The Catecholamine–Cytokine Balance

Interaction between the Brain and the Immune System

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ABSTRACT: Cytokines are involved both in various immune reactions and in controlling certain events in the central nervous system (CNS). In our earlier studies, it was shown that monoamine neurotransmitters, released in stress situations, represent a tonic sympathetic control on cytokine production and on the balance of proinflammatory/anti-inflammatory cytokines. Basic and clinical studies have provided evidence that the biophase level of monoamines, determined by the balance of their release and uptake, is involved in the pathophysiology and treatment of depression, while inflammatory mediators might also have a role in its etiology. In this work, we studied the role of changes in norepinephrine (NE) level on the lipopolysaccharide (LPS) evoked tumor necrosis factor (TNF)-α and interleukin (IL)-10 response both in the plasma and in the hippocampus of mice. We demonstrated that the LPS induced TNF-α response is in direct correlation with the biophase level of NE, as it is significantly higher when the release of NE of vesicular origin was completely inhibited in an animal model of depression (reserpine treatment) and it is significantly lower in the case of increasing biophase levels of NE by genetic (NET–KO) or chemical (desipramine) disruption of NE reuptake. IL-10 was changed inversely to TNF-α levels only in the desipramine-treated animals. Our results showed that depression is related both to changes in peripheral and in hippocampal inflammatory cytokine production and to monoamine neurotransmitter levels. Since several anti-inflammatory drugs also have antidepressant effects, we hypothesized that antidepressants are also able to modulate the LPS-induced inflammatory response, which might contribute to their antidepressant effect.

KEYWORDS: cytokine; catecholamine; neuroimmunomodulation; inflammation; depression

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INTRODUCTION

Cytokines are low molecular weight proteins or glycoproteins that were initially thought to be restricted to the immune system as mediators of communication between immune cells. Recently, however, increasing attention has been paid to the fact that many cytokines also play a key role in the central nervous and endocrine systems. It was reported that cytokines were also produced by brain cells, and that they interact closely with neurohormones and neurotransmitters. The regulatory pathways that control the immune system include mediators, most notably catecholamines (CA), from the brain, and various hormones produced by the endocrine system, for example, corticosteroids (Fig. 1).

A large number of external and/or internal stimuli, including bacterial endotoxin (LPS), viral infections, differentiating factors, prostaglandins, cytokines, etc., are able to activate the immune system via macrophages, resulting in increased production of proinflammatory cytokines, such as interleukin (IL)-1, IL-12, and tumor necrosis factor (TNF)-α. As all normal immune responses are temporary, both antigen-specific and nonspecific immune responses are well controlled. Sympathetic neurotransmitters have an important role in the fine-tuning of immune and inflammatory responses. Recent studies have also supplied ample evidence of the relationship between stress or other situations that influence catecholamine levels and cytokine production, and also between the production of inflammatory mediators and the development of some diseases of the central nervous system (CNS), for example, depression, Alzheimer disease, etc.

OCCURRENCE OF CYTOKINES IN THE BRAIN

Cytokines are involved both in the immune response and in controlling various events in the CNS; that is, they are equally immunoregulators and modulators of neural functions and neuronal survival. There is ample evidence to indicate the constitutive expression of numerous Th1- and Th2-type cytokines and also their functionally active receptors in “normal” adult brain. There is also a diurnal rhythm in the expression of at least IL-1β and TNF-α that is under complex neuroendocrine control, probably mainly by physiological variations of the corticotropin-releasing factor (CRF) levels.

