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SYMPOSIUM REVIEW

The intestinal cholinergic anti-inflammatory pathway

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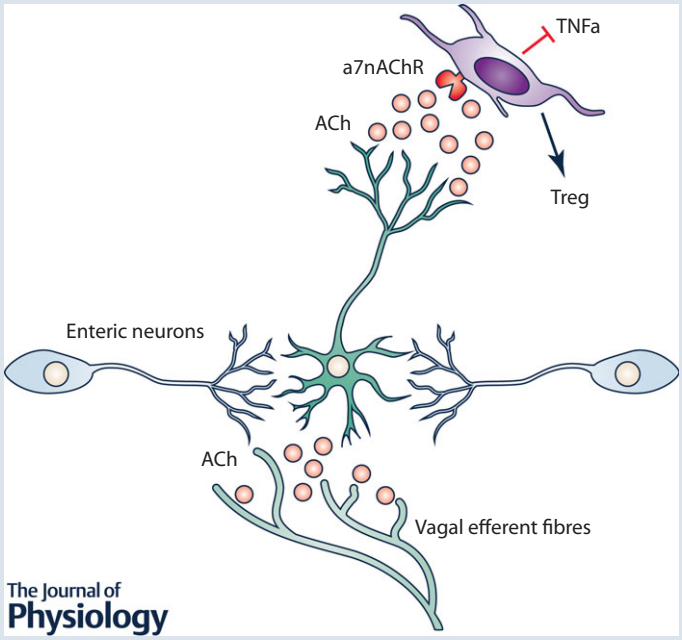

Abstract The main task of the immune system is to distinguish and respond accordingly to 'danger' or 'non-danger' signals. This is of critical importance in the gastrointestinal tract in which immune cells are constantly in contact with food antigens, symbiotic microflora and potential pathogens. This complex mixture of food antigens and symbionts are essential for providing vital nutrients, so they must be tolerated by the intestinal immune system to prevent aberrant inflammation. Therefore, in the gut the balance between immune activation and tolerance should be tightly regulated to maintain intestinal homeostasis and to prevent hypersensitivity to harmless luminal antigens. Loss of this delicate equilibrium can lead to abnormal activation of the intestinal immune system resulting in devastating gastrointestinal disorders such as inflammatory bowel disease (IBD). Recent evidence supports the idea that the central nervous

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system interacts dynamically via the vagus nerve with the intestinal immune system to modulate inflammation through humoral and neural pathways, using a mechanism also referred to as the intestinal cholinergic anti-inflammatory pathway. In this review, we will focus on the current understanding of the mechanisms and neuronal circuits involved in the intestinal cholinergic anti-inflammatory pathway. Further investigation on the crosstalk between the nervous and intestinal immune system will hopefully provide new insights leading to the identification of innovative therapeutic approaches to treat intestinal inflammatory diseases.

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Abstract figure legend Activation of the intestinal cholinergic anti-inflammatory pathway is resulting in reduction of mucosal inflammation. Vagal efferent fibers are able to increase release of acetylcholine (ACh) from cholinergic enteric neurons and thus, modulated activation of macrophages residing in the gut wall. Hereby, enteric neurons are able to modulate the local immune system and establish immune tolerance in the gut.

Abbreviations 5-HT_4R, 5-hydroxytryptamine 4 receptor; α7nAChR, α7-subtype of the nicotinic acetylcholine receptor; CAIP, cholinergic anti-inflammatory pathway; CD, Crohn's disease; CSF-1, colony stimulating factor 1; DMV, dorsal motor nucleus of the vagus; DNBS, 2,4-dinitrobenzenesulfonic acid; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; IL-6, interleukin-6; IL-10, interleukin-10; JAK2, Janus kinase 2; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; POI, postoperative ileus; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor β; TNF-α, tumour necrosis factor α; UC, ulcerative colitis; VNS, vagus nerve stimulation.

Introduction

The gastrointestinal system is one of the largest vulnerable surfaces of our body continuously facing the external environment, including microbiota, nutrients, pollutants, and harmful pathogens. This result in mutual benefits represented by co-habitation and at the same time it provides protection against pathogens. To maintain intestinal homeostasis, the mucosal immune system is able to recognize pathogenic insults and mount an inflammatory response; however, at the same time it is able to be 'tolerant' to innocuous food antigens and microbiota. This education and maturation of the intestinal immune system is the result of millions of years of co-evolution with host-specific microbiota and dietary intake. Disruption of these homeostatic mechanisms can result in undesired immune reactions leading to intestinal disorders, such as inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD) (Veldhoen & Brucklacher-Waldert, 2012; Thorburn *et al.* 2014).

In the last two decades, it has become evident that the communication between the nervous and immune system can influence the inflammatory response via the release of neurotransmitters, cytokines and hormones (Steinman, 2004; Sternberg, 2006). Several mechanisms have been described within the nervous system that can execute this anti-inflammatory effect. For example, the release of glucocorticoids via the hypothalamic–pituitary–adrenal axis and the adrenergic nervous system have been shown to modulate the immune system by reducing inflammation (Haddad *et al.* 2002; Silverman *et al.* 2005; Bellinger *et al.* 2008).

