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Review

Neural regulators of innate immune responses and inflammation

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Abstract. The nervous system regulates immune function and inflammation. Experimental evidence shows an important role of the autonomic nervous system in the bidirectional communication between the brain and the immune system, underlying the ability of the brain to monitor immune status and control inflammation. Here we review the involvement of the autonomic nervous system in regulating inflammation, with a focus on the vagus nerve. The clinical implications of the recently discovered anti-inflammatory role of the efferent vagus nerve are also discussed.

Key words. Neuroimmunomodulation; TNF; autonomic nervous system; vagus nerve; innate immunity; inflammation.

Introduction

Inflammation is a local response to infection and tissue damage mediated by activated macrophages, monocytes and other immune cells. Those cells release cytokines [including tumor necrosis factor (TNF), interleukin-1β (IL-1β)] and other mediators of inflammation [1, 2]. Inflammation must be precisely regulated, because either insufficient or excessive responses can lead to pathological complications. Immunodeficiency, the lack of adequate inflammatory responses, results in increased rates of infections and cancer [1, 3]. On the other hand, the excessive release of TNF, high mobility group box 1 (HMGB1) and other pro-inflammatory mediators leads to systemic inflammation, associated with the development of serious pathological complications including sepsis and autoimmune diseases [1, 4–8]. The control of inflammation, which prevents extension of local inflammation into the systemic circulation, is realized by two major mechanisms: self-controlling innate immune mechanisms and brain-derived immunoregulatory output.

Activated immune cells release anti-inflammatory cytokines interleukin-10 (IL-10) and interleukin-4 (IL-4), soluble TNF receptors and transforming growth factor β, that play a counter-regulatory role in inflammation [1, 9]. The hypothalamic-pituitary-adrenal (HPA) axis, which controls the release of glucocorticoids is a well-characterized brain-derived neuroendocrine immunomodulatory mechanism [10–12]. The initial, brain response to inflammation is mediated by activation of autonomic nervous system pathways [6, 13–16]. Afferent vagus nerve fibers play a role in rapidly signaling the brain to trigger immunomodulatory responses in the early phases of inflammation [14, 17, 18]. Recent studies indicate that the efferent vagus nerve inhibits the release of pro-inflammatory cytokines and regulates inflammation in real time, similar to its effects on the heart rate and gastrointestinal function [6, 19–22]. This function of the efferent vagus nerve has been termed the cholinergic anti-inflammatory pathway [19]. The activated vagus nerve exerts anti-inflammatory action through peripheral

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mechanisms that require signaling via α7 nicotinic acetylcholine receptors [22].

This review outlines neural mechanisms that regulate innate immunity and inflammation. The focus is on the anti-inflammatory role of the vagus nerve, the mechanisms of suppression of TNF by activation of the efferent vagus nerve and the potential for vagus nerve stimulation as a therapeutic strategy.

Innate immune responses and the role of TNF

Innate immunity is a major component of the defensive strategy of the organism against pathogen invasion, injury and trauma. The immediate activation of the innate immune system by infection is influenced by the ability to recognize non-self motifs. The host recognizes pathogen-associated molecular patterns by pattern-recognition receptors, many of which are members of the family of the TOLL-like receptors (TLRs) [23]. For example lipopolysaccharide (LPS, endotoxin), a component of the cell wall of Gram-negative bacteria, and a prototype activator of innate immune responses [24], activates TLR4, expressed on macrophages, dendritic and other immune cells. Receptor-ligand interaction induces intracellular downstream pathways, leading to release of cytokines and other pro-inflammatory mediators [1]. Innate immunity can also be activated by tissue injury and trauma without accompanying infection. Recent evidence indicates that necrotic cell damage activates immune cells through release of HMGB1, heat-shock proteins and oxidized lipoproteins by signaling via TLR [1, 25]. Pro-inflammatory mediators, including TNF, IL-1β, HMGB1, reactive oxygen and nitrogen intermediates, and adhesion molecules [1, 2], mediate vasodilation, recruitment of white blood cells and increased vascular permeability. The pro-inflammatory immune response is balanced by the release of anti-inflammatory mediators, including the cytokines IL-10 and IL-4, soluble TNF receptors, interleukin-1 receptor antagonists and transforming growth factor β [9]. The polyamine spermine, released at sites of inflammation, is also capable of suppressing pro-inflammatory responses [26]. Macrophages and dendritic cells, as antigen-presenting cells, link the innate immunity with the induction of T and B lymphocytes, underlying slower adaptive (specific) immune responses [1].

While beneficial at early phases of inflammation, the overexpression of TNF and other pro-inflammatory mediators may lead to conversion of local inflammation into systemic and chronic inflammation, associated with secondary tissue damage and death. Many disorders, including rheumatoid arthritis, Crohn's disease, ischemia-reperfusion injury and sepsis, are classified as inflammatory because of the critical contribution of excessive inflammation to the pathological complications [1]. TNF fulfills central functions during the local response to infection and injury and the subsequent induction of adaptive immune responses. TNF plays a role in the extracellular killing of the pathogen, and contributes to localization of the microbial invasion and repairing tissue damage [27, 28]. If its release is uncontrolled, however, TNF is toxic, mediating microvascular coagulation and capillary leakage syndrome, impaired cardiac output and the development of lethal shock [4, 29]. TNF amplifies the immune response by stimulating its own synthesis and the synthesis of other pro-inflammatory mediators, including IL-1β, HMGB1, eicosanoids, and reactive oxygen and nitrogen intermediates. Some of these substances are in turn capable of stimulating TNF synthesis [29, 30].

Due to its essential role in the pro-inflammatory immune response, TNF represents an important therapeutic target in treatment of inflammatory diseases, including rheumatoid arthritis and Crohn's disease [31–33]. Recent attention has focused on other potential therapeutic targets of inflammation, downstream of TNF in the cytokine cascade [7, 30, 34, 35]. For instance HMGB1 antibodies are effective therapies for arthritis and sepsis in animal models [34, 35].

Crosstalk between the immune system and the brain during inflammation: role of the autonomic nervous system

The influence of the brain on health and disease is a topic that originated in ancient times. We have attempted to outline here some aspects of this subject from the perspective of the brain control of innate immune function and cytokine release. The immune system can interact with the brain to mobilize brain-derived responses to control inflammation. TNF and other pro-inflammatory cytokines elicit signals in brain which in turn exert strong regulatory effects on the innate immune responses underlying inflammation. Brain immunoregulatory output is mediated by the autonomic nervous system; sympathetic and vagus nerve innervation of the thymus, liver, heart, gastrointestinal tract, lungs, pancreas and kidneys represents hard-wired pathways of CNS-derived regulation of innate immunity. Neural regulation is faster than endocrine processes; thereby the vagus nerve and the sympathetic division of the autonomic nervous system can mediate an initial and rapid brain immunomodulatory response. Slower neuroendocrine mechanisms, including HPA axis activation and α-melanocyte-stimulating hormone, additionally regulate inflammation and protect against the deleterious effects of the pro-inflammatory mediators.