Cytokines might interact with the CNS in numerous ways, in which it is not negligible whether these mediators originate from peripheral immunocompetent cells or are produced within the CNS. The peripheral and central cytokine compartments appear to be integrated, and their effect might either inhibit or synergize each other; however, it should always be taken into account that they are differentially regulated. On the other hand, cytokine production is under the tonic control of the peripheral nervous system and the CNS.
FIGURE 1. Bidirectional interactions between the central nervous system (CNS) and cytokines. Varieties of psychological or physical stimuli alter the biophase level of catecholamines and activate the production of cytokines, both in the immune- and the CNS. Cognitive stimuli like stress (a) induce both catecholamine (i) and cytokine (d) production in the brain (A). Catecholamines are able to modulate the immune response via receptors expressed on the immune cells (e). This regulation might be either attenuative or intensifying, depending on the costimulus. Physical stimuli (a) (e.g., infections, tissue damages, etc.) induce cytokine production, usually in the periphery (B), however, their effect might also influence the events in the CNS (b) (c). These cytokines also promote production of each other, change the level of stress hormones, and are under the regulation of the HPA axis via corticosteroids (g) (h). These interactions might also be either synergistic or attenuative. Many of these mediators (e.g., IL-1, NGF, TNF-α, NO, etc.) are upregulated by neuronal activity and promote behavioral changes and CNS diseases. The activity of the immune system and the production of catecholamines are also under diurnal control, therefore, the type and pattern of their biophase concentration provide feedback control on their own activities by tuning the levels of cytokines, hormones, and other mediators. The motor vagus might also influence these interactions (f). Collectively, these actions form a biochemical cascade involved in physiological and pathological psychological-, and immune regulation, as well as changes in their responses to external or internal challenges. CNS = central nervous system; HPA axis = hypothalamic-pituitary-adrenal axis; IL-1 = interleukin-1; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-alpha; NE = norepinephrine; DA = dopamine; SHT, 5-hydroxy-tryptamine; CRH = corticotropin-releasing hormone; βAR = beta-adrenergic receptor; MAPKs = mitogen-activated protein kinases.
and the cytokine balance can be modulated by the action of neurotransmitters released from nonsynaptic varicosities.\(^9\)

The various cytokines affecting directly the CNS might originate from:

— Peripheral immune cells that are the main source of cytokines The cytokines produced by the immune cells in the periphery are able to cross the blood–brain barrier,\(^10\) even in healthy, basal conditions. Active, saturable, and specific transport of certain cytokines across the blood–brain barrier was also demonstrated earlier.\(^11\)

— Neuronal cells within the CNS are also able to produce cytokines even in the healthy state. Most of the cytokines and their receptors have been demonstrated and/or postulated in various cell types of the CNS, both under healthy and diseased circumstances. 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 brain injuries originated by trauma, ischemia, infection, or inflammation.\(^12–14\) Once in the brain, there is a CNS–cytokine network that is made up of its own cells (neurons and glial elements). These cells not only produce cytokines and express cytokine receptors, but also amplify cytokine signals, which in turn can have profound effects on neurotransmitter and corticotropin-releasing hormone (CRH) function, as well as on behavior.\(^12–16\)

**CONTRIBUTION OF CATECHOLAMINE AND CYTOKINE RECEPTORS IN THE INFLAMMATORY RESPONSE**

The sympathetic nervous system (SNS) innervates immune organs and, when activated, releases its signaling molecules in the vicinity of immune cells. Accordingly, cytokine balance can be modulated by sympathetic neurotransmitters, both in the CNS and in the periphery. Immune cells express various neurotransmitter receptors that are sensitive to monoamines, and the production of cytokines (and other immune/inflammatory mediators (chemokines and free radicals) is modulated by activation of these receptors.\(^1\) Once the neurotransmitters have reached the target cells, they occupy their appropriate receptors and the initiated signal transduction modulates the cytokine production of the cell.

The catecholamines norepinephrine (NE) and epinephrine (EPI) exert their effects by binding to 7 transmembrane spanning G-protein-coupled cell-surface receptors termed adrenoceptors. Adrenoceptors can be classified into three major groups: α1—, α2—, and β-adrenoceptor types.\(^17\) Each of these three major types can be subdivided further into at least three subtypes: α1A, α1B, α1C; α2A, α2B, α2C; and β1, β2, and β3 and at least the presynaptic α2- and the peripheral β2-adrenoceptors play major roles in the regulation of cytokine balance.
There is ample evidence that in the immunomodulatory effect of catecholamines, cAMP plays one of the key roles.\textsuperscript{7,18–20} Thus, occupation of neurotransmitter receptors that stimulate or inhibit adenylate--cyclase also influence the cytokine profile of the system. NE and the adrenergic drugs may influence the immune response directly, through adrenergic receptors expressed on macrophages and also on other immunologically competent cells, as well as indirectly via alteration of endogenous NE levels by influencing the activity of release-regulating presynaptic α2-adrenoceptors (α2-AR) located on sympathetic nerve terminals.\textsuperscript{9,21–24} Activation of the presynaptic α2-adrenoceptors results in a negative feedback effect on NE release, leading to decreased extracellular NE concentration.\textsuperscript{25,26} The majority of the direct effect of NE prevails via the β2-adrenoceptors expressed by various immune cells.

