Abstract: The inflammatory reflex is a neurophysiological mechanism that regulates the immune system. The efferent branch of the reflex the cholinergic anti-inflammatory pathway, which inhibits inflammation by suppressing cytokine synthesis via release of acetylcholine in organs of the reticuloendothelial system, including the spleen, liver, and gastrointestinal tract. Acetylcholine binds to α7 nicotinic acetylcholine receptors expressed by macrophages and other cytokine-producing cells. Receptor-ligand engagement suppresses proinflammatory cytokines and prevents tissue damage. Herein is a review of some of the experimental studies that define the inflammatory reflex and its anatomic and physiologic components.

Key Words: cytokine · TNF · inflammation · cholinergic anti-inflammatory pathway

INTRODUCTION

Inflammation, defined clinically as heat, pain, redness, and edema, is the physiological response to injury and infection. Although it is integral to restoring tissue homeostasis during damage as a result of invading pathogens, foreign bodies, and trauma, inflammation has also been implicated in the pathogenesis of acute and chronic diseases. This list includes inflammatory bowel disease, rheumatoid arthritis, type 2 diabetes, atherosclerosis, multiple sclerosis, Alzheimer’s disease, sepsis, hemorrhagic shock, and ischemia/reperfusion injury [1, 2]. Experimental studies in animals and clinical results with drugs that target the activity of cytokines indicate that cytokines are necessary and sufficient for the development of disease signs and symptoms. Health is maintained in part by counter-regulatory, physiological mechanisms that inhibit cytokine activity and limit toxicity.

Recent evidence indicates that cytokine release by the immune system is regulated by neural pathways. The major components of this pathway, termed “the inflammatory reflex,” function in concert with the humoral anti-inflammatory response. Here, we review the physiology, neuroscience, and immunology of inflammatory reflex with a focus on some of the results that helped to define this physiological cytokine controller. The original observations that led to the identification of the inflammatory reflex came from studies of CNI-1493, an experimental, anti-inflammatory agent. Recent evidence implicates the vagus nerve and the nicotinic acetylcholine receptor α7 (α7nAChR) subunit as components.

HARD-WIRING OF THE INFLAMMATORY REFLEX

The inflammatory reflex (Fig. 1) maps the concept that a neural mechanism can coordinate and modulate cytokine responses [4]. It is comprised of an afferent (sensory) and an efferent (motor) branch, which function in concert to form a discrete and rapid mechanism in which the motor branch opposes the action that activated the sensory branch [1]. Inflammatory mediators activate afferent vagus nerve signals, which are transmitted to the medullary reticular formation, locus ceruleus, hypothalamus, and dorsal vagal complex, and ultimately lead to an increase in adrenocorticotropic hormone (ACTH) from the anterior pituitary gland [1]. Afferent neural signals stimulate an increase in systemic glucocorticoid levels which can inhibit proinflammatory cytokines [5–7]. Melanocyte-stimulating hormone, a potent anti-inflammatory protein that inhibits the synthesis of cytokines, is also released in inflammatory conditions [8]. Ascending sensory fibers of the vagus nerve that synapse in the nucleus tractus solitarius of the upper medulla can also activate nerve signals to inhibit cytokine release [9].

The motor, neural arc of the reflex, termed the cholinergic anti-inflammatory pathway, inhibits inflammation by suppressing cytokine synthesis [1, 10, 11] via release of acetylcholine in organs of the reticuloendothelial system, including the spleen, liver, and gastrointestinal tract [1, 12]. Acetylcholine binds to the α7nAChR expressed on the surface of activated macrophages and other cytokine-producing cells, whereby cytokine synthesis and release are prevented by inhibiting NF-κB (i.e., decreasing transcription activity of the transcription factor NF-κB subunit p65) and by stimulating the JAK-STAT anti-inflammatory pathway [10, 13–16]. Together, evidence that vagotomized animals and animals lacking the α7 receptor develop exaggerated responses to invasive stimuli indicates...
that the inflammatory reflex controls the set point for cytokine production by the immune system.

