The inflammatory reflex

Kevin J. Tracey

Laboratory of Biomedical Science, North Shore-LIJ Research Institute, 350 Community Drive, Manhasset, New York 11030, USA (e-mail: kjtracey@sprynet.com)

Inflammation is a local, protective response to microbial invasion or injury. It must be fine-tuned and regulated precisely, because deficiencies or excesses of the inflammatory response cause morbidity and shorten lifespan. The discovery that cholinergic neurons inhibit acute inflammation has qualitatively expanded our understanding of how the nervous system modulates immune responses. The nervous system reflexively regulates the inflammatory response in real time, just as it controls heart rate and other vital functions. The opportunity now exists to apply this insight to the treatment of inflammation through selective and reversible 'hard-wired' neural systems.

"*The mind has great influence over the body, and maladies often have their origin there.*" Molière (1622–1673).

Survival is impossible without vigilant defence against attack and injury. The innate immune system continuously surveys the body for the presence of invaders. When it encounters an attack, it involuntarily sets in motion a discrete, localized inflammatory response to thwart most pathogenic threats. The magnitude of the inflammatory response is crucial: insufficient responses result in immunodeficiency, which can lead to infection and cancer; excessive responses cause morbidity and mortality in diseases such as rheumatoid arthritis, Crohn's disease, atherosclerosis, diabetes, Alzheimer's disease, multiple sclerosis, and cerebral and myocardial ischaemia. If inflammation spreads into the bloodstream, as occurs in septic shock syndrome, sepsis, meningitis and severe trauma, the inflammatory responses can be more dangerous than the original inciting stimulus. Homeostasis and health are restored when inflammation is limited by anti-inflammatory responses that are redundant, rapid, reversible, localized, adaptive to changes in input and integrated by the nervous system.

The nervous system is composed of sensory systems (which detect the state of the body and organs) and motor systems (which transmit signals to the body and organs). Whereas the somatic motor system controls voluntary movements, the autonomic motor system controls visceral body functions and innervates glands (involuntary). The autonomic nervous system has two principal divisions, the parasympathetic pathway and the sympathetic pathway, which act either in synergy or in opposition to mediate basic physiological responses in real time. The autonomic system continuously controls heart rate and blood pressure, respiratory rate, gastrointestinal motility, body temperature and other constantly changing, essential life functions. The autonomic nervous system interacts with the primitive brain, including the limbic system (serving important memory functions), brain stem and hypothalamus. Hypothalamic neural output is relayed to sympathetic and parasympathetic nuclei in the brain stem and spinal cord. Hormonal input also controls the release of pituitary hormones, which in turn regulate basic functions of the endocrine organs. Autonomic nervous functions are normally subconscious, but essential basic autonomic functions can be placed under conscious control from signals originating in the higher brain (cerebral cortex). For example, subjects can be trained through biofeedback to decrease their heart rate by increasing parasympathetic outflow.

Recent insights have identified a basic neural pathway that reflexively monitors and adjusts the inflammatory response. Inflammatory stimuli activate sensory pathways that relay information to the hypothalamus. Like a knee-jerk reflex, in which the stretching of a patellar tendon elicits a rapidly opposing motor action, inflammatory input activates an anti-inflammatory response that is fast and subconscious. This prevents spillage of inflammatory products into the circulation. The nervous system integrates the inflammatory response: it gathers information about invasive events from several local sites, mobilizes defences and creates memory to improve chances for survival.

Here I review evidence showing that the neural control of acute inflammation is reflexive, directly interconnected and controllable. Special emphasis is placed on cholinergic anti-inflammatory mechanisms that inhibit the activation of macrophages and the release of cytokines (Fig. 1). I also discuss evidence indicating that stimulation of the vagus nerve, by either electrical or pharmacological means, prevents inflammation and inhibits the release of cytokines that are clinically relevant drug targets for treating inflammatory disease.

Inflammation mediated by TNF

Tumour-necrosis factor (TNF), a cytokine with a relative molecular mass of 17,000 (M_r 17K), is produced by activated macrophages in response to pathogens and other injurious stimuli, and is a necessary and sufficient mediator of local and systemic inflammation[1,2]. Local increases in TNF cause the cardinal clinical signs of inflammation, including heat, swelling, pain and redness. Systemic increases in TNF mediate tissue injury by depressing cardiac output, inducing microvascular thrombosis and mediating systemic capillary leakage syndrome. TNF amplifies and prolongs the inflammatory response by activating other cells to release both cytokines such as interleukin 1 (IL-1) and high mobility group B1 (HMGB1), and mediators such as eicosanoids, nitric oxide and reactive oxygen species, which promote further inflammation and tissue injury[3]. TNF is essential for the complete expression of inflammation during invasion, and self-limited inflammation is normally characterized by decreasing TNF activity.