More recently, Tracey and coworkers demonstrated that stimulation of the parasympathetic nervous system via the vagus nerve led to lower cytokine production and increased survival in a rodent model of sepsis, introducing the novel concept of the 'cholinergic anti-inflammatory pathway' (CAIP) (Borovikova *et al.* 2000; Tracey, 2002). This was the first experimental proof of the direct interaction between the central nervous system (CNS) and the immune system, with a direct regulation of inflammation by means of neuronal circuitry in peripheral tissues.

Currently, it is proposed that sensory neurons are activated by inflammatory mediators, released during local tissue inflammation, and subsequently send signals regarding the microenvironment to the CNS. In turn, efferent nerve fibres release neuromediators in the periphery to modulate local immune cells (Tracey, 2009). Taking into account the extensive innervation of the gastrointestinal tract, it is not surprising that the nervous system, via the vagus nerve, appears to play a major role in modulating immune activation in the gut wall (Fig. 1). In this review, we will focus on the anti-inflammatory role of the parasympathetic nervous system in the gastrointestinal tract and discuss current knowledge and clinical implication of the intestinal CAIP.

The cholinergic anti-inflammatory pathway

In 2000, during the investigation of the anti-inflammatory properties of the p38 MAP kinase inhibitor, semapimod (CNI-1493), Tracey and colleagues revealed for the first time the vagal anti-inflammatory effect using a model of carrageenan-induced paw oedema (Borovikova *et al.* 2000). Both intracerebroventricular application of CNI-1493 and electrical stimulation of the transected peripheral vagus nerve protected against the development of acute inflammation resulting in oedema. These anti-inflammatory effects were abrogated by bilateral vagotomy, suggesting that the intact vagus nerve is necessary for CNI-1493 activity. A few years later, Wang and coworkers identified the missing link between the vagus nerve and macrophage activation as the anti-inflammatory action of vagal stimulation was absent in mice lacking the α7-subtype of the nicotinic acetylcholine receptor (α7nAChR) (Wang *et al.* 2003). The cholinergic regulation of the inflammatory response seems to be mediated by the presence of the classical receptor from the neurotransmitter-gated superfamily of ion channels, α7nAChR, on non-neuronal cells such as macrophages (de Jonge & Ulloa, 2007). Besides the classical induction of ion channel fluxes, α7nAChR stimulation in macrophages results in the inhibition of lipopolysaccharide-mediated activation of the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) transcription factor (Wang *et al.* 2004). A critical component of the nicotinic anti-inflammatory effect, at least in macrophages, is mediated via the activation of the Janus kinase 2 (JAK2)–signal transducer and activator of transcription 3 (STAT3) signalling pathway. Stimulation of α7nAChR with nicotine triggers activation of its catalytic intracellular domain leading to recruitment and phosphorylation of the tyrosine kinase JAK2, and