**CYTOKINES AND NEURONAL SURVIVAL**

Cytokines can be expressed under resting physiological conditions in resident CNS cells, but are also induced during injury and development. In addition, under pathological conditions, cytokines can be expressed in infiltrating macrophages in the brain. Inflammatory processes have been implicated in both acute and chronic neurodegenerative conditions. The inflammatory response of the CNS is weaker and delayed over other tissues,\textsuperscript{27} however, many inflammatory responses (e.g., activation of microglia and release of inflammatory mediators) can be induced rapidly. Physiologically, the cellular expression of cytokines in the CNS is strictly controlled; however, under certain pathological conditions, the expression of various cytokine genes may become spatially and temporally modified. Literary data indicate, for example, that the expression of tumor growth factor (TGF)-β1 by hippocampal CA1 neurons is upregulated during the first hours after ischemia.\textsuperscript{28} Proinflammatory cytokine production and signaling results in important changes in the neurons long before their ultimate cell death.

Neurotoxic and neuroprotective mechanisms are both closely related to the balance between the proinflammatory and anti-inflammatory cytokines. The process of neurodegeneration is closely related to the shift of cytokine production toward the side of proinflammatory cytokines like IL-1 or TNF--α, regardless of the fact that they are produced within the CNS or have systemic origins. On the other hand, anti-inflammatory cytokines in the CNS maintain homeostasis and protect cell viability by inhibiting inflammatory responses.\textsuperscript{29–31} Since there is an inhibitory cross-regulation between the two groups of cytokines that indirectly suppress the synthesis of each other, this provides a further fine-tuning of the balance between neurodegenerative and neuroprotective effects.

The role of proinflammatory cytokines like IL-1 or TNF-α in neurotoxicity is considerable but controversial. *In vivo* animal experiments have revealed
that interferon (IFN)-γ/LPS administration into the rat hippocampus induces delayed neuronal apoptosis.\textsuperscript{32} Concerning IL-1, it was demonstrated that this cytokine itself was not toxic to healthy brain tissue or to normal neurons, but even in very low concentrations, it augmented traumatic, ischemic, or inflammatory brain injuries.\textsuperscript{31,33} This dual effect of cytokines expressed within the CNS is similar to the role played by cytokines in the periphery, that of helping to select populations of mature cell types by enhancing survival of some and eliminating others through apoptosis. In immune cells, and similarly, in neurons, the transcription factor nuclear factor (NF)-κB may play a pivotal role in these context-dependent effects on cell survival or death, acting much like a switch that can block death signals when induced and can allow death signal activation of the apoptotic pathway when suppressed or blocked. \textit{In vitro} studies in mixed neuronal/glial cultures show that several cytokines play dual context-dependent maturation roles in either promoting or preventing apoptotic neuronal cell death.

Considering that proinflammatory cytokines are not neurotoxic per se, it is possible that they alter neuronal survival not only by intracellular receptor–receptor interactions but also by cell–cell interactions.\textsuperscript{34} The primary source of IL-1 and TNF-α after brain injury is microglia, but astrocytes, oligodendrocytes, and other cells in the CNS may also produce cytokines, which can interact with each other. This is supported by another concept of neurodegeneration that implies the existence of a different level of regulation between pro- and anti-inflammatory cytokines in the brain. This concept is based on the intracellular cross-talk between heterologous receptors on a single cell that can either promote or inhibit receptor activity. If a receptor representing a death signal (e.g., TNF-α receptor) interacts with a receptor for survival signal (e.g., IGF1 receptor) it might inhibit IGF1-mediated neuroprotection; that is, the receptor–receptor interaction is a “silencing of the survival signal.”\textsuperscript{35}

Thus, activation of neurotransmitter receptors that stimulate adenylate–cyclase accompanied by LPS stimulus of the immune system leads to a shift toward T helper 2 (Th2)-type responses, which are both neuroprotective and anti-inflammatory, whereas downregulation of intracellular cAMP stimulates a T helper 1 (Th1)-type response, resulting in cell destructive effects and inflammation.