Low amounts of TNF can contribute to host defence by limiting the spread of pathogenic organisms into the

Table 1 **'Diffusible' anti-inflammatory mediators**

Cytokines	IL-10 TGF-β TNF-binding protein IL-1Rα
Hormones	Glucocorticoids Adrenaline Noradrenaline α-MSH
Local effectors	Spermine Prostglandin E2 Fetuin Heat-shock proteins Acute phase proteins

circulation, promoting coagulation to localize the invader, and stimulating the growth of damaged tissues[4]. In a typical 'successful' inflammatory response, the duration and magnitude of TNF release is limited, its beneficial and protective activities predominate, and it is not released systemically. Studies of the inflammatory action of TNF in non-malignant disease have led to widespread investigation of both the 'normal' mechanisms that regulate inflammation and the therapeutic potential of monoclonal antibodies specific for TNF.

Monoclonal antibodies against TNF

Early studies using monoclonal antibodies against TNF showed that this approach effectively prevents lethal tissue injury during bacterial invasion[1]. Subsequent clinical trials led to the registration of both monoclonal antibodies against TNF, and TNF-binding proteins for treating rheumatoid arthritis and Crohn's disease. Many individuals with these debilitating inflammatory illnesses have enjoyed disease remissions and an improved quality of life. Crippling joint pain has been alleviated in children with rheumatoid arthritis treated with TNF antibodies; some of the youngest patients have even experienced 'catch-up growth' and normalization of development (U. Andersson, personal communication). These and other clinical successes with TNF monoclonal antibodies have proved that cytokine responses can be manipulated to specific therapeutic advantage for inflammatory disease.

But strategies using TNF antibodies have not been translated successfully into treatments for bacterial invasion or sepsis, for reasons that have been reviewed extensively elsewhere[5]. Most notably, serum concentrations of TNF were undetectable in most of the individuals recruited into clinical sepsis trials, because the study group comprised a heterogeneous population with diverse diseases at varying stages of illness. In early experiments of the use of TNF monoclonal antibodies in bacteraemia, it became clear that TNF is an early mediator of inflammation and that TNF antibodies are ineffective if therapy is initiated after serum TNF has been cleared[1]. Continued interest in understanding the use of TNF antibodies for individuals with sepsis is now focused on identifying a homogenous study population with increased serum TNF for treatment early in the course of illness.

An alternative therapeutic strategy is to target 'late' mediators of lethality that are produced after TNF in the inflammatory pathway[3]. HMGB1 has been implicated as an experimental therapeutic target that is produced relatively late in the course of endotoxaemia[3,6]. Antibodies specific for HMGB1 confer significant protection against the lethality of endotoxaemia, even when the first antibody doses are administered after the early TNF concentrations have been cleared. Reducing serum concentrations of HMGB1 by administering ethyl pyruvate as late as 24 h after the onset of sepsis rescues animals from lethality, indicating that it may now be possible to develop therapies for sepsis that cover a significantly wider, clinically relevant treatment window[7,8].

Other cytokines have been implicated as therapeutic targets in the pathogenesis of inflammatory diseases, and it is likely that future treatment plans will target mediators in addition to TNF. Although I focus the discussion of neural regulation of inflammation primarily on cholinergic inhibition of TNF, evidence indicates that these neural anti-inflammatory mechanisms also inhibit the release of IL-1, IL-18 and HMGB1.

Anti-inflammatory responses normally inhibit inflammation

Highly conserved, counter-regulatory mechanisms normally limit the acute inflammatory response and prevent the spread of inflammatory mediators into the bloodstream (Table 1). Activated immunologically competent cells release TNF receptor fragments that bind and neutralize its inflammatory and potentially toxic actions[9]. Anti-inflammatory cytokines, such as IL-10 and transforming growth factor-β (TGF-β), specifically inhibit the release of TNF and other proinflammatory mediators[10]. Adrenal glucocorticoids, adrenaline, α-melanocyte-stimulating hormone (α-MSH) and other 'classical' stress hormones inhibit cytokine synthesis and intracellular signal transduction[11–14]. Spermine accumulates at sites of tissue injury and infection and inhibits macrophage activation and cytokine synthesis[15].