CNI-1493 AND IMPLICATIONS OF A NEURAL REFLEX

The hypothesis that inflammation is under neural control stemmed from observations made during studies of the anti-inflammatory mechanism of action of CNI-1493. This compound, a tetravalent guanylhydrazone, was initially developed during efforts to create a pharmaceutical agent capable of selectively inhibiting macrophage-derived inflammatory mediators of inflammation, including TNF and NO [17]. CNI-1493 inhibits production of pro-inflammatory cytokines in macrophages through the inhibition of the phosphorylation of p38 MAPK, which plays an integral role in the translation of mRNA of pro-inflammatory cytokines such as TNF [4, 18–22]. CNI-1493 effectively attenuates inflammatory responses in a variety of animal models. For example, CNI-1493 administration protects rats from IL-2 toxicity, inhibits endotoxin-induced shock, reduces endotoxin-induced elevation of TNF-α, IL-1β, and IL-6 tissue levels in lung and spleen, and protects against lethal sepsis [23, 24].

During the course of investigating the use of CNI-1493 in animal models of cerebral ischemia, Tracey and colleagues [9] were surprised to observe that intracerebral administration of CNI-1493 significantly inhibited serum (systemic) TNF responses to i.v. (systemic) endotoxemia. Administration of extremely low doses of CNI-1493 directly into the brain significantly inhibited serum TNF levels during endotoxemia. The TNF-suppressing activity of CNI-1493 in vivo required an intact vagus nerve, as TNF production was normalized by vagus nerve lesions in animals receiving CNI-1493 [9].

EXPLORATION AND CHARACTERIZATION OF THE LINK BETWEEN THE NERVOUS AND IMMUNE SYSTEMS

The cholinergic anti-inflammatory pathway

Having considered that signals in the vagus nerve may control cytokine responses, it next became possible to identify the essential mechanisms of this pathway [9, 11, 13]. The principal neurotransmitter of the vagus nerve is acetylcholine, and it significantly inhibited TNF release from primary human macrophage cultures [11]. These studies also implicated the α-bungarotoxin nicotinic acetylcholine receptor family as mediating the cholinergic signal that inhibited cytokine release. In addition to inhibiting TNF release, acetylcholine inhibited the release of other macrophage-derived, LPS-stimulated cytokines, including IL-1β, IL-6, and IL-8 in a dose-dependent manner, but not IL-10, an anti-inflammatory cytokine [11].

To study the physiology of this pathway, rats were subjected to vagotomy. During endotoxemia, serum TNF levels in vagotomized rats were significantly higher than sham-operated control rats. Direct activation of efferent vagus nerve signals in animals with intact vagus nerves significantly inhibited serum TNF and prevented shock and tissue injury during endotoxemia. Electrical stimulation of the vagus nerve did not augment corticosteroid or IL-10 levels, indicating that the observed immune responses to electrical stimulation were not a result of stimulating humoral anti-inflammatory mechanisms. Rats that were subjected to vagotomy without electrical stimulation had a significantly worsened severity of hypotension as compared with sham-operated controls. This indicates that the vagus nerve and the inflammatory reflex normally work in concert to maintain the set point on cytokine release from the immune system. Vagus nerve stimulation conferred significant protec-
tion against the onset of endotoxin-induced shock, as it inhibited TNF release, which is the primary mediator of hypotension during endotoxemia [11].

CNI-1493 revisited

In addition to electrical stimulation of the vagus nerve, CNI-1493 is capable of inhibiting systemic inflammation during ischemia reperfusion injury [9]. Intracerebroventricular application of CNI-1493 or vagus nerve stimulation conferred significant protection against TNF release, hypotension, and tissue injury during aortic clamp-induced ischemia reperfusion. These protective effects were abrogated by vagotomy, indicating that an intact, cholinergic, anti-inflammatory pathway is required for the anti-inflammatory effects of CNI-1493.

IDENTIFICATION OF THE \( \alpha_7 \)nACh SUBUNIT

Early studies [9, 11] implicated the \( \alpha \)-bungarotoxin class of nicotinic receptors in the regulation of cytokine release, but subsequent work revealed the molecular identity of the acetylcholine receptor as required for the \( \alpha_7 \)nAChR [13]. FITC-tagged \( \alpha \)-bungarotoxin was used to identify specific cholinergic receptors on macrophages, and RT-PCR revealed \( \alpha_1 \), \( \alpha_7 \), and \( \alpha_{10} \) mRNA expression. Western blotting revealed expression of \( \alpha_7 \) subunits. To confirm that \( \alpha_7 \) was indeed the receptor subunit responsible for binding \( \alpha \)-bungarotoxin, \( \alpha \)-bungarotoxin-conjugated beads were used to isolate proteins from human macrophages, which expressed an \( \alpha \)-bungarotoxin-binding \( \alpha_7 \) subunit that had identical exons 1–10, as the \( \alpha_7 \) subunit expressed in neurons (as determined via comparison of cDNA). The function of \( \alpha_7 \) on TNF release was revealed by addition of antisense oligonucleotides: \( \alpha_7 \) “knockout” macrophages were significantly less responsive to the inhibitory effect of nicotine on TNF synthesis, as compared with macrophages exposed to “sense” RNA. Together, these results indicated that the \( \alpha_7 \) subunit is necessary for inhibition of TNF release by acetylcholine [13].

