Vagal-Immune Interactions Involved in Cholinergic Anti-Inflammatory Pathway

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Summary
Inflammation and other immune responses are involved in the variety of diseases and disorders. The acute response to endotoxemia includes activation of innate immune mechanisms as well as changes in autonomic nervous activity. The autonomic nervous system and the inflammatory response are intimately linked and sympathetic and vagal nerves are thought to have anti-inflammation functions. The basic functional circuit between vagus nerve and inflammatory response was identified and the neuroimmunomodulation loop was called cholinergic anti-inflammatory pathway. Unique function of vagus nerve in the anti-inflammatory reflex arc was found in many experimental and pre-clinical studies. They brought evidence on the cholinergic signaling interacting with systemic and local inflammation, particularly suppressing immune cells function. Pharmacological/electrical modulation of vagal activity suppressed TNF-α and other proinflammatory cytokines production and had beneficial therapeutic effects. Many questions related to mapping, linking and targeting of vagal-immune interactions have been elucidated and brought understanding of its basic physiology and provided the initial support for development of Tracey’s inflammatory reflex. This review summarizes and critically assesses the current knowledge defining cholinergic anti-inflammatory pathway with main focus on studies employing an experimental approach and emphasizes the potential of modulation of vagally-mediated anti-inflammatory pathway in the treatment strategies.

Key words
Lipopolysaccharide • Endotoxemia • Autonomic nervous system • Vagus nerve • Cholinergic anti-inflammatory pathway • Inflammatory reflex • Cytokines

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Introduction

One of the major functions of the immune system is to provide defense against pathogens. Defense response has to be controlled in order to avoid excessive inflammation and successive tissue/organ impairment. Many diseases result from inappropriate inflammatory response characterized by the complex interaction between pro- and anti-inflammatory cytokines, and other inflammatory mediators. On the local level, inflammatory response is mainly controlled through cytokine production by immune cells while systemic inflammatory response is regulated particularly through neuroendocrine mechanisms (Maier et al. 1998, Elenkov et al. 2000). It was clearly shown that inflammatory cytokines signal the brain via the afferent vagus nerve, resulting in fever and causing activation of the stress response including the hypothalamic-pituitary-adrenal axis (Maier et al. 1998), hypothalamic-pituitary-gonadal axis, hypothalamic-pituitary-thyroid axis, and the sympathetic nervous system (Elenkov et al. 2000, Bellinger et al. 2008). According to Hasko and Szabo (1998), the sympathetic division of the autonomic nervous system is associated with a dual mode of regulation of inflammatory responses.
and both adrenaline and noradrenaline modulate the release of cytokines and inflammation through adrenergic receptors on immune cells. Last two decades brought a strong interest in modulation of the immune system by autonomic nervous system. Many studies confirmed that the release of tumor necrosis factor (TNF), interleukin (IL)-1 high mobility group box 1 (HMGB1) protein, and other pro- and anti-inflammatory cytokines from immune cells is at least partially regulated through autonomic nervous system (Elenkov et al. 2000, Tracey 2002, Pavlov et al. 2003, Pavlov and Tracey 2004).

The research has been focused particularly on parasympathetic nervous system. It was shown that afferent arm of vagus nerve may sense inflammation in the periphery and relay the signals to the brain, which in turn dampens sickness response (Watkins et al. 1995, Goehler et al. 2000). In 2000 Tracey and coworkers elegantly demonstrated powerful relation between efferent vagus nerve and the innate immune system. Strong link has been discovered and Tracey’s experiments established a new concept of the „inflammatory reflex“ as a well-defined nervous-immune systems interplay regulating inflammation (Borovikova et al. 2000).

The cholinergic anti-inflammatory pathway

Tracey’s experiments clearly showed anti-inflammatory mechanisms mediated by efferent vagus nerve. Pharmacological/electrical stimulation of vagus nerve significantly reduced inflammation in endotoxemic rats while this effect was blocked by vagotomy and atropin administration (Borovikova et al. 2000). The release of acetylcholine following vagus activation inhibited the LPS-induced production of systemic pro-inflammatory cytokines (TNF-α, IL-1, 6, 18) in human macrophages, but circulating levels of anti-inflammatory cytokines were not suppressed (Gaykema et al. 1995, Fleshner et al. 1998).

