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# The cholinergic anti-inflammatory pathway: an innovative treatment strategy for respiratory diseases and their comorbidities

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Over the past few decades, it has been clarified that the nervous system and immune system have overlapping distributions and their interactions are critical in the regulation of immunological and inflammatory responses. The cholinergic anti-inflammatory pathway, including the parasympathetic nerve systems and humoral factors orchestrate the immune responses to protect the body during infection and tissue injury. Recent investigations have attempted to clarify how the parasympathetic nerve systems attenuate the systemic inflammatory responses and identified the α7 nicotinic acetylcholine receptor (α7nAChR) as a crucial target for attenuating the release of inflammatory cytokines from inflammatory cells including macrophages and dendritic cells. This modulatory circuit pathway possibly exists in the lungs and might be involved in regulating inflammation and immunity during infection and other inflammatory lung diseases including asthma and COPD, which means that modulation of the cholinergic anti-inflammatory pathway is a possible therapeutic target for lung diseases.

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## Introduction

In the neuroimmune system, the nervous system and immune system cooperate to maintain the physiological condition as well as to regulate inflammatory responses during pathological conditions including infection and tissue injury [1–4]. The neuroimmune reflex system consists of sensory nerves, the autonomic nervous system, and immune cells with mediators generated by neurons and immune cells. As another type of autonomous reflex for circulation and blood pressure, the role of sensory afferent neurons is to sense changes in the body and transmit the information to the central nervous system (CNS) during the neuroimmune reflex. However, during inflammation or tissue injury, the stimuli to activate afferent nerves are inflammatory mediators including pathogen-associated molecular patterns (PAMPs) originating from pathogens, danger associated molecular patterns (DAMPs) from injured cells, and cytokines/chemokines generated by immune/inflammatory cells [5,6]. The signals transmitted by the afferent nerves are then integrated in the CNS. Then, appropriate responses are made through transmitting signals to the periphery via first, humoral pathways including activation of the hypothalamic–pituitary–adrenal axis [7], second, the activation of the sympathetic nervous system [7,8], and third, the activation of cholinergic efferent vagus nerve [2–4]. In this review, we focus on the cholinergic anti-inflammatory pathway that includes both the cholinergic vagus nerve and the cholinergic system of the immune cells. We also summarize the present knowledge about the effect of modulation of the cholinergic anti-inflammatory pathway during inflammation or injury in animal disease models including lung, and then discuss the neuroimmune pathway as a possible therapeutic target for inflammatory lung diseases.

## The cholinergic anti-inflammatory pathway in systemic inflammation

The idea that cholinergic vagus nerves attenuate inflammatory responses in the body during inflammation was based on the findings of animal experiments. Watkins *et al.* reported that the effect of intraperitoneally injected IL-1β, which is one of the pro-inflammatory cytokines, in inducing fever is dependent on intact afferent vagus neurons, which means IL-1β induces hyperthermia thorough the activation of the subdiaphragmatic afferent vagus neurons, not by directly stimulating the brain by blood-borne IL-1β [9]. Thereafter, Borovikova *et al.* reported a basic finding concerning the cholinergic anti-inflammatory pathway [10••]. In a rat model of septic shock induced by intravenous injection of lipopolysaccharide (LPS), the systemic inflammatory responses including the elevation of both the serum and liver TNF-α levels and hypotension were attenuated by electrical stimulation of the efferent vagus nerve. They further evaluated the effects of acetylcholine (ACh) on

the production of LPS-induced pro-inflammatory cytokines including TNF-α from human macrophages *in vitro* culture experiments, and found that ACh stimulated the macrophages to cause a concentration-dependent inhibition of the production of pro-inflammatory cytokines. Based on their study, the authors termed this anti-inflammatory effect as resulting from the 'cholinergic anti-inflammatory pathway'.

After the discovery of the cholinergic anti-inflammatory pathway, Wang *et al.* performed experiments using α7 nicotinic ACh receptor (α7nAChR) deficient mice and revealed that this receptor is essential for attenuating the inflammatory response to LPS by stimulating the efferent vagus nerve [11••]. They also found the expression of α7nAChR mRNA by human macrophages and the presence of these receptors on the cell surface using FITC-labelled α-bungarotoxin, a competitive antagonist to α7nAChR [11••]. In *in vitro* experiments with human macrophages and mouse peritoneal macrophages, they further revealed that ACh and nicotine inhibited the LPS-induced release of TNF-α from macrophages through stimulating α7 receptors. Thereafter, subsequent experiments revealed that splenectomy as well as selective abdominal vagotomy abrogated the anti-inflammatory effects by both vagus nerve stimulation and nicotine, suggesting that the spleen is a critical target of the cholinergic anti-inflammatory pathway in terms of the vagus nerve-mediated inhibition of the production of pro-inflammatory cytokines [12–14].