The importance of these endogenous anti-inflammatory pathways has been shown by experimentally impairing isolated pathways. For example, animals subjected to hypophysectomy or adrenalectomy are significantly sensitized to the lethal effects of endotoxin[16]. In the absence of an adequate adrenocorticotropic hormone (ACTH) and glucocorticoid response, TNF is significantly overexpressed during endotoxaemia[16–18]. Functional deficiencies in the release of corticotropin-releasing factor (CRF) predispose Lewis rats to developing experimental arthritis induced by streptococcal antigens because of an insufficient glucocorticoid response[13,19,20]. Animals deficient in IL-10 develop a chronic inflammatory bowel disease that predominately affects the colon[21] and are susceptible to a more severe form of collagen-induced arthritis[22]. Administration of specific pharmacological spermine antagonists significantly increases local TNF activity and carrageenan-induced oedema formation, and amplifies the inflammatory response[15]. Together, these findings show that loss of endogenous anti-inflammatory mechanisms converts a normally protective, self-limited inflammatory response into an excessive, potentially deleterious response.

Communication between immune and nervous systems

The activation of pituitary-dependent adrenal responses after endotoxin administration[23] provided early evidence that inflammatory stimuli can activate anti-inflammatory signals from the central nervous system (CNS). Subsequently, Besedovsky *et al.*[24] showed directly that inflammation in peripheral tissues alters neuronal signalling in the hypothalamus. Extensive work has identified a common molecular basis for communication, with cells from each system expressing signalling ligands and receptors from the other[25]. For example, neurons in the CNS can synthesize and express TNF and IL-1, and these cytokines may participate in neuronal communication[26,27]. This communication is bi-directional, because cytokines can activate hypothalamic-pituitary release of glucocorticoids and, in turn, glucocorticoids suppress further cytokine synthesis[28]. In addition, cells of the immune system can produce neuropeptides (including endorphins), acetylcholine and other neurotransmitters.

The importance of the interaction between the nervous system and immune system signalling has been demonstrated recently in the development of pathological pain. Watkins and Maier[29] have proposed that cytokines produced by inflammatory and glial cells change neuronal excitability and that this link contributes directly to the development of intractable pain.

Cholinergic anti-inflammatory pathway

Our understanding of the basic mechanisms that regulate inflammation has been advanced by the identification of a neural mechanism that inhibits macrophage activation through parasympathetic outflow[30]. Called the 'cholinergic anti-inflammatory pathway' because acetylcholine is the principle parasympathetic neurotransmitter, macrophages that are exposed to acetylcholine are effectively

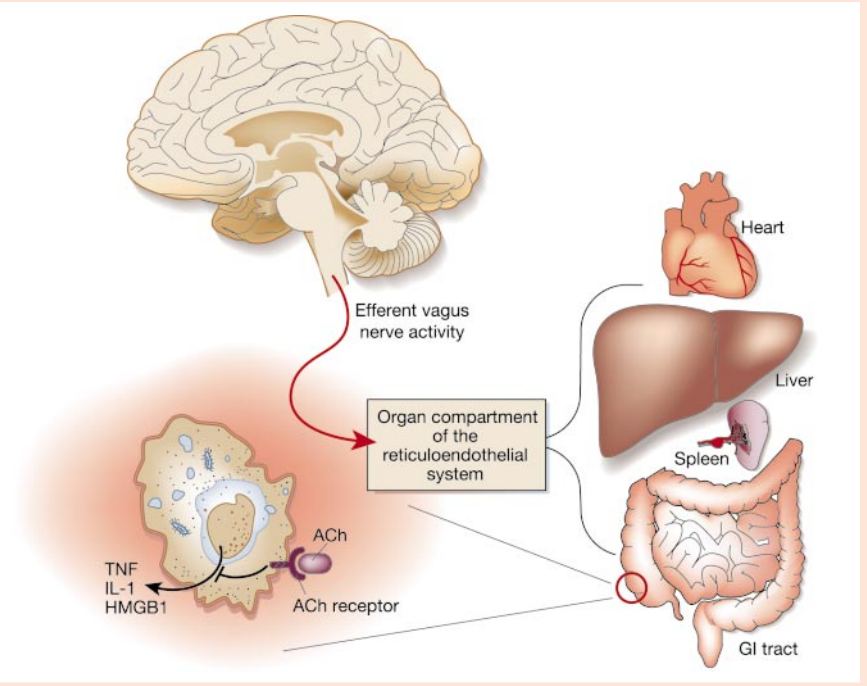

Figure 1 The cholinergic anti-inflammatory pathway. Efferent activity in the vagus nerve leads to acetylcholine (ACh) release in organs of the reticuloendothelial system, including the liver, heart, spleen and gastrointestinal tract. Acetylcholine interacts with α-bungarotoxin-sensitive nicotinic receptors (ACh receptor) on tissue macrophages, which inhibit the release of TNF, IL-1, HMGB1 and other cytokines.

