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NONSYNAPTIC NORADRENALINE RELEASE IN NEURO-IMMUNE RESPONSES*

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Evidence has recently been obtained that the branches of the autonomic nervous system, mainly, the sympathetic [25], regulate cytokine production. Not only the primary (thymus, bone marrow) and secondary (spleen, tonsils, and lymph nodes) lymphoid organs, but also many other tissues are involved in immune responses and are heavily influenced by noradrenaline (NA) derived from varicose axon terminals of the sympathetic nervous system [25, 100]. Besides NA released from nonsynaptic varicosities of noradrenergic terminals [92], circulating catecholamines (adrenaline, dopamine, NA) are also able to influence immune responses, the production of pro- and anti-inflammatory cytokines by different immune cells. The sympathetic nervous system (catecholamines) and the hypothalamic-pituitary-adrenal (HPA) axis (cortisol) are the major integrative and regulatory components of different immune responses. In our laboratory convincing evidence has been obtained that NA released non-synaptically [90, 92] from sympathetic axon terminals and enhanced in concentration in the close proximity of immune cells is able to inhibit production of proinflammatory (TNF-α, IFN-γ, IL-12, IL-1) and increase antiinflammatory cytokines (IL-10) in response to LPS [25, 91], indicating a fine-tuning control of the production of TNF-α and other cytokines by sympathetic innervation under stressful conditions. This effects are mediated *via* β_2-adrenoceptors expressed on immune cells and coupled to cAMP levels.

Keywords: Immune response – sympathetic outflow – cytokines – nonsynaptic

INTRODUCTION

It is generally accepted that the brain communicates with the immune system *via* two pathways: (i) the neuroendocrine humoral axis [16] and (ii) direct innervation through autonomic neuronal efferents. The interaction between the immune responses and emotional states was probably first noted almost 2000 years ago. The Greek physician Galen, who is also reputed to be the first to describe the morphology of the thymus gland, observed that melancholy women were more prone to develop disease than were sanguine women. Although the concepts of cellular and humoral immunity (Metchnikoff, Ehrlich), and of chemical neurotransmission

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(Elliott, Loewi) emerged at the beginning of the previous century, neurosciences and immunology developed for many years without serious consideration of the possibility of interactions between the brain and the immune system. Until now the emphasis has been placed on the humoral axis, in light of the role of glucocorticoids and other hormones, in the immune system [8, 25, 45, 86]. Evidence has recently been obtained that the branches of the autonomic nervous system, namely, the sympathetic [25] and parasympathetic [11], regulate blood flow and cytokine production. Not only the primary (thymus, bone marrow) and secondary (spleen, tonsils, and lymph nodes) lymphoid organs, but also many other tissues are involved in immune responses and are heavily influenced by noradrenaline (NA) derived from varicose axon terminals of the sympathetic nervous system [25, 91]. Besides NA released from nonsynaptic varicosities of noradrenergic terminals [92], circulating catecholamines (adrenaline, dopamine, NA) are also able to influence immune responses, the production of pro- and anti-inflammatory cytokines [25, 60, 91] by different immune cells (Table 1).

RESULTS AND DISCUSSION

Immune cells

As far as the origin of cytokines is concerned, there are several cells able to produce cytokines. Macrophages are essential for host defense and play an important role in orchestrating immune responses [59]. They are the first cells to receive signals. During phylogeny, lymphocytes take over the duties of macrophages using their recognition structures. This shift is very important. The T or other types of lymphocytes control the type of immune responses. Th1 immune responses are associated with TNF-α, IFN-γ, and Th2 responses with IL-4, IL-5, and IL-10 (Table 1). IL-12 secreted by myelomonocytic cells is an important factor promoting the development of Th1 cells. These immune cells receive sympathetic innervation, but noradrenergic boutons do not make synaptic contacts with these cells. The varicosities are about 50–500 nm from the surface of immune cells.