The mechanisms by which efferent vagus nerve stimulation induces anti-inflammatory responses in spleen was still unclear, because there is no neuroanatomical evidence for direct innervation of parasympathetic or vagus nerves to any immune organs including spleen [15]. Because branches of the vagus nerve provide preganglionic cholinergic input and prevertebral sympathetic ganglia provide major sympathetic input to the spleen via the splenic nerve [8,16], it has been proposed that efferent vagus nerve stimulation activates sympathetic neurons that innervate the spleen. However, the source of ACh that activates α7nAChR on macrophages in the spleen was still unclear because innervated sympathetic neurons to the spleen only provide norepinephrine (NE), not ACh.

To answer this question, Rosas-Ballina *et al.* postulated that NE released by innervated sympathetic neurons can stimulate solenocytes, resulting in the production ACh by splenocytes [17••]. Then, they showed that electrical vagus stimulation elevated the ACh concentrations in the spleen. They further showed that NE can stimulate the release of ACh from solenocytes at high concentrations. To determine which types of immune cells in the spleen release ACh, they first used nude mice lacking functional T cells and found that vagus nerve stimulation did not suppress serum TNF-α after LPS treatment in nude mice. They further performed the experiments using transgenic mice that express eGFP controlled by the promoter of choline acetyltransferase (ChAT), the synthetic enzyme of ACh. They observed that only 3% of the total splenic T cells expressed eGFP in the transgenic mice. The adoptive transfer of these ChAT positive T cells to nude mice restored the anti-inflammatory response of vagus nerve stimulation while the transfer of ChAT negative T cells did not. A subsequent study revealed that β2-adrenergic receptors on these ChAT positive T cells are essential for the anti-inflammatory response to vagus nerve stimulation [18•]. These investigations revealed that efferent vagus nerve signals are transferred to ChAT positive T cells through NE released from the splenic sympathetic nerve that innervates the spleen, and then ChAT positive T cells increase the production of ACh through the activation of β-adrenoceptors. The elevated ACh in the spleen stimulates α7 nAChRs in macrophages, resulting in the suppression of the synthesis and release of TNF-α (Figure 1).

## The cholinergic anti-inflammatory pathway in the lung

Unlike the spleen, organs including the gut and lung have different cholinergic anti-inflammatory pathways because these organs have efferent vagus nerve projections. These preganglionic vagus nerves provide preganglionic cholinergic input to the gut or lung ganglia in which nicotinic receptors mediate ganglionic neurotransmission [19–21]. Postganglionic cholinergic neurons innervate effector tissues of the gut or lung. In human lungs, the parasympathetic postganglionic fibres originate in plexuses within the airway wall associated with mucosa, submucosa, smooth muscle, peri-tracheal, and peribronchial layers [22,23]. At the endings of these nerves, the receptors that can sense mechanical, chemical, and biological stimuli are expressed. The afferent vagus nerves transmit signalling to the CNS. The vagus nociceptors, a type of receptor that responds to damaging stimuli, can also be activated by inflammatory cytokines and transmit inflammatory signals from the lung to the CNS [24]. Moreover, receptors for pro-inflammatory cytokines are expressed in the pulmonary nerve endings [25,26], which may suggest that stimuli by inflammation can activate pulmonary sensory neurons and transmit the signals to the CNS, resulting in modulation of the inflammation by pulmonary vagal inflammatory reflex. Activation of the efferent vagus nerves by this reflex could stimulate postganglionic cholinergic neurons that innervate the lung tissue, resulting in an increase of the ACh concentration in the lungs. The elevated ACh activates α7nAChR on infiltrated inflammatory cells, including macrophages and neutrophils, during acute lung injury, which induce the suppression of NF-kB activation and secretion of pro-inflammatory cytokines and chemokines from inflammatory cells including alveolar macrophages, therefore resulting