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The central and autonomic nervous systems: Essential regulators of the immune response

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“The brain is the last and grandest biological frontier, the most complex thing we have yet discovered in our universe. The brain boggles the mind.”

James D. Watson (from *Discovering the Brain*, National Academy Press, 1992)

Of all the organs in our body, the brain is the most versatile and poses the greatest mystery. It contains hundreds of billions of cells interlinked through trillions of connections which generate our thoughts, houses our “soul”, makes us sense, feel and move through the somatic and sensory nervous system and orchestrates essential life functions through the autonomic nervous system. The brain consists of extensively connected subsystems, such as the brain stem, limbic system, hypothalamus and cerebral cortex and many others which are all directly or indirectly connected to sensory (afferent) and effector (efferent) pathways reaching virtually any location in the body. The autonomic nervous system relays input on all vital processes in the body to the brain and controls our heart rate, respiratory rate, blood pressure, gut motility, body temperature and virtual any essential involuntary process. One could say that in any vital process that takes place there is “always a brain attached”. Although it is widely accepted that virtually any vital process is supervised and controlled by the central nervous system (CNS), our innate immune response has long been regarded as a totally peripheral system regulated by the interaction of immune competent cells with pathogens and with each other. In this article, we will review data that suggest that communication between the immune, nervous and endocrine systems is in fact an essential homeostatic system that regulates the innate immune response. First we will discuss the classic regulation of innate immunity and the physiological anatomy of the autonomic nervous system; second we will discuss new insights in immune to brain and brain to immune communication and finally focus on the cholinergic anti inflammatory pathway and the important physiological insights and therapeutic opportunities that arise from the concept that activation of innate immunity is, at least in part, regulated in the CNS.

Classic regulation of innate immunity, the balance between pro and anti inflammation

Inflammation is a physiologic response of the host to either invasion of micro organisms or local injury. The innate immune system is the first line of defense against

invading pathogens. Invading pathogens activate the cells of the innate immune system by a variety of, pathogen class specific, stimuli¹. As a result of these triggers immune competent cells are activated to release a plethora of pro-inflammatory soluble mediators, such as cytokines (e.g. tumor necrosis factor (TNF- α)- α , Interleukin (IL)-1) and chemokines (e.g. IL-8)². Goal of this inflammatory reaction is to restrain and localize the infection to the infected compartment of the body and ultimately clear the pathogen and remove the injury.

However, the release of all these mediators can be detrimental to the host³. Inflammatory reactions must be fine-tuned and regulated in a precise manner since exaggerated inflammation may lead to tissue damage and morbidity. Indeed, in recent years many common diseases have been recognized as “inflammatory conditions”, including atherosclerosis, ischemia reperfusion injury, rheumatoid arthritis, inflammatory bowel disease and fulminant sepsis^{2,4}. In these diseases, there is an induction of activation of the innate immune system, activation and migration of neutrophils and the cytokine network. To keep the potentially detrimental effects of the pro-inflammatory system in check an anti-inflammatory cytokine system (e.g. IL-10, IL-4) counterbalances the pro inflammatory systems. In health and disease, the balance between pro and anti inflammatory systems is essential to maintain a delicate homeostasis which ensures an adequate host defense with minimal collateral damage due to over aggressive responses of the innate immune system. On the basis of this concept, new therapies have been developed in recent years. Elimination of TNF- α by monoclonal antibodies restores the inflammatory balance and effectively treats “pro inflammatory diseases” such as Crohn’s disease and rheumatoid arthritis⁵.

Physiological anatomy of the autonomic nervous system

The autonomic nervous provides constant and extremely rapid control of the visceral functions, such as arterial pressure, gastrointestinal motility and secretion, urinary bladder emptying, sweating, body temperature and many other activities of which some are totally and others partially controlled by the autonomic nervous system. The autonomic nervous system consists of sensory neurons and motor neurons that run between the CNS (especially the hypothalamus and medulla oblongata) and internal organs. It differs from the sensory-somatic system in using two groups of motor neurons

to stimulate the effectors instead of one. Preganglionic neurons arise in the CNS and run into a ganglion where they synapse with a postganglionic neuron that runs all the way into the effector region. The autonomic nervous system has two subdivisions that usually act reciprocally of each other; the sympathetic and the parasympathetic nervous system.