Classical teaching suggests that the two divisions of the autonomic nervous system usually act in opposition. In some cases the sympathetic and vagus nerve regulation of innate immunity is an example of synergistic mode of ac-

tion to suppress cytokine release. The cellular targets of the autonomic neural regulation of inflammation are tissue macrophages, Kupffer cells (in the liver), dendritic cells, mast cells, and other immune and non-immune cytokine-producing cells in the innervated tissues. Sympathetic induction can cause suppression of the pro-inflammatory cytokine synthesis by β-adrenoceptor mechanisms; vagus nerve stimulation inhibits inflammatory responses through activation of α7 subunit-containing nicotinic acetylcholine receptors [22]. In some cases sympathetic activation also can result in upregulation of TNF synthesis by α2-adrenoceptor-mediated mechanisms [13, 15], but the activation of the vagus nerve counteracts excessive TNF release.

Sympathetic regulation of innate immune responses and inflammation

Activation of the sympathetic division of the autonomic nervous system is an important immunomodulatory mechanism. Experimental evidence has revealed that sympathetic output inhibit inflammation. The sympathetic division has a critical role in stroke-provoked immunosupression, bacterial infection and mortality [36]. Preganglionic sympathetic fibers receive neuronal input from the locus coeruleus (LC) and rostral ventrolateral medulla (RVM), brainstem nuclei associated with the brain's ability to monitor and respond to immune signaling [13, 16] (fig. 1). Sympathetic regulation of cytokine synthesis/release and inflammation has been extensively investigated during the past 2 decades and outlined in several reviews [13, 15, 37]. Sympathetic postganglionic neurons innervate lymphoid organs, blood vessels, heart, liver and gastrointestinal tract, and this is the anatomical basis of the sympathetic regulation of innate and adaptive immunity, and inflammation [13, 38]. Norepinephrine is the principal mediator of sympathetic postganglionic neurons; some sympathetic postganglionic neurons also release neuropeptide Y and epinephrine. Activation of the preganglionic sympathetic fibers innervating the chromaffin cells in the adrenal medulla leads to secretion of epinephrine, and to a lesser extent norepinephrine and dopamine, which also exert sympathetic effects acting as hormones. The interaction of norepinephrine, and epinephrine with α- and β-adrenoreceptors (adrenoceptors), mediates the sympathetic regulation of physiological functions. These adrenoceptors, expressed on macrophages, monocytes and other immune cells, mediate the sympathetic regulation of the cytokine levels and immune inflammatory responses. Norepinephrine, released from axon terminals of sympathetic postganglionic fibers, interacts with immune cells, surrounding the axon vicinity through two mechanisms: (i) synaptic-like interactions and (ii) diffusion and interaction with extrasynaptic receptors on cytokine-secreting cells [13]. The first

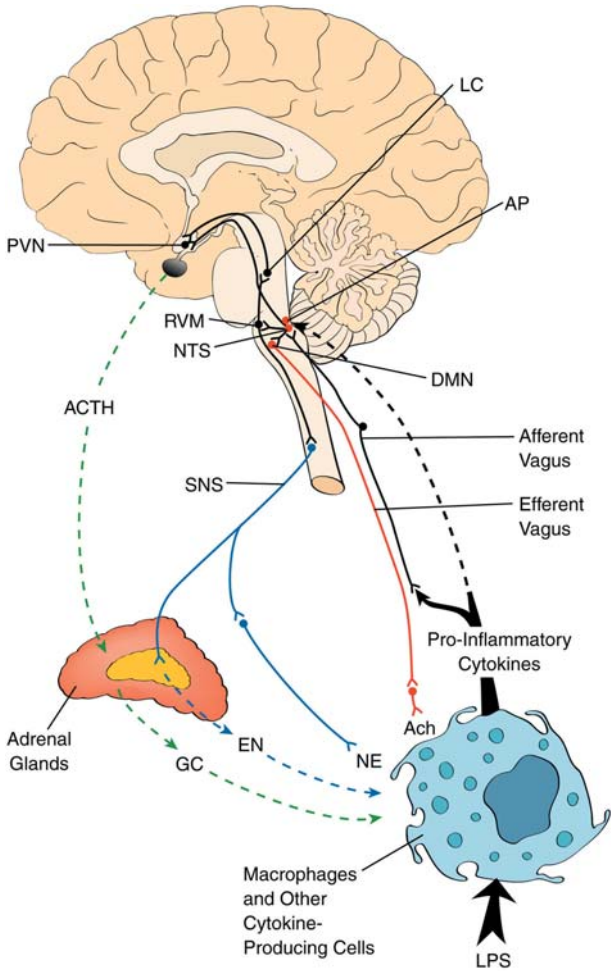

Figure 1. Neural and neuro-hormonal pathways of regulation of innate immunity and inflammation. During immune challenge activated macrophages and other immune and non-immune cells release cytokines that signal the brain for activation of immunomodulatory mechanisms. Central immunomodulation is achieved by the efferent vagus nerve, the sympathetic part of the autonomic nervous system and the HPA axis (see the text for details). Abbreviations: area postrema (AP); nucleus tractus solitarius (NTS); dorsal motor nucleus of the vagus (DMN); paraventricular nucleus (PVN); rostral ventrolateral medulla (RVM); locus coeruleus (LC); sympathetic division of the autonomic nervous system (SNS); adrenocorticotropin hormone (ACTH); glucocorticoids (GC); epinephrine (EN); norepinephrine (NE); acetylcholine (ACh); LPS (lipopolysaccharide, endotoxin). Taken from [16, *Molecular Medicine*, 2003, 9(5–8): 125–134] with *Molecular Medicine's* copyright permission.

mechanism seems to play a role in the spleen, where noradrenergic nerve terminals have been found to appose macrophages and other immune cells, thus forming synaptic-like junctions [13, 15, 38]. The second mechanism is based on the non-synaptic release of norepinephrine from free nerve endings, which do not form further synaptic contacts. It transmits sympathetic immunoregulatory signals to macrophages and other immune cell types in the thymus and other innervated organs [13]. The suppression of endotoxin-induced TNF release by nor-

epinephrine and epinephrine is achieved by activation of β2-adrenoceptors on macrophages and monocytes [13, 37, 39–41]. Catecholamines also upregulate the anti-inflammatory cytokine IL-10 [13, 40, 42–44]. Pro-inflammatory cytokine levels appear to be under tonic sympathetic control [13]. The possibility of central activation of sympathetic routes of immunosuppression has been suggested by the observation that brain-spinal cord-sympathetic pathways mediate the α-melanocyte-stimulating hormone immunosuppressive effects [45]. In early phases of some cases of inflammation, TNF expression can also be upregulated by catecholamines acting through the α2-adrenoceptor mechanism on cytokine-producing cells [46], or by activation of α2-presynaptic adrenoceptors, negatively regulating norepinephrine release [15, 46]. Epinephrine administration suppresses TNF and increases IL-10 in LPS-treated volunteers [40].