Effects of the sympathetic and parasympathetic nervous systems on immune responses

Effect of sympathetic transmitter (NA) on immune responses. Catecholamines (adrenaline, dopamine, NA), in concert with the adrenal cortical hormone cortisol, act on a wide variety of tissues to maintain the integrity of the internal environment, both at rest and in response to internal and external challenges. The sympathetic nervous system (catecholamines) and the hypothalamic-pituitary-adrenal (HPA) axis (cortisol) are the major integrative and regulatory components of this response (Fig. 1). In contrast to adrenal cortical hormones, which act only after a lag period of 30 min, the

effects of catecholamines are expressed within seconds, and thus are ideally suited for making the rapid short-term adjustments demanded by a changing environment. At rest catecholamines maintain *homeostasis,* as major regulator of fuel metabolism, heart rate, blood vessel tone, thermogenesis, etc. When *homeostasis* is disturbed or threatened, by internal or external challenges, both the sympathetic nervous system and HPA axis become activated, resulting in increased peripheral levels of catecholamines and glucocorticoids that act in concert to keep the steady state of the internal milieu. This reaction is called the general adaptation syndrome or stress response. Centrally, the two principal components of the general adaptational response are the corticotropin-releasing hormone (CRH) and the locus coeruleus-noradrenaline (LC/NA) autonomic (sympathetic) nervous system. The CRH system is widespread throughout the brain but is best characterized in the paraventricular nucleus (PVN) of the hypothalamus. The neuroanatomical basis for these interactions is complex and includes projections of CRH-secreting neurons from the lateral PVN to the sympathetic systems in the hindbrain, and conversely, projections of catecholaminergic fibers from the LC-NA system, *via* the ascending noradrenergic bundle, to the PVN in the hypothalamus. Activation of the LC-NA system leads to release of NA from an extraordinarily dense network of neurons throughout the

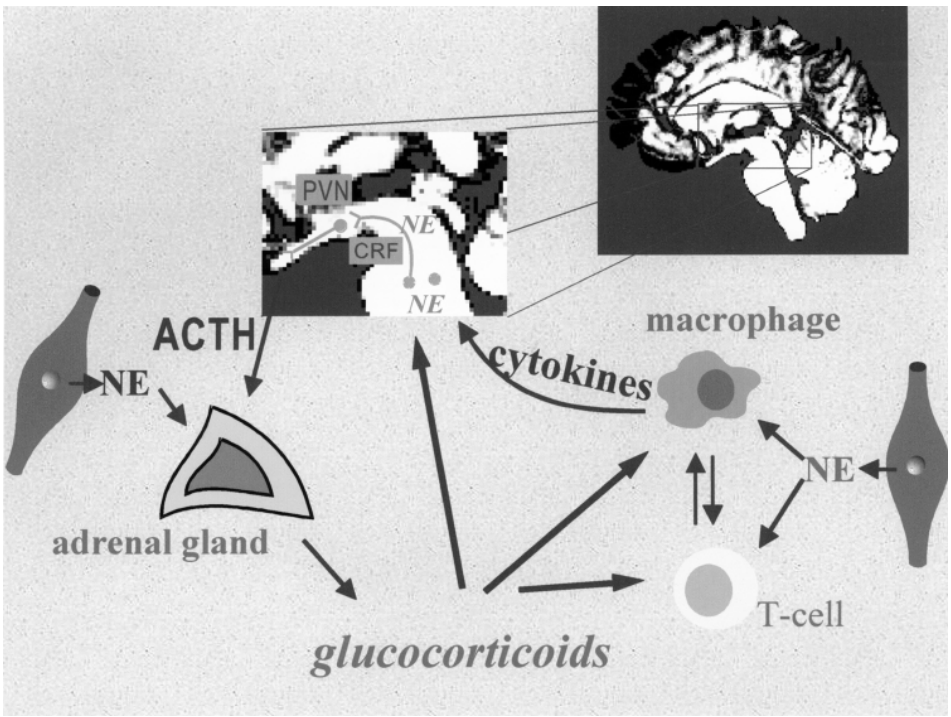


Fig. 1. Interactions between brain, immune responses and endocrine system. Noradrenaline influences cytokine production and glucocorticoid production [91]. For details see the text

brain, resulting, centrally, in enhanced arousal and vigilance, and peripherally, in increased sympathetic output, i.e., increase of the release of NA from the sympathetic nerve terminals and adrenaline from the adrenal medulla.

