MINIREVIEW

How the Blood Talks to the Brain Parenchyma and the Paraventricular Nucleus of the Hypothalamus During Systemic Inflammatory and Infectious Stimuli (44459)

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Abstract. **There are exciting new developments regarding the molecular mechanisms involved in the influence of circulating proinflammatory molecules within cells of the blood-brain barrier (BBB) during systemic immune challenges. These molecules, when present in the circulation, have the ability to trigger a series of events in cascade, leading to either the mitogen-activated protein (MAP) kinases/nuclear factor kappa B (NF-κB) or the janus kinase (JAK)/signal transducer and activator of transcription (STAT) transduction pathways in vascular-associated cells of the central nervous system (CNS). The brain blood vessels exhibit both constitutive and induced expression of receptors for different proinflammatory ligands that have the ability to stimulate these signaling molecules. Depending on the challenges and the cytokines involved, the transduction signal(s) solicited in cells of the BBB may orient the neuronal activity in a very specific manner in activating the transcription and production of soluble factors, such as prostaglandins (PGs). It is interesting to note that cytokines as well as systemic localized inflammation stimulate the cells of the BBB in a nonselective manner (i.e., within both large blood vessels and small capillaries across the brain). This nonselectivity raises several questions with regard to the localized neuronal activation induced by different experimental models of inflammation and cytokines. It is possible that the selectivity of the neuronal response is a consequence of the fine interaction between nonparenchymal synthesis of soluble mediators and expression of specific receptors for these ligands within parenchymal elements of different brain nuclei. This review will present the recent developments on this concept and the mechanisms that take place in cells of the BBB, which lead to the neuronal circuits involved in restoring the body's homeostasis during systemic immunogenic challenges. The induction of fever, the hypothalamic-pituitary adrenal (HPA) axis, and other autonomic functions are among the physiological outcomes necessary for the protection of the mammalian organism in the presence of foreign material.** [P.S.E.B.M. 2000, Vol 223]

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The discovery that functional bilateral pathways exist between the immune and neuroendocrine systems has been one of the most fascinating developments in better understanding the regulation of the homeostatic balance of living organisms challenged by antigens. Physical, emotional, and environmental stimuli, including infection, can harm body integrity, and a complex network operating at the different levels of the brain coordinates the appropriate metabolic, behavioral, and endocrine changes necessary for the restoration of homeostasis.

Bacterial Endotoxins and Signaling Events in the Brain

Proinflammatory cytokines are produced by different cells of myeloid lineage upon presentation of an antigen, and their secretion into the bloodstream is believed to be the key step in the neuronal activity and the subsequent neurophysiological responses that take place during immune stimuli. However, this depends on the type, the cytokine(s) involved, and the severity of the challenge, which may overlap the progressive activation of macrophages, neutrophils, and lymphocytes reflected by the circulating levels of different proinflammatory molecules. Systemic injection with the endotoxin lipopolysaccharide (LPS) is a good example of this concept; the component of the outer membrane of Gram-negative bacteria is a powerful immune challenge associated with an increase in the circulating levels of different cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 (1). Bacterial toxins constitute a group of many virulent factors by which bacteria cause disease, and their interaction with the host's immune system, in particular by inducing the release of cytokines, has been the object of a large number of studies. The endotoxin LPS is capable of inducing fever, septic shock, and the acute-phase response in several animal species, including humans (1). This treatment (2–7) also causes a wide expression of the immediate *early* genes (IEGs) c-*fos* and nerve growth factor inducible gene B (NGFI-B) mRNA (indexes of cellular activation) throughout the rat brain, suggesting a complex and redundant neuronal circuitry involved to activate the endocrine hypothalamus and interfere with other systems (see Fig. 1).

The theory that LPS-induced neuronal activity *via* the endogeneous production of circulating cytokines remains controversial and once again dependent on the dose and the route of administration. Although systemic injections of IL-1β, IL-6, or TNF-α are capable of mimicking the effects of LPS on different physiological responses (8–13), proinflammatory cytokines of systemic origin may not be essential for the effects of LPS on neuronal activation, the corticotrophic axis and increase in body temperature. Despite some differences between studies for the exact temporal profile of secretion and detection, numerous physiological responses to the bacterial endotoxin precede detection of cytokines into

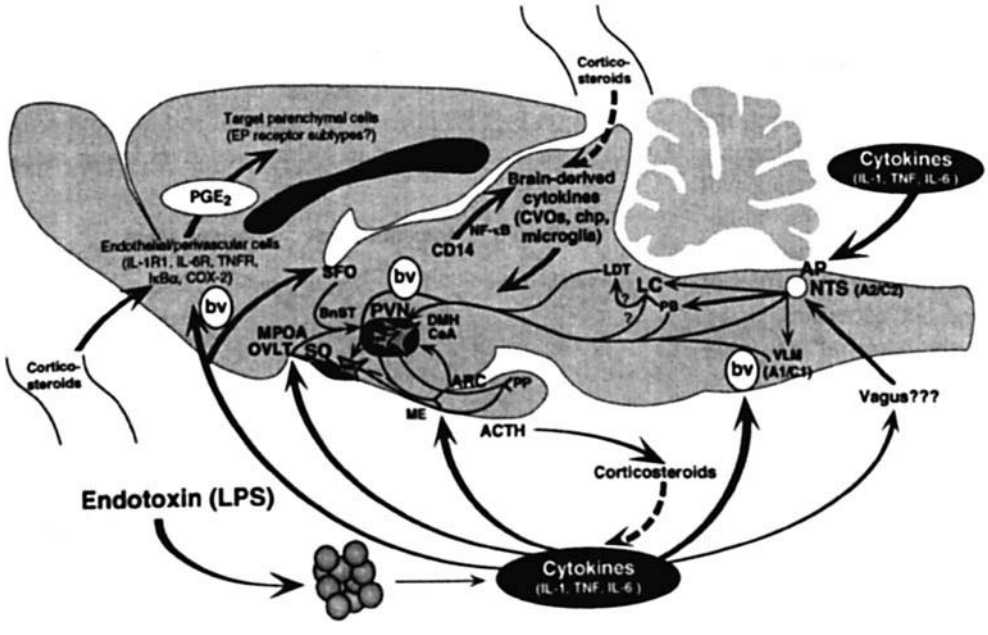

Figure 1. Schematic illustration of the possible circuitry mediating the activation of neuroendocrine PVN and the hypothalamic-pituitary-adrenal (HPA) axis during the acute-phase response of an immune challenge. The immune system most likely uses several pathways and sites of entry to communicate with the brain and neuroendocrine functions. It is suggested that circumventricular organs (organs devoid of blood-brain barrier) and the blood vessels (bv) are crucial target sites of cytokines of systemic origin produced during the acute-phase response, whereas activated regions of the brain stem and deep limbic system might play a determinant role in the integration of information received from the periphery. Among these integrative structures, the PVN may be central to the appropriate control of homeostasis during immune challenge in directly controlling various neuroendocrine functions, such as the activity of the HPA axis. The fact that most of the corticotropin-releasing factor (CRF) neurons of the parvocellular PVN expressed c-*fos* mRNA and that transcription of the gene coding CRF is activated essentially in this hypothalamic nucleus confer the importance and the specificity of this neuroendocrine nucleus in endotoxin-treated animals. The mechanisms and the circuitry controlling the CRF release and the activity of the HPA axis might also be different from those involved in the biosynthetic machinery of CRF during immune challenge. AP, area postrema; ARC, arculate nucleus; BnST, bed nucleus of the stria terminalis; bv, blood vessels; chp, choroid plexus; CeA, central nucleus of the amygdala; DMH, dorsomedial nucleus of the hypothalamus; ME, median eminence; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; LPS, lipopolysaccharide; LRNm, lateral reticular nucleus medial; MPOA, medial preoptic area; NTS, nucleus of the solitary tract; OVLT, organum vasculosum of the lamina terminalis; PB, parabrachial nucleus; PP, posterior pituitary; PVN, paraventricular nucleus of the hypothalamus (parvocellular (pc) and magnocellular divisions (mc)); SFO, subfornical organ; SON, supraoptic nucleus; VLM, ventrolateral medulla.

the systemic circulation. It has indeed been demonstrated that the surge of fever and plasma ACTH and corticosterone levels obtained after intra-arterial infusion of LPS precedes by at least 30 min measurable proinflammatory cytokines into the bloodstream (14). Moreover, systemic administration of IL-1-receptor antagonist (IL-1ra) protein failed to prevent the increase of corticotropin-releasing factor (CRF) mRNA in the paraventricular nucleus (PVN) of the hypothalamus and circulating corticosterone levels (15, 16), whereas mice receiving TNF-α antibody, either alone or in combination with IL-1ra, still exhibited robust activation of the hypothalamic-pituitary-adrenal axis (HPA) in response to LPS (15). These results clearly indicate that cytokines of systemic origin secreted in response to LPS administration are dispensable in the early stages of the fever and HPA axis stimulation. However, the possibility remains that these cytokines contribute in the prolonged and sustained responses to systemic endotoxemia. Pretreatment with antibodies specific for either IL-1 or TNF-α was indeed able to prevent LPS-induced ACTH release, but at times not earlier than 4 hr post-LPS challenge, whereas simultaneous administration of both antibodies diminished, but did not eliminate, the ACTH release at 2 hr (17). In consequence, systemic production of cytokines may not be an essential step in the early changes provoked by the bacterial endotoxin, a fact that has been further supported in proinflammatory cytokine-deficient mice (18).