deactivated (Fig. 1). The vagus nerve (which was named for its wandering course) innervates the principal organs, including those that contain the reticuloendothelial system (liver, lung, spleen, kidneys and gut)[31]. Experimental activation of the cholinergic anti-inflammatory pathway by direct electrical stimulation of the efferent vagus nerve inhibits the synthesis of TNF in liver, spleen and heart, and attenuates serum concentrations of TNF during endotoxaemia[30,32]. Vagotomy significantly exacerbates TNF responses to inflammatory stimuli and sensitizes animals to the lethal effects of endotoxin.

This 'hard-wired' connection between the nervous and immune systems functions as an anti-inflammatory mechanism in other models of systemic and local inflammation. Direct stimulation of the vagus nerve *in situ* inhibits proinflammatory cytokine synthesis in liver and cardiac tissue obtained from animals subjected to ischaemia-reperfusion by transient aortic clamping. Stimulation of either the right or the left cervical vagus nerves protects against the development of hypotension and inhibits serum TNF responses to ischaemia reperfusion[32]. The protection conferred by stimulation of the vagus nerve is dependent on the applied voltage and is associated with normalization of tachycardia during the reperfusion-induced hypotensive phase[32]. In a standardized model of experimental murine arthritis induced by the application of carrageenan, vagus nerve stimulation inhibits the inflammatory response and suppresses the development of paw swelling, indicating that the cholinergic anti-inflammatory pathway can inhibit localized inflammation specifically[33].

The molecular dovetail between the cholinergic nervous system and the innate immune system is a nicotinic, α-bungarotoxin-sensitive macrophage acetylcholine receptor[30]. Exposure of human macrophages, but not peripheral blood monocytes, to nicotine or acetylcholine inhibits the release of TNF, IL-1 and IL-18 in response to endotoxin. Tissue macrophages, but not circulating monocytes, produce most of the TNF that appears systemically during an excessive inflammatory response. Interaction between the macrophage cholinergic receptor and its ligand inhibits the synthesis of proinflammatory cytokines (TNF, IL-1 and IL-18) but not anti-inflammatory cytokines (such as IL-10)[30]. Acetylcholine inhibits the expression of TNF protein in macrophages, but not the induction of TNF messenger RNA levels, indicating that activation of the cholinergic receptor transduces intracellular signals that inhibit cytokine synthesis at a post-transcriptional stage.

As compared with macrophages, monocytes are refractory to the cytokine-inhibiting effects of acetylcholine: only supraphysiological concentrations of cholinergic agonists inhibit cytokine synthesis in monocytes[30]. The macrophage acetylcholine receptor is distinct from the muscarinic receptor activities identified on lymphocytes, peripheral blood mononuclear cells and alveolar macrophages[34,35]. The exquisite sensitivity of macrophages to acetylcholine suggests that other non-neuronal cells that produce acetylcholine (such as epithelial cells, T lymphocytes and endothelial cells) might also participate in modulating the function of adjacent tissue macrophages[36,37].

Vagus nerve stimulation suppresses inflammation

Stimulation of efferent vagus nerve activity has been associated classically with slowing heart rate, induction of gastric motility, dilation of arterioles and constriction of pupils. Inhibition of the inflammatory response can now be added to this list (Fig. 1). From an oversimplified, teleological engineering perspective, there are many reasons why a neural-based anti-inflammatory pathway is advantageous. The diffusible anti-inflammatory network, which includes glucocorticoids, anti-inflammatory cytokines and other humoral mediators, is slow, distributed, non-integrated and dependent on concentration gradients. By contrast, the cholinergic anti-inflammatory pathway is discrete and localized in tissues where invasion and injury typically originate (Fig. 2).