Role of the vagus nerve in neuroimmunomodulation

Until recently, most research on neuroimmunomodulation was focused on the role of the sympathetic division of the autonomic nervous system. It recently became clear that the parasympathetic division is ultimately involved in the control of immunity and inflammation. Afferent vagal neurons play a role in transmitting immune signals to the brain during inflammation, and activation of the vagal efferent fibers leads to suppression of inflammation. The sensory afferent and motor efferent vagal neurons form a centrally integrated reflex mechanism that controls inflammation in real time [6]. The sympathetic immunoregulatory output is also an integral component of the efferent part of the inflammatory reflex [6].

Sensory functions of afferent vagus nerve fibers during inflammation

Pro-inflammatory cytokines function as signaling molecules by which the immune system informs the brain about peripheral inflammation (fig. 1). Sensory vagal neurons, residing in the nodose ganglion, are involved in transmitting cytokine signals to the brain [14, 18]. These neurons can monitor the presence of inflammation. Intraperitonealy administered LPS induces IL-β protein expression not only in macrophages and dendritic cells within connective tissues, surrounding abdominal afferent vagal fibers, but also within these fibers [47]. IL-1β causes brain-derived sickness symptoms and fever through activation of abdominal vagal afferents [48]; subdiaphragmatic vagotomy eliminates the effect of low-dose IL-1β, but does not effectively block the effects of high concentrations [49]. Subdiaphragmatic vagotomy significantly attenuates the activation of key components of the HPA immunosuppressive axis following endotoxin infusion [50]. The neural pathway of immune-to-brain communication seems to play a primary role during moderate localized inflammatory responses. The released TNF, IL-1β and other pro-inflammatory cytokines predominantly activate afferent vagus fibers, distributed in the abdominal cavity [18, 47]. The vagus nerve also can monitor blood-borne cytokines through vagal paraganglia, associated with sensory vagal neurons [18]. These vagal neurons terminate in the nucleus tractus solitarius (NTS) in the brainstem medulla oblongata (fig. 1). The NTS has an important role in the coordination of autonomic function. The NTS, together with the dorsal motor nucleus of the vagus (DMN) and area postrema (AP), form the dorsal vagal complex (DVC). The interaction between the NTS and the DMN neurons can be associated with activation of preganglionic vagal efferents originating from the DMN. Efferent vagus nerve activation during inflammation has been observed as neuronal activation in the DMN, NTS and AP. Endotoxin activates the vagal fibers innervating the thymus after IL-1β infusion [51, 52]. Vagotomized endotoxemic rats develop enhanced inflammation, indicating the role of the vagus nerve in the tonic supression of cytokine responses [19]. The NTS neurons also project to the nucleus ambiguous, where some of the cardiovascular vagal neurons originate. The neuronal contacts between the NTS and the RVM and LC (brain functional components of the sympathetic route of immunomodulation) are the anatomical basis for subsequent activation of sympathetic preganglionic neurons in the spinal cord. Bidirectional neural communications also exist between the RVM and LC, and the hypothalamic paraventricular nucleus. The key role of the NTS in the interaction and integration of the autonomic function with endocrine and behavioral responses is attributed to bidirectional neuronal circuits between the NTS and hypothalamic nuclei, including the paraventricular nucleus.

The transmission of cytokine signals to the brain through the vagal sensory neurons depends upon the magnitude of the immune challenge. The cholinergic anti-inflammatory pathway and other brain-derived immunomodulatory circuits can also be activated by pro-inflammatory cytokines through humoral mechanisms. The humoral cytokine signal during robust and systemic inflammatory responses is transmitted to the brain via several mechanisms: (i) saturable carrier-mediated transport through the blood-brain barrier; (ii) binding to receptors at the surface of the endothelium of the brain capillaries, and subsequent release of soluble mediators such as prostaglandins and nitric oxide, and; (iii) via circumventricular organs that lack normal blood-brain barrier function [53–57]. The AP (which is one of the circumventricular organs) is an important site for the third mechanism [57]. The close proximity of the AP to the NTS and DMN, ascending projections of the NTS neurons, and cytokine-induced production of prostaglandins within the AP and

NTS, contributes to triggering brain immunomodulatory responses (fig. 1).

Control of innate immune responses and inflammation by the efferent vagus nerve: the cholinergic anti-inflammatory pathway

Acetylcholine – the major parasympathetic neurotransmitter – is an inhibitor of TNF release in LPS-treated macrophages [19]. Acetylcholine negatively affects the release of IL-1, IL-6 and IL-18, but has no effect on the level of the anti-inflammatory cytokine IL-10 in LPS-stimulated macrophages [19]. In vivo studies revealed that efferent cholinergic vagal neurons play a critical role in controlling TNF levels and innate immune responses. Electrical stimulation of the distal end of the vagus nerve effectively decreases serum and hepatic TNF levels, attenuating the development of shock (hypotension) during lethal endotoxemia [19]. Vagotomy without stimulation is accompanied with higher serum TNF levels in response to endotoxin administration and more rapid development of shock, compared with sham-operated control rats [19]. This observation indicates that the vagus nerve exerts a tonic suppression on TNF release. Electrical vagus nerve stimulation significantly inhibits TNF concentrations in the heart and liver during endotoxemia [21, 58]. Anti-inflammatory effects of electrical vagus nerve stimulation have been observed in animal models of ischemia reperfusion [59] and hemorrhagic shock [60] – pathological complications in which TNF overproduction is a recognized mediator of inflammation. Electrical vagus nerve stimulation significantly attenuates TNF synthesis and protects rats against shock in an aortic occlusion-caused reperfusion injury [59]. Electrical stimulation of the efferent vagus nerve attenuates hypotension and improves survival rate of rats during severe hypovolemic hemorrhagic shock [60].