The autonomic nervous system relays information from the CNS to visceral target tissues through the sympathetic and parasympathetic nervous systems [17, 55]. In our laboratory convincing evidence has been obtained that NA released nonsynaptically [90, 92] from sympathetic axon terminals and enhanced in concentration in the close proximity of immune cells is able to influence TNF-α response to LPS [25, 91], indicating a fine-tuning control of the production of TNF-α and other cytokines by sympathetic innervation under stressful conditions. Neural regulation of immune function through the sympathetic nervous system is modulated by the release of NA at autonomic nerve endings ([91], Fig. 1) and adrenaline from the adrenal medulla [69]. The major lymphoid organs, (i.e., spleen, lymph nodes, thymus, and intestinal Peyer's patches) are extensively innervated by noradrenergic sympathetic nerves [27, 100]. Noradrenaline and other catecholamines have a wide range of direct effects on immune cells, particularly monocytes and lymphocytes [14, 54, 55]. These neurotransmitters regulate the functions of immune cells through interactions with cell surface adrenergic (α_{2B} and β_2) and dopamine receptors [6, 21, 25, 38, 39, 86]. Both the adrenergic and dopamine receptors are members of the superfamily of guanosine triphosphate-binding protein (G-protein)-coupled receptors [53]. These receptors are coupled intracellularly to the adenylate cyclase complex, which, when activated, leads to increased levels of cAMP [13]. It is possible that manipulation of cAMP metabolism through adrenergic (β_2) and dopaminergic receptors, or through phosphodiesterase inhibition, is the crucial element of the immunomodulatory potential of vasoactive pharmacological agents used for circulatory support.

All primary and secondary lymphoid organs are innervated by the sympathetic division of the autonomic nervous system and when activated by stress or other stimuli, these neurons release micromolar quantities of NA (Fig. 2A) into the vicinity of immune cells, such as T lymphocytes and macrophages [27, 28, 29, 78, 100]. β_2-adrenoceptors expressed on the lymphocyte plasma membrane bind NA and signal the cellular interior *via* the $G_{\alpha S}$ subunit of a heterotrimetric G protein linked to the adenylyl cyclase-cAMP-protein kinase A (PKA) signaling cascade [46, 50, 51].

It has also been shown that cytokines are capable of influencing transmitter release. TNF-α inhibits the release of NA from isolated median eminence [23], IL-6 administration releases serotonin in the striatum [104], and TNF-α influences the uptake of taurine [15]. The fact that brain-derived neurotrophic factor modulates serotonin transporter function [62] suggests that not only the release mechanism but also the inactivation process is affected.

Strong evidence is available that the effect of the central nervous system on immune responses is mediated by nonsynaptically released NA from noradrenergic varicosities *via* activation of α_{2B} and β_2 adrenoceptors [21, 24, 25, 30, 76, 79, 81, 100]. Attention has been focused recently on α- and β-receptor-mediated modulation by endogenous NA of pro- and anti-inflammatory cytokine production [21, 30, 38]. For example, the β-adrenoceptor antagonist propranolol, removing the stimulatory

effect of endogenously released NA on β_2-adrenoceptors expressed on cytokine-producing cells, increases the TNF-α [21] and inhibits the IL-10 production (Fig. 2B) normally induced by LPS [86]. On the other hand, when the sympathetic outflow, i.e., the release of endogenous NA, is increased by α_2-adrenoceptor inhibitor, LPS-induced TNF-α levels are reduced and IL-10 levels are increased [21, 25, 38, 76, 79, 81, 82, 86], (Fig. 1C). These facts indicate that NA *via* activation of β_2-adrenoceptors present on the surface of the immune cells reduces the production of proinflammatory (TNF-α, IL-1, IFN-γ, IL-12) and increases that of antiinflammatory (IL-10) compounds [91].