As compared with the routine, biological pace of a typical, diffusible inflammatory response (hours to days), neural signalling is like lightning. This regulatory attribute is highly advantageous for containing immune activation at the crucial stages of a nascent response. Neural control of biological functions is short-lived: after a brief refractory period, responding cells can resume function as required in the absence of further neural input. Recovery of immune function after transient inhibition enables necessary local inflammatory responses to be mobilized during persisting threat or infection. The impact of sensitization or desensitization developing after, respectively, denervation or repeated neural firing to an inflammatory site has not been explored, but it would be predicted to influence anti-inflammatory function.

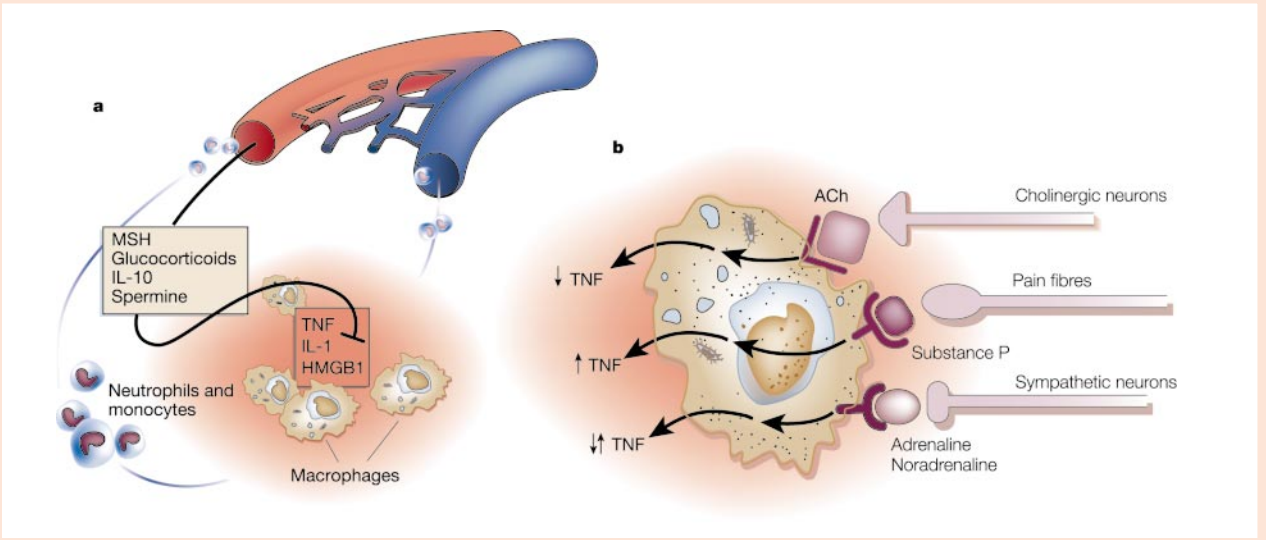

Figure 2 Diffusible versus neural anti-inflammatory pathways. **a**, Diffusible pathways. The circulation delivers inflammatory cells (monocytes and neutrophils) and cytokines to and from the inflammatory site; these responses are concentration gradient-dependent, slow and not integrated. Inflammatory products produced in the damaged tissue (TNF, IL-1, HMGB1) diffuse into the bloodstream, and anti-inflammatory hormones and cytokines (glucocorticoids, α-MSH, IL-10, spermine) diffuse into the zone. **b**, Neural pathways. Neural anti-inflammatory regulation of tissue macrophages is local, fast and integrated through the CNS. Acetylcholine inhibits the release of TNF from macrophages. Adrenaline and noradrenaline predominately inhibit TNF release but can, under some circumstances, stimulate TNF release. Substance P can stimulate cytokine synthesis to amplify the local inflammatory response and can also mediate pain.

Neural regulation of discrete, distributed, localized inflammatory sites provides a mechanism for integrating responses in real time. It is intriguing to consider that, in addition to the development of immunological memory, the involvement of the cholinergic anti-inflammatory pathway might also modulate processing events that promote neural memory of the peri-inflammatory events (that is, the 'hissing snake' or 'charging lion' that caused the wound and/or infection). Clark *et al.*[38] recently discovered that electrical stimulation of the vagus nerve in humans significantly enhanced word-recognition memory, indicating that memory formation and vagus nerve activity are closely linked.