Pavlov et al. (2006) demonstrated that antiinflammatory effect of vagal stimulation cannot be blocked by muscarinic antagonist and these findings show involvement of central muscarinic link in the cholinergic anti-inflammatory pathway. Subsequent studies helped to identify the peripheral structures involved in anti-inflammatory activity. In 2003 Wang and coworkers described a key role of nicotinic receptor and its α7 subunit (α7nAchR) as the main receptor allowing macrophage modulation.

The concept of α7nAchR as a peripheral „immune“ nicotinic component has been subsequently applied in a number of studies confirming attenuation of inflammatory response during vagus nerve stimulation with beneficial effects in sepsis, ischaemia-reperfusion, shock and gastrointestinal inflammation models (Borovikova et al. 2000, Bernik et al. 2002, Guarini et al. 2004, Lubers et al. 2010, Lubers et al. 2011).

In 2006 Huston et al. proposed spleen as a crucial organ, where cytokines production is suppressed by stimulation of vagus. However, considering minimal cholinergic innervation of the spleen in rodents, this concept required revision and disynaptic pathway was suggested by Rosas-Ballina et al. (2008). In this model, vagal preganglionic fibers synapse with postganglionic sympathetic neurons in celiac ganglion subsequently traveling in splenic nerves. Disynaptic model has been extended by recognition of acetycholine-synthesizing T-cells (Rosas-Ballina et al. 2011, Gautron et al. 2013) where essential α7 nicotinic receptor is located on splenic macrophages. In 2012 Bratton and coworkers critically questioned disynaptic concept. They did not confirm any functional or anatomical connection between vagus and splenic nerves. For explanation of this apparent contradiction, Martelli et al. (2014) have proposed the concept of non-neural link between vagus and splenic sympathetic nerves with α7 nicotinic receptors situated on peripheral terminals of splenic nerves. When α7 subunits are stimulated by acetylcholine released from T-cells, terminals secrete noradrenaline which subsequently binds to β-adrenergic receptors of splenic macrophages to attenuate TNF-α production. As a mechanism of predicted non-neural communication between vagus and spleen following vagal stimulation, the authors propose a role of mobilized lymphocytes including acetycholine-synthesizing T-cells (Martelli et al. 2014).

Assessment of cholinergic anti-inflammatory pathway

Excessive activation of immune system may lead to organ damage/failure in shock, sepsis, trauma and autoimmune diseases. Numerous animal studies demonstrated beneficial outcomes of the cholinergic anti-inflammatory pathway stimulation. These effects were achieved by electrical stimulation of vagal fibers and/or by administration of specific muscarinic and α7nAchR agonists. However, for human use, both approaches have
some limitations. Electrical stimulation is not appropriate in acute situations while pharmacological method is limited because of its specificity, toxic side effects, routes of administration and possible physical addiction (nicotine). Lubbers et al. in series of studies (Lubbers et al. 2010, Lubbers et al. 2011, Lubbers et al. 2013) demonstrated that also physiological stimulation of anti-inflammatory pathway, predominantly of its afferent vagal limb by enteral feeding, can reduce cytokines levels and improve organ functions.

Generally, vagal nerve activity and its immune-modulatory effect are difficult to be assessed in human. However, analysis of heart rate variability (HRV) gives a clear idea about autonomic regulation of cardiorespiratory function (Fernandez et al. 2014). Reduced HRV was found in endotoxemic patients (Rassias et al. 2005) and HRV measurement may indicate inverse relationship between inflammation biomarkers and vagal HRV measures also in animal models (Crandall et al. 2000, Matthew et al. 2004, Tateishi et al. 2007). In our recent study, short-term heart rate variability was evaluated as an index of cardiac autonomic control in rats with LPS-induced endotoxemia. Endotoxemia was accompanied by a significant decrease in HRV spectral activity in high-frequency range at maximal body temperature and increased IL-6 level was present in the cardiac tissue of endotoxemic rats. We concluded that decreased HRV in HF band may indicate a reduced parasympathetic activity in LPS-induced endotoxemia as basic characteristics of altered cardiac control during response to endotoxemia (Zila et al. 2015). In healthy human, low vagal tone was reported in independent association with increased IL-6 and TNF levels (Marsland et al. 2007, Sloan et al. 2007, von Känel et al. 2008, Weber et al. 2010, Tonhajzerova et al. 2013, Visnovcova et al. 2015). Several clinical studies provided evidence on inverse correlation between HRV and inflammatory markers, mostly in immune-mediated diseases, brain injury and cardiovascular diseases (Goldstein et al. 2007, Haensel et al. 2008, Huston and Tracey 2011, Kox et al. 2012). On the contrary, study of Papaioannou et al. (2009) did not report any correlation between IL-6 and vagal tone suggesting pluripotency of IL-6 possessing both pro-inflammatory and anti-inflammatory features at the same time. Kox et al. (2011) also reported no HRV – cytokines association in human endotoxemia. Moreover, because of organ specific vagal outflow (heart versus inflammatory organs) the authors have questioned HRV analysis as an appropriate method to evaluate activation of the cholinergic anti-inflammatory pathway (Kox and Pickkers 2015).