Acetylcholine exerts its effects by interacting with two types of acetylcholine receptors – muscarinic and nicotinic – which can be further subclassified. All pre- and most of the postganglionic vagal neurons are cholinergic. Acetylcholine released from the postganglionic axon terminals binds to different subtypes muscarinic (metabotropic) acetylcholine receptors on smooth muscle cells, cardiac myocytes and glandular cells in the innervated organs. The presence of muscarinic acetylcholine receptors has also been shown on immune and non-immune TNF-producing cells [61–67]. Interestingly, muscarinic receptors are most likely not involved in the mediation of the anti-inflammatory effects of the vagus nerve [16, 22]. The nicotinic receptors are the ionotropic type of acetylcholine receptors, forming pentamer structures with different subunit compositions. Nicotinic receptors ($\alpha 1_2/\beta 1/\varepsilon/\delta$) are implicated in transmitting the somatic nervous system cholinergic output in the neuromuscular junction [68]. Different α/β subunit hetero- and $\alpha 7$-, $\alpha 8$- or $\alpha 9$-subunit homopentamer combinations of nicotinic receptors are distributed in the CNS, autonomic ganglia, and extraneuronal sites. The main function of the nicotinic receptors ($\alpha 4/\beta 2$ and $\alpha 7$ homopentamer) in the brain is modulation of acetylcholine and other neurotransmitter release [69]. In sympathetic and parasympathetic ganglia, nicotinic receptors (the $\alpha 3$ subunit in combination with $\beta 4$) play a critical role in mediation of fast synaptic transmission [68].

We recently gained important insight into the receptor mechanisms mediating the acetylcholine effects on macrophage TNF release by demonstrating the critical role of the $\alpha 7$ subunit of nicotinic acetylcholine receptor. $\alpha 7$ subunit-containing nicotinic receptors are expressed on human macrophages, and this nicotinic receptor subunit plays a critical anti-inflammatory role in vivo [22]. $\alpha 7$ subunit-deficient mice have higher sensitivity to immune challenge; LPS exposure leads to significantly higher elevation of serum TNF, IL-1 and IL-6 levels in these mice, as compared to the wild type [22]. Electrical stimulation of the vagus nerve does not significantly suppress serum and organ TNF levels in the $\alpha 7$ subunit-deficient endotoxemic mice, in contrast to the wild type [22]. This finding suggests that the $\alpha 7$ subunit of the nicotinic acetylcholine receptors plays an essential role in mediating the anti-inflammatory effects of cholinergic vagal fibers. The fact that the cytokine suppressive effects of the vagal cholinergic fibers are mediated by nicotinic $\alpha 7$ receptors, rather than the classical muscarinic receptors, indicates the high specificity of this function of the vagus nerve.

The activated vagus nerve exerts its immunomodulatory effects on tissue macrophages and other cytokine-synthesizing cells in the organs of the reticuluendothelial system, including the heart, liver, gastrointestinal tract and spleen (fig. 2). Electrical stimulation of the vagus nerve is not effective in suppressing lung TNF levels [21], which demonstrates organ specificity of the immunosuppressive function of the activated vagus nerve. Although the liver has been suggested to be the main source of TNF during experimental endotoxemia, we recently demonstrated the essential role of the spleen in the LPS-induced TNF synthesis. Vagal innervation of the spleen has been a subject of numerous studies, with inconsistents results. We recently demonstrated that the vagus nerve exerts a strong modulatory effect on serum TNF levels by suppressing TNF synthesis in the spleen [unpublished observations]. The gastrointestinal tract is heavily innervated by the vagus nerve, making it a potential target of the vagus nerve regulation of TNF. Vagal regulation of the gastrointestinal secretion and motility is achieved by postganglionic enteric excitatory neurons, releasing acetylcholine and substance P, and inhibitory neurons, releasing ATP, vasoactive intestinal peptide (VIP) and nitric oxide [70]. The release

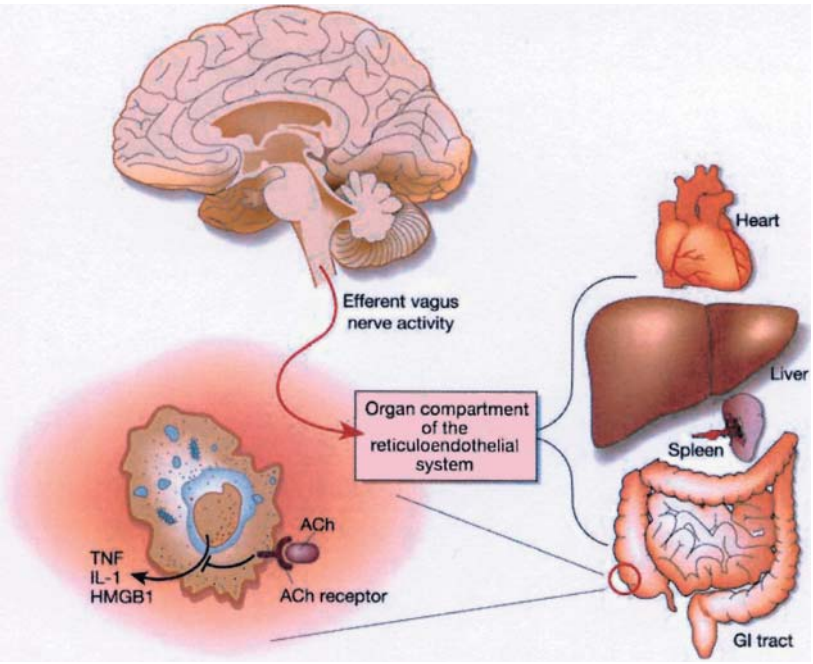

Figure 2. The cholinergic anti-inflammatory pathway. Efferent activity in the vagus nerve leads to acetylcholine (ACh) release in organs of the reticuloendothelial system, including the liver, heart, spleen and gastrointestinal tract. Acetylcholine interacts with the α-7 subunit containing nicotinic acetylcholine (Ach) receptors on tissue macrophages, which inhibit the release of TNF, IL-1, HMGB1 and other cytokines. Taken from [6].

of gastrointestinal local mediators and hormones, including pepsinogen, histamine, gastrin, somatostatin and leptin, is under vagal control [70–73]. Apart from their effects on the gastrointestinal tract, some of these substances have other functions, including immunomodulation. Recent studies have demonstrated the ability of histamine to inhibit LPS-induced TNF production in peripheral blood mononuclear cells [74] and interferon (IFN)-α and TNF from dendritic cells [75]. Histamine also negatively regulates neutrophil infiltration via H2 receptors in allergic inflammation [76]. A very recent study has demonstrated that the vagus nerve innervates adipose tissue; the physiological impact of this innervation is associated with selective induction of endocrine function and leptin release [77]. Although the implication of leptin in chronic inflammation and rheumatoid diseases, respectively, remains controversial [78], a number of studies demonstrate a protective role for leptin in inflammation [79–83].