Do Cytokines Mediate the Effect of LPS on the Brain?

Secretion of cytokines by circulating monocytes/neutrophils and tissue macrophages by LPS requires a series of mechanisms in cascade; the endotoxin must reach the bloodstream to bind with the serum protein LPS-binding protein (LBP) or septins. The newly formed complex will bind to the membrane CD14 receptor located on mononuclear cell surface and therefore induce the release of cytokines (19). We have recently reported that the effect of LPS in the brain may be direct and not necessarily *via* a monocyte/macrophage stimulation; the bacterial endotoxin has a profound stimulatory influence on CD14 expression in both parenchymal and nonparenchymal elements of the brain, which may subserve a direct binding ability of the endotoxin to modulate different brain functions (20). Brain CD14 expression is likely to be a key step in the transcription of proinflammatory cytokines (21–23) first within accessible structures from the blood (such as the circumventricular organs, CVOs) and thereafter through scattered parenchymal cells during severe sepsis. In agreement with this concept is the rapid induction of TNF-α within all the CVOs and the choroid plexus (chp), structures that are highly vascularized and devoid of BBB. The CVOs contain a rich vascular plexus with specialized arrangements of blood vessels. The tight junctions normally present between the endothelial cells are shifted in part to the ventricular surface and partly to the boundary between the CVOs and the adjacent structures explaining the diffusion of large molecules into the perivascular region (24). It is very likely that LPS injected into the general circulation penetrates the organum vasculosum of the lamina terminalis (OVLT), subfornical organ (SFO), median eminence (ME), and area postrema (AP) tissues, which then allow the endotoxin to trigger locally the synthesis of its own receptor.

This mechanism that is illustrated in Figure 2 and that seems dependent on the production of cytokines from phagocytes has been reported in various peripheral tissues (25). Of interest is the data that systemic injection of the bacterial endotoxin induced strong expression of CD14 (20) mRNA in a pattern that was closely related to the induction of TNF-α (21) and IL-1β (22) transcript with a rapid and delayed response. Although there is a large body of evidence that CD14 is necessary for the role of LPS on the induction of cytokine transcription from different myeloid cells, the possibility remains that the cytokine itself acts as an autocrine and paracrine factor to upregulate the LPS receptor. TNF and IL-1 are potent inducers that lead to the activation and translocation of p50/65 NF-κB into the nucleus (26), and these proinflammatory cytokines have been reported to modulate CD14 expression. Indeed, TNF-α is able to induce a transient increase in plasma CD14 levels with a peak at 6–8 hr, and this elevation in levels of CD14 antigen was shown to be accompanied by increased levels of CD14 mRNA in lung, liver, and kidney (27). A similar pattern of LPS receptor expression was found following systemic IL-1β injection (28), and pretreatment of mice with anti-IL-1β and/or anti-TNF-α antibodies significantly prevented LPS-induced mCD14 transcription (27, 28). However, these authors found that regulation of CD14 gene expression by LPS differed in epithelial and myeloid cells, with epithelial responses in kidney and liver being medi-

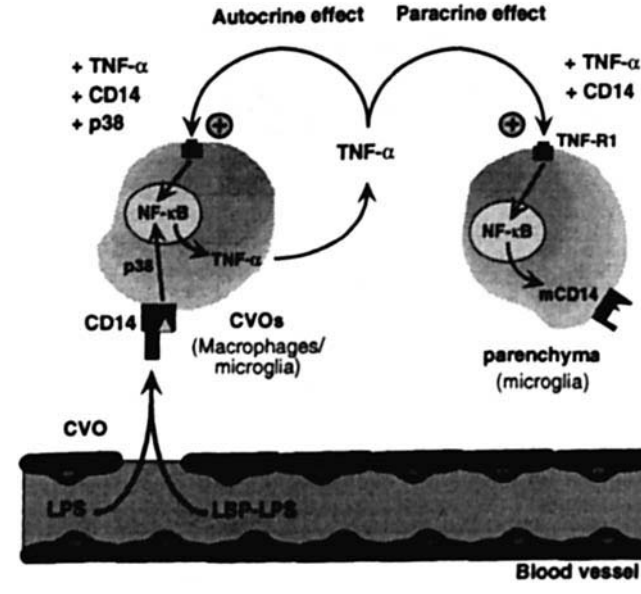

Figure 2. Autocrine and paracrine roles of TNF-α in mediating the synthesis of the LPS receptor CD14 in the brain microglial cells during blood endotoxemia. See text for details.

ated, in part, by TNF-α. Whether such a mechanism is indeed taking place in the brain has yet to be investigated, but it is possible that the cytokine is one of the paracrine factors that activates adjacent cells within the parenchymal brain. In this regard, intracerebroventricular (icv) TNF-α injection caused a robust and rapid induction of mCD14 mRNA in the rat brain (Nadeau S, Rivest S, unpublished data). The physiological relevance of TNF-α-induced CD14 within deep structures is unknown, although it is possible that mCD14 acts as an opsonic receptor to enhance the clearance of the endotoxin particles that may reach the CSF and be toxic for the neurons. This complex machinery may be of great importance to maintain the body homeostasis and protect neurons during infectious diseases and severe blood sepsis. The potential direct action of LPS on different groups of cells in the brain is summarized in Figure 3.

NF-κB Signaling in Response to Systemic Inflammation

It is not well known how cell activation is triggered after binding between the LBP-LPS complex and the glycosyl-phosphatidylinositol (GPI)-anchored mCD14, although there is now evidence that activation of tyrosine kinase leads to transduction signal and cytokine gene transcription through nuclear factor-κB (NF-κB). The recent characterization of human homologs of Toll (TLR1/6) may be the missing link for the transduction events leading to NF-κB activity, but at present there is still no strong evidence for a direct and meaningful interaction of LPS with any member of the Toll family (29). As shown by Figure 4, NF-κB is normally present in the cytoplasm forming an inactive complex with an inhibitor known as IκBα. Following extracellular stimulation by growth factors, mitogens, and cytokines that activate mitogen-activated protein (MAP) kinases, IκBα is phosphorylated by NF-κB-inducible kinase (NIK)/IκBα kinases (IKK), ubiquitinated and degraded by cytoplasmic proteasome (30, 31). Free active NF-κB (the commonest complex is the p50/p65 heterodimer) is then translocated into the nucleus where it is able to regulate transcription of various genes in binding to its κB consensus sequence. Following its degradation, IκBα is rapidly re-synthesized to act as an endogenous inhibitory signal for NF-κB and is a powerful tool to investigate the activity of the transcription factor within the CNS.

Systemic LPS and circulating proinflammatory molecules have the ability to cause a robust transcriptional activity of IκBα in different structures of the brain (32, 33). Indeed, the endotoxin injected either intravenously (iv) or intraperitoneally (ip), and intravenous IL-1β and TNF-α administration provoked a rapid (within 30–60 min) expression of IκBα mRNA first in the endothelium of the cerebral blood vessels and afterward within parenchymal microglia across the rat brain (32). The signal for IκBα in the parenchymal microglia remained positive up to 6 hr post ip LPS injection, whereas it vanished 3 hr after the single iv bolus with either IL-1β or TNF-α (32). On the other hand, a selective expression of IκBα was detected along the cerebral endothelium of intramuscular (i.m.) turpentine-injected rats (32), which is a model of sterile and localized inflammatory insult that provokes a robust swelling at the site of injection (the left hind paw) and plasma release of IL-1β and IL-6 (18). In contrast to LPS, IL-1, and TNF, intramuscular turpentine insult induced IκBα transcript only in cells associated with the microvasculature and not within parenchymal elements of the brain (32). The rapid and transient induction of IκBα indicates a strong NF-κB activity in cells of the BBB by circulating proinflammatory cytokines, which may lead to the transcription of target genes.