It was interesting to learn that in pregnant animals the modulation of both IL-10 and TNF-α production by NA via α_2- or β-adrenoceptors does not operate [96] and the LPS-induced production of proinflammatory cytokines (TNF-α, IL-6, and IFN-γ) is enhanced while IL-10 production is depressed.

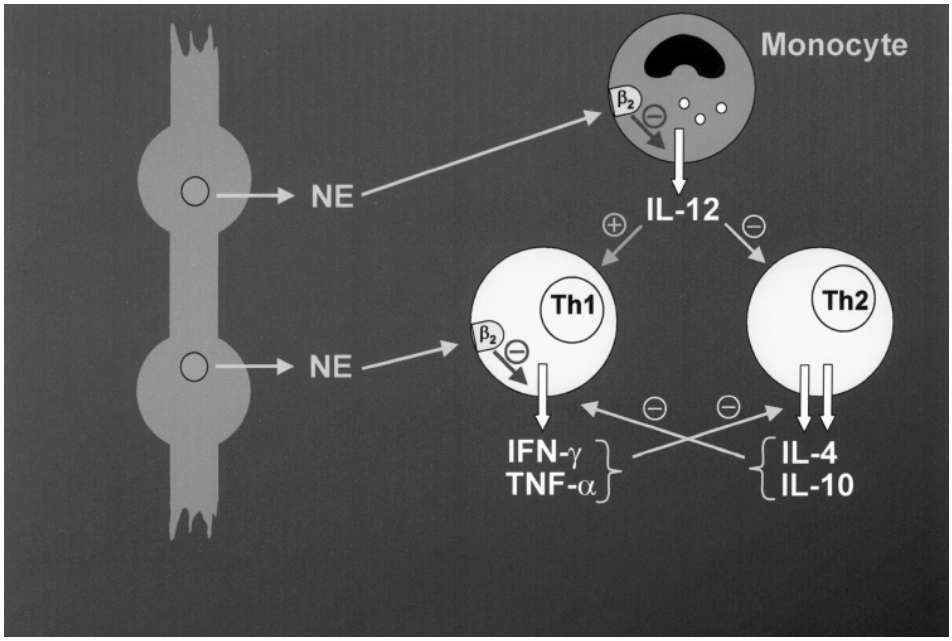
Isoproterenol pretreatment reduced cytokine and NO production induced by bacterial lipopolysaccharide (LPS, 4–10 mg/kg). Pretreatment with isoproterenol (10 mg/kg) blunted the LPS-induced TNF response, increased the LPS-induced formation of interleukin-10 and interleukin-6 and reduced the LPS-induced production of NO in conscious mice [82]. Beta-adrenergic agonists exert many of their effects by elevation of intracellular cyclic AMP (cAMP) concentration. Cyclic AMP can modulate endotoxin-induced cytokine and NO production.

Cholinergic inhibitory pathway. Cytokines and LPS stimulate afferent neural signals in the vagus nerve that increase acute-phase responses (fever, upregulation of the expression of interleukin (IL)-1β) in the brain [31, 57, 99]. Afferent vagus neural signals are rapidly transmitted to the hypothalamic-pituitary axis, which releases adrenocorticotropin hormone, and subsequently, by the increase in glucocorticoid levels inhibits cytokine release by the innate immune system [80]. Thus, afferent neural sig-