The cholinergic anti-inflammatory pathway can be centrally activated [21]. CNI-1493, a tetravalent guanylhydrazone, protects against systemic shock in endotoxemic rats by inhibiting systemic TNF levels, and its effects are mediated by a central mechanism, which leads to activation of the efferent vagus nerve [21]. CNI-1493 can also modulate local peripheral inflammation; the paw edema development in rats is suppressed by CNI-1493 through an efferent vagus nerve-dependent mechanism [20].

As described above, a non-synaptic 'diffusion' model of sympathetic immunoregulation may predominate in vivo [13, 31]. This mechanism is based on the existence of postganglionic sympathetic fibers, which do not make synaptic contact with smooth muscle, cardiac or glandular cells. Norepinephrine, released non-synaptically from axon terminals of these fibers, is capable of diffusing through the parenchyma and interacting with the adrenoceptors on the immune cells surrounding the axon vicinity [13]. Anatomo-physiological considerations do not support the possibility that cholinergic immunomodulation could be realized by such a mechanism: (i) to the best of our knowledge, no non-synaptic cholinergic vagal fibers in target organs have been identified so far; and (ii) acetylcholine is rapidly hydrolyzed by the acetylcholinesterase into choline and acetate, following its release from the axon terminals. An intriguing fact, which might be relevant to the cholinergic mechanisms of immunomodulation, is that apart from acetylcholine, its stable metabolite choline is capable of activating α7 subunit-containing nicotinic receptors [84]. Sensitivity to choline is one of the pharmacological characteristics of the α7 subunit, together with high Ca^{2+} permeability, fast desensitization and blockade by α-bungarotoxin. It is theoretically possible that choline produced as a result of acetylcholine hydrolysis by acetylcholinesterase diffuses from the synaptic cleft and interacts with perisynaptic and extrasynaptic α7 subunit-containing nicotinic receptors. While considering that vagal postganglionic fibers are the source of acetylcholine (choline) capable of sup-

pressing the pro-inflammatory cytokine release, we cannot exclude a contribution of preganglionic neurons. In contrast to sympathetic ganglia (located some distance from the innervated organs), parasympathetic/vagal ganglia are located either in close proximity or within the innervated organs.

Therapeutic implications of the cholinergic anti-inflammatory pathway

The discovery of the anti-inflammatory function of the activated vagus nerve provides the possibility for using novel, unconventional strategies to control unrestrained inflammation. Studies aimed at finding parameters of vagus nerve stimulation resulting in maximal protection against exaggerated inflammation are in progress in our laboratory. A novel anti-inflammatory strategy could be developed by means of optimal vagus nerve stimulation generated by a special device. Surgically implanted vagus nerve stimulators have been successfully used in the treatment of epilepsy [85, 86]. It is plausible that surgically implanted devices programmed to specifically stimulate efferent vagus nerve fibers could have a successful clinical implication in the treatment of inflammatory disorders. The major requirements for this therapy would be related to preventing deleterious effects of TNF and other pro-inflammatory mediators and preserving the beneficial impact of anti-inflammatory substances, without increasing the risk of secondary infections. Based on the experimental finding that electrical stimulation of the vagus nerve results in lower heart TNF levels [21, 58] during endotoxemia, this treatment could be explored for modulating the local cardiac TNF levels to attenuate the development of myocardial depression and shock. Vagus nerve stimulation could also be explored in the treatment of disorders that are accompanied by autonomic dysfunction associated with decreased parasympathetic component, including perhaps rheumatoid arthritis.

It is possible that not only direct (electrical) stimulation of the vagus nerve, but some alternative approaches for vagus nerve activation, can modulate inflammation. It is interesting to consider the impact of biofeedback, acupuncture, hypnosis, meditation and Pavlovian conditioning of immune responses on immune function and inflammatory diseases through parasympathetic/vagal mechanisms. Pavlovian conditioning of immunosuppression has been shown in rodent models and recently in humans [87]. Interestingly, the suggested role for the sympathetic part and the HPA axis in the immune-to-brain communication underlying the conditioning in rodents has not been demonstrated in humans [87]. It is an open question whether the vagus nerve plays a role in the Pavlovian conditioning in rodents and humans.

The recent discovery that the nicotinic acetylcholine receptor α7 subunit on macrophages is a peripheral component of the cholinergic anti-inflammatory pathway provides the possibility of suppressing inflammation by specifically targeting this receptor. Our preliminary studies with specific cholinomimetics confirm the validity of this approach [88]. The aforementioned distribution of α7 subunit-containing nicotinic receptors suggests that a therapeutic strategy based on activation of this subunit could be specific, without interfering with ganglionic neurotransmission and sympathetic activation, respectively. Despite the presence of the α7 subunit in some autonomic ganglia [89, 90], no critical role for this subunit in fast ganglionic transmission, mediated by α3/β4 nicotinic receptors, has been shown [68]. Another intriguing possibility of achieving peripheral immunosupression is based on exploring central mechanisms of activation of the cholinergic anti-inflammatory pathway. Identifying brain receptors involved in the central activation of the vagus nerve may allow the development of therapeutic approaches to treat inflammation.

Conclusions

Experimental evidence allows building a model of neuroimmunomodulation in which the brain monitors immune status and actively responds to challenge. The communication between the brain and the immune system is bidirectional, involving neural and humoral mechanisms. The two major divisions of the autonomic nervous system are strongly involved in this communication; the brain is capable of sensing fluctuations in peripheral immune status by afferent vagal neurons and controlling the innate immune response to injury and pathogen invasion by activation of efferent vagal (parasympathetic) and sympathetic neural and neuro-hormonal mechanisms. The rapid, real-time brain control of innate immune mechanisms underlying inflammation is based on autonomic neuronal projections to sites of inflammation. Our growing knowledge about the molecular and receptor mechanisms of the neural regulation of immune function may lead to treatments for inflammatory diseases.