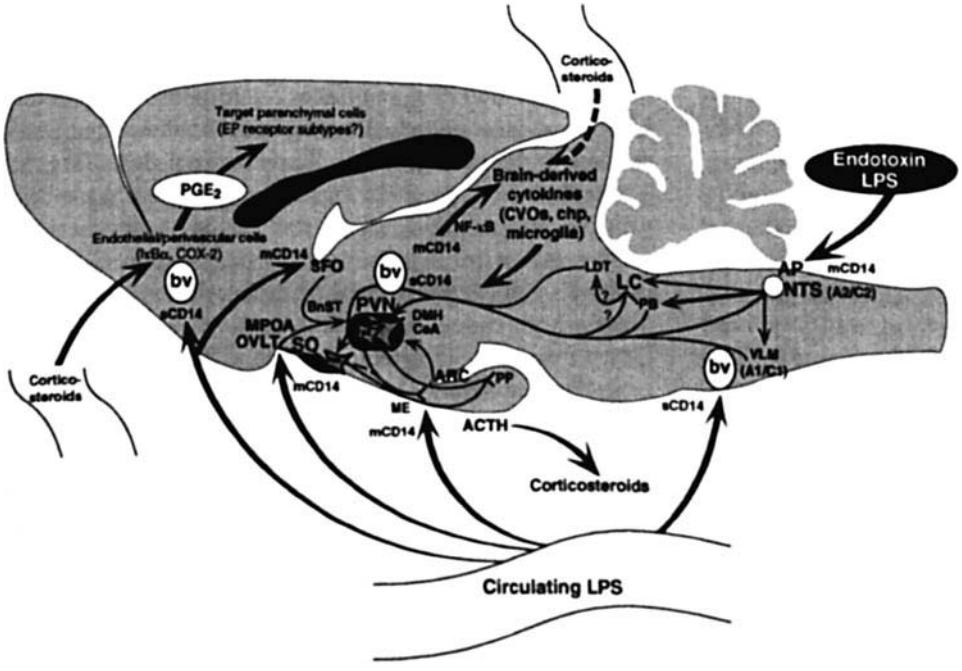

Figure 3. The bacterial endotoxin lipopolysaccharide (LPS) has the ability to trigger accessible organs through its membrane CD14 (mCD14) receptor. This phenomenon is likely to induce the transcription of proinflammatory cytokines into the central nervous system. On the other hand, the soluble form of CD14 (sCD14) may allow a direct action of the bacterial endotoxin on the endothelium of the brain blood vessels (bv) to stimulate the mitogen-activated proteins, then NF-κB and COX-2 genes. These effects are likely to be independent of systemically produced cytokines. For details, see Figure 1 and text.

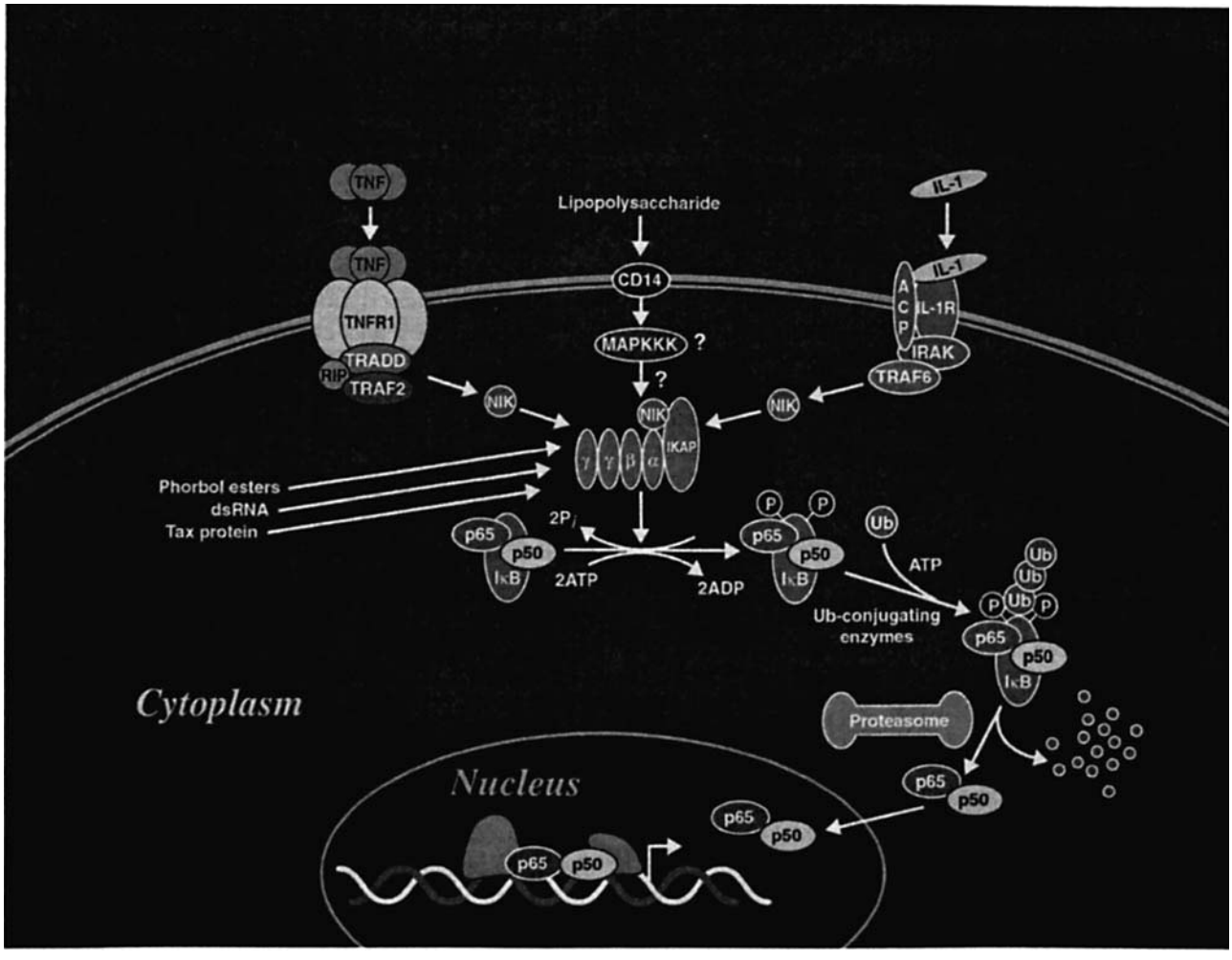

Figure 4. The proinflammatory signal transduction pathways evolving the nuclear factor kappa B (NF-κB). p50 and p65 are the two most common DNA-binding subunits of the NF-κB dimer that can trigger the transcription of numerous proinflammatory genes. See text for details and abbreviations.

One potential candidate is the gene encoding cyclooxygenase-2 (COX-2), the limiting enzyme for the formation of prostaglandins (PGs). It has recently been shown that IL-1β produces a 10-fold induction of COX-2 mRNA and an 8-fold increase in COX-2 transcription that was temporally preceded by activation of the transcription factor NF-κB in type II A459 cells (34). Two putative NF-κB motifs from the COX-2 promoter were found to bind p50/p65 NF-κB heterodimers in an IL-1β-dependent manner, whereas the two NF-κB subunits synergistically activate a –917/+49 COX-2 promoter construct (34, 35). The NF-κB site (nucleotide –223 to –214) is also involved in LPS-induced expression of COX-2 in monocytic differentiated U937 cells (36), whereas hypoxia induces COX-2 transcription *via* p65 binding to 3′ NF-κB consensus element in the enzyme upstream promoter region in vascular endothelial cells (37). Of interest is the fact that systemic LPS, intravenous injection of both IL-1β and TNF-α and intramuscular turpentine insult cause a profound transcriptional activation of the gene encoding COX-2 along vascular cells (38). It is therefore likely that circulating proinflammatory molecules stimulate prostaglandin (PG) production *via* transcriptional activation of COX-2 through NF-κB signaling pathways within cells of the BBB.