The motor neurons of the sympathetic nervous system arise in the spinal cord and pass into two large chains of perivertebral ganglia. Here the preganglionic neuron can synapse directly with a postganglionic neuron, travel up or down the chain to synapse in a ganglion at another level with postganglionic neurons or travel up or downward and terminate in one of the prevertebral ganglia. Either way, from the ganglion the postganglionic fibers travel all the way to their destination in the various organs where norepinephrine (NE) is released by the postganglionic neuron. One exception is the sympathetic innervation of the adrenal medulla. Here, preganglionic fibers travel all the way to the medulla without synapsing and end directly on neuronal cells that secrete epinephrine and NE into the blood stream. The release of NE results in stimulation of the heartbeat, a raise in blood pressure, dilatation of the bronchi, a shunt of blood away from non-vital organs and an inhibition of bladder and gastrointestinal peristalsis. Activation of the sympathetic nervous system results in generalized responses since preganglionic neurons usually synapse with many postganglionic neurons and also because the release of epinephrine by the adrenal medulla ensures that even body sites that are not reached by postganglionic sympathetic neurons will be drenched with epinephrine. In short, stimulation of the sympathetic branch of the autonomic nervous system prepares the body for emergency: which are usually referred to as "fight or flight" reactions.

About 75% of parasympathetic nerves fibers arise from the tenth cranial nerves, the vagus nerves. Other sympathetic innervation comes from cranial nerves III, VII and IX and from the second and third sacral spinal nerves. The two vagus nerves originate in the medulla oblongata and wander all the way through the cervical area (where one vagus nerve travels right and the other left of the trachea) and the thoracic and abdominal regions of the body. At several locations both vagus nerves meet and share fibers with each other. The vagus nerve supplies parasympathetic innervation to the heart, the lungs, the esophagus, the small intestine, the proximal half of the colon, the liver, the pancreas, the ureters and the spleen. Just as the sympathetic nervous system the vagus nerve has both preganglionic and postganglionic neurons. However, the preganglionic

fibers travel uninterrupted to their destination and synapse with postganglionic fibers inside the target organ where short postganglionic fibers spread throughout the organ. Parasympathetic stimulation causes slowing of the heartbeat, lowering of blood pressure and activation of gastrointestinal peristalsis. In short, parasympathetic innervation returns the body to its resting state after it has been activated by the sympathetic nervous system.

Sympathetic and parasympathetic neurons all secrete one of the two synaptic neurotransmitters, acetylcholine (ACh, cholinergic neurons) or NE (adrenergic neurons). All preganglionic neurons as well as the postganglionic neurons of the parasympathetic division are cholinergic whereas (except for sweat glands) postganglionic sympathetic neurons are adrenergic. Before ACh or NE can exert effects on the target organ they need to bind to specific receptors. Usually binding of a neurotransmitter to a receptor results in either a change in cellular membrane permeability or in the alteration of intracellular enzymes, such as in the case of NE binding where cyclic AMP (cAMP) is formed in response to receptor binding⁶. ACh activates two different receptors, muscarinic and nicotinic ACh receptors. Muscarinic receptors are found in all effector cells stimulated by postganglionic neurons whereas nicotinic receptors are found in the synapses between pre and postganglionic neurons. Adrenergic receptors can be divided in α and β receptors. Stimulation of α and β receptors have sometimes contrary effects on target organs, implicating that the effects of epinephrine and NE on organs is dependent on the type of receptors present in the particular organ.

Interactions between the CNS and the immune system

The CNS and the immune system have several important features in common. Both systems are designed to constantly survey the body for danger and mount an appropriate response to these threats. In contrast to classical thinking, these two systems act together in orchestrating the immune response in response to infection or injury. Stimulation or ablation of several regions of the brain can alter immune responses; secondly, inflammatory processes can alter the firing rate of CNS neurons. Thus, there is a cross talk between inflammatory cells and the CNS that can go towards as well as from an inflamed site of the body.

Immune to brain communication

The CNS receives sensory input from the immune system through both humoral and neural routes (Figure 1). IL-1 β , TNF- α , and other immunological active mediators, can signal the brain in circumscribed areas⁷. These so called circumventricular organs include specific sites in the hypothalamus as well as the dorsal vagal complex (DVC)⁸. The DVC consists of the nucleus tractus solitarius (NTS), the dorsal motor nucleus of the vagus (DMN) and the area postrema (AP) (Figure 1)⁹.

