constitutive and IL-1-induced NO. ICAM-1 can be upregulated by IL-1 to a greater degree in hypertensive rat brain endothelial cells, when compared with cells from normotensive rats; therefore, differential sensitivity to the effects of IL-1 in vascular cells may predispose to atherosclerosis and stroke. This concept is supported by the finding that IL-1 $\alpha$  protein, measured by immunohistochemistry, is consistently overexpressed in atherosclerotic human blood vessels (9). Thus, functional, pathological, and neuroanatomical data, such as the localization of the mRNAs encoding several components of the IL-1 system within brain vasculature, suggest that constitutive vascular IL-1 may have a role in early pathophysiological events, ultimately leading to atherosclerosis and stroke (for review see reference 7). We have shown that IL-1ra is constitutively localized in the brain (10) and that immune neutralization of IL-1 bioactivity results in markedly increased stroke volume after middle cerebral artery occlusion (MCAo) (11); we concluded that IL-1ra is an endogenous neuroprotective agent. IL-1ra treatment inhibits IL-1-induced endothelial cell activation and induction of adhesion molecules (12). Those functional data and the finding of constitutive IL-1ra mRNA in blood vessels in the brain have led us to suggest that IL-1ra might be an endogenous vascular protective agent (7).

In addition to these indirect routes by which cytokines signal the CNS, peripheral cytokines may actually reach brain parenchyma by crossing the BBB. Recent data showing significant quantities of human recombinant IL-1 $\alpha$ , but not murine IL-1 $\alpha$ , in mouse cortex after subcutaneous injection, strongly suggest that such direct access of peripheral cytokines to the brain can play a role in the central effects of cytokines (13). Even in healthy basal conditions, cytokines may enter the brain at areas that lack a tight BBB. Those areas are collectively referred to as the circumventricular organs. Included in this designation are the pineal body, the subcommissural organ, the subfornical organ, the organum vasculosum of the lamina terminalis (OVLT), the median eminence, the neurohypophysis, and the area postrema. Cytokines can also be carried across the BBB by active transport (13). Such transport is saturable and specific for individual cytokines, does not apply to all cytokines (13), and is not modified by dexamethasone, morphine, indomethacin, or  $\alpha$ -melanocyte stimulating hormone (14). However, the degree to which this mechanism can explain all the central effects of cytokines is still not determined.

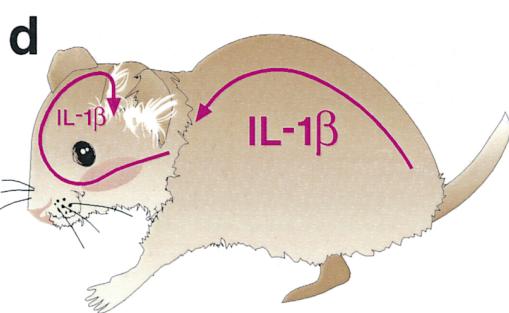
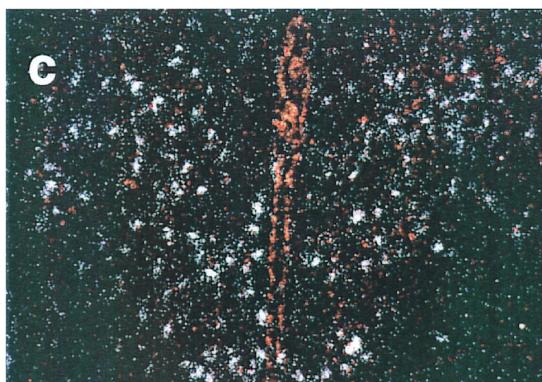
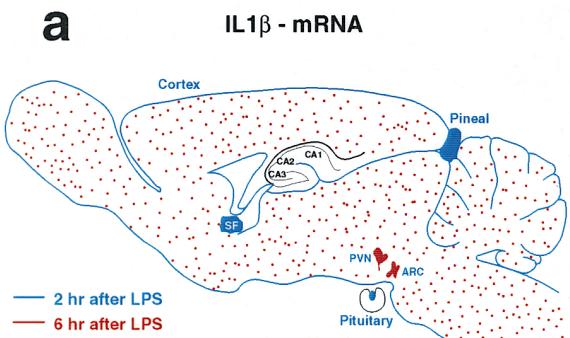
The functioning of the CNS during systemic inflammation is modulated not only by cytokines originating from peripheral sites, but also by cytokines that are synthesized by the brain. The genes encoding for a variety of cytokines and their receptors, initially identified in the peripheral immune system, are constitutively expressed in the brain, indicating that some cytokines may contribute to the normal functioning of the CNS (Fig. 2 and reference 15) (for review see reference 16). Moreover, peripheral cytokines also cause the synthesis of cytokines within the brain. After peripheral LPS injection, TNF- $\alpha$  mRNA is initially induced in perivascular cells, meningeal cells, and neurons in circumventricular organ neurons and ventral surface of the medulla. This is followed by TNF- $\alpha$  mRNA induction in paraventricular and arcuate hypothalamic nuclei and the nucleus of the solitary tract (17). Our group and others have shown that during the course of systemic inflammation