Although the induction of proinflammatory cytokines from systemic phagocytes may contribute to activate the endothelium of brain capillaries in response to systemic LPS, the effects of the endotoxin may be direct and independent of the production of circulating molecules (Fig. 3). The endothelium does not express mCD14, but these cells were shown to play a major role in the pathogenesis of gram-negative bacterial sepsis *via* free soluble CD14. In fact, LPS can trigger tyrosine phosphorylation of MAP kinases within endothelial cells despite the lack of mCD14 (39). However, such direct action on cells of the BBB may not take place during a systemic and localized inflammatory response, such as intramuscular turpentine injection. This model of localized tissue damage provokes a specific induction of IL-1β and IL-6 without any detectable IL-1α and TNF-α production, suggesting the existence of a common cascade of cytokine release, characteristic of sterile inflammation where IL-1β and IL-6 might play a critical role (18). Interestingly, IL-1β-deficient mice respond normally following systemic LPS injection, whereas the mutant mice

exhibit an impaired acute-phase inflammatory response and are completely resistant to fever development when challenged with turpentine (40).

Interleukin-1 Is a Potential Key Player During Systemic Inflammatory Stimuli

A clear example of the bilateral communication linking the immune and the neuroendocrine systems is the fact that cytokines, especially IL-1β, are profound stimulators of neuroendocrine corticotropin-releasing factor (CRF) neurons (8, 10, 12, 41–44), which lead to a subsequent activation of adrenocorticotropic hormone (ACTH) and adrenal steroid release. Stimulation of the HPA axis has been the subject of great interest because of its major impact on regulation of the immune response. Glucocorticoids, specifically cortisol in humans and corticosterone in rats, have long been recognized as being among the most powerful anti-inflammatory agents known, and their use in restraining excessive immune responses, such as in individuals afflicted with rheumatoid arthritis, has been common in clinical practice for more than 50 years. The importance of a timely release of glucocorticoids is indicated by the high mortality observed in untreated Addisonian patients (45) and in experimentally adrenalectomized animals (46–48). Inappropriate plasma levels of glucocorticoids may therefore play a crucial role in contributing to deviant regulation of the immune response, indicating the importance of identifying and characterizing the mechanisms through which local inflammatory process interacts with the neuroendocrine system. The recent evidence that corticoids may inhibit proinflammatory molecules *via* a direct genomic effect in stimulating the transcription of IκBα (49, 50) or in interfering with the transactivation potential of the NF-κB p65 subunit (51, 52) are exciting new developments, and it is possible that such events occur within specific populations of cells in the CNS during systemic and central inflammatory challenges.

Although it has been suggested that lymphocyte-derived ACTH can play a role in triggering corticosterone release in immune-challenged animals (53), this hypothesis remains highly controversial, and solid evidence supports the concept of neuroendocrine CRF-mediated mechanisms in this interface (54–57). Recombinant preparations of various cytokines can activate the HPA axis *in vivo* by stimulating CRF secretion from the hypothalamus, but several arguments favor IL-1β as a key mediator of the immune response to interfere with this neuroendocrine function. The proinflammatory cytokine is considered one of the most potent secretagogues of ACTH and glucocorticoid secretion (55, 58, 59). Secretion of neuroendocrine CRF is an important step in IL-1β-induced HPA axis activation, because the subsequent increase in plasma ACTH and corticosterone levels can be significantly prevented by administration of CRF antisera and lesions of the paraventricular nucleus (PVN, the hypothalamic structure responsible for delivering CRF into the infundibular system) in the rat (8, 10, 60, 61). Moreover, IL-1 induces expression of the immediate *early* genes (IEGs) c-*fos* within CRF neurons of the rat PVN (43, 62, 63), increases the transcriptional activity of the neuropeptide in this hypothalamic nucleus (62–64), and stimulates the release of CRF from the median eminence (ME) (8, 10, 12, 41, 42, 44).

The exact mechanisms and site(s) of action through which this cytokine interacts with the neuroendocrine system remains unclear, but a strong body of evidence now supports the concept that IL-1 acts on the endothelium of brain blood vessels to activate the release of intermediate molecules, such as PGs. Indeed, brain microvessels exhibit a robust constitutive expression of the IL-1 type 1 receptor transcript (IL-1R1) (65), whereas systemic intravenous injection of recombinant rat IL-1β causes a profound transcriptional activation of the gene encoding COX-2 (38), and IκBα (32) within endothelial cells of the CNS blood vessels. It is interesting to note that the cytokine as well as systemic localized inflammation stimulates these genes in a nonselective manner throughout the entire brain microvasculature. This nonselectivity raises several questions with regard to the localized neuronal activation induced by different experimental models of inflammation and intravenous injection with recombinant rat IL-1β (23). It is possible that the selectivity of the neuronal response is a consequence of the fine interaction between nonparenchymal PG synthesis and expression of specific PG receptors within parenchymal elements of different brain nuclei (see below).

As mentioned, IL-1β-deficient mice responded normally following LPS-induced inflammation, whereas the mutant mice exhibited an impaired acute-phase inflammatory response and were completely resistant to fever development when challenged with turpentine into the left hind paw (40). We have recently observed a strong transcriptional activation of both IκBα and COX-2 genes in the mouse brain in response to different models of systemic immunogenic stimuli (66). The bacterial endotoxin caused an interesting pattern of IκBα expression; a robust signal was first detected in blood vessels and then within microglial cells across the brain parenchyma, whereas COX-2 was only induced along vascular cells of the brain. Dual-labeling procedure provided the anatomical evidence that both IκBα and COX-2 transcripts were expressed within endothelial markers, although another study reported that COX-2-immunoreactive cells were perivascular microglia in the rat brain (67). However, there is now a large body of evidence that supports the endothelium as being the essential source of PG production in the brain during immunogenic stimuli (68–70). The pattern of expression and intensity of signal in response to intraperitoneal LPS injection was similar in IL-1β-deficient mice and their control littermates (66). In contrast to the effects of the endotoxin that induced IκBα in both vascular and parenchymal elements of the brain, intramuscular turpentine injection stimulated the inhibitory factor only within cells of the BBB in wild-type mice. This model of sterile and localized inflammation also provoked a

transcriptional activation of the gene encoding COX-2 in the cerebral endothelial cells of wild-type animals, whereas IL-1β-deficient mice did not exhibit notable induction of both IκBα and COX-2 genes. Taken together, these data provided the very first evidence that circulating IL-1β is responsible for triggering the endothelium of the brain capillaries during a systemic and localized inflammatory response and not in response to endotoxemia.

The binding of IL-1β to its cognate type I receptor leads to the formation of the IL-1 receptor-associated kinases (IRAK)/TNF receptor-associated factor 6 (TRAF6) complex, which activates NIK/IKK kinases involved in the phosphorylation and degradation of IκBα (30). NF-κB is then translocated into the nucleus and may bind to its κB consensus sequence on target genes (31). The nuclear factor binding to the COX-2 promoter is able to influence the enzyme transcription in response to different immunogenic ligands, including IL-1β (34, 35). We have recently shown that both IκBα and COX-2 are expressed within the same cells indicating a potential interaction between the transcription factor and COX-2 expression in the brain vascular cells of immune-challenged animals (66). Obviously, this remains speculative from these anatomical data, and the signaling pathways that lead to the NF-κB nuclear translocation and COX-2 transcription have yet to be determined in the cells of the BBB. *In vivo* approaches are quite limited in the investigation of the intracellular events taking place within specific cellular populations of the CNS, but they provide an essential integration of the systems that interact during the acute-phase response. In this regard, the results that both IκBα and COX-2 transcripts were induced only in wild-type and not in IL-1β-deficient mice in response to intramuscular turpentine injection strongly support the hypothesis that the circulating proinflammatory cytokine, when released by the site of inflammation, has a key role in leading to the NF-κB signaling pathway and COX-2 transcription in the cerebral endothelial cells.

These results highlight a central role for IL-1β as a mediator of the acute-phase response in an experimental model of localized inflammation associated with an increase in the circulating levels of IL-1β and IL-6, but not IL-1α and TNF-α. Circulating IL-6 may not contribute to the induction of either COX-2 or IκBα in response to systemic inflammation because high doses of recombinant IL-6 fail to stimulate these genes in vascular cells of the brain (32, 38). IL-6 is actually involved in activating a different transduction signaling pathway in cells of the CVOs and blood vessels (see below). Moreover, mice deficient in either IL-1α or IL-1 receptor antagonist (IL-1ra) genes did not exhibit a suppressed acute-phase response upon injection with turpentine (71). This latter study also reported regional differences in the induction of COX-2 in large regions of the brain by Northern blot analysis (71). However, COX-2 mRNA is induced only in cells associated with the brain vasculature (38, 72, 73) and regional changes may simply reflect the structures that are either highly or poorly vascularized. As depicted by Figure 5, circulating IL-1β has the ability to target the endothelium of brain blood vessels that allow NF-κB nuclear translocation (possibly *via* the IL-1R1/IRAK/TRAF6/NIK/IKK pathway), then COX-2 gene transcription and brain PGE_2 production in response to systemic and localized inflammatory processes. These intracellular mechanisms are likely to be the critical link between the circulating immunogenic molecules and parenchymal elements of the brain to activate the neuronal circuits needed to restore the homeostatic balance. Induction of fever, autonomic and neuroendocrine functions, such as the increase in plasma glucocorticoid levels, may have determinant functional consequences on the molecular mechanisms that take place in cells of the BBB.