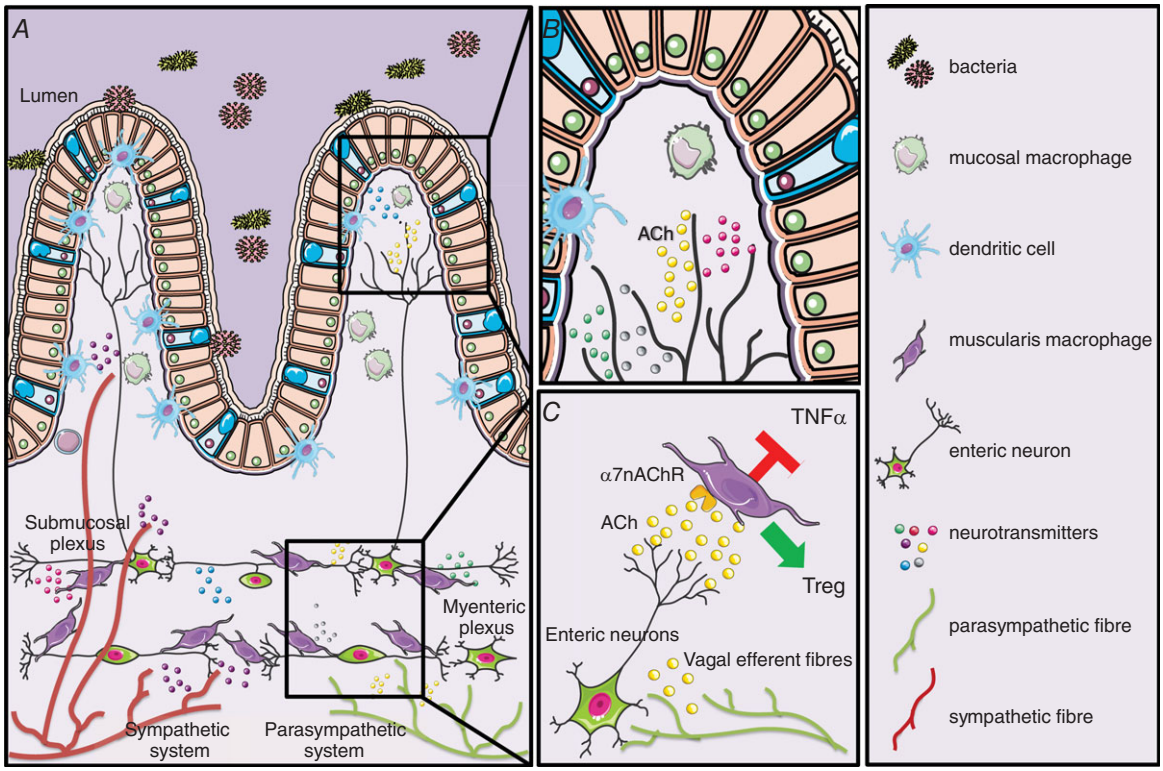

Figure 1. Schematic overview of the crosstalk between the nervous and immune system in the gastrointestinal tract
The gastrointestinal tract has extensive innervation provided by the enteric nervous system and by extrinsic fibres from the sympathetic and parasympathetic nervous system. *A*, schematic representation of the intestinal wall with its different layers, showing the distribution of the intrinsic and extrinsic innervation and their relationship with the intestinal immune cells. Parasympathetic efferent fibres innervate the intestinal wall by contacting exclusively the enteric neurons located in the myenteric plexus region. Instead, sympathetic efferent fibres are also in direct contact with the mucosa and with intestinal immune cells located in the submucosal and mucosal compartment. *B*, in the mucosal villi, several immune cells such as macrophages, dendritic cells and T cells are affected by the release of neurotransmitters, such as ACh, produced by neuronal fibres. *C*, in the myenteric plexus, close proximity between enteric neurons and resident macrophages has been described in several publications. Based on anatomical and functional evidence, it has been hypothesized that secretion of ACh by cholinergic enteric neurons influences the phenotype of resident macrophages resulting in inhibition of TNF-α and induction of regulatory T cells.

subsequent activation of the transcription factor STAT3, resulting in direct inhibition of inflammatory cytokine production (de Jonge *et al.* 2005).

Since the spleen is one of the major sources of inflammatory mediators during systemic inflammation such as sepsis (Huston *et al.* 2006; Qin *et al.* 2006; Huston *et al.* 2008), it represents an ideal target for modulation of the immune response. Initial experiments indeed indicated the spleen as a target organ of the vagus nerve controlling tumour necrosis factor α (TNF-α) production during sepsis (Huston *et al.* 2006). The anatomy of the splenic innervation, in particular the cholinergic or vagal component, is, however, still a matter of debate. Close interposition of immune cells with nerve terminals could be identified, but these fibres are of an adrenergic nature. Although these data indicate adrenergic modulation of splenic macrophages, *in vitro* data strongly suggest cholinergic inhibition of splenic macrophages via the expression of α7nAChR. In contrast to the initial hypothesis proposing direct contact between vagal nerve fibres and splenic macrophages (Tracey, 2002), it is now clear that in the spleen the vagus nerve rather indirectly modulates the innate immune response by activating adrenergic neurons in the prevertebral ganglia. In line with this hypothesis, only in mice with an intact and innervated spleen, vagus nerve stimulation (VNS) is able to exert its anti-inflammatory effect (Huston *et al.* 2006). Recent studies suggest that ACh released by the vagus nerve in the celiac mesenteric ganglia activates post-synaptic α7nAChR on adrenergic neurons of the splenic nerve, leading to the release of noradrenaline in the spleen (Rosas-Ballina *et al.* 2011). There, adrenergic nerve fibres stimulate ACh synthesis by memory splenic T cells interacting with α7nAChR located on adjacent macrophages (Rosas-Ballina *et al.* 2011).

Thus, it is supposed that in the case of systemic inflammation, cytokines and/or endotoxins are present in detectable amounts in the circulation resulting in the activation of different areas of the CNS. As these areas project to the autonomic motor neurons in the brain stem connected to peripheral organs via the vagus nerve, this will result in activation of the cholinergic pathway, thereby modulating inflammatory response via the so-called 'inflammatory reflex' (Tracey, 2002). In the case of more localized peripheral inflammation, circulating cytokines are absent or too low to activate the circumventricular organs. Yet, the CNS is informed on the presence of inflammation through 'sensory' neuronal fibres innervating the periphery (Andersson & Tracey, 2012).

The intestinal cholinergic anti-inflammatory pathway

The intestinal immune system needs to preserve a delicate equilibrium between tolerance to harmless antigens and an effective immune response to pathogens. When this equilibrium is disrupted, an immune response against innocuous antigens can be induced leading to