the gene encoding for IL-1 $\beta$  is likewise expressed in high levels in the brain first in areas of the brain that lack a BBB, such as the subfornical organ, and subsequently within brain parenchyma in areas that have an intact BBB, such as paraventricular and arcuate nuclei of the hypothalamus (Fig. 3).

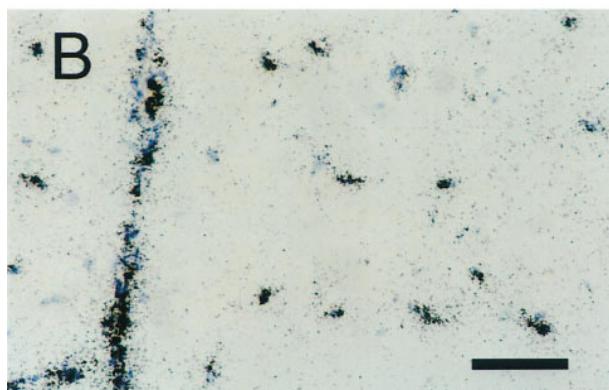
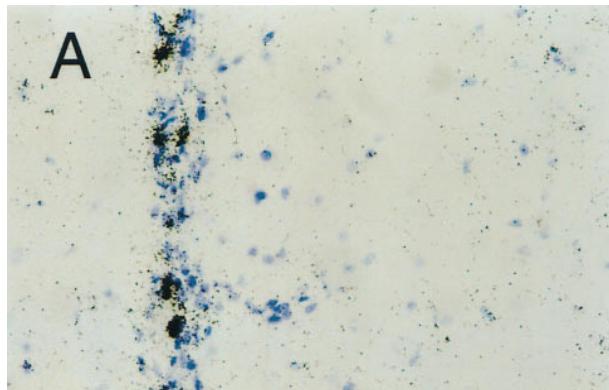
Other second messengers may also modulate the diffusion of a cytokine-mediated signal from barrier areas into brain parenchyma. The NF- $\kappa$ B/I $\kappa$ B system is involved in the cellular responsiveness to immune system molecules. The NF- $\kappa$ B family of transcription factors is a primary regulatory component of the intracellular signal pathways in cells of the immune system (these factors respond to immune challenges by increased gene transcription). Furthermore, the expression of IK $\kappa$ B mRNA parallels both NF- $\kappa$ B activity and the duration of extracellular activation. This temporal parallelism between IK $\kappa$ B mRNA expression and the duration of extracellular stimulation is different from other transcription factors, such as *c-fos*, that are transiently expressed only at the onset of cellular stimulation. For those reasons, a neuroanatomical study has used IK $\kappa$ B $\alpha$  induction to infer the extent and cellular localization of immune responsiveness within the brain after peripheral immune stimulation (Fig. 4). The finding of IK $\kappa$ B $\alpha$  mRNA induction beginning in cells lining the blood side of the BBB and progressing to cells inside the brain supports the hypothesis that cells of the BBB synthesize immune signal molecules, such as NO (18) or prostanooids (19), to activate cells inside the CNS in response to peripheral inflammatory stimuli (20).

Saper and colleagues have proposed that cytokines produced by a cascade of neurons (and glial cells) within the brain may participate in the complex autonomic, neuroendocrine, metabolic, and behavioral responses to infection and inflammation (21). However, it should be emphasized that, in contrast to the periphery, the genes encoding cytokines that decrease IL-1 bioactivity, such as IL-1ra, IL-10, and IL-13, are expressed in the brain in levels that are far lower than those of IL-1 $\beta$ . Thus, the peripheral and central cytokine compartments appear to be integrated but differentially regulated.