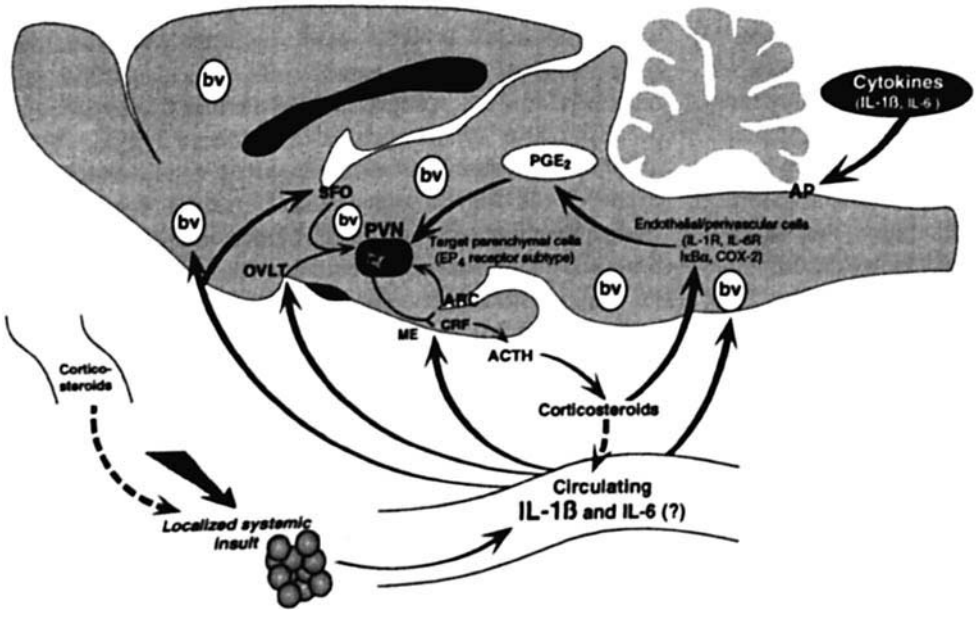

Figure 5. Possible mechanisms involved in the effects of a localized systemic inflammatory challenge on the brain and the activity of the neurons that control the hypothalamic-pituitary adrenal axis. Circulating interleukin-1β (IL-1β) is likely to play a crucial role in triggering the endothelium of the brain blood vessels (bv) and thereafter the production of soluble mediators, such as PGE_2 within parenchymal elements of the brain. These vascular-derived PGs may contribute to activate a selective circuit leading to CRF neuronal activity and the secretion of plasma glucocorticoids. For abbreviations, see Figure 1.

Tumor Necrosis Factor-α

TNF-α, previously known as cachectin, is a pleiotropic cytokine originally recognized for its antitumor activity (74), but now is referred to as a proinflammatory factor that plays a central role in initiating the cascade of other cytokines all together involved in a timely controlled immune response to infection. This cytokine is produced by a variety of cell types including neutrophils, activated monocyte/macrophages (75), T and B lymphocytes (76), NK cells, astrocytes (77), mast cells, endothelial cells (78), smooth muscle cells, synovial cells (79), brain ependymal cells (80), and neurons (81, 82). During inflammatory processes, TNF-α is one of the primary secreted cytokines by different myeloid cells, and a rapid increase in circulating TNF followed by IL-1β and IL-6 is detected in response to Gram-negative bacteria (1). TNF has been found to be a major factor inducing shock, and passive immunization against the cytokine can attenuate the appearance of other proinflammatory molecules, such as IL-1β and IL-6 (83). Surprisingly, however, cytokine production and circulating IL-6 and IL-1β induced by intraperitoneal LPS injection are intact in TNF-deficient mice (84), which suggests that TNF is certainly not the sole primary event that leads to production of subsequent cytokines in response to the endotoxin and other models of inflammation. As shown previously (11) and confirmed by a recent study (85), circulating TNF-α is able to stimulate the corticotroph axis. Although the cytokine is capable of stimulating the ACTH release from dispersed pituitary cells incubated *in vitro* (86), the primary site of action of the cytokine appears to be the hypothalamic CRF-secreting neurons (9). The stimulatory influence of the proinflammatory cytokine on the ACTH release is blunted in animals bearing a bilateral lesion of the hypothalamic PVN suggesting that neuroendocrine CRF mediates ACTH-inducing activity of TNF (87). Other studies have also provided evidence that the acute activation of the pituitary-adrenal axis following *in vivo* IL-1, IL-6, and TNF administration is not mediated by a direct action of these cytokines at the level of the pituitary and/or adrenal gland (88). Our data that intravenous TNF-α-activated transcription of the neuroendocrine CRF and the strong expression of the IEG *c-fos* in the paravocellular PVN are clear evidence that the neurons controlling the HPA axis are activated by the circulating cytokine (85).

Constitutive expression of the p55 TNF receptor mRNA was found in the hypothalamic PVN, but the signal was low and did not allow convincing dual-labeling procedure to reveal the type(s) of cells that may be expressing the transcript (85). Moreover, it is unlikely that circulating TNF has the ability to bind to its receptor expressed at the surface of potential neurons of the endocrine hypothalamus, which are protected by the BBB and nonpermeable to such products of high molecular weight. Like IL-1β, intravenous TNF-α injection has a profound stimulating influence on the endothelium of the brain blood vessels including those penetrating the PVN. Indeed, a strong induction of IκBα and COX-2 mRNA was found within endothelial cells lining the CNS vascular system in response to intravenous TNF-α injection (32, 38, 85). The binding of TNF-α to its cognate p55 receptor leads to the formation of the TNF-R1-associated death domain (TRADD)/TRAF2 complex, which activates the NF-κB signaling events. TNF-α is actually one of the most potent effectors of NF-κB activity through the 55 kd TNF type I receptor.

The distribution and regulation of the genes encoding p55 and p75 have recently been reported in the rat brain under basal conditions and following systemic injections with the bacterial endotoxin LPS and the exogenous ligand for these TNF receptors (85). Both p55 and p75 mRNAs were visible in unchallenged brains, but the TNF p55 receptor was clearly the most abundant. A convincing hybridization signal was detected in the chp, leptomeninges, ependymal lining cells of the ventricular walls, PVN, supraoptic nucleus, OVLT, ME, AP, cerebellum, and along the blood vessels, whereas p75 mRNA was present only in the hippocampus of vehicle-treated animals. Systemic treatment with the bacterial endotoxin LPS and recombinant rat TNF-α provoked a notable increase in the expression of p55 in barrier-associated populations of cells, including the CVOs, chp, leptomeninges, and along the microvasculature. Circulating TNF-α caused c-*fos* mRNA induction in specific nuclei involved in autonomic control as well as CVOs in patterns similar to intravenous LPS and IL-1β injection. Moreover, transcriptional activation of CRF in the parvocellular PVN paralled the activity of the HPA axis, as reflected by the raise in plasma corticosterone levels found in IV TNF-injected rats (85). These data provide the anatomical evidence that barrier-associated cells have the ability to express TNF receptors (in particular the p55 subtype), which may subserve the influence of the circulating cytokine onto the endothelium and/or perivascular cells of organs that are devoid of BBB, such as the CVOs. The fact that these structures are profoundly activated in response to circulating TNF supports this concept, and intermediate molecules produced by these cells may be responsible for informing the brain parenchyma and the neurophysiological functions necessary to restore the homeostasis during immunogenic insults.

As mentioned, the cytokine is also produced in the brain during endotoxemia; animals injected with LPS exhibited an interesting pattern of positive cells that was dependent on the time and the dose of the endotoxin (21). Whether such induction is involved in the effects of the bacterial endotoxin on the regulation of the corticotroph axis is still an open question that needs to be clearly addressed. Like IL-1 and IL-6, central and systemic TNF administration stimulates hypothalamic CRF release and the HPA axis (11, 13, 82), and pretreatment with TNF-α antisera into the circulation (89) or directly within the brain (90) prevents the increase of the HPA axis caused by systemic

LPS insult. Inhibition of TNF-α action within the CNS has also been shown to markedly reduce the plasma ACTH response to peripheral local inflammation using intramuscular turpentine insult (82). These data are nevertheless quite surprising because this model of sterile localized inflammation does not, in contrast to LPS, stimulate TNF-α production from neither the periphery (18) nor the brain (21). As depicted by Figure 6, it is possible that the proinflammatory cytokine of central origin contributes to activate the neurons that control the HPA axis during endotoxemia, but not in response to a localized systemic inflammation. The exact mechanisms mediating the influence of TNF, either produced locally by myeloid cells of the brain or circulating in the blood, on the neuronal activity as well as the physiological relevance of LPS-induced TNF-α transcription within the CNS have yet to be clarified.