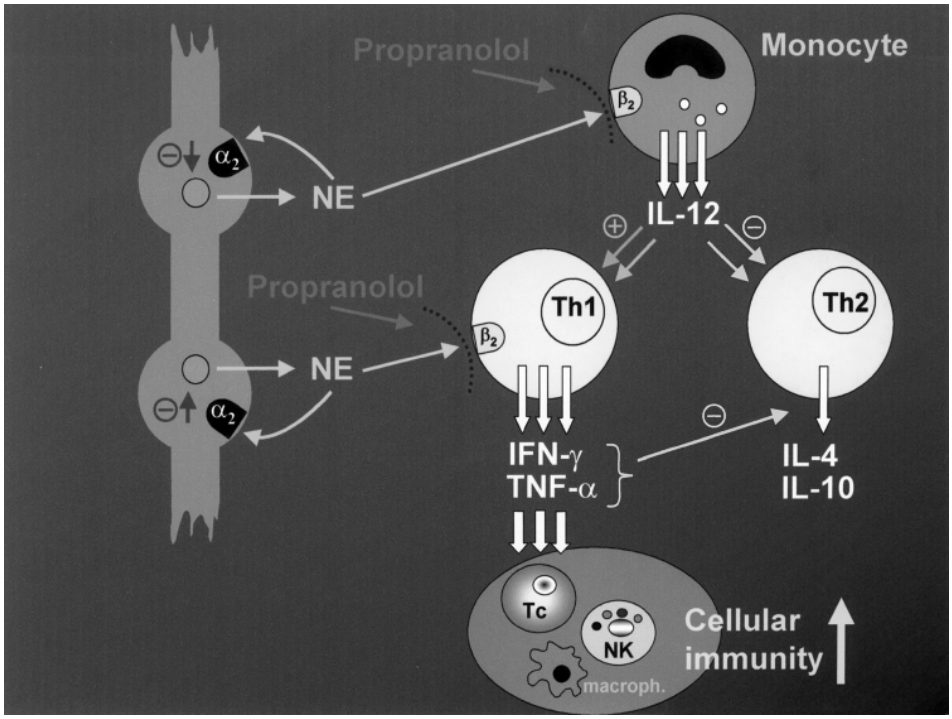
Table 1
Major pro- and antiinflammatory cytokines

Proinflammatory cytokines	Source
IL-12	APCs
TNF-α	APCs
IL-1	APCs, fibroblasts, endothelium
IFN-γ	Th1 and NK cells
Antiinflammatory cytokines	**Source**
IL-4	Th2 cells
IL-10	APCs, Th2 cells
TGF-β	Different cells

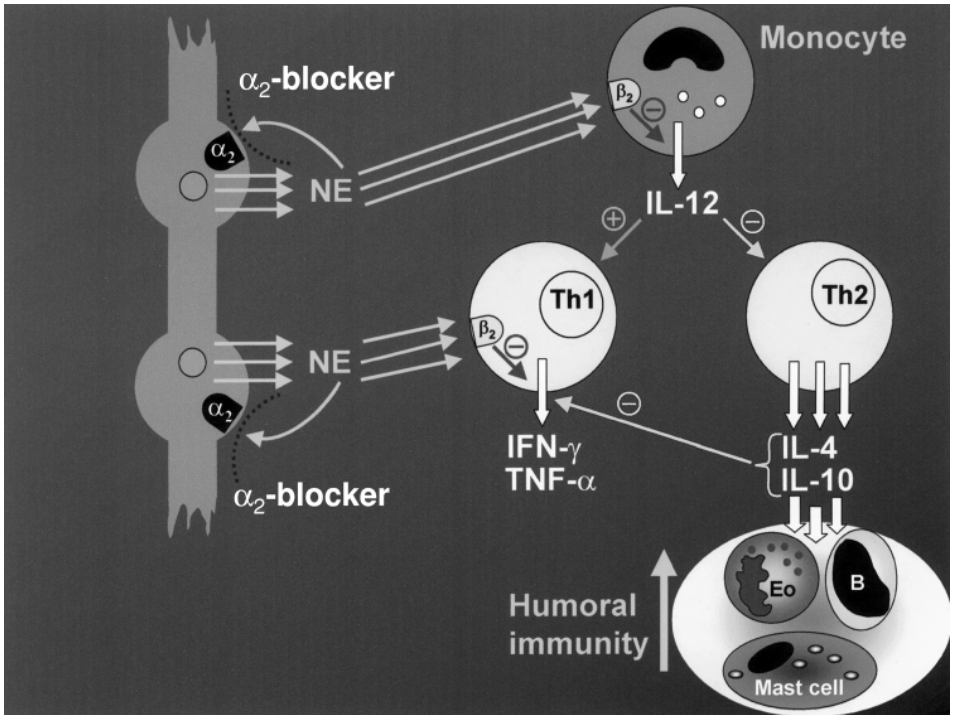
APCs, antigen-presenting cells; NK, natural killer cell; Th, helper lymphocyte