The concept that cytokines acting within the brain may regulate key elements of the CNS response to peripheral inflammation has been substantiated by the discovery that those responses can be attenuated or even in some cases abolished by central administration of IL-1ra. The induction of CRH gene expression in the paraventricular nucleus (PVN) of the hypothalamus in response to peripheral LPS administration can be abolished by central (but not peripheral) administration of IL-1ra and suggests that the actions of IL-1ra within the brain are required for CRH mRNA induction during peripheral inflammation (22). Likewise, in mice, IL-1 $\beta$  administered centrally or peripherally causes a decrease in social behavior and loss of body weight that can be attenuated by pretreatment with intracerebroventricular administration of IL-1ra, indicating that those effects of peripheral IL-1 $\beta$  are centrally mediated (23). However, central blockade of IL-1 receptors by intracerebroventricular IL-1ra does not completely abrogate the effects of intraperitoneal LPS on social behavior in rats, indicating that other cytokines can compensate for the lack of action of IL-1 in the brain of LPS-treated animals. This possibility has been confirmed by the demonstration that mice deficient for IL-1RI no longer respond to intracerebroventricular rat IL-1 $\beta$ , but are still sensitive to the central effects of intracerebroventricular LPS on social behavior. Their sensitivity to intraperitoneal LPS is due to TNF- $\alpha$  because pretreatment with intracere-



**Figure 3.** IL-1 $\beta$  gene expression in the brain during systemic inflammation. In an experimental model of sepsis (26) we used *in situ* hybridization histochemistry to show a distinct pattern of IL-1 $\beta$  gene expression in the brain. At baseline we found no constitutive IL-1 $\beta$



**Figure 4.** Bright-field photomicrographs of emulsion-coated sections of rat cingulate cortex showing localization of I $\kappa$ B mRNA over cells at 0.5 h (A) or 2 h (B) after an intraperitoneal injection of 2.5 mg/kg LPS. Nissl counterstain shows blue staining of the endothelial and small glial cells of the brain and interhemispheric sulcus. Accumulations of silver grains mark I $\kappa$ B mRNA-positive cells in the large blood vessels of the sulcus at 0.5 h and positive cells (mostly endothelia and occasional astrocytes) throughout the brain parenchyma at 2 h. Bar, 50  $\mu$ m. Figure provided by N. Quan, M. Whiteside, L. Kim, and M. Herkenham (20).

broventricular TNF- $\alpha$  binding protein (TNF- $\alpha$ bp) abrogates the depressing effects of intraperitoneal LPS on social behavior in IL-1RI $^{-/-}$  mice (Bluthé, R.M., and R. Dantzer, personal communication). Mice that are deficient in IL-6 are less sensitive to the depressing effects of both IL-1 and LPS on social behavior when these molecules were administered either in-

mRNA in brain parenchyma. (a) Using hybridization with a  $^{35}$ S-labeled IL-1 $\beta$  riboprobe, we show that 2 h after intraperitoneal LPS administration, IL-1 $\beta$  mRNA is found in meninges, neurohypophysis, pineal gland, and circumventricular organs; at 6 h, IL-1 $\beta$  mRNA is found in a diffuse pattern in glial structures throughout the brain and also in neuronal structures such as the paraventricular and arcuate nuclei of the hypothalamus. (b) Dark-field photomicrograph of subfornical organ 2 h after intraperitoneal endotoxin injection. (c) Dark-field photomicrograph of PVN 6 h after intraperitoneal endotoxin administration. In b and c white dots represent silver grains overlying IL-1 $\beta$  mRNA; bar, 78  $\mu$ m. (d) Cartoon showing that fever in a mouse can be caused by a sequence of events: peripheral IL-1 $\beta$  induction results in IL-1 $\beta$  production in brain areas that lack BBB (b), followed by IL-1 $\beta$  synthesis in areas of the brain with intact BBB (c).