Sensory function of vagus nerve signals in inflammation

The CNS receives sensory input from the immune system through both humoral and neural routes. Blalock[39,40] originally suggested that the immune system functions as a 'sixth sense' that detects microbial invasion and produces molecules that relay this information to the brain. TNF and other immunological mediators can gain access to brain centres that are devoid of a blood–brain barrier in the circumventricular region. Indeed, the dorsal vagal complex, comprising the sensory nuclei of the solitary tract, the area postrema and the dorsal motor nucleus of the vagus, responds to increased circulating amounts of TNF by altering motor activity in the vagus nerve[41–43]. This humoral route for communication between the immune system and the nervous system has been implicated in the development of fever, anorexia, activation of hypothalamic-pituitary responses to infection and injury, and other behavioural manifestations of illness.

Sensory innervation of immune organs by ascending fibres travelling in the vagus nerve, as well as by other pain and ascending sensory pathways, provides important input about the status of invasive and injurious challenges in distributed body compartments. Notably, these neural inflammation-sensing pathways can function at low thresholds of detection and can activate responses even when the inflammatory agents are present in tissues in quantities that are not high enough to reach the brain through the bloodstream. Watkins and colleagues[44–47] have provided insight into the sensory role of afferent vagus nerve fibres by observing that vagotomy blunts the development of fever in animals exposed to intra-abdominal IL-1. The afferent vagus pathway is activated by very low doses of endotoxin or IL-1; but higher doses of these agents can directly activate thermogenic responses through the humoral route to the brain[48]. It is not completely clear how the vagus nerve 'detects' the presence of low doses of endotoxin or other inflammatory agents, but neurons in the vagus nerve express IL-1 receptor mRNA and discrete IL-1-binding sites have been identified on glomus cells in the vagus nerve proper[41,49].

Electrophysiological studies indicate that vagus nerve signals also can be activated by TNF, other cytokines, mechanoreceptors, chemoreceptors, temperature sensors and osmolarity sensors that might be activated at an inflammatory locus[50]. Somatic sensory input into the CNS is organized somatotopically, such that sensory input from a discrete peripheral site is localized precisely in the ascending fibre pathways and brain. The first CNS synapse for afferent vagus signals lies in the nucleus tractus solitarius, and electrolytic lesioning of this region impairs the development of IL-1-induced fever[51]. Thus, inflammation-derived sensory input can be processed differentially in the brain, depending on the location of the inflammatory site and the nature of the sensory signal.

Reflex inhibition of inflammation

The inflammation-sensing and inflammation-suppressing functions outlined above provide the principal components of the inflammatory reflex (Fig. 3). The appearance of pathogenic organisms in a local wound, or at the site of epithelial barrier dysfunction, activates innate immune cells that release cytokines. These activate sensory fibres that ascend in the vagus nerve to synapse in the nucleus tractus solitarius. Increased efferent signals in the vagus nerve suppress peripheral cytokine release through macrophage nicotinic receptors and the cholinergic anti-inflammatory pathway. The 'inflammatory reflex' is described as localized, rapid and discrete; but it can also induce systemic humoral anti-inflammatory responses. This occurs because vagus nerve activity can be relayed to the medullary reticular formation, to the locus ceruleus and to the hypothalamus, leading to increased release of ACTH from the anterior pituitary.

Increased cytokine production in tissues causes pain, providing another mechanism for transferring information from the immune