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1 Nathan C. (2002) Points of control in inflammation. Nature **420:** 846–852
2 Sell S. (2001) Inflammation and wound healing. In: Immunology, Immunopathology and Immunity, 6th ed, pp. 33–96, Sell S. (ed.), ASM Press, New York
3 Coussens L. M. and Werb Z. (2002) Inflammation and cancer. Nature **420:** 860–867
4 Tracey K. J., Beutler B., Lowry S. F., Merryweather J., Wolpe S., Milsark I. W. et al. (1986) Shock and tissues injury induced by recombinant human catchectin. Science **234:** 470–474
5 Tracey K. J., Fong Y., Hesse D. G., Manogue K. R., Lee A. T., Kuo G. C. et al. (1987) Anti-cachectin/TNF-monoclonal anti-

bodies prevent septic shock during lethal bacteraemia. Nature **330:** 662–664
6 Tracey K. J. (2002) The inflammatory reflex. Nature **420:** 853–859
7 Wang H., Bloom O., Zhang M., Vishnubhakat J. M., Ombrellino M., Che J. et al. (1999) HMG-1 as a late mediator of endotoxin lethality in mice. Science **285:** 248–251
8 Wang H., Yang H. and Tracey K. J. (2004) Extracellular role of HMGB1 in inflammation and sepsis. J. Intern. Med. **255:** 320–331
9 Opal S. M. and DePalo V. A. (2000) Anti-inflammatory cytokines. Chest **117:** 1162–1172
10 Reichlin S. (1993) Neuroendocrine-immune interactions. New Engl. J. Med. **329:** 1246–1253
11 McEwen B. S., Biron C. A., Brunson K. W., Bulloch K., Chambers W. H., Dhabhar F. S. et al. (1997) The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. Brain Res. Rev. **23:** 79–133
12 Webster J. I., Tonelli L. and Sternberg E. M. (2002) Neuroendocrine regulation of immunity. Annu. Rev. Immunol. **20:** 125–163
13 Elenkov I. J., Wilder R. L., Chrousos G. P. and Vizi E. S. (2000) The sympathetic nerve – an integrative interface between two supersystems: the brain and the immune system. Pharmacol. Rev. **52:** 595–638
14 Watkins L. R., Maier S. F. and Goehler L. E. (1995) Cytokine-to-brain communication: a review and analysis of alternative mechanisms. Life Sci. **57:** 1011–1026
15 Hasko G. and Szabo C. (1998) Regulation of cytokine and chemokine production by transmitters and co-transmitters of the autonomic nervous system. Biochem. Pharmacol. **56:** 1079–1087
16 Pavlov V. A., Wang H., Czura C. J., Friedman S. G. and Tracey K. J. (2003) The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. Mol. Med. **9:** 125–134
17 Elmquist J. K., Scammell T. E. and Saper C. B. (1997) Mechanisms of CNS response to systemic immune challenge: the febrile response. Trends Neurosci. **20:** 565–569
18 Goehler L. E., Gaykema R. P. A., Hansen M. K., Anderson K., Maier S. F. and Watkins L. (2000) Vagal immune-to-brain communication: a visceral chemosensory pathway. Auton. Neurosci. **85:** 49–59
19 Borovikova L. V., Ivanova S., Zhang M., Yang H., Botchkina G. I., Watkins L. R. et al. (2000) Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature **405:** 458–461
20 Borovikova L. V., Ivanova S., Nardi D., Zhang M., Yang H., Ombrellino M. et al. (2000) Role of vagus nerve signaling in CNI-1493-mediated suppression of acute inflammation. Auton. Neurosci. **85:** 141–147
21 Bernik T. R., Friedman S. G., Ochani M., DiRaimo R., Ulloa L., Yang H. et al. (2002) Pharmacological stimulation of the cholinergic antiinflammatory pathway. J. Exp. Med. **195:** 1–9
22 Wang H., Yu M., Ochani M., Amella C. A., Tanovic M., Susarla S. et al. (2003) Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. Nature **421:** 384–388
23 Aderem A. and Ulevitch R. J. (2000) Toll-like receptors in the induction of the innate immune response. Nature **406:** 782–787
24 Ulevitch R. J. and Tobias P. S. (1999) Recognition of gram-negative bacteria and endotoxin by the innate immune system. Curr. Opin. Immunol. **11:** 19–22
25 Scaffidi P., Misteli T. and Bianchi M. E. (2002) Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature **418:** 191–195
26 Zhang M., Wang H. and Tracey K. J. (2000) Regulation of macrophage activation and inflammation by spermine: a new chapter in an old story. Crit. Care Med. **28 Suppl.** N60–N66
27 Tracey K. J., Vlassara H. and Cerami A. (1989) Cachectin/tumour necrosis factor. Lancet. **1:** 1122–1126
28 Echtenacher B., Falk W., Mannel D. N. and Krammer P. H. (1990) Requirement of endogenous tumor necrosis factor/cachectin for recovery from experimental peritonitis. J. Immunol. **145:** 3762–3766
29 Cohen J. (2002) The immunopathogenesis of sepsis. Nature **420:** 885–891
30 Riedemann N. C., Guo R. F. and Ward P. A. (2003) Novel strategies for the treatment of sepsis. Nat. Med. **9:** 517–524
31 Taylor P. C. (2003) Anti-cytokines and cytokines in the treatment of rheumatoid arthritis. Curr. Pharm. Des. **9:** 1095–1106
32 Feldmann M. and Maini R. N. (2003) TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. Nat. Med. **9:** 1245–1250
33 Abuzakouk M., Feighery C. and Jackson J. (2002) Tumour necrosis factor blocking agents: a new therapeutic modality for inflammatory disorders. Br. J. Biomed. Sci. **59:** 173–179
34 Kokkola R., Li J., Sundberg E., Aveberger A. C., Palmblad K., Yang H. et al. (2003) Successful treatment of collagen-induced arthritis in mice and rats by targeting extracellular high mobility group box chromosomal protein 1 activity. Arthritis Rheum. **48:** 2052–2052
35 Yang H., Ochani M., Li J., Qiang X., Tanovic M., Harris H. E. et al. (2004) Reversing established sepsis with antagonists of endogenous high-mobility group box1. Proc. Natl. Acad. Sci. USA **101:** 296–301
36 Prass K., Meisel C., Hoflich C., Braun J., Halle E., Wolf T. et al. (2003) Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1-like immunostimulation. J. Exp. Med. **198:** 725–736
37 Vizi E. S. and Elenkov I. J. (2002) Nonsynaptic noradrenaline release in neuro-immune responses Acta Biol. Hung. **53:** 229–244
38 Bellinger D. L., Lorton C. L., Lubahn C. and Felten D. L. (2001) Innervation of lymphoid organs-association of nerves with cells of the immune system and their implications in disease. In: Psychoneuroimmunology, 3rd edn, vol. 1, pp. 55–111, Ader R., Felten D. L. and Cohen N. (eds), Academic Press, New York
39 Severn A., Rapson N. T., Hunter C. A. and Liew F. Y. (1992) Regulation of tumor necrosis factor production by adrenaline and beta-adrenergic agonists. J. Immunol. **148:** 3441–3445
40 van der Poll T., Coyle S. M., Barbosa K., Braxton C. C. and Lowry S. F. (1996) Epinephrine inhibits tumor necrosis factor-alpha and potentiates interleukin-10 production during human endotoxemia. J. Clin. Invest. **97:** 713–719
41 Szelenyi J., Kiss J. P., Puskas E., Szelenyi M. and Vizi E. S. (2000) Contribution of differently localized alpha 2- and beta-adrenoceptors in the modulation of TNF-alpha and IL-10 production in endotoxemic mice. Ann. N. Y. Acad. Sci. **917:** 145–153
42 Woiciechowsky C., Asadullah K., Nestler D., Eberhardt B., Platzer C., Schoning B. et al. (1998) Sympathetic activation triggers systemic interleukin-10 release in immunodepression induced by brain injury. Nat. Med. **4:** 808–813
43 Le Tulzo Y., Shenkar R., Kaneko D., Moine P., Fantuzzi G., Dinarello C. A. et al. (1997) Hemorrhage increases cytokine expression in lung mononuclear cells in mice: involvement of catecholamines in nuclear factor-kappaB regulation and cytokine expression. J. Clin. Invest. **99:** 1516–1524
44 Szelenyi J., Kiss J. P., Puskas E., Selmeczy Z., Szelenyi M. and Vizi E. S. (2000) Opposite role of alpha2- and beta-adrenoceptors in the modulation of interleukin-10 production in endotoxaemic mice. Neuroreport **11:** 3565–3568
45 Macaluso A., McCoy D., Ceriani G., Watanabe T., Biltz J., Catania A. et al. (1994) Antiinflammatory influences of α-MSH molecules: central neurogenic and peripheral action. J. Neurosci. **14:** 2377–2382