To confirm these findings in vivo, serum TNF levels were measured in normal and \( \alpha_7 \) knockout mice following the administration of endotoxin (LPS). The \( \alpha_7 \) knockout mice had significantly higher levels of TNF than the wild-type counterparts. Further, macrophages derived from the knockout mice were nonresponsive to the TNF-inhibiting effects of nicotine and acetylcholine. Additional in vivo studies revealed that electrical stimulation of the vagus nerve significantly decreased TNF serum levels in wild-type mice but not in the knockout mice. Thus, the \( \alpha_7 \) subunit is an essential component of the inflammatory reflex in vivo [13].

MODULATING THE INFLAMMATORY REFLEX: SUMMARY OF PRECLINICAL STUDIES

A number of experiments have been conducted to determine whether it is possible to impact the inflammatory reflex in a variety of disease processes. Preclinical studies performed in animal models of local (e.g., arthritis) or systemic (e.g., sepsis, shock) diseases have focused on stimulating the vagus nerve or activating the cholinergic anti-inflammatory pathway (CAP) to ultimately minimize the detrimental effects of cytokines.

Sepsis

Sepsis, a lethal syndrome that can develop following infection or injury, results from an imbalance in cytokines that contribute to the development of multi-systemic organ failure. In animal studies of sepsis induced via cecal perforation, mice that received nicotine had significantly decreased serum levels of HMGB1 as compared with vehicle-treated controls. Further, this inhibition of HMGB1 occurred when treatment with nicotine was delayed until after the onset of peritonitis, even when nicotine administration was delayed for 24 h [14]. In a separate study, animals were injected i.p. with *Escherichia coli* to induce a bacterial peritonitis [25]. Although vagotomized animals developed significant increases in inflammatory cells in the peritoneum, peripheral cytokine levels, and extensive hepatic tissue damage, administration of nicotine alleviated each of these effects. Further studies have also identified the control of endothelial activation, recruitment of leukocytes, and the process of coagulation and fibrinolysis as under the modulation of the cholinergic anti-inflammatory pathway [26, 27].

Hemorrhagic shock

Tissue damage and organ dysfunction observed during hemorrhagic shock are attributable, at least in part, to the overproduction of cytokines [28–30]. Stimulation of the vagus nerve in rats with hemorrhagic shock resulted in significant protection against hypotension, prolonged survival, and decreased production of TNF. Administration of a nicotine receptor antagonist ablated these protective effects observed following vagus nerve stimulation [31]. Administration of ACTH\(_{1–24}\), which can stimulate the efferent arm of the inflammatory reflex by significantly increasing vagus nerve signaling, was evaluated in rats [32]. The protective effects of ACTH\(_{1–24}\) were ablated in rats with hemorrhagic shock, indicating that ACTH\(_{1–24}\) increases activity in the cholinergic anti-inflammatory pathway and suppresses cytokine-mediated damage [32].

Ischemia/reperfusion

Cytokines such as TNF, IL-1, and HMGB1 are overproduced during ischemia and reperfusion [33–35]. In rats with an aortic ischemia/reperfusion injury, stimulation of the vagus nerve resulted in a significant inhibition of cytokine responses [9]. Further, intracerebral administration of CNI-1493 increased vagus nerve activity and protected animals against tissue damage [9, 35]. In vagotomized animals, however, the protective effects of CNI-1493 were ablated. This indicates that an intact vagus nerve is required for the integrity of this CNI-1493 mechanism. In another model involving a splanchnic artery reperfusion injury, vagus nerve stimulation protected animals against the development of shock and inhibited TNF (and other cytokine) synthesis [36]. This also conferred protection against leukocyte accumulation in the ileum and lung and reduced the severity of tissue damage in these organs. In contrast, these beneficial effects were reversed following administration of nicotinic receptor antagonists [36]. Vagus nerve stimulation is
also beneficial in studies of myocardial ischemia/reperfusion, where it significantly reduced free radical accumulation, death rate, and incidence of severe arrhythmias. Administration of ACTH1–24 protected rats against myocardial damage in this model via stimulation of the vagus nerve [37].