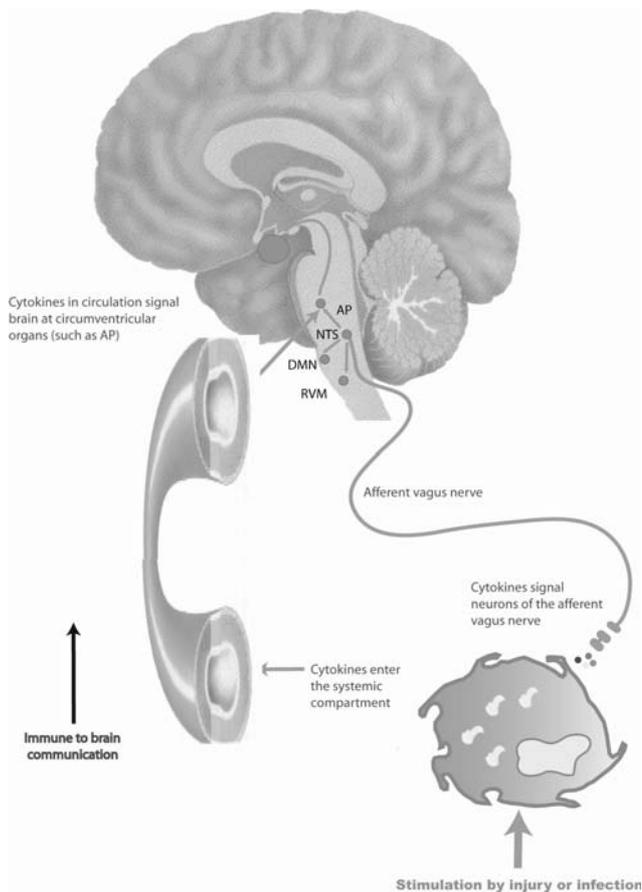


Figure 1.

Immune to brain communication.

The brain receives information from the immune system by humoral as well as neural pathways. Cytokines elicit signaling in the afferent vagus nerve which reach the nucleus tractus solitarius (NTS). From here, projections lead via the area postrema (AP) to the hypothalamus and subsequently the pituitary gland, and also to the dorsal motor nucleus (DMN) of the vagus nerve and the rostroventrolateral medulla (RVM). All these nuclei are involved in generating responses of the brain back to the immune system (see figure 2). Via the humoral route cytokines can signal through circumventricular organs such as the AP.

The DMN is the major site of origin of efferent vagal neurons whereas the main portion of vagal sensory input is received by neurons in the NTS. The AP, which lacks a tight

blood-brain barrier, is an important circumventricular organ and site for humoral immune-to-brain communication⁷. In fact, reversible inactivation of the DVC completely blocks endotoxin induced behavioral changes and expression of c-FOS (neuronal activation marker) in forebrain regions of endotoxemic animals⁸. Exactly how cytokines are able to cross the blood brain barrier at these sites and activate the CNS is a matter of debate. Some studies suggest there is active transport of cytokines across the blood brain barrier⁶. Others implicate receptors for cytokines and for bacterial fragments that are constitutively expressed in cells within circumventricular organs and upregulated during inflammation⁷. Binding of cytokines to these receptors induces responses including changes in electrical activity of neurons, induction of transcription factors leading to modifications in gene expression during inflammation and to a localized release of secondary signal molecules which are able to cross the blood brain barrier⁷.

Neural pathways, predominantly the vagus nerve, also signal the brain for danger (Figure 1)¹⁰. Cytokines and bacterial products such as endotoxin stimulate afferent neural fibers in the vagus nerve that are processed in the brain and result in the initiation of an acute phase response, induction of fever and upregulation of IL-1 β in the brain¹⁰⁻¹².

Immunogenic stimuli activate vagal afferents either directly by cytokines released by inflammatory cells at the site of infection or injury or indirectly through chemoreceptive cells located in vagal paraganglia¹³. After stimulation by cytokines, vagal afferent fibers transmit signals to the DVC^{13, 14} where most sensory information is relayed to the NTS.