Anyway, although there is only limited number of studies evaluating anti-inflammatory potential of vagus in human, even with some controversial results, HRV analysis is currently the only available method to assess vagal activity.

Clinical implications

Most preclinical studies indicate the suppression of systemic or local inflammation via anti-inflammatory pathway has the potential of an effective treatment strategy. Unilateral cervical (Van Westerloo et al. 2005) or subdiaphragmatic vagotomy (Kessler et al. 2006) increased plasma levels of pro-inflammatory cytokines, tissue damage and mortality in sepsis. On the other hand, activation of anti-inflammatory pathway via stimulation of vagus nerve or by α7nAchR agonists clearly demonstrated the effective reduction in cytokine/HMGB1 levels and increased survival rate in sepsis models (Borovikova et al. 2000, Wang et al. 2003). Recent observations indicate the future potential mostly for non-invasive techniques of activation (electroacupuncture) and highlight several pharmacological approaches (α7nAchR agonists, β2 adrenergic agonists) in inhibition of the initial phase of sepsis minimizing an impact on specific innate immune mechanisms eliminating bacteria (Torres-Rosas et al. 2014, Rosas-Balina et al. 2015, Kanashiro et al. 2017, Pinheiro et al. 2017). Besides sepsis model, the cholinergic anti-inflammatory pathway was reported to be capable in reducing ischemic tissue and organ damage in intestine, heart, brain and kidneys (de Haan et al. 2008, Ottani et al. 2009, Yeboah et al. 2008, Kong et al. 2011).

Anti-inflammatory pathway and its effect on chronic inflammatory diseases of gastrointestinal tract were widely studied in preclinical animal models. Herein, particularly treatment of ulcerative colitis seems to be promising, since both α7nAchR agonists and acetylcholinesterase inhibitors reduced severity of the disease (Miceli and Jacobson 2003, Bai et al. 2009). However, current clinical observations yielded in controversial data, as selective α7nAchR agonists used in treatment of ulcerative colitis and Crohn’s disease gave ambiguous results (Nikfar et al. 2010, Kox et al. 2011, Bonaz et al. 2016). Administration of α7nAchR agonists to animals with another chronic inflammatory disease, rheumatoid arthritis, also leads to a significant improvement (Koopman et al. 2011). In the same
experimental model, stimulation of the vagus nerve ameliorated arthritis symptoms, reduced serum cytokine levels, and protected against joint destruction (Koopman et al. 2014). Recent data also suggest a possible role of cholinergic anti-inflammatory pathway in the prevention of type 1 diabetes mellitus (George et al. 2016). Anti-inflammatory pathway was suggested to be of benefit in heterogeneous group of diseases including postoperative ileus, asthma, acute respiratory distress syndrome, as well as cognitive impairments in schizophrenia and Alzheimer’s disease (Costes et al. 2014, Galle-Treger et al. 2016, Pinheiro et al. 2017, Lewis et al. 2017).

Conclusions

Although better understanding of vagal-immune interactions involved in cholinergic anti-inflammatory pathway would require further insights, at present, well-defined Tracey’s concept of vagal-immune communication has been accepted. The current knowledge suggests a huge potential of modulation of a vagally-mediated anti-inflammatory pathway in the treatment strategies.

Conflict of Interest

There is no conflict of interest.

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