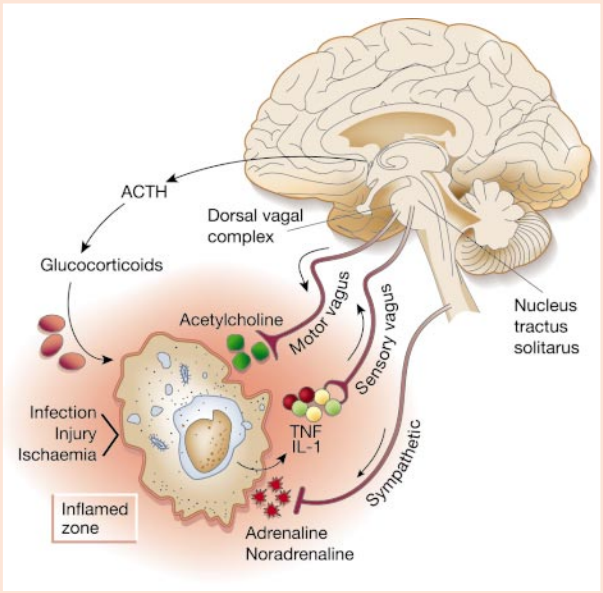

Figure 3 Wiring of the inflammatory reflex. Inflammatory products produced in damaged tissues activate afferent signals that are relayed to the nucleus tractus solitarius; subsequent activation of vagus efferent activity inhibits cytokine synthesis through the cholinergic anti-inflammatory pathway ('the inflammatory reflex'). Information can also be relayed to the hypothalamus and the dorsal vagal complex to stimulate the release of ACTH, thereby activating the humoral anti-inflammatory pathway. Activation of the sympathetic outflow by flight-or-fight responses or pain, or through direct signalling, can increase local concentrations of adrenaline and noradrenaline, which can suppress inflammation further.

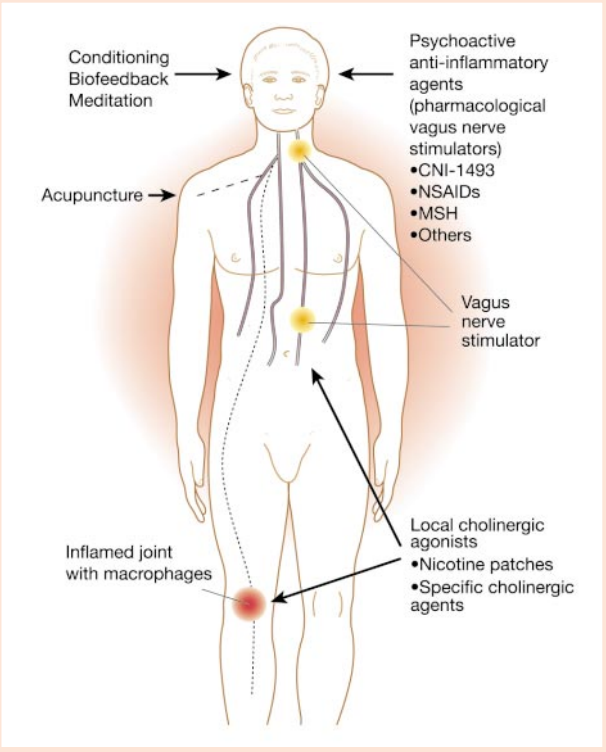

Figure 4 Targeting therapies to the cholinergic anti-inflammatory pathway. The physiological basis of the cholinergic anti-inflammatory pathway could guide the development of therapies based on either modulating the activity of the vagus nerve or targeting specific components of the pathway. For example, biofeedback, conditioning, meditation, hypnosis or acupuncture could be potentially used to modulate vagus output, 'psychoactive' drugs could be tailor-made to increase vagus output ('pharmacological vagus nerve stimulators'; NSAIDs, non-steroidal anti-inflammatory drugs), and other agents could be used to target macrophage cholinergic receptors in the periphery. Unbroken lines represent known vagus nerve pathways; dotted lines are hypothetical.

system to the brain. This information can be relayed to other brain centres that influence motor output in the vagus nerve. Pain and stress can activate the flight-or-fight responses, and the resultant increase of adrenaline and noradrenaline also can inhibit macrophage activation and suppress synthesis of TNF and other cytokines[13,52,53]. High sympathetic activity and resultant increases in catecholamines stimulate the β-adrenergic-receptor-dependent release of IL-10, a potent anti-inflammatory cytokine, from monocytes[11,54]. Thus, the anti-inflammatory effects of the sympathetic and parasympathetic nervous systems seem to be synergistic in this setting.

Classical teaching stresses that actions of the sympathetic and parasympathetic nervous systems are usually in opposition. But in many situations the two systems function synergistically. For example, simultaneous stimulation of both sympathetic and vagus nerves produces a higher increase in cardiac output than does isolated stimulation of either nerve alone[55]. Flight-or-fight activation of sympathetic responses also stimulates increased vagus nerve output. The combined action of these neural systems is significantly anti-inflammatory and is positioned anatomically to constrain local inflammation by preventing spillover of potentially lethal toxins into the circulation through both local (neural) and systemic (humoral) anti-inflammatory mechanisms.

Implications of the inflammatory reflex

Knowledge of the inflammatory reflex and the cholinergic anti-inflammatory pathway is yielding insight into both physiological pathways and therapeutic strategies (Fig. 4). For example, it may be possible to activate neural anti-inflammatory mechanisms using small molecules that initiate signals in proximal components of the pathway in the CNS. One such molecule is CNI-1493, a tetravalent guanylhydrazone that was originally described as an inhibitor of macrophage activation and TNF release[56,57].