Whether humoral or neural pathways are essential in relaying information on the presence of inflammation to the brain is largely dependent on the magnitude of the inflammatory response. In experimental studies it has been shown that when the level of inflammation is low, such as when a low dose of endotoxin is injected intraperitoneally, vagotomy inhibits the stimulation of the hypothalamus-pituitary-adrenal (HPA) axis and the induction of IL-1 in the brain^{10, 11} whereas high doses of endotoxin induce responses by the brain independent of the vagus nerve^{15, 16}. This implicates that neural pathways are essential in the relay of localized inflammation whereas information about severe systemic inflammation reaches the brain predominantly through humoral pathways.

Brain to immune communication

Neuro-endocrine pathways

When notified of ongoing inflammation, either by humoral or neural pathways as described above, the brain exerts strong anti inflammatory effects through activation of the HPA axis (Figure 2). Information received via the afferent vagus in the NTS is relayed to the hypothalamus which may induce release of α -melanocyte stimulating hormone (α -MSH) and corticotrophin releasing hormone (CRH). CRH induces adrenocorticotropin hormone release (ACTH) by the pituitary gland and activates a neural-endocrine anti inflammatory pathway^{11, 13, 17}. Upon ACTH stimulation, cortisol is released by the adrenal medulla. Cortisol inhibits pro inflammatory gene expression by immune cells by binding to an intracellular receptor and subsequent suppression of nuclear factor kappa B (NF κ B) activity as well as activation of transcription of anti inflammatory genes¹⁸.

Hard wired connections

The CNS and the immune system are directly linked through the autonomic nervous system (Figure 2). Direct contact between postganglionic neurons of the autonomic nervous system and immune cells, either with immune cells in lymphoid organs or with residential or migrated immune cells located in an inflamed area, provides a direct hard wired link for the CNS to modulate inflammatory responses in vivo. Lymphoid organs are innervated by the parasympathetic nervous system as well as the sympathetic nervous system¹⁹. Furthermore, lymphocytes, granulocytes and macrophages have been shown to carry receptors for Ach^{20, 21} as well as NE but also for various other substances released by neurones such as vaso intestinal peptide²², α -MSH²³ and leptin²³.

Sympathetic nervous system, stimulation of beat receptors on immune cells

The autonomic nervous system is activated upon detection of inflammation either directly or via activation of the NTS through the afferent vagus nerve. With regard to the sympathetic nervous system, there are connections of the NTS with nuclei, such as the rostral ventrolateral medulla (RVM), that activate the sympathetic nervous system

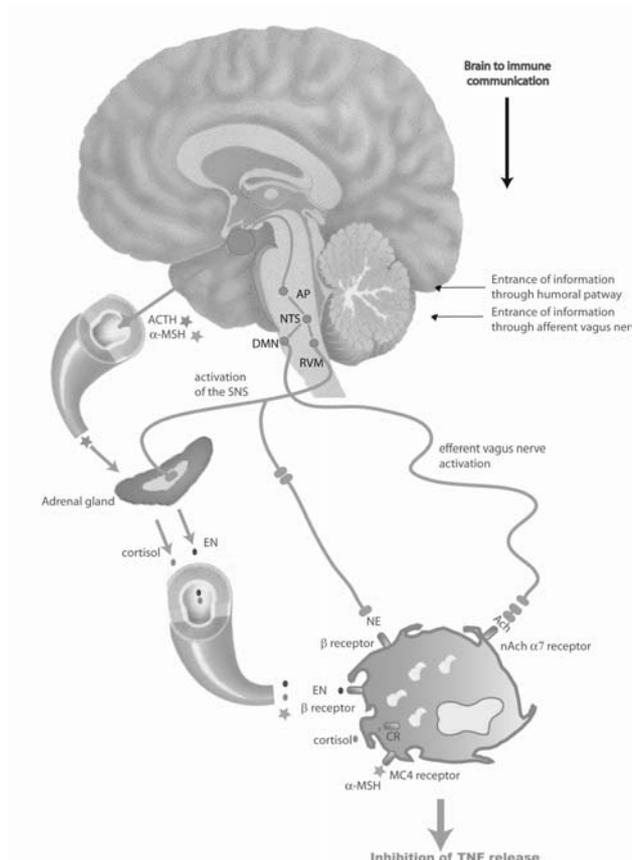


Figure 2.