traperitoneally or intracerebroventricularly, and their sensitivity to these treatments was entirely due to IL-1 as pretreatment with intracerebroventricular IL-1ra abrogates the depressing effects of intraperitoneal LPS on social behavior in IL-6<sup>-/-</sup> mice (Bluthé, R.M., and R. Dantzer, personal communication). Thus, a complex cascade of cytokines that include IL-1, IL-6, and TNF- $\alpha$  is expressed in the brain to modulate the central effects of peripheral inflammatory mediators. Additionally, peripheral LPS administration causes protracted increases in locus ceruleus firing rates, that can be attenuated by microinfusion of IL-1ra directly into the locus ceruleus (Borsody, M., and J. Weiss, personal communication). These different lines of evidence seem to indicate that IL-1 within the brain acts during peripheral inflammation to mediate sickness behaviors induced by peripheral cytokines. However, because the signal-transducing IL-1RI has not been identified in brain areas whose activities are modulated by IL-1ra, it is difficult to explain how central IL-1 might be in mediating these effects at the cellular and molecular levels.

Thus, the key question in the field remains unanswered. In response to peripheral inflammation, peripheral cytokines such as IL-1 $\beta$  are synthesized and may circulate in the blood stream. This is followed by the synthesis of IL-1 $\beta$  in key areas of the brain, such as the PVN, which potently respond to that cytokine; however, IL-1 receptors do not seem to be present in those areas. We can propose three explanations for this interesting paradox. First, it is possible that novel IL-1 receptors exist in brain. However, despite considerable effort by several groups, such receptors have not been identified. Second, because IL-1 receptors are downregulated by inflammation, it is possible that existing studies have not identified IL-1RI in the PVN because previous studies were not specifically designed to rigorously eliminate infection or inflammation as a confounding variable. Alternatively, it is possible that IL-1 $\beta$ , acting in (a) areas that send inputs to the PVN, (b) areas that are adjacent to the PVN, or (c) at the level of brain vasculature within the PVN, might result in the generation of other informational molecules, such as prostanoids or NO, that would modulate the effects of IL-1 on PVN function. Thus, it is possible that IL-1 synthesis and actions may occur in areas, such as the PVN, that apparently lack IL-1 receptors. IL-1 binding to receptors localized in sites other than PVN neurons would stimulate the production of prostanoids or NO; those molecules would then mediate the effects of IL-1 $\beta$  on PVN neurons.

IL-1 $\beta$  synthesized by the PVN might not act directly at that site, and could be instead secreted into hypophyseal portal blood as a hypothalamic neurohormone that regulates pituitary function. IL-1 $\beta$ , at levels that are achieved in the circulation during inflammation, directly stimulates pituitary cells to secrete ACTH, LH, GH, and TSH, and inhibits the secretion of prolactin (24). These pituitary effects have led Bernton and colleagues (25) to propose that IL-1, acting directly at several sites that include the pituitary gland, may be an important regulator of the metabolic adaptations to infectious stressors. The pituitary is particularly responsive to IL-1 $\beta$  during stress or inflammation because CRH, a hormone that is synthesized by the PVN and secreted into hypophyseal portal blood, induces the expression of IL-1RI receptors in the pituitary gland (24), thereby increasing the pituitary responsiveness to IL-1 $\beta$ .