Monocyte
NE
IL-12
Th1
Th2
NE
IFN-γ
TNF-α
IL-4
IL-10

2A


Propranolol
Monocyte
NE
IL-12
Propranolol
Th1
Th2
NE
IFN-γ
TNF-α
IL-4
IL-10
Tc
NK
macroph.
Cellular
immunity

2B



2C

Fig. 2. Noradrenaline (NE) is a link between nervous system and immune cells. There is a balance between proinflammatory (TNF-α, IFN-γ, IL-1) and antiinflammatory (IL-10) cytokin production (Fig. 2A). Noradrenaline released from varicosities diffuses far away and stimulates β_2-adrenoceptors expressed on immune cells (monocytes, T helper (Th), lymphocyte subclasses Th1 and Th2, that are parts of adaptive immunity strong activation (e.g. in stress) of β_2-adrenoceptors results in increase of CAMP level and reducation of IL-12, IFN-γ and TNF-α production (Fig. 2B), but the production of IL-10 is increased (note the lack of IL-12-induced inhibition of IL-10 production). Under this condition humoral immunity is increased. In the presence of β-adrenoceptor inhibition by propranolol (Fig. 2C) TNF-α level is increased and IL-10 level reduced (cellular immunity)

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nals alert the CNS that there are microbial threats or cytokine excess and thereby stimulate an anti-inflammatory counter-response to prevent systemic inflammation.

The transmitter between the vagus nerve and the innate immune system is acetylcholine [11, 12]. Macrophages express cholinergic receptor activity; acetylcholine significantly inhibits LPS-induced TNF protein release through a post-transcriptional mechanism [11]. Acetylcholine significantly inhibits the release of other pro-inflammatory cytokines, including IL-1β, IL-6, and IL-18, but not IL-10, an anti-inflammatory cytokine. Other cholinergic agonists (nicotine and muscarine) also inhibit LPS-induced TNF release; macrophage cholinergic receptor activity is exquisitely sensitive to α-conotoxin, implicating nicotinic-type receptor activity in the transduction of the cytokine-inhibiting signal. Collectively, these observations

implicate cholinergic signals from the CNS as direct and rapid modulators of the inflammatory response [11, 12]. This mechanism has been termed the "cholinergic anti-inflammatory pathway" [87].