Brain to immune communication.

Brains to immune pathways are activated by signals reaching the brain through the systemic compartment and the vagus nerve. The brain responds by activating a neuroendocrine pathway through the release of corticotropin releasing hormone (CRH), which activates cortisol release by the adrenal medulla as well as α -melanocyte stimulating hormone (α -MSH). Cortisol inhibits immune cells through intracellular receptors, α -MSH through the MC4 receptor. Also, vagal efferent activity is activated through the dorsal motor nucleus (DMN), which results in the release of Acetylcholine (ACh) at vagal post synaptic neurons, inhibiting immune cells through the nicotinic ACh α 7 receptor. Furthermore the sympathetic nervous system (SNS) is activated and epinephrine (EN) is released by the adrenal cortex and norepinephrine (NE) at postsynaptic SNS neurons. Both EN and NE inhibit immune cells through β -receptors.

(Figure 2)²⁴. Activation of preganglionic neurons of the sympathetic nervous system induces the release of epinephrine by the adrenal medulla into the bloodstream, converting a neural pathway into an endocrine anti-inflammatory pathway, since in response to catecholamines monocytes release less pro inflammatory mediators and are stimulated to produce IL-10^{6, 25, 26}. The major importance of this pathway is shown by experiments where the infusion of β agonists in humans and animals reduces inflammation during experimental endotoxemia whereas β receptor antagonists stimulate pro-inflammatory responses. The importance of the sympathetic nervous system in inhibiting the immune response is illustrated by several elegant experiments. In a mouse model of stroke, the hypothesis that a stroke-induced immunodeficiency increases the susceptibility to bacterial infections was tested. Indeed, mice developed spontaneous pneumonia within three days after the induction of stroke. Administration of the β

adrenoreceptor antagonist propranolol drastically reduced the incidence of pneumonia, the defect in lymphocyte activation and mortality after stroke²⁶. This suggests that immunosuppression during stroke is actually catecholamine-mediated. Furthermore, NE is released by postganglionic neurons which directly affects nearby immune cells through β receptors (Figure 2).

Parasympathetic nervous system; the cholinergic anti-inflammatory pathway

Upon activation of the NTS, projections to the AP stimulate the HPA axis and projections to the RVM the sympathetic nervous system (Figure 3). However, direct connections between the NTS and the DMN ensure that when signals reach the NTS vagal efferent activity is stimulated as well (Figure 3). Release of Ach by postganglionic neurons of the vagus nerve inhibits the release of pro inflammatory cytokines by immune cells (Figure 3).

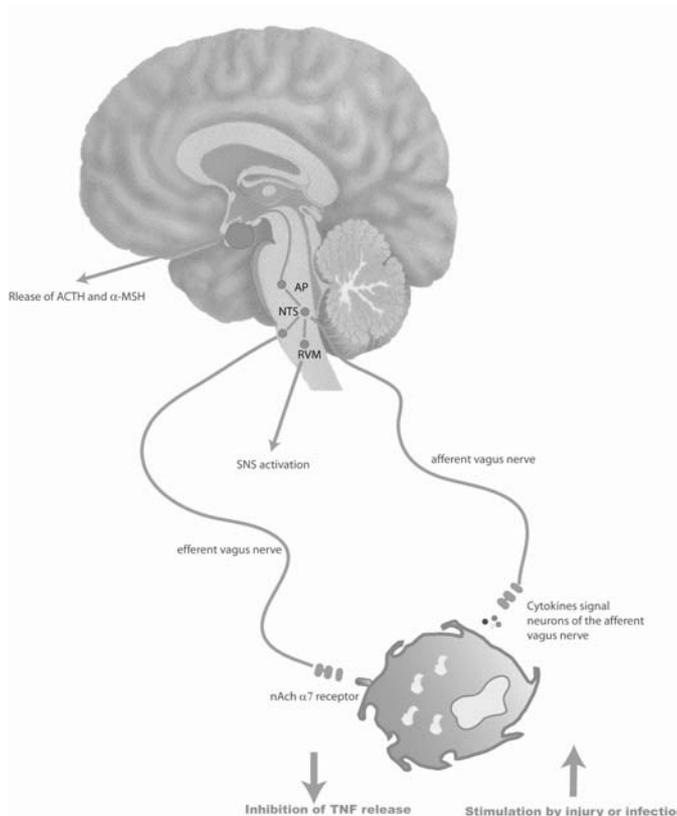


Figure 3.
The cholinergic anti-inflammatory pathway.