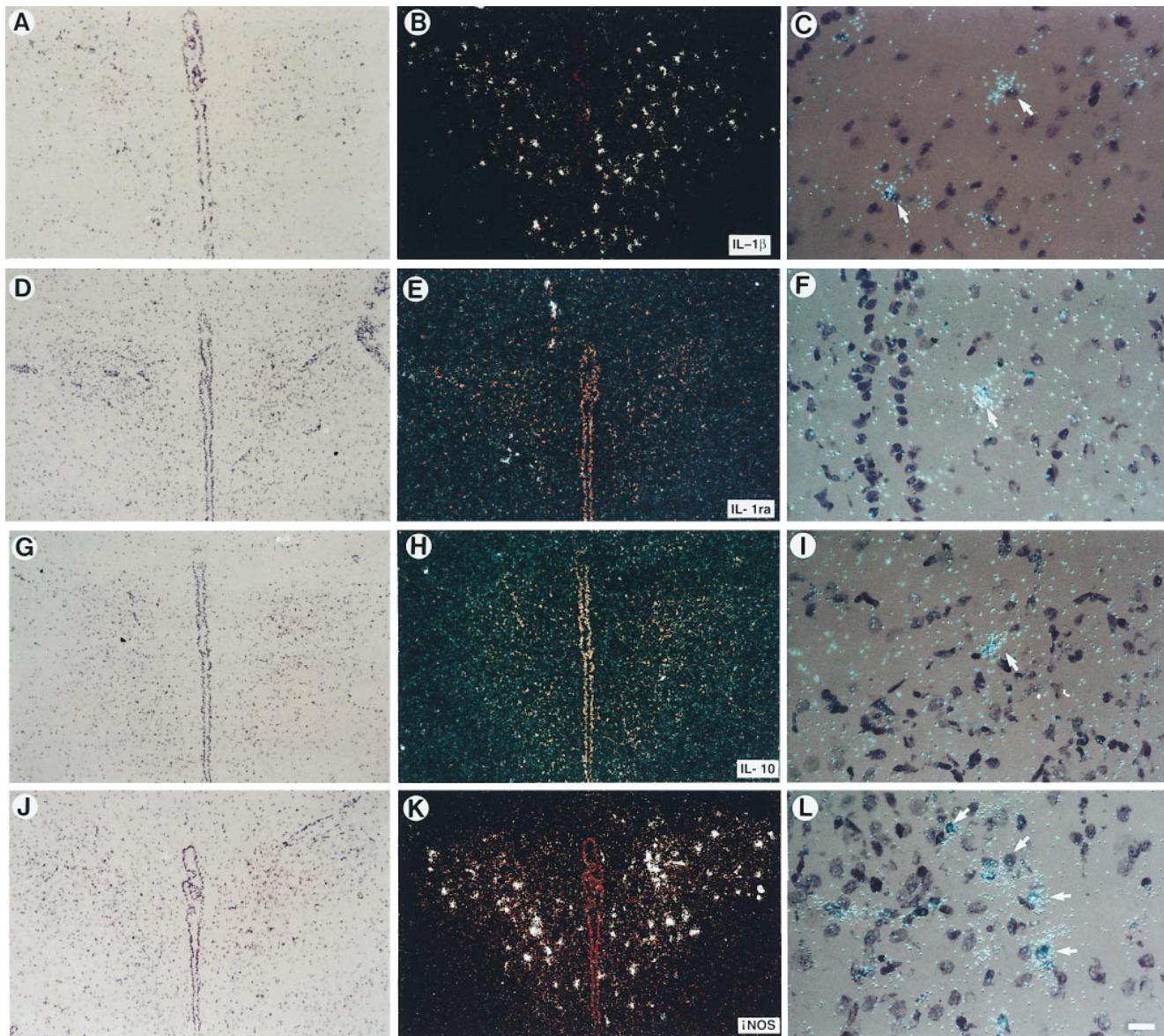
We have shown that during systemic inflammation, key areas that drain to the anterior pituitary, such as PVN, arcuate

nucleus, median eminence, and posterior pituitary, all express high levels of IL-1 $\beta$  mRNA; in the same study we showed that the anterior pituitary expresses moderate levels of IL-1 $\beta$  followed by very high levels of expression of the mRNA for the secreted isoform of IL-1ra, sIL-1ra, which is known to be induced by IL-1 $\beta$ . Therefore, we have proposed that the pituitary secretion of IL-1ra might represent a novel systemic hormonal antiinflammatory mechanism that is elicited during systemic inflammation by IL-1 $\beta$  that reaches the anterior pituitary originating from multiple sources that include PVN, arcuate nucleus, median eminence, posterior pituitary, systemic circulation, and the anterior pituitary itself (26, 27).

It has been demonstrated recently by Reichlin and colleagues (28) that the brain and/or its supporting structures are activated by intracerebroventricular IL-1 $\beta$  to release IL-6 into the blood, and that such an effect is not dependent on peripheral sympathetic activity or central mobilization of CRH. Direct secretion of IL-6 and possibly of other cytokines from the brain has been postulated to be a pathway of neuroimmuno-modulation (28) in much the same way that the hormonal cascade released from the hypothalamic-pituitary-adrenal axis modulates peripheral endocrine effects. Thus, the relationship between peripheral and central cytokines is such that peripheral cytokines, originating from immune cells, affect the functioning of the brain, and central cytokines may be secreted by the brain to modulate peripheral immune function.