Nonsynaptic release of noradrenaline

Since Sherrington's classic work (1906) it has become a doctrine of neurophysiology that the synapse, a part of the surface of separation between neurons, is the primary site of neuronal information processing. This type of communication is relevant for some regions of CNS [32, 35, 36, 37] and particularly the neuro-muscular junction [94]. However, this concept has recently been seriously challenged [89, 90, 92]. The varicose noradrenergic, serotonergic and dopaminergic terminals *(boutons en passant)* that lack synaptic contact with the target cell are still able to release transmitters in response to axonal electrical activity. Functionally, the first neurochemical evidence of non-synaptic "cross-talk" between neurons was provided by [67], when it was shown that NA released from the noradrenergic axon terminals tonically inhibits, through presynaptic α-adrenoceptors, the release of acetylcholine from cholinergic varicose axon terminals. Thus, many of the neurotransmitters, especially the monoamines and peptides, show a release profile that is halfway between specific synaptic neurotransmission and relatively non-specific endocrine secretion [90, 94]. This release profile is referred to as "non-synaptic" [89, 90, 93], a term meant to imply a type of neuron that could release its neurotransmitters locally and then have these neurotransmitters diffuse some distance away from the release site. Such a diffusion of neurotransmitters could interact with a large number of target cells within a local area. Nonsynaptically, the neurotransmitter is released from free nerve endings into a large extraneuronal space [64], with no post-junctional specializations and, hence, the neurotransmitter diffuses a considerable distance before interacting with its receptors (sometimes this could be more than 50–100 μm).

Effect on the hypothalamic-pituitary-adrenal (HPA) axis

Endotoxin, IL-1 and TNF-α activate the HPA axis [20]. The site of their action is in the hypothalamus through the production of corticotropin-releasing factor resulting in an increase of ACTH and corticosterone secretion. Our previous results [22] showed that lesions of the hypothalamic paraventricular nucleus did not completely block the effect of endotoxin (lipopolysaccharide, LPS) on plasma ACTH levels in the late phase of the response. Because the lesions of the median eminence totally abolished the effect of LPS, it is conceivable that the median eminence is a target for the effect of LPS and/or related cytokines. In addition to hypophyseal control, steroid synthesis in and secretion from the adrenal cortex is under direct local neural modulation. Morphological and neurochemical evidence [97, 98] is available that NA and DA released from sympathetic nerve endings. Dopamine diffuses far away from

release site to the zona gromerulosa cells and inhibits the secretion of glucocorticoid *via* activation of D_2-receptors.

Effect on nerve cells, neurodegeneration

It has been shown that TNF-α released from different immune cells activates IL-1 production and induces inflammation, fever, necrosis or apoptosis, i.e., swelling, destruction, and lysis of the cell and cell shrinkage. Inflammation and subsequent cytotoxicity can also be mediated by reactive oxygen species, such as peroxides and nitric oxide (NO), which can be produced by TNF-α [43, 73]. TGF-β released from immune cells acts in a different way (Fig. 1); it deactivates macrophages and reduces the release of reactive oxygen species and NO [105]. In addition, TNF-α has also been shown to be mitogenic for a number of normal cells. Evidence is now available that specific cytokines, particularly IL-1, are involved directly in neurodegeneration, neuronal death in the CNS [71]. Neuronal death is associated with excitotoxicity, i.e., excessive release of glutamate and subsequent activation of NMDA and AMPA receptors [95]. There is some evidence that IL-1β is the predominant form of IL-1 induced by brain insults. It is likely that IL-1 interacts in some way with this cascade to modify glutamate release, reuptake, or action [71]. Under certain conditions, resident cells in the CNS, particularly astrocytes and microglia, may operate as immunocompetent cells. Glia undergo inflammatory activation in most CNS pathologies, able to release NO and glutamate [3, 72], and is also involved in immune responses to bacterial LPS and cytokines [44, 47, 49, 52, 61, 102]. Nitric oxide is partly responsible for microglia-induced neurodegeneration [3, 41] and inhibition of catecholamine reuptake [48, 56].

Pro-inflammatory cytokines and endotoxin (bacterial LPS) induce the expression of a distinct inducible isoform of NO synthase (iNOS) in various cell types [34, 63, 82]. The production of large amounts of NO by iNOS is cytotoxic. Although it is believed that NO *per se* mediates cytotoxicity in nerve cells expressing iNOS, there is evidence that pro-inflammatory cytokines and LPS induce the production of both oxygen free radicals in macrophages and neurons [2, 5, 26, 42, 88]. NO is known to react with superoxide anion to form a potentially even more toxic species, peroxynitrite [4, 5, 68], a potent suppressor of mitochondrial respiration [106].

