Modulation of the Innate Immune Response through the Vagus Nerve

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Introduction

The innate immune system plays a pivotal role in host defense. However, not only in a variety of inflammatory conditions, such as major surgery, sepsis, trauma and ischemia-reperfusion injury, but also in autoimmune diseases, excessive or unwanted activation of the innate immune system can lead to organ damage. For example, cardiac surgery induces a systemic immune response the extent of which is related to postoperative complications such as hemodynamic instability as well as pulmonary and renal injury [1]. In this respect, therapeutic measures aimed at limiting the innate immune response could be beneficial.

Classically, the innate immune response is believed to be self-governing. However, in the past few decades, strong links between the brain, in particular, the vagus nerve, and the innate immune system have been established. The afferent arm of the vagus nerve can sense inflammation in the periphery and relay this information to the brain. More recently, an anti-inflammatory mechanism that is mediated by the efferent vagus nerve has been identified. This mechanism is known as the cholinergic anti-inflammatory pathway (CAP). Since its discovery, numerous animal studies have shown beneficial effects of stimulation of this pathway in models of inflammatory diseases, either through (electrical) stimulation of the vagus nerve or pharmacological approaches. However, human data are very scarce. In this review, we present an overview of the molecular and anatomical bases of the cholinergic anti-inflammatory pathway, but mainly focus on human studies. We discuss the difficulties and drawbacks associated with investigating this pathway in humans, and finally, we provide future perspectives.

Key Words
Vagus nerve · Innate immune system · Cholinergic anti-inflammatory pathway · Inflammation · Cytokines · Lipopolysaccharide · Heart rate variability

Abstract
The innate immune system is a defense mechanism that is of vital importance to our survival. However, excessive or unwanted activation of the innate immune system, which can occur in major surgery, sepsis, trauma, ischemia-reperfusion injury and autoimmune diseases, can lead to damage of the kidneys and other organs. Therefore, therapeutic approaches aimed at attenuating the innate immune response could have beneficial effects in these conditions. The vagus nerve exerts anti-inflammatory effects through the so-called cholinergic anti-inflammatory pathway. Since its discovery, numerous animal studies have shown beneficial effects of stimulation of this pathway in models of inflammatory diseases, either through (electrical) stimulation of the vagus nerve or pharmacological approaches. However, human data are very scarce. In this review, we present an overview of the molecular and anatomical bases of the cholinergic anti-inflammatory pathway, but mainly focus on human studies. We discuss the difficulties and drawbacks associated with investigating this pathway in humans, and finally, we provide future perspectives.

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identified. This mechanism was coined the cholinergic anti-inflammatory pathway, after acetylcholine, the neurotransmitter of the vagus nerve.

The Interplay between the Vagus Nerve and the Innate Immune Response

It has long been known that inflammatory cytokines in an inflamed part of the body signal the brain via the afferent vagus nerve, resulting in fever, activation of the stress response consisting of the hypothalamic–pituitary–adrenal axis [2] and the sympathetic nervous system [3]. This signaling pathway was elegantly demonstrated in animal studies, where subdiaphragmatic vagotomy reduced the increase in temperature and cortisol response (the end product of the hypothalamic–pituitary–adrenal axis) induced by intraperitoneal administration of lipopolysaccharide (LPS) [4], IL-1β [5] and TNF-α [5].

Approximately 10 years ago, Tracey et al. [6] discovered that electrical stimulation of the efferent vagus nerve (VNS) attenuated the LPS-induced inflammatory response in rats, while transecting the vagus nerve (vagotomy) enhanced inflammation. Furthermore, it was shown that acetylcholine inhibited LPS-induced release of pro-inflammatory cytokines in primary human macrophages [6]. Further studies using knockout mice for different nicotinic acetylcholine receptors identified the α7 nicotinic acetylcholine receptor (α7nAChR) on macrophages as the principal mediator of the anti-inflammatory effects of the vagus nerve [7]. As the afferent arm of the vagus nerve can sense inflammation in the body and the efferent arm attenuates it, this pathway has been proposed as a reflex-type mechanism to counteract excessive inflammation [8]. In this vago-vagal reflex, afferent vagus nerve fibers terminating in the nucleus tractus solitarius synapse with fibers in the dorsal motor nucleus, where the efferent vagus nerve originates, a mechanism well-described in regulation of digestive functions [9]. The effects of efferent vagus nerve activity on the heart can be measured by heart rate variability (HRV) analysis which will be discussed later on in this article.