The pronounced expression of IL-1 $\beta$  within the brain during systemic inflammation, in the context of limited expression of the genes encoding for cytokines that limit IL-1 bioactivity, such as IL-1ra, IL-10, and IL-13, should result in marked actions of IL-1 $\beta$  within the brain (Fig. 5). That hypothesis has been supported by the fact that systemic inflammation is associated with alterations in temperature, neuroendocrine function, and behavior, phenomena that could be attributed to the effects of cytokines such as IL-1 $\beta$  in the brain. In the brain, IL-1 $\beta$  and TNF- $\alpha$  are potent stimuli for iNOS production (29). We have shown that at baseline there is no detectable iNOS gene expression in the brain, but a detailed neuroanatomical study revealed that early in the course of systemic inflammation there is a profound induction of iNOS mRNA in vascular, glial, and neuronal structures of the rat brain. Two neuronal hypothalamic nuclei showed strikingly high induction of iNOS mRNA: the PVN (Fig. 5) and the arcuate nucleus. iNOS mRNA levels were also markedly induced in both endocrine glands that are situated in close proximity to the brain, the pituitary and the pineal. During systemic inflammation, iNOS gene expression was accompanied by the production of NO metabolites in brain parenchyma and cerebrospinal fluid (8). We have proposed that the spillover of nitrites into cerebrospinal fluid has the potential to be a diagnostic marker for systemic inflammation and sepsis. Thus, the metabolic by-products of cytokine action within the brain may be of diagnostic relevance in human disease.

Serious technical problems, which have been overcome only recently, have limited progress in the study of the central effects of peripheral immune mediators. Those include limited availability of reagents and the confounding effects of experimental conditions. Cytokines and their receptors were first identified, purified, and cloned by immunologists. The preferred animal used in preclinical studies in immunology is the mouse, although human immune tissue can be easily obtained. Thus, cytokines and their receptors were first cloned in murine



**Figure 5.** Expression of the genes encoding IL-1 $\beta$  (A–C), IL-1ra (D–F), IL-10 (G–I), and iNOS (J–L) in the PVN of the rat hypothalamus 6 h after intraperitoneal LPS administration. Images in the first column show the histology of the PVN for each row. The second column shows dark-field microscopy, and the third column shows bright-field, high-magnification photomicrographs, with mRNA localization at the cellular level. Silver grains overlying mRNA are identified by white dots on the second column and by blue dots in the third column (arrows). Figures represent peak of mRNA induction. Marked induction of IL-1 $\beta$  mRNA (B and C), in the context of limited induction of IL-1ra (E and F) and IL-10 (H and I), is associated with high levels of iNOS gene expression (K and L). Bar, 126  $\mu$ M in the first and second columns; bar, 15  $\mu$ M in the third column.

and human tissues. Recombinant molecules for cytokines and their receptors, as well as molecular probes, monoclonal and polyclonal antibodies, and immunoassays were all first developed for human or murine cytokines. However, due to its bigger brain and larger blood volume, the experimental animal of choice for neurobiologists has traditionally been the rat. Even though human and murine cytokines are bioactive in the rat, molecular and immune probes directed against murine or human cytokines do not work in the rat due to species differences in the order of 20–30% in the structure of these molecules. Rat cytokines have been cloned just recently, and commercially available immunoassays for rat cytokines have reached the

market only within the last year. An additional key technical point is related to the confounding effects of stress and infection. Both stress and infection can induce cytokine gene expression in the brain (for review see reference 7). Immunological work is not usually controlled for level of animal stress. On the other hand, neurobiological work, conducted in conventional animal facilities, is usually not controlled for infection. Thus, conflicting results on the localization of cytokine gene expression in the brain have been reported in studies that did not rigorously control for the confounding factors of stress and infection. Animal work in this field is only credible, and reproducible, if conducted in virus- and antibody-free animals that

are housed in carefully monitored animal facilities and which are subjected to experimental procedures specifically designed to eliminate stress and infection as confounding variables. Additional factors that have limited the progress of research in this field include the virtual absence of stable, nonpeptide agonists and antagonists, the very low level of constitutive expression of most cytokines and their receptors, and discrepancies between bio- and immunoassays due to the presence of naturally occurring inhibitors, binding proteins, and soluble receptors in tissues and extracellular fluids (30).

Very rapid progress has been achieved recently in our understanding of the mechanisms by which peripheral immune mediators can affect the functioning of the brain through various routes and mechanisms. The integration and differential regulation of central and peripheral cytokine compartments is a key element for the optimal functioning of the immune and nervous systems. Furthermore, it is relevant for the pathophysiology not only of diseases that have been conceptualized traditionally as disorders of the peripheral immune system, but also for brain disorders such as multiple sclerosis, stroke, brain trauma, neuroAIDS, Alzheimer's disease, and psychiatric disorders (31).