Interleukin-6 and gp130-Related Cytokines

As shown in Figure 7, the first step in the induction of the transduction signals by IL-6 is the binding of the ligand to its IL-6 receptor subunit (IL-6R), which is either located at the cell surface or present in soluble form in the liquids of the organism. The association of these two molecules with the membrane subunit gp130 forms a high-affinity complex that triggers specific transduction signals (91). The gp130 protein does not serve as signal transducer only for IL-6, but also for the ciliary neurotrophic factor (CNTF), leukemia-inhibitory factor (LIF), oncostatin M (OSM), CT-1, and IL-11 (92, 93). However, the actions of these proinflammatory cytokines are limited by the mechanisms that control their synthesis, as they are produced in a tissue-specific manner in response to different immunogenic stimuli. Three members of the janus kinase family, JAK1, JAK2, and TYK2, are closely related to gp130 and rapidly activated in the presence of IL-6 (94–96). These kinases phosphorylated the tyrosine residues of the gp130 cytoplasmic domain allowing the recruitment and phosphorylation of at least two transcription factors of the signal transducers and activators of transcription family (STAT1, STAT3) and one tyrosine phosphatase (SHP-2) (96–101). Once activated, the STAT proteins may activate different genes in combining their SH2 domains and forming homodimers (102, 103). Moreover, SHP-2 is able to activate the membrane protein *ras*, which leads to the induction of the MAP kinase ERK-1 and

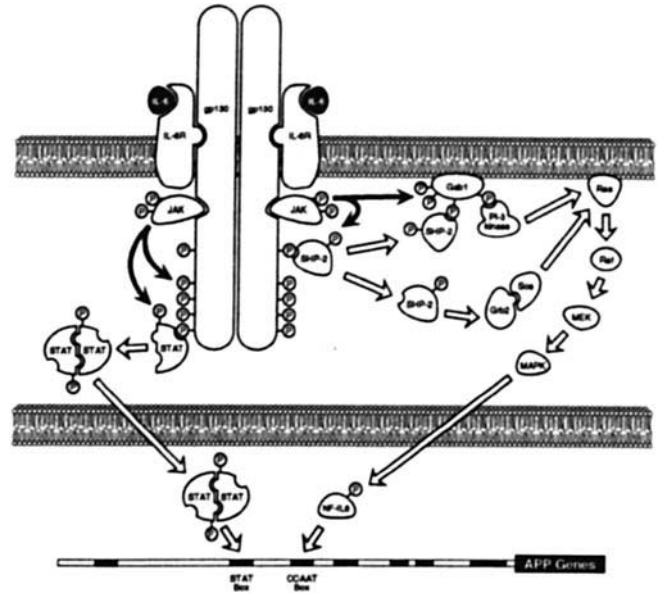

Figure 7. Interleukin 6-induced transduction signals leading to the transcription of acute-phase protein (APP). See text for details.

ERK-2 (104–107). Two pathways exist relating SHP-2 and *ras*, one using the adapter protein Gab1 associated with phosphatidylinositol-3 kinase and another *via* the Grb2-Sos complex (98, 108). The activated MAP kinases may in turn induce nuclear proteins, such as NF-IL6. This short description of the signaling events that take place in response to IL-6 is quite simplistic because gp130 may also be coupled to several other kinases, including Shc, Hck, Btk, and Tec (109–112) or the receptor ErbB2 to stimulate other MAP kinases (113).

Like the NF-κB signaling that is inhibited by IκBα, the JAK/STAT pathway is inhibited by at least two intracellular systems to avoid exaggerated responses. The first is the internalization of the IL-6/IL-6R/gp130 complex and its degradation by specific enzymes (114–116). This process does not require activation of the JAK kinases and is transduced by a degradation of the receptor at the level of the cell surface (117). The second involves activation of the transduction signals and the *de novo* production of inhibitory proteins that prevent phosphorylation of the transcription factor STAT and activation of the MAP kinases by interacting with the catalytic domain of the JAK kinases (118). These cytokine-inducible proteins named as suppressors of

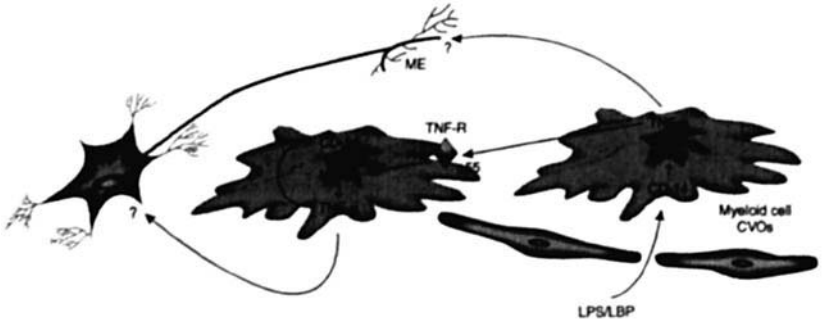

Figure 6. Possible interaction between centrally produced TNF-α and neuronal material in the central nervous system, including corticotropin-releasing factor (CRF) neurons in the paraventricular nucleus of the hypothalamus.

cytokine signaling (SOCS-1, SOCS-2, SOCS-3), JAK binding protein (JAB), STAT-induced STAT inhibitors (SSI), and cytokine-inducible SH2 protein (CIS) are rapidly induced by IL-6 and other members of the gp130 cytokine family (119, 120). Expression of these inhibitory proteins may therefore be very useful tools to investigate the signaling events occuring in the brain during immunogenic stimuli that evolve IL-6. In this regard, we have recently observed that systemic LPS treatment is capable of stimulating IL-6R transcription in cells of the BBB (23), an event associated with a selective expression of SOCS-3 in the CVOs and blood vessels across the CNS (Lebel E, Vallières L, Rivest S, unpublished data). The endogenous production of the cytokine appears to be an essential prerequisite, as LPS-induced SOCS-3 transcription is prevented in vascular elements of the brain in IL-6-deficient mice (Lebel E, Vallières L, Rivest S, unpublished data). Moreover, SOCS-3 has recently been shown to function as an intracellular regulator of proopiomelanocortin (POMC) gene expression and ACTH secretion within AtT-20 corticotroph cells (121). The idea that SOCS-3 acts as a potential negative feedback for the synthesis and production of ACTH from the adenohypophysis in response to locally produced gp130 cytokines is very interesting, although such a mechanism is likely to take place only during chronic and prolonged systemic immune challenges or induction of proinflammatory molecules locally within the anterior pituitary gland.

Several pleiotropic effects of IL-1β are mediated by IL-6, a cytokine previously identified as a B cell-stimulating factor (122). These cytokines are strongly synergistic in various immunologic processes, including induction of cell proliferation and acute-phase protein synthesis by hepatocytes (123, 124), whereas IL-1β is an important stimulator of IL-6 production in peripheral organs (125). IL-6 has been defined as one of the principal endogenous pyrogens from observations that IL-6-deficient mice are unable to develop normal fever in response to both LPS and IL-1β (126). Based on physiological and neuroanatomical studies, it has been proposed that IL-6 induces fever by signaling thermoregulatory neurons of the anterior hypothalamic area through PG-dependent pathways. Unfortunately, depending on the experimental procedure and the tissue studied, there are, in the literature, as many studies that prove their involvement in mediating the central effects of IL-6 as there are studies that are in disagreement with it. For example, it has been demonstrated that prostaglandins mediate IL-6-induced fever (127–129) and HPA axis stimulation (42, 130), but IL-6 is unable to activate prostaglandin formation in cerebral microvessels (131) and induce COX-2 mRNA synthesis in rat brains (38) or in cultured microglial cells (132). It is clear, however, that IL-6 does not stimulate the production of prostaglandins in peripheral organs and that, conversely, its own synthesis is induced by them (133, 134). In agreement with the latter, it has recently been shown that IL-6 expression in astrocytes can be induced *in vitro* by prostaglandin E_2 (135). These observations suggest that IL-6 does not stimulate COX-2 gene transcription, but the possibility that IL-6 may influence prostaglandin synthesis at post-transcriptional levels or may cooperate with them to activate CRF neurons cannot be ruled out.