The afferent vagus nerve is stimulated by cytokines. Information is relayed to nucleus tractus solitarius (NTS). From here, projections lead to the area postrema (AP); this nucleus connects to the hypothalamus which activates the HPA axis. Second, projections to the rostral ventrolateral medulla (RVM) activate the sympathetic nervous system and epinephrine (EN) is released by the adrenal cortex and norepinephrine (NE) at postsynaptic SNS neurons. Finally, through the dorsal motor nucleus (DMN) vagal efferent activity is activated through the dorsal motor nucleus (DMN), which results in the release of acetylcholine (ACh) at vagal post synaptic neurons, inhibiting immune cells through the nicotinic acetylcholine $\alpha 7$ receptor.

In vitro studies have shown that immune cells are susceptible to Ach. When macrophages are exposed to Ach, the principle parasympathetic neurotransmitter, these cells are effectively deactivated²⁰. This Ach-induced deactivation is characterized by a dose-dependent reduction in the release of a series of proinflammatory cytokines, including TNF- α , IL-1 β , IL-6 and IL-18, by macrophages stimulated with endotoxin²⁰. Ach acts through two types of receptors: muscarinic and nicotinic. In addition to the brain and “wire-innervated” peripheral structures, these Ach receptor subtypes are also expressed by immune cells^{17, 21, 27-29}. Evidence indicates that the anti-inflammatory effects of Ach are mediated by nicotinic Ach receptors, and in particular by the $\alpha 7$ subunit of the nicotinic Ach receptor²¹. In vitro studies have shown that Ach and nicotine inhibit endotoxin-induced proinflammatory cytokine release by macrophages; this Ach effect can be prevented by nicotine receptor antagonists, and macrophages deficient for the $\alpha 7$ subunit of the nicotinic Ach receptor can not be inhibited with regard to cytokine release by Ach or nicotine^{20, 21}. In vivo studies in endotoxemia and other models of inflammation have shown that macrophages are directly influenced by vagus nerve derived Ach, suggesting that the vagus nerve provides a hard wired anti inflammatory pathway called the “cholinergic anti inflammatory pathway” (Figure 3)²⁷. In these studies, electrical stimulation of the efferent vagus nerve inhibits TNF release induced by injection of endotoxin into rats and mice and prevents shock; however, electrical stimulation of the vagus nerve in mice deficient for the $\alpha 7$ subunit of the nicotinic Ach receptor does not result in a reduced cytokine release upon endotoxin administration²⁰. Besides inflammation induced by endotoxin, the cholinergic anti-inflammatory pathway can also inhibit other types of inflammation in vivo. Direct stimulation of the vagus nerve diminished shock and proinflammatory cytokine synthesis in liver and heart obtained from animals subjected to ischemia-reperfusion injury induced by transient aortic occlusion³⁰. Furthermore, in hypovolemic hemorrhagic shock in rats, stimulation of the vagus nerve increased survival time, reverted hypotension, blunted NF κ B activity in the liver and reduced TNF levels³¹. Localized inflammation is also affected by the cholinergic anti inflammatory pathway, as shown in experimental murine arthritis induced by carrageenan where vagus nerve stimulation inhibited the inflammatory response and suppressed the development of paw swelling³². In, as of yet unpublished, studies by our group we have shown that the severity of experimental pancreatitis is dependent on nicotinic Ach receptors and the vagus nerve and that the cholinergic anti inflammatory pathway regulates host defense and the inflammatory response during experimental gram

negative sepsis. Another line of evidence comes from studies in which vagus nerve activity was stimulated centrally. CNI-1493, a tetravalent guanyldrazone, has been shown to induce efferent vagus nerve firing when injected intracerebroventrically³². CNI-1493 significantly suppressed carrageenan-induced paw edema, even in doses at least 6-logs lower than those required for a systemic effect. Bilateral cervical vagotomy or atropine blockade abrogated the anti-inflammatory effects of CNI-1493 indicating that the intact vagus nerve is required for CNI-1493 activity. Taken together, activation of efferent vagus nerve activity provides the CNS with a fast and powerful anti-inflammatory pathway that is mediated by the release of Ach by postganglionic vagal neurons which inhibits the release of pro-inflammatory mediators by immune cells in the area of inflammation.