Some data have raised the possibility that some of the previously ascribed cytotoxic actions of NO may not, in fact, result from the effect of NO *per se,* but rather *via* the production of $ONOO^-$ a reactive oxidant species, and an important mediator of cell damage under conditions of inflammation and oxidant stress. Recent data suggest that NO may lower cellular ATP in neurons *via* an indirect mechanism [103]. A high concentration of NO has been shown to cause DNA injury and trigger a repair process by the nuclear enzyme poly(ADP ribose) synthetase (PARS), which consumes cellular energy stocks, resulting in the depletion of NAD^+ and ATP and in irreversible cellular injury [84]. Therefore antioxidants are proposed for the treatment of shock, inflammation, and ischemia/reperfusion injury [18].

Autoimmune disorders

Rheumatoid arthritis, multiple sclerosis [70], and insulin-dependent diabetes mellitus are diseases which seem to have little in common. All three are inflammatory disorders [100]. TNF-α is involved in a number of autoimmune and inflammatory conditions (rheumatoid arthritis, Crohn's disease, [40] inflammatory polyarthritis), and in demyelination and oligodendrocyte toxicity in multiple sclerosis [74, 75]. Patients with active psoriasis often have increased TNF-α and IL-6 levels in the psoriatic plaques, and even in their plasma [65]. Corticosterone inhibits production and downregulates transcription and translation of TNF-α. In accordance with this, the Lewis strain of rat (with low corticosterone response to stress) is particularly susceptible to such chronic inflammatory models as arthritis and experimental allergic emcephalomyelitis.

Traumatic brain injury

Traumatic brain injury in humans is associated with immunosuppression and is often accompanied by infectious complications. Brain tumor resection frequently results in localized brain damage and inflammation. The sympathetic activation (stress) resulting in catecholamine release is presumably due to acute decompression, brain stem manipulation, lesion, and irritation during the neurosurgical procedure. As a result of sympathetic activation (excess of NA release), IL10 production is increased (Fig. 1C). This iatrogenic brain injury is associated with sympathetic activation, which results in catecholamine release, i.e., increased release of NA, and activation of β-adrenergic receptors that are coupled to the cyclic AMP-protein kinase A signal transduction pathway. Protein kinase A induces release of preformed IL-10 from monocytes. It was found in a rat model of acute brain injury that propranolol, a β-adrenoceptor antagonist, prevented the increase of IL-10 plasma levels. Interleukin-10 not only downregulates the MHC class II expression on monocytes but also inhibits monocyte production of proinflammatory cytokines, including IL-1, IL-2, IL-6, IL-8, TNF-α, and IFN-γ [19, 33]. Woiciechowsky and colleagues [101] demonstrated that catecholamines trigger the release of IL-10 from unstimulated monocytes without costimulation *via* β-adrenoceptors [81].

Sepsis

Besides pro-inflammatory cytokines, such as TNF-α and free radicals, such as nitric oxide (NO), are mediators of endotoxaemia. Catecholamines are in clinical use to treat the haemodynamic consequences of severe septic shock. Sepsis is characterized by peripheral vasodilatation, myocardial dysfunction, and disrupted intracellular energetic processes [9, 10, 18, 58]. The haemodynamic changes in sepsis are mediated by the production of pro-inflammatory cytokines [1, 25] and by the overpro-

duction of free radical and oxidant mediators of shock (such as superoxide, nitric oxide (NO) peroxynitrite and hydroxyl radical [83, 84, 85]). A large number of studies indicate the protective effect of superoxide dismutase and NOS inhibitors in animal models of sepsis.

Alzheimer disease

Evidence has recently been obtained that proinflammatory cytokines (IL-1, TNF-α) are also involved in the pathogenesis of Alzheimer disease [7, 41, 66, 77].

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