Concerning the anatomical basis of the effects of the cholinergic anti-inflammatory pathway on effector organs, it is thought that in organs that are innervated by the vagus nerve, such as the liver [10], lungs [11] and gut [12], acetylcholine released from cholinergic nerve terminals activates α7nAChRs on tissue resident macrophages [13]. A special role for the spleen, an important cytokine-producing organ, has been proposed. The spleen is not innervated by cholinergic fibers; it solely receives sympathetic input [14]. However, it has been reported to be essential for the systemic anti-inflammatory effects of the vagus nerve, as splenectomy abrogated the TNF-α-suppressing effects of VNS in LPS-treated rats [15]. It is suggested that the effects of VNS on the spleen rely on synaptic activation of the splenic (sympathetic) nerve by the vagus nerve in the celiac-superior mesenteric plexus ganglion [16]. In turn, the splenic nerve releases norepinephrine which can directly attenuate cytokine production in splenic macrophages via β-receptors [17], or enhance splenic acetylcholine levels through acetylcholine-synthesizing T-cells, resulting in α7nAChR activation and inhibition of cytokine production [16, 18]. In contrast, a recent study showed that the anti-inflammatory effects of VNS in the intestine are independent of the spleen or T-cells [19]. These seemingly discrepant results might be due to differences between models of localized and systemic inflammation.

Human Studies into the Anti-Inflammatory Effects of the Vagus Nerve

Although numerous animal studies have demonstrated the beneficial anti-inflammatory effects of VNS and/or pharmacological stimulation of the cholinergic anti-inflammatory pathway in models of endotoxemia [20, 21], sepsis [22, 23], trauma [24], (renal) ischemia-reperfusion injury [25, 26], hemorrhagic shock [27] and arthritis [28–30], human data are scarce. Several reasons for this can be put forward. While VNS is approved by the FDA for the treatment of epilepsy and depression using an implantable VNS system consisting of a cuff electrode wrapped around the left vagus nerve [31, 32], this requires surgery and is therefore less feasible in acute situations. As such, it represents a less relevant treatment option for acute inflammatory conditions. Nevertheless, a small number of observational studies have been performed, yielding conflicting results. Two studies demonstrated no change in plasma cytokine levels 3 [33] or 7 [34] months after VNS, while another study found that plasma cytokine levels had actually increased after 3 months of VNS [35]. The interpretation of these studies is hampered by the fact that no inflammatory trigger was present in these patients with epilepsy or depression. As such, circulating cytokine levels in many cases were undetectable or very low [33–35]. Another study investigated the effects of VNS for 6 months and 3 weeks on cytokine-
kine production by ex vivo stimulation of monocytes with LPS. Out of the 5 measured cytokines at 2 different time points, only ex vivo stimulated production of IL-8 was significantly lower 6 months after the implantation of the stimulator device [36]. Only one published study evaluated the effects of VNS in an inflammatory setting, namely the coronary artery bypass graft surgery [37]. In this study, the epicardial vagal ganglionated plexus was stimulated using a temporary wire electrode placed into the vagal fat pad on the right ventricle. Indeed, a reduction of serum levels of IL-6, TNF-α, vascular endothelial growth factor and epidermal growth factor were found after 6 hours of vagal stimulation. However, the approach employed to stimulate vagal fibers in this particular study is very different from that used in all other clinical and animal studies. As such, other effects, such as direct effects on the right ventricle, may play a role in the observed results. Also, similar to the cuff electrode system, this approach is not feasible in acute situations.

Second, pharmacological stimulation of the cholinergic anti-inflammatory pathway with acetylcholine is not possible because it is immediately degraded by acetylcholinesterases upon parenteral administration. The therapeutic potential of acetylcholine is further hampered by its non-specificity and unwanted side effects such as vasodilatation. Nicotine, another α7nAChR agonist, has limited therapeutic potential because of its lack of pharmacologic specificity, toxic side effects and its potential to produce physical dependence (addiction). There is one report on the effects of nicotine on the inflammatory response in humans [38]. In this study, transdermal nicotine administration during human endotoxemia, a highly standardized, controlled model to investigate the innate immune response in humans in vivo, did not affect pro-inflammatory cytokine levels, although it did potentiate IL-10 levels [38]. The lack of an effect on proinflammation probably resulted from the relatively low plasma nicotine levels achieved by transdermal administration. More promising and feasible treatment options are represented by specific α7nAChR agonists. Many specific agonists have shown beneficial effects in animal models; however, most of these compounds are at the moment not suitable for human use. We have previously investigated the anti-inflammatory potential of GTS-21, a specific α7nAChR agonist available for human use. We showed that GTS-21 exerts potent anti-inflammatory effects in human leukocytes [39]. However, oral administration of GTS-21 (the only administration route available) did not modulate the immune response during experimental human endotoxemia [40]. These disappointing results may be explained by the fact that plasma concentrations of GTS-21 were relatively low and also highly variable between subjects [40]. Of interest, within the GTS-21–treated group, higher GTS-21 plasma concentrations correlated with lower levels of TNF-α, IL-6 and IL-1RA, but not that of IL-10 [40]. This suggests that GTS-21 can limit the innate immune response in humans in vivo at higher concentrations.