Therapeutic Implications

We have reviewed data that show that the CNS and the immune system are actually two tightly linked systems. The CNS is informed about ongoing inflammation by humoral and neural networks and responds in a reflex like manner by the release of hormones and through activation of the autonomic nervous system. This “inflammatory reflex” is a powerful endogenous system designed to restrain the potential detrimental effects of excessive inflammatory responses to the body. The identification of these links between the CNS and the nervous system provides an opportunity to study new therapeutic approaches for diseases in which unrestrained inflammation is essential. Most current strategies for treatment of unrestrained inflammation are based on direct suppression of pro-inflammatory cytokines or cytokine activity. The identification of the cholinergic anti-inflammatory pathway now suggests several new approaches to modify cytokines and inflammatory responses to therapeutic advantage. Such potential new approaches include electrical stimulation of the vagus nerve which may represent a novel strategy to inhibit the production of TNF and to protect against pathological inflammation. In this regard it is important to realize that permanently implanted vagus nerve stimulators are clinically approved devices for treatment of epilepsy and depression³³⁻³⁶. So far, more than 15,000 patients have been implanted with a vagus nerve stimulator for these indications with only moderate side effects³⁷. It is conceivable to treat patients with inflammatory diseases, severe infections and overshoot inflammatory syndromes, such as sepsis and the systemic inflammatory response syndrome, with vagus nerve stimulation. Especially in

TNF- α mediated diseases, such as Crohn's disease, rheumatoid arthritis and sepsis vagus nerve stimulation alone or as a supplemented to treatment with anti TNF- α strategies might be a valuable treatment. Since the anti inflammatory effects of the vagus nerve are carried out through nicotinic Ach receptors of the $\alpha 7$ subtype, pharmacological stimulation of the $\alpha 7$ subunit of the nicotinic Ach receptor may be another approach to modulate inflammatory disorders. We have obtained proof of principle that compounds specifically stimulating the $\alpha 7$ subunit of the nicotinic Ach receptor can inhibit endotoxin-induced TNF release by macrophages in vitro and in mice in vivo. A third target might be the development of small molecules, such as CNI-1493, that stimulate proximal components of the cholinergic anti-inflammatory pathway in the CNS and induce vagal efferent firing. Of note, many anti inflammatory drugs such as aspirin, indomethacin and ibuprofen have also been shown to increase vagus nerve activity which may indeed contribute to their mode of action³⁸. Finally, it is intriguing to note that the functions of the autonomic nervous system are carried out involuntary and often in a reflex like manner, however, due to the connections of the autonomic nervous system with the cerebral cortex a certain amount of conscious control is possible. An elegant example for this is the control certain individuals can exert over their heart rate and blood pressure in deep meditation, which exceeds the amount of maximal change observed during sleep or hypnosis. Knowledge of the link between the vagus nerve and regulation of the inflammatory response makes it conceivable that inhibition of vagal activity, which for example may be associated with chronic stress, contributes to the development of mild inflammatory syndromes. On the other hand, one might postulate that behavioral techniques that induce vagal activity could be effective as an anti inflammatory treatment.

Conclusion

The CNS and the immune system are tightly linked through humoral, endocrine and hard wired connections. The autonomous nervous system provides the CNS with real time information on the status of immunological activation in the body and the CNS responds to this information by generating a series of generalized behavioral and endocrine (fever, anorexia, ACTH release) as well as hard wired responses. These hard wired responses include the release of epinephrine and NE through the sympathetic nervous system as well as through activating efferent activity in the vagus nerve. Both systems function to

suppress inflammation in order to prevent inflammatory responses to become generalized. Especially the vagus nerve is an essential neural circuit for immunomodulation since its efferent activity is stimulated upon the detection of inflammation and subsequently inflammation is controlled in a reflex like manner by the anti inflammatory effects of Ach on immune cells. Knowledge of these newly discovered connections between the nervous system and the immune system, and especially of the cholinergic anti-inflammatory pathway, provides new insights in the regulation of the immune response and may pave the way for new options for the treatment of inflammatory diseases.

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