## Acknowledgments

We are grateful to Dr. Esther Sternberg for her comments.

This work has been supported in part by grants from the Stanley Foundation (J. Licinio) and from NARSAD (M.-L. Wong).

## References

1. Fleshner, M., L.E. Goehler, J. Hermann, J.K. Relton, S.F. Maier, and L.R. Watkins. 1995. Interleukin-1 beta induced corticosterone elevation and hypothalamic NE depletion is vagally mediated. *Brain Res Bull.* 37:605–610.
2. Goehler, L.E., C.R. Busch, N. Tartaglia, J. Relton, D. Sisk, S.F. Maier, and L.R. Watkins. 1995. Blockade of cytokine induced conditioned taste aversion by subdiaphragmatic vagotomy: further evidence for vagal mediation of immune-brain communication. *Neurosci. Lett.* 185:163–166.
3. Bluthé, R.-M., B. Michaud, K.W. Kelley, and R. Dantzer. 1996. Vagotomy blocks behavioural effects of interleukin-1 injected via the intraperitoneal route but not via other systemic routes. *NeuroReport.* 7:2823–2827.
4. Goehler, L.E., J.K. Relton, D. Dripps, R. Kiechle, N. Tartaglia, S.F. Maier, and L.R. Watkins. 1997. Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Res Bull.* 43:357–364.
5. Hashimoto, M., Y. Ishikawa, S. Yokota, F. Goto, T. Bando, Y. Sakakibara, and M. Iriki. 1991. Action site of circulating interleukin-1 on the rabbit brain. *Brain Res.* 540:217–223.
6. Wong, M.-L., and J. Licinio. 1994. Localization of interleukin 1 type I receptor mRNA in rat brain. *Neuroimmunomodulation.* 1:110–115.
7. Wong, M.-L., P.B. Bongiorno, P.W. Gold, and J. Licinio. 1995. Localization of interleukin-1 beta converting enzyme mRNA in rat brain vasculature: evidence that the genes encoding the interleukin-1 system are constitutively expressed in brain blood vessels. Pathophysiological implications. *Neuroimmunomodulation.* 2:141–148.
8. Wong, M.-L., V. Rettori, A. Al-Shekhlee, P.B. Bongiorno, G. Canteros, S.M. McCann, P.W. Gold, and J. Licinio. 1996. Inducible nitric oxide synthase gene expression in the brain during systemic inflammation. *Nat. Med.* 2:581–584.
9. Brody, J.I., N.J. Pickering, D.M. Capuzzi, G.B. Fink, C.A. Can, and F. Gomez. 1992. Interleukin-1 alpha as a factor in occlusive vascular disease. *Am. J. Clin. Pathol.* 97:8–13.
10. Licinio, J., M.-L. Wong, and P.W. Gold. 1991. Localization of interleukin-1 receptor antagonist mRNA in rat brain. *Endocrinology.* 129:562–564.
11. Loddick, S.A., M.-L. Wong, P.B. Bongiorno, P.W. Gold, J. Licinio, and N.J. Rothwell. 1997. Endogenous interleukin-1 receptor antagonist is neuroprotective. *Biochem. Biophys. Res. Commun.* 234:211–215.
12. Bertini, R., M. Sironi, I. Martin-Padura, F. Colotta, S. Rambaldi, S. Bernasconi, P. Ghezzi, S.J. Haskill, D. Liu, and A. Mantovani. 1992. Inhibitory effect of recombinant intracellular interleukin 1 receptor antagonist on endothelial cell activation. *Cytokine.* 4:44–47.
13. Banks, W.A., and A.J. Kastin. 1997. Relative contributions of peripheral and central sources to levels of IL-1 $\alpha$  in the cerebral cortex of mice: assessment with species-specific enzyme immunoassays. *J. Neuroimmunol.* 79:22–28.
14. Banks, W.A., L. Ortiz, S.R. Plotkin, and A.J. Kastin. 1991. Human interleukin (IL) 1 alpha, murine IL-1 alpha and murine IL-1 beta are transported from blood to brain in the mouse by a shared saturable mechanism. *J. Pharmacol. Exp. Ther.* 259:988–996.
15. Wong, M.-L., and J. Licinio. 1994. Localization of stem cell factor mRNA in adult rat hippocampus. *Neuroimmunomodulation.* 1:181–187.