The relative contribution of IL-6 in modulating the HPA axis during endotoxemia and its exact mechanisms of action are not yet fully understood. It has been shown that IL-6 can increase glucocorticoid production by stimulating the secretion of ACTH from the anterior pituitary (136, 137) and by acting directly onto the adrenal gland (138–140). However, the increase in plasma ACTH levels after intravenous IL-6 administration has been shown to be abolished totally by immunoneutralization of CRF, which suggests a role of the neuropeptide in the effect of the proinflammatory cytokine on the activity of the HPA axis (141). Although IL-6 was shown to trigger the release of CRF from hypothalamic explants *in vitro* (42, 130), this cytokine seems unable, in contrast to IL-1β and TNF-α, to induce neuronal activation and CRF gene transcription in the hypothalamic PVN (142, 143). This lack of effect might be explained by the fact that the IL-6 receptor (IL-6R) is not present in the nucleus under basal conditions; systemic immunogenic stimuli cause a profound transcriptional activation of the gene encoding IL-6R in vascular and parenchymal cells of the rat PVN (23). We therefore proposed that induction of IL-6R synthesis may be an essential step taking place early during inflammation and allowing IL-6, when it becomes available in the circulation, to trigger neuronal activity.

Quite interestingly, our recent observations suggest that IL-6 signaling is enhanced during endotoxemia, and IL-6 modulates PVN functions only after preinduction of its receptor in immune-challenged animals (144). Using a dual labeling technique, we also found that some CRF neurons express IL-6R suggesting that IL-6 may target these cells directly to trigger neuronal activation and CRF secretion. In this regard, a systemic LPS insult caused a profound transcriptional activation of the gene encoding IL-6 selectively in the sensorial CVOs and the choroid plexus (23), which provided solid evidence that the cytokine is produced in the brain and may act on neurons or other parenchymal elements of the CNS. In addition, our latest data obtained from the experiment performed in wild-type and IL-6-deficient mice support the concept that IL-6, although not involved during the initial phases of endotoxemia, is necessary during the later phases for maintaining the stimulation of CRF neurons controlling the HPA axis and prolonging the activation of neural cells throughout the brain (144). This phenomenon might be of great importance to protect the brain and to restore homeostasis during bacterial septic shock. The increase in circulating corticosterone levels has recently been found to be lower in IL-6–/– than IL-6+/+ mice in response to intraperitoneal LPS injection, but not during restrain stress (145). This suggests that the involvement of IL-6 in the control of the HPA axis is quite specific to the immune stimuli and not neurogenic stresses. The participation of IL-6 was further suggested by the fact that pretreat-

ment with anti-IL-6 antibody abrogated the ACTH secretion both 2 hr and 4 hr after LPS administration, but not after 1 hr (17), whereas anti-IL-6 antibody significantly prevented the IL-1-induced increase in plasma ACTH (146). Interestingly, this determining role has been confirmed in models using inflammatory agents lacking the intrinsic capacity to stimulate the HPA axis, but able to induce cytokine production. It was concluded that IL-6 is an obligate factor to increase glucocorticoid production during cytomegalovirus infection or after the injection of a synthetic analog of viral nucleic acid (145).

Role of Cyclooxygenase Pathways

Activation of PG synthesis by cyclooxygenase pathways is believed to play a key role in the cascade of events mediating the effects of circulating cytokines on the numerous functions, including fever, activation of neuroendocrine CRF neurons, and the HPA axis as well as the sympathetic nervous system of immune-challenged animals. Indeed, blockage of the eicosanoid cyclooxygenase pathways can prevent the stimulation of CRF release by proinflammatory cytokines from *in vitro* hypothalamic explants (42, 130) and ME (147), and IL-1- (148, 149) and TNF-α- (150) induced ACTH release *in vivo*. Inhibition of PG production has been reported to prevent IL-1-induced alteration of other neuroendocrine functions, such as luteinizing hormone-releasing hormone (LHRH) and luteinizing hormone (LH) release (151) as well as hypothalamic arginine vasopression (AVP) and oxytocin (OT) release (152). The role of PGs in mediating the stimulatory influence of the acute-phase response seems, however, to depend on the severity of the systemic stressful situation, the brain regions and cell groups, as well as the activated target genes (153). Systemic intraperitoneal injection of a low dose of endotoxin (2.5 μg/100 g b.w.) induced c-*fos* mRNA expression quite selectively within the sensorial CVOs, whereas 25 and 250 μg of the endotoxin LPS/100 g b.w. caused strong transcription of the IEG in numerous structures throughout the rat brain. Administration of the eicosanoid cyclooxygenase inhibitor indomethacin attenuated IEG induction in very specific brain nuclei and the leptomeninges of rats treated with 25 μg/100 g b.w., but not in animals receiving the highest dose of LPS intraperitoneally. In a similar manner, pretreatment with indomethacin was significantly effective to prevent the transcription of neuroendocrine CRF and its type 1 receptor in rats treated with a moderate dose of LPS (153, 154). Finally, PG production in response to such a dose of endotoxin seems largely responsible for the activation of A1/C1, but not A2/C2 groups of brainstem catecholaminergic neurons (153).

The exact PG subtype(s) and the site(s) of action within the brain involved in these effects remain unclear, but a large body of evidence indicates that PG of E_2 type might be involved in several changes observed during immune challenge and treatment with cytokines. IL-1 increases the release of PGE_2 from rat hypothalamic explants *in vitro* (155), medial preoptic area (MPOA)/OVLT, PVN, dorsal hippocampus, lateral ventricle *in vivo* (156), rat astrocyte cultures (157), isolated pancreatic islets (158), and papillary collecting duct (159). Mapping of PGE_2 binding sites in the rat brain using quantitative autoradiography revealed binding sites in numerous brain structures including the hypothalamus (160, 161). Moreover, intracerebroventricular administration of PGE_2 (162) or directly into the MPOA/OVLT (163) elevated plasma ACTH and corticosterone in rats, an effect most likely mediated through neuroendocrine CRF neurons. Central treatment with PGE_2 is not only associated with an increase in HPA axis activity, but is also known to produce many other physiological responses such as the alteration of the cardiovascular and sympathetic nervous system functions (164, 165) and hyperthermia in rats (166). When injected into the brain, PGE_2 causes a selective cellular activation as indicated by the rapid and transient expression of the IEG c-*fos* mRNA and protein within multiple regions of the brain recognized to be activated during the acute-phase response of an immune challenge (167, 168). In a similar manner, the PG-triggered transcription of CRF and its type-1 receptor essentially in the hypothalamic PVN (167). Local production of PGE_2 might therefore be a crucial step within the CNS to mediate the effects of cytokines and other immune-related systemic factors on the neuronal circuitry involved in the activation of the HPA axis. The PG is produced by vascular-associated elements, as indicated by the robust and transient expression of COX-2 within the microvessels in response to systemic inflammation (38, 72, 169) and the selectivity of the neuronal responses may depend on the expression of specific PGE_2 receptor subtypes or isoforms within defined parenchymal structures of the brain.

The actions of PGE_2 are mediated by their specific seven transmembrane receptors that can be divided on the basis of their responses to different agonists and antagonists into four subtypes, EP_1, EP_2, EP_3, and EP_4. Three isoforms of the EP_3 receptors have been identified ($EP_{3\alpha}$, $EP_{3\beta}$, $EP_{3\gamma}$), which share common extracellular and membrane-spanning regions, but differ in intracellular and carboxyterminal domains. These receptor variants are generated by alternative splicing of the primary transcript and are associated with different G proteins, like the other subtypes. This family of rhodopsin receptors triggers different transduction signals; EP_1, EP_2 and EP_3 are presumably coupled to stimulation of phospholipase C, and stimulation and inhibition of adenylate cyclase, respectively. The most recently identified subtype, EP_4, is like the EP_2 receptor positively coupled to adenylate cyclase (170, 171). *In situ* hybridization histochemical signal for mRNA encoding the EP_3 PGE_2 receptor subtypes and binding sites were observed over neurons of numerous regions of the rat brains, including the ventral septal area (VSA), the nucleus of the solitary tract (NTS), the ventrolateral medulla (VLM), and numerous hypothalamic nuclei and areas (160, 161, 172, 173). It has also been suggested that EP_1 receptors mediate

the PG-induced hyperthermia (166) whereas EP_3 is involved in the regulation of thermal hyperalgesia (174) as well as fever generation in response to both exogenous and endogenous pyrogens (175). However, until recently, there was very little existing documentation on the distribution, regulation, and function of the genes encoding EP_2 and EP_4 receptor subtypes in the brain under both basal and immune-challenged conditions.