BIDIRECTIONAL COMMUNICATION BETWEEN CYTOKINES AND NEUROTRANSMITTERS

The modulation of cytokine balance by catecholamines is governed by the biophase concentration of these monamines. The amount of catecholamines in the extracellular space is a function of the balance between their vesicular release and their reuptake by the monoamine transporter system.
Stimulation of $\beta_2$-AR is the classical example of activation of adenylyl cyclase via stimulatory G proteins (Gs), resulting in the subsequent increase in intracellular cAMP. Although both $\alpha_2$- and $\beta_2$-adrenoceptors are expressed on the surface of various immune cells,$^{23,36,37}$ macrophagic $\alpha_2$-adrenoceptors have only a minor role. Occupation of $\alpha_2$-adrenoceptors on macrophages results in the suppression of the intracellular cAMP level, because these receptors are associated with a Gi-type protein.$^{17}$ Since cAMP has generally been proven to suppress the inflammatory immune response, sympathetic control of the innate immune response is believed to be necessarily immunosuppressive,$^{1,38,39}$ while the exact mechanism is not yet fully understood.

As the immunoregulatory effect of catecholamines becomes effective practically as a costimulus, the most common way of studying it is to apply an agonist together with a known immune activator. Cytokine production by macrophages or macrophage-like cells (e.g., microglia) is most commonly induced by bacterial endotoxin (LPS). Evidence is available that the monocyte/macrophage system orchestrates the innate immune response to LPS by producing cytokines and other mediators, like TNF-$\alpha$, IL-1$\beta$, IL-8, or nitric oxide (NO). However, no data are available on the effect of sympathetic signals on the cytokine production evoked by other known stimuli, such as cytokines, tumor promoters, or viral components.

In earlier studies, we investigated whether the immunomodulatory effect of the SNS would also be effective, together with stimuli other than LPS.$^{40}$ LPS stimulation via the CD14 receptor complex activates several intracellular pathways that include the IκB kinase NF-κB pathway and the MAPK pathways: ERK1 and 2, and p38.$^{41}$ We presented evidence that the $\beta$-adrenergic agonist isoproterenol had opposite immunomodulatory effects on TNF-$\alpha$, IL-12, and NO production in macrophages stimulated by phorbol esters (PMA; a PKC activator) versus LPS (FIG. 1B). We also demonstrated that these opposite effects of $\beta_2$-AR stimulation on LPS- versus PMA-induced mediator production correlated with their effects on MAPK activation. These results show that the ERK and p38 MAPKs may act as molecular switches and that, depending on the applied stimulus, they can regulate sympathetic immunomodulation induced by $\beta$-adrenergic agonists/antagonists.$^{40,42}$

Effects of cytokines on the noradrenergic system have also been described (FIG. 1A). In addition to the effects on neurotransmitter metabolism, it was shown that inflammatory cytokines exerted profound stimulatory effects on the hypothalamic-pituitary-adrenal (HPA) axis hormones as well as on CRH (mRNA and protein), both in the hypothalamus and amygdala, brain regions that have an important role in fear and anxiety.$^{43,44}$ Changes in the catecholamine metabolism in brain regions being essential to the regulation of emotion, including the limbic system (amygdala, hippocampus, and nucleus accumbens), might influence sickness behavior.$^{45,46}$ These effects are, in large part, mediated by the cross-talk of cytokines and their receptors within the HPA axis tissues that facilitate the integration of cytokine signals.$^{47}$ As an example,
IL-1 was shown to stimulate hypothalamic and preoptic noradrenergic neurotransmission, similar to the effects observed after administration of various forms of IL-1. There is inconsistent data for other proinflammatory cytokines and their influence on noradrenergic neurotransmission. IL-2 showed similar effects as IL-1, while other studies report that TNF-α inhibited NE release from the median eminence. It is now well established that immune activation triggers the SNS to release the neurotransmitters noradrenaline (NA), adrenaline, and dopamine.

THE CATECHOLAMINE–CYTOKINE INTERACTION IN STRESS AND DEPRESSION

Growing evidence suggests that overactivation of innate immune responses following stress and during depression might come at the expense of decreased cellular and humoral acquired immune responses. Activation of the stress system might promote cytokine production through several mechanisms. Despite suppressing certain immune processes, activation of the SNS is linked in several studies to proinflammatory activation in the periphery, which might, in turn, influence inflammatory processes in the CNS. It was demonstrated that stress-induced activation of NF-κB in peripheral blood mononuclear cells appeared to be dependent on noradrenaline.