46 Szelenyi J., Kiss J. P. and Vizi E. S. (2000) Differential involvement of sympathetic nervous system and immune system in the modulation of TNF-alpha production by alpha2- and beta-adrenoceptors in mice. J. Neuroimmunol. **103:** 34–40
47 Goehler L. E., Gaykema R. P., Nguyen K. T., Lee J. E., Tilders F. J., Maier S. F. et al. (1999) Interleukin-1 in immune cells of the abdominal vagus nerve: a link between the immune and nervous system? J. Neurosci. **19:** 2799–2806
48 Watkins L. R., Goehler L. E., Relton J. K., Tartaglia N., Silbert L., Martin D. et al. (1995) Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. Neurosci. Lett. **183:** 27–31
49 Hansen M. K., O'Connor K. A., Goehler L. E., Watkins L. R. and Maier S. F. (2001) The contribution of the vagus nerve in interleukin-1beta-induced fever is dependent on dose. Am. J. Physiol. Regul. Integr. Comp. Physiol. **280:** R929–R934
50 Gaykema R. P., Dijkstra I. and Tilders F. J. (1995) Subdiaphragmatic vagotomy suppresses endotoxin-induced activation of hypothalamic corticotropin-releasing hormone neurons and ACTH secretion. Endocrinology **136:** 4717–4720
51 Hermann G. E., Emch G. S., Tovar C. A. and Rogers R. C. (2001) C-Fos generation in the dorsal vagal complex after systemic endotoxin is not dependent on the vagus nerve. Am. J. Physiol. Regul. Integr. Comp. Physiol. **280:** R289–R299
52 Niijima A., Hori T., Katafuchi T. and Ichijo T. (1995) The effect of interleukin beta on the efferent activity of the vagus nerve to the thymus. J. Auton. Nerv. Syst. **54:** 137–144
53 Hopkins S. J. and Rothwell N. (1995) Cytokines and nervous system I: expression and recognition. Trends Neurosci. **18:** 83–88
54 Rivest S., Lacroix S., Vallieres L., Nadeau S., Zhang J. and Laflamme N. (2000) How the blood talks to the brain parenchyma and the paraventricular nucleus of the hypothalamus during systemic inflammatory and infectious stimuli. Proc. Soc. Exp. Biol. Med. **223:** 22–38
55 Banks W. A., Kastin A. J. and Broadwell R. D. (1995) Passage of cytokines across the blood-brain barrier. Neuroimmunomodulation **2:** 241–248
56 Ek M., Engblom D., Saha S., Blomqvist A., Jacobsson P.-J. and Ericsson-Dahlstrand A. (2001) Inflammatory response: pathway across the blood-brain barrier. Nature **410:** 430–431
57 Buller K. M. (2001) Circumventricular organs: gateways to the brain. Role of circumventricular organs in pro-inflammatory cytokine-induced activation of the hypothalamic-pituitary-adrenal axis. Clin. Experiment. Pharmacol. Physiol. **28:** 581–589
58 Shi D. G., Hu S., Jiang X. G., Sun D., Jin H., Li J. Y. et al. (2003) Effects of efferent vagus nerve excitation on inflammatory response in heart tissue in rats with endotoxemia. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue **15:** 26–28
59 Bernik T. R., Friedman S. G., Ochani M., DiRaimo R., Susarla S., Czura C. J. et al. (2002) Cholinergic antiinflammatory pathway inhibition of tumor necrosis factor during ischemia reperfusion. J. Vasc. Surg. **36:** 1231–1236
60 Guarini S., Altavilla D., Cainazzo M. M., Giuliani D., Bigiani A., Marini H. et al. (2003) Efferent vagal fibre stimulation blunts nuclear factor-kappaB activation and protects against hypovolemic hemorrhagic shock. Circulation **107:** 1189–1194
61 Mita Y., Dobashi K., Suzuki K., Mori M. and Nakazawa T. (1996) Induction of muscarinic receptor subtypes in monocytic/macrophagic cells differentiated from EoL-1 cells. Eur. J. Pharmacol. **297:** 121–127
62 Hellstrom-Lindahl E. and Nordberg A. (1996) Muscarinic reeptor subtypes in subpopulations of human blood mononuclear cells as analyzed by RT-PCR technique. J. Neuroimmunol. **68:** 139–144
63 Costa P., Auger C. B., Traver D. . and Costa L. G. (1995) Identification of m3, m4 and m5 subtypes of muscarinic receptor mRNA in human blood mononuclear cells. J. Neuroimmunol. **60:** 45–51
64 Tayebati S. K., El-Assouad D., Ricci A. and Amenta F. (2002) Immunochemical and immunocytochemical characterization of cholinergic markers in human peripheral blood lymphocytes. J. Neuroimmunol. **132:** 147–155
65 Fujii T. and Kawashima K. (2000) Ca^{2+} oscillation and c-fos gene expression induced via muscarinic acetylcholine receptor in human T- and B-cell lines. Naunyn. Schmiedebergs Arch. Pharmacol. **362:** 14–21
66 Masini E., Fantozzi R., Blandina P., Brunelleschi S. and Mannaioni P. F. (1983) Muscarinic cholinergic receptor binding in rat mast cells. Agents Actions **13:** 327–332
67 Blandina P., Fantozzi R., Mannaioni P. F. and Masini E. (1980) Characteristics of histamine release evoked by acetylcholine in isolated rat mast cells. J. Physiol. **301:** 281–293
68 Wang N., Orr-Urtreger A. and Korczyn A. D. (2002) The role of neuronal nicotinic acetylcholine receptor subunits in autonomic ganglia: lessons from knockout mice. Prog. Neurobiol. **68:** 341–360
69 Vizi E. S. and Lendvai B. (1999) Modulatory role of presynaptic nicotinic receptors in synaptic and non-synaptic chemical communication in the central nervous system. Brain Res. Brain Res. Rev. **30:** 219–235
70 Chang H. Y., Mashimo H. and Goyal R. K. (2003) Musings on the wanderer: what's new in our understanding of vago-vagal reflex? IV. Current concepts of vagal efferent projections to the gut. Am. J. Physiol. Gastrointest. Liver Physiol. **284:** G357–G366
71 Debas H. T. and Carvajal S. H. (1994) Vagal regulation of acid secretion and gastrin release. Yale J. Biol. Med. **67:** 145–151
72 Gottwald T. P., Hewlett B. R., Lhotak S. and Stead R. H. (1995) Electrical stimulation of the vagus nerve modulates the histamine content of mast cells in the rat jejunal mucosa. Neuroreport **7:** 313–317
73 Sobhani I., Buyse M., Goiot H., Weber N., Laigneau J. P., Henin D. et al. (2002) Vagal stimulation rapidly increases leptin secretion in human stomach. Gastroenterology **122:** 259–263
74 Morichika T., Takahashi H. K., Iwagaki H., Yoshino T., Tamura R., Yokoyama M. et al. (2003) Histamine inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production in an intercellular adhesion molecule-1- and B7.1-dependent manner. J. Pharmacol. Exp. Ther. **304:** 624–633
75 Mazzoni A., Leifer C. A., Mullen G. E., Kennedy M. N., Klinman D. M. and Segal D. M. (2003) Cutting edge: histamine inhibits IFN-alpha release from plasmacytoid dendritic cells. J. Immunol. **170:** 2269–2273
76 Hirasawa N., Ohtsu H., Watanabe T. and Ohuchi K. (2002) Enhancement of neutrophil infiltration in histidine decarboxylase-deficient mice. Immunology **107:** 217–221
77 Kreier F., Fliers E., Voshol P. J., Van Eden C. G., Havekes L. M., Kalsbeek A. et al. (2002) Selective parasympathetic innervation of subcutaneous and intra-abdominal fat – functional implications. J. Clin. Invest. **110:** 1243–1250
78 Palmer G. and Gabay C. (2003) A role for leptin in rheumatic diseases? Ann. Rheum. Dis. **62:** 913–915
79 Takahashi N., Waelput W. and Guisez Y. (1999) Leptin is an endogenous protective protein against the toxicity exerted by tumor necrosis factor. J. Exp. Med. **189:** 207–212
80 Gabay C., Dreyer M. G., Pellegrinelli N., Chicheportiche R. and Meier C. A. (2001) Leptin directly induces the secretion of interleukin 1 receptor antagonist in human monocytes. J. Clin. Endocrinol. Metab. **86:** 783–791
81 Faggioni R., Moser A., Feingold K. R. and Grunfeld C. (2000) Reduced leptin levels in starvation increase susceptibility to endotoxic shock. Am. J. Pathol. **156:** 1781–1787
82 Bornstein S. R., Licinio J., Tauchnitz R., Engelmann L., Negrão A. B., Gold P. et al. (1998) Plasma leptin levels are increased in survivors of acute sepsis: associated loss of diurnal rhythm in