We have recently reported a very distinct distribution of both EP_2 and EP_4 receptors across the brain under basal conditions (176); an hybridization signal for the type 2 receptor was detected in the bed nucleus of the stria terminalis, lateral septum, SFO, ventromedial hypothalamic nucleus, central nucleus of the amygdala (CeA), locus ceoruleus (LC), and the AP, whereas the ventral septal/anterior preoptic area, the magnocellular PVN, supraoptic nucleus, parabrachial nucleus, LC, nucleus of the solitary tract (NTS), and ventrolateral medulla (VLM) exhibited moderate to strong levels of the EP_4 mRNA under basal conditions. Upregulation of the genes encoding EP_2 and EP_4 receptors was detected in selective regions and neuronal populations during systemic inflammatory challenges (176). The most dramatic one was the robust transcriptional activation of the EP_4 subtype within CRF neurons of the parvocellular PVN following intravenous LPS and IL-1β injection as well as in response to intramuscular turpentine insult. These neurons of the endocrine hypothalamus as well as those of numerous nuclei involved in autonomic control were activated by the proinflammatory cytokine as they were immunoreactive (ir) to Fos nuclear protein (176). The EP_4 transcript was also present in activated catecholaminergic neurons of the LC, NTS, and VLM, although only the A1 cell group exhibited an increase in EP_4 transcription in response to circulating IL-1β. Moreover, the systemic immunogenic insults caused a significant increase in the EP_2 mRNA levels in the CeA, SFO, AP, and leptomeninges. These data provided a distinct pattern of EP_2 and EP_4 expression throughout the rat brain under both basal and immune-challenged conditions and underlie the possible role of the EP_4 subtype in mediating the effects of PGE_2 on different autonomic and neuroendocrine functions (176). The presence of Fos-ir nuclei in various populations of EP_4 neurons of IL-1-treated animals clearly supports this concept and suggests that the selectivity of the neuronal response during systemic inflammation may depend on the expression of specific PGE_2 receptors in key structures of the brain.

Concluding Remarks

We and other groups have contributed to establish the potential brain circuitry solicited in response to different immunogenic challenges (see Figs. 1, 3, and 5). Although the regions and population of activated cells are somewhat similar following systemic treatment with IL-1β, TNF-α, and LPS, the effects of IL-6 are quite different. The most likely targets of plasma molecules are structures that are devoid of BBB and the microvasculature itself, and recent evidence clearly supports this hypothesis. Systemic injection of LPS, IL-1β, and TNF-α causes a rapid expression of c-*fos* mRNA within cells associated with the microvessels and a delayed response within the parenchymal brain in both neuronal and non-neuronal elements (6, 23, 62, 177). These phenomena also take place in the CVOs, choroid plexus, and meninges, although the activated cells are more heterogeneous and not only of the endothelial type at early times post challenge (177). The exact pattern and time course of IEG expression in the brain following intramuscular injection of turpentine that causes a more restricted cytokine response than LPS insult is unclear, but seems distinct from the previous models (Lacroix S, Rivest S, unpublished data). Among many activated groups of cells, CVOs and related structures (OVLT/MPOA, SFO, ARC/ME, AP), PB, NTS/X (A2/C2) and VLM (A1/C1) might contribute to relay the information received from the periphery to activate the PVN and the corticotroph axis. As presented in this review, these relays differ among the cytokines and models of acute-phase response, and the PVN itself may be targeted by the PGs that are produced by vascular lining cells penetrating the endocrine hypothalamus. The vagus nerve has also been reported to play a role in mediating the effects of LPS, although this would occur only when the endotoxin is injected intraperitoneally and at a very low dose. Indeed, vagotomy can prevent neuronal activation in response to fairly low doses of LPS and IL-1β injected intraperitoneally but not following moderate to high doses and not when the endotoxin and proinflammatory cytokines are injected directly into the circulation (178). The participation of vagal sensory mechanisms in mediating the neuronal response to immune challenge is therefore quite limited to local peritoneal inflammation and may not be considered as a general mechanism by which circulating proinflammatory molecules trigger the cascade of events taking place in the CNS during systemic innate immunogenic stimuli.

The fundamental mechanisms underlying the influence of the immune system on the CNS and neuroendocrine functions are now better understood since the early reports presenting the evidence that cytokines may possibly talk to neurons. However, a great deal of research is needed to determine the signaling events and the exact players involved in both vascular and parenchymal elements of the brain by circulating proinflammatory molecules during infectious and inflammatory diseases. Understanding this basic machinery is of great importance not only because of the elegant concept of bi-directional communication already presented, but also because these mechanisms may help us uncover how disorders of this fine interplay may contribute to the onset and progression of various pathological states characterized by an exaggerated immune response. Stimulation of the HPA axis has been the subject of great interest because of its major impact on the regulation of the immune response, and it is likely that inappropriate plasma levels of

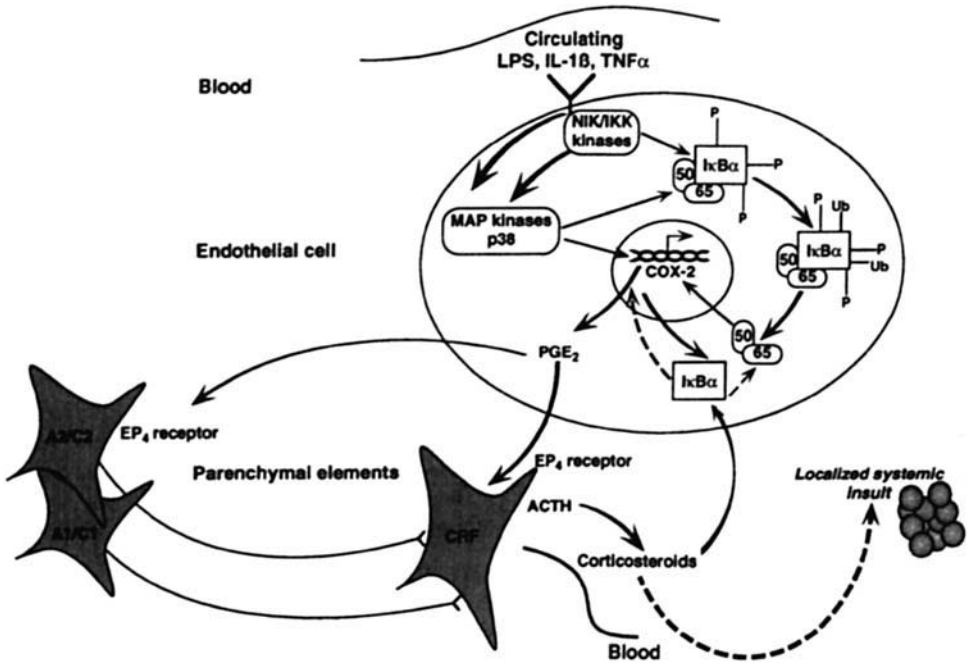

Figure 8. Hypothetical intracellular mechanisms mediating the influence of circulating inflammatory molecules on the transcription of the cyclooxygenase-2 (COX-2) within an endothelial cell of the blood brain barrier. Although overly simplistic, both the MAP kinases and NF-κB pathways may be transduction/transcription signals in these processes. The production of the prostaglandin of E_2 type is believed to be a key mediator to diffuse through the parenchymal brain and the neurons that control the hypothalamic-pituitary adrenal axis. The subsequent release of glucocorticoids is determinant for the immunosuppression of the systemic inflammation and the downregulation COX-2 transcription. Glucocorticoids may increase IκBα transcription and/or interfere with NF-κB binding ability on COX-2 promoter in cerebral vascular cells. For abbreviations, see Figure 1.

corticoids contribute to deviant regulation of the immunosuppression, which may lead to chronic inflammatory diseases. The intracellular signaling events that take place in specific populations of cells depend on the models, severity, and cytokine involved, but the genetic transcription of the limiting enzyme for the PG synthesis COX-2 along the cerebral endothelium may have a leading role in stimulating parenchymal elements of the brain. A localized neuronal activation occurs despite the robust COX-2 transcriptional activation along the entire brain vascular system, although this general induction depends on the severity of the challenge and the dose of the recombinant cytokines used. As shown by Figure 8, the EP_4 subtype is likely to subserve the effects of circulating inflammatory molecules on specific activated populations of cells, including selective catecholaminergic neurons of the VLM and neuroendocrine CRF neurons. The cytokine IL-1β may play a key role as the circulating factor mediating these effects during systemic and localized inflammatory stimuli, but not in response to endotoxemia that encompasses a more complex and redundant circuitry.

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