16. Wong, M.-L., A. Al-Shekhlee, P.W. Gold, and J. Licinio. 1996. Cytokines in the brain. In *Cytokines in the Nervous System.* N.J. Rothwell, editor. Landers Bioscience Publishers, Georgetown, TX. 3–20.
17. Breder, C.D., M. Tsujimoto, Y. Terano, D.W. Scott, and C.B. Saper. 1993. Distribution and characterization of tumor necrosis factor-alpha-like immunoreactivity in the murine central nervous system. *J. Comp. Neurol.* 337:543–567.
18. Wong, M.-L., P.B. Bongiorno, A. Al-Shekhlee, A. Esposito, P. Khatri, and J. Licinio. 1996. IL-1 $\beta$ , IL-1 receptor type I, and iNOS gene expression in rat brain vasculature and perivascular areas. *NeuroReport.* 7:2445–2448.
19. Saper, C.B. 1994. The neurologic basis of fever. *N. Engl. J. Med.* 330:1880–1886.
20. Quan, N., M. Whiteside, L. Kim, and M. Herkenham. 1997. Induction of inhibitory factor kB mRNA in the central nervous system after peripheral lipopolysaccharide administration: an *in situ* hybridization study in the rat. *Proc. Natl. Acad. Sci. USA.* 94:10985–10990.
21. Breder, C.D., C. Hazuka, T. Ghayur, C. Klug, M. Huginin, K. Yasuda, M. Teng, and C.B. Saper. 1994. Regional induction of tumor necrosis factor alpha expression in the mouse brain after systemic lipopolysaccharide administration. *Proc. Natl. Acad. Sci. USA.* 91:11393–11397.
22. Kakucska, I., Y. Qi, B.D. Clark, and R.M. Lechan. 1993. Endotoxin-induced corticotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus is mediated centrally by interleukin-1. *Endocrinology.* 133:815–821.
23. Bluthé, R.M., R. Dantzer, and K.W. Kelley. 1997. Central mediation of the effects of interleukin-1 on social exploration and body weight in mice. *Psychoneuroendocrinology.* 22:1–11.
24. Takao, T., C. Tojo, T. Nishioka, S. Makino, K. Hashimoto, and E.B. De Souza. 1996. Stress-induced upregulation of pituitary interleukin-1 receptors is mediated by corticotropin releasing factor. *Life Sci.* 59:PL165–PL168.
25. Bernton, E.W., J.E. Beach, J.W. Holaday, R.C. Smallridge, and H.G. Fein. 1987. Release of multiple hormones by a direct action of interleukin-1 on pituitary cells. *Science.* 238:519–521.
26. Wong, M.-L., P.B. Bongiorno, V. Rettori, S.M. McCann, and J. Licinio. 1997. Interleukin (IL) 1 $\beta$ , IL-1 receptor antagonist, IL-10, and IL-13 gene expression in the central nervous system and anterior pituitary during systemic inflammation: pathophysiological implications. *Proc. Natl. Acad. Sci. USA.* 94:227–232.
27. Licinio, J., and M.-L. Wong. 1997. Interleukin 1 receptor antagonist gene expression in rat pituitary in the systemic inflammatory response syndrome: pathophysiological implications. *Mol. Psychiatry.* 2:99–103.
28. Romero, L.I., I. Kakucska, R.M. Lechan, and S. Reichlin. 1996. Interleukin-6 (IL-6) is secreted from the brain after intracerebroventricular injection of IL-1 beta in rats. *Am. J. Physiol.* 270:R518–R524.
29. Romero, L.I., J.B. Tatro, J.A. Field, and S. Reichlin. 1996. Roles of IL-1 and TNF-alpha in endotoxin-induced activation of nitric oxide synthase in cultured rat brain cells. *Am. J. Physiol.* 270:R326–R332.
30. Rothwell, N.J., G. Luheshi, and S. Toulmond. 1996. Cytokines and their receptors in the central nervous system: physiology, pharmacology, and pathology. *Pharmacol. Ther.* 69:85–95.
31. Licinio, J., J.P. Seibyl, M. Altemus, D.S. Charney, and J.H. Krystal. 1993. Elevated CSF levels of interleukin-2 in neuroleptic-free schizophrenic patients. *Am. J. Psychiatry.* 150:1408–1410.