A dysregulation of the cytokine balance could induce depressive symptoms, due to lower levels of anti-inflammatory cytokines and higher levels of proinflammatory cytokines. Anti-inflammatory cytokines are known to evoke an anti-inflammatory state, both on their own (IL-10, TGF-β) receptors and also by the blockade of the binding of proinflammatory stimuli to their cell-surface receptors (IL-1RA, soluble TNF receptors II).

In our earlier studies, it was shown that cytokine production was under tonic, sympathetic control. We could demonstrate that the amount of catecholamines in the extracellular space was a function of the balance between their vesicular release and their reuptake by the monoamine transporter system. The release of NE, for example, is controlled by the negative feedback mechanism of presynaptic α2-ARs, as discussed above. For the rapid removal of noradrenaline released from sympathetic neurons, its reuptake via noradrenaline-transporter (NET) is responsible.

Low levels of extracellular NE can be achieved experimentally by reserpine treatment. This results in an animal model of depression, where we could demonstrate that the TNF-α response was significantly higher than that in the healthy mice. On the other hand, the extracellular NE level could be increased either by genetic removal or by a chemical blockade of the NET. Recently, it was demonstrated that genetically modified mice, which became NET-deficient (NET–KO) resulted in a significant decrease in the LPS-evoked TNF-α response, confirming the assumption that the balance between
NE release and uptake is one of the key modulators of inflammatory mediator production (Fig. 2).\textsuperscript{6,57}

According to the mostly accepted theory of depression, the extent and/or the duration of monoamine neurotransmitter action are key points in the development and therapy of depression. A number of effective antidepressants have been reported to inhibit the activity of monoamine transporters that resulted in an increased biophase level of monoamines. A similar effect could be achieved by genetic removal of monoamine transporter genes, as it was realized in NET–KO,\textsuperscript{56} DAT–KO,\textsuperscript{58} and 5HTT–KO mice.\textsuperscript{59} These animals behave like chronically antidepressant treated ones, exhibiting high extracellular monoamine levels. Long-term inhibition of the NET by antidepressants has been reported to change the density and function of pre- and postsynaptic α2-ARs, which may contribute to the antidepressant effects of NET inhibitors, such as desipramine. In the NET–KO animals, it was demonstrated that density of α2-AR was upregulated in the brainstem, hippocampus, and striatum.\textsuperscript{60} In these mice, the α2-AR autoreceptors are not desensitized and the inhibitory tone on NE release is stronger as a consequence of elevated extracellular NE concentration.\textsuperscript{61}
Since the extracellular level of monoamines is highly dependent on the activity of their transporters, it was assumed that, in acute treatment of mice, not only desipramine but also other monoamine transporter inhibitors, exhibiting antidepressant characteristics (dopamine transporter and serotonin transporter inhibitors), had modulatory effects on the inflammatory immune response. Whereas an approximately 3-week long chronic treatment is necessary to start the therapeutic antidepressant effect of these drugs, it was studied whether the immunomodulatory effect was also present after chronic treatments.

Concerning the crucial role that cAMP plays in cytokine production, it may be assumed that tricyclic antidepressants (TCAs) produce their immunomodulatory effects through this signaling mechanism. The antidepressant effects of phosphodiesterase inhibitors (like rolipram) and the overexpression of the cAMP response element-binding protein (CREB) in the hippocampus after chronic antidepressant administration, might support this assumption. However, an increasing amount of evidence is available that chronic treatment with TCAs has pleiotropic effects in addition to blocking the monoamine uptake systems, which contribute to exert their antidepressive action in the CNS.

In our recent experiments, we demonstrated significant differences between peripheral and hippocampal cytokine production. As is shown in Figure 3, there are differences in both basal and induced TNF-α production. An induction of TNF-α production with LPS resulted in a significantly higher TNF-α production in the plasma of wild-type (WT) than that in the NET–KO animals, while there was no difference in their basal levels. In the hippocampus of NET–KO animals, the basal TNF-α content was significantly higher than that of the WT mice, while upon induction, both strains responded similarly.

In conclusion, recognition of the interactions between the catecholamine- and inflammatory systems might be important, since certain anti-inflammatory drugs also have antidepressant effects, and most of the antidepressant drugs can modulate the immune response. Considering the essential role of sympathetic neurotransmitters and the fact that β-adrenergic agonists/antagonists are...
widely used in the therapy of many diseases, the recognition that inhibitors of the monoamine uptake system might have multiple targets and are also able to modulate the inflammatory response is highly important, both in future therapy and in the development of new drugs.

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