Pancreatitis
The pathogenesis of pancreatitis is in part attributable to the overproduction of cytokines, which contributes to tissue damage and mortality [38–40]. In an experimental murine pancreatitis model, a significant increase in tissue damage was observed in vagotomized mice as compared with mice with intact vagus nerves. In this same model, administration of an α7nAChR agonist resulted in a significant decrease in disease severity and limited pulmonary inflammation, even in vagotomized mice. These results indicate that even in cases where vagus nerve stimulation is not achievable, systemic administration of nicotinic receptor agonists may be beneficial in inflammatory conditions [41].

Experimental arthritis
In addition to diseases or conditions involving systemic inflammation, vagus nerve stimulation appears to be beneficial in modulating local inflammatory processes as well. In animals with carrageenan-induced paw inflammation, vagus nerve stimulation inhibited formation of foot-pad edema, as well as the cytokine production in inflamed tissue areas [42]. In a second carrageenan-induced inflammatory model, mice injected with carrageenan into an air pouch had significantly fewer polymorphonuclear leukocytes recruited to the inflamed area following vagus nerve stimulation [26]. The vagus nerve does not directly innervate these areas. It is possible that stimulation of the vagus nerve dampens local inflammatory responses by inhibiting cytokine production in the reticuloendothelial system redirecting leukocyte recruitment peripherally [43].

DIETARY MANIPULATION OF THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY
The inflammatory reflex and the cholinergic anti-inflammatory pathway in particular can be modulated via ingestion of a high fat diet [44]. Reduced circulating levels of TNF and IL-6 were observed in rats fed a high fat diet and subjected to a standard, nonlethal hemorrhagic shock model known to cause elevated blood cytokine levels. Vagotomy and administration of cholecystokinin and nicotinic receptor antagonists each significantly blunted the inhibitory effect of the high fat diet on circulating levels of TNF and IL-6. These findings are important in describing a mechanistic role for cholecystokinin and delineating a pathway that may explain why gastrointestinal microorganisms do not stimulate an exaggerated immune response.

THE IMMUNOLOGICAL HOMUNCULUS: FUTURE IMPLICATIONS OF THE INFLAMMATORY REFLEX
Identification of the basic anatomic and physiologic components of the inflammatory reflex has changed the paradigm of how acute and chronic inflammatory conditions may regulate neural activity. Knowledge of the inflammatory reflex raises the theoretical possibility that specific regions of the brain can control specific immunological responses [4]. Brain regions may be organized to control specific components of the immune system, akin to an “immunological homunculus” as depicted in Figure 2 [4]. In this case, the CNS may be organized somatotopically, such that neural networks, including the inflammatory reflex via the cholinergic anti-inflammatory pathway regulates the immune system. It is likely that the higher brain regions that serve to control immune responses may operate below the level of consciousness, akin to other controller mechanisms that regulate heart rate and other visceral functions. Nonetheless, these other systems are also somatotopically organized and can be manipulated and dissected anatomically and functionally in animal studies. With clinical advances in brain imaging and methods to detect brain function, the concept of an immunological homunculus, although theoretical, represents a testable hypothesis, and studies are now in progress to identify the neural pathways that control other activities of the immune system in addition to cytokine release.

![Fig. 2. Akin to the classic map of the brain that identifies which neural networks regulate or coordinate specific actions in the periphery, it is possible that additional research may identify that specific regions of the brain control other immune responses, potentially via the inflammatory reflex and its efferent arm, the cholinergic, anti-inflammatory pathway, as depicted in this pictorial hypothesis. DC, Dendritic cell.](image-url)
CN1-1493, although initially developed as an inhibitor of p38 MAPK, led to the discovery of neural mechanisms that control cytokine release. Elucidation of the physiology and immunology of the inflammatory reflex reveals that cholinergic signals carried via the vagus nerve to the immune system exert a crucial controller function during inflammation. Future studies and clinical trials may elucidate whether the cholinergic anti-inflammatory pathway can be manipulated via pharmaceutical or electrical methods to alleviate inflammation in humans.

REFERENCES