cortisol and leptin secretion. J. Clin. Endocrinol. Metab. **83:** 280–283
83 Xiao E., Xia-Zhang L., Vulliémoz N. R., Ferin M. and Wardlaw S. L. (2003) Leptin modulates inflammatory cytokine and neuroendocrine responses to endotoxin in the primate endocrinology. Endocrinology **144:** 4350–4353
84 Alkondon M., Pereira E. F., Cortes W. S., Maelicke A. and Albuquerque E. X. (1997) Choline is a selective agonist of alpha7 nicotinic acetylcholine receptors in the rat brain neurons. Eur. J. Neurosci. **9:** 2734–2742
85 George M. S., Sackeim H. A., Rush A. J., Marangell L. B., Nahas Z., Husain M. M. et al. (2000) Vagus nerve stimulation: a new tool for brain research and therapy. Biol. Psychiatry **47:** 287–295
86 Valencia I., Holder D. L., Helmers S. L., Madsen J. R. and Riviello J. J. (2001) Vagus nerve stimulation in pediatric epilepsy: a review. Pediatr. Neurol. **25:** 368–376
87 Goebel M. U., Trebst A. E., Steiner J., Xie Y. F., Exton M. S., Frede S. et al. (2002) Behavioral conditioning of immunosuppression is possible in humans. FASEB J. **16:** 1869– 1873
88 Han J. L., Amella C. A., Tanovic M., Wang H. G., Yang H., Wang H. et al. (2003) Cholinergic suppression of cytokine release from human macrophages. Shock **19 (S1):** 22
89 Skok M. V., Voitenko L. P., Voitenko S. V., Lykhmus E. Y., Kalashnik E. N., Litvin T. I. et al. (1999) Alpha subunit composition of nicotinic acetylcholine receptors in the rat autonomic ganglia neurons as determined with subunit-specific anti-alpha (181–192) peptide antibodies. Neuroscience **93:** 1427–1436
90 Kirchgessner A. L and Liu M. T. (1998) Immunohistochemical localization of nicotinic acetylcholine receptors in the guinea pig bowel and pancreas. J. Comp. Neurol. **390:** 497–514