CNI-1493 inhibits TNF synthesis and inflammatory responses in animal models of local and systemic inflammation[58]. It also significantly reduced disease severity in a small clinical trial of severe Crohn's disease and is currently being evaluated in a large phase II trial of Crohn's disease[59]. Unexpectedly, recent evidence has shown that the TNF-suppressing activities of CNI-1493 *in vivo* are dependent on the cholinergic anti-inflammatory pathway, and that CNI-1493 functions as a pharmacological stimulator of the vagus nerve[32,60]: intracerebral application of small doses of CNI-1493 significantly inhibited peripheral TNF synthesis, and intact vagus nerves were required to prevent increases in serum TNF. The mechanism through which CNI-1493 activates the vagus nerve is unknown, but increased vagus nerve firing has been observed after either intracerebral or intravenous administration of CNI-1493 — an effect that seems to be dependent on specific CNS receptors[33].

It is likely that other experimental and clinically approved therapeutic agents suppress peripheral inflammation by activating pathways in the CNS. Small doses of α-MSH applied intracerebrally inhibited pulmonary myeloperoxidase activity in mice exposed to endotoxin[61] and suppressed the development of intradermal oedema induced by exposure to TNF or IL-1 (ref. 62). Specific anti-inflammatory responses have been observed in response to intracerebral application of salicylates, but not dexamethasone[63]. The cardiac anti-arrhythmic drug amiodarone has been identified as an inhibitor of TNF synthesis in monocytes *in vitro*[64], but it also functions as a potent stimulator of vagus nerve activity[65]. Systemic administration of the non-steroidal anti-inflammatory drugs aspirin, indomethacin and

ibuprofen substantially increases vagus nerve activity[66]. Although this vagus nerve response had been studied in the context of increasing gastric acidity and ulcer formation, knowledge of the cholinergic anti-inflammatory pathways raises the possibility that the vagus-nerve-stimulating activity of these agents may also contribute to their anti-inflammatory action. A better understanding of the CNS receptors, pathways and neural mechanisms that activate the vagus nerve to inhibit production of TNF should facilitate development of this pharmacological 'vagus-nerve-stimulating' approach.

Another experimental therapeutic approach is based on direct electrical stimulation of the vagus nerve. So far, more than 10,000 individuals have received implantable vagus nerve stimulators for the treatment of epilepsy[67,68]. Vagus nerve stimulation in humans with small, pacemaker-like devices is safe, well tolerated and not associated with increased rates of infection. But the immunological effects of this approach have not been reported and, indeed, it will interesting to assess whether stimulating the vagus nerve in humans modulates TNF synthesis and inflammation. In place of implantable devices, it should be possible to develop pharmacological approaches that target the peripheral macrophage receptor to inhibit TNF synthesis. A precedent for this approach has been already achieved in the clinic, because nicotine administration is significantly efficacious in reducing the severity of ulcerative colitis[69]. Other preclinical studies using standard murine models of diabetes have shown that nicotine reduces the incidence of diabetes by reducing pancreatic concentrations of TNF and other cytokines[70]. Unanticipated activities of the cholinergic anti-inflammatory pathway in inflammatory disease and in non-immune cells might be determined by further studies.

Some of the earliest studies of the nervous system and inflammation examined the effects of pavlovian conditioning on intra-abdominal inflammatory responses[71]. Behavioural conditioning using models of learned association can reproducibly influence acute inflammatory responses and alter the course of experimental inflammatory diseases in animals and humans[72–74]. Hypnosis and meditation can significantly increase vagus nerve output and have been observed to inhibit immediate-type and delayed-type hypersensitivity responses[75,76]. Biofeedback and acupuncture have been used to modulate vagus nerve activity to alter bowel function, gastric acidity and heart rate[77,78]. Each of these approaches has been used to reduce experimental inflammation, but the relationships between vagus nerve activity and anti-inflammatory action had not been defined previously. Autonomic dysfunction occurs as a classical complication of rheumatoid arthritis, diabetes and other autoimmune disorders[79–81]. It is now intriguing to consider whether vagus nerve dysfunction underlies the progression of inflammation, owing to impairment of the cholinergic anti-inflammatory pathway. It is reasonable to propose that, one day, the rational modulation of vagus nerve activity using these or other approaches may provide a therapeutic advantage for inflammatory disease. □

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