Third, measuring vagus nerve activity in humans in vivo is notoriously difficult. The only method available in humans is HRV. Several observational studies have found inverse relationships between vagal HRV parameters and markers of inflammation in patients with rheumatoid arthritis [41] and brain injury [42], as well as in cross-sectional community samples [43–45]. However, in similar (patient) populations, these observations were not confirmed by others [46, 47]. Moreover, in the ‘positive’ studies, relationships between other HRV parameters, such as those reflecting sympathetic nervous system activity, and inflammatory markers were found as well, and levels of inflammatory markers were mostly very low, hampering interpretations [41, 43, 44]. We assessed the relationship between HRV and the inflammatory response during experimental human endotoxemia and found no relationship among any of the HRV parameters and any inflammatory markers [48]. The lack of a clear-cut relationship between vagal HRV parameters and inflammatory markers might have several reasons. Vagal outflow might be organ specific, as has been shown for sympathetic nerves [10, 49]. Therefore, vagal or sympathetic input to the heart may not represent vagal input to inflammatory organs such as the spleen, lungs, gut or liver. Furthermore, animal studies have shown that electrical stimulation at levels below the threshold required to change heart rate, and thus alter HRV, is sufficient to activate the cholinergic anti-inflammatory pathway [21]. Another confounding factor is represented by medication, particularly sedatives, as they are known to affect HRV [50]. HRV analysis as a measure of autonomic nervous system activity is therefore highly debated [51]; however, currently it remains the only tool available to measure vagus nerve activity in humans.

**Future Perspectives**

Although there are relatively few human studies on the anti-inflammatory effects of VNS and data are conflicting, a search on ClinicalTrials.gov indicates that more than 60 clinical studies are currently underway investi-
gating VNS as a possible therapy for rheumatoid arthritis, Crohn’s disease, stroke, pain, heart failure, obesity, epilepsy, depression, headache, tinnitus, fibromyalgia, fear extinction and schizophrenia. The rationale for many of these studies is based on the belief that VNS has an anti-inflammatory effect. However, this is yet to be proven in humans.

Except for direct electrical VNS, future research should also focus on alternative ways to stimulate the vagus nerve/cholinergic anti-inflammatory pathway. For instance, in mice, transcutaneous vagus nerve stimulation was shown to be as equally effective as electrical stimulation in reducing inflammation [21], and a recent study in patients with paroxysmal atrial fibrillation suggests that this approach might be effective in attenuating inflammation [52]. Furthermore, high-fat enteral nutrition has been shown to activate the vago-vagal anti-inflammatory reflex in animal models [53, 54]. In accordance, we recently showed that lipid- and protein-rich nutrition attenuates proinflammatory cytokine levels and augments the anti-inflammatory response during experimental human endotoxemia [55]. Transvenous VNS might represent another promising approach to dampen inflammation that is more suitable in acute settings. Our group has recently performed a randomized sham-controlled study to evaluate the effects of transvenous VNS, using a stimulation catheter placed temporarily in the internal jugular vein, on the innate response during experimental human endotoxemia (ClinicalTrials.gov NCT01944228). The manuscript describing the study results is currently under review.

Selective pharmacological stimulation of the α7nAChR might also have therapeutic potential. In case of GTS-21, higher dosages or other routes (parenteral) of administration should be evaluated. Furthermore, the anti-inflammatory potential of other specific α7nAChR agonists that are suitable for human use, such as TC-5619 [56], should be evaluated in humans. Along these lines, specific α7nAChR agonists that have been shown to exert anti-inflammatory effects in animals, such as AR-R17779 [30, 57], CAP55 [58] and PNU-282987 [59], should be explored if or when they are safe and suitable for human use.

Finally, as previously alluded to, investigation of the cholinergic anti-inflammatory pathway is seriously hindered by the lack of a proper readout for vagus nerve activity. Nevertheless, as a very recent study showed that α7nAChR mRNA levels in peripheral blood mononuclear cells of septic patients were increased as well as directly related with vagal HRV parameters and inversely with the extent of inflammation, severity of disease and clinical outcome [60], this might serve as an alternative end point to determine vagus nerve activity.

**Disclosure Statement**

The authors have no conflicts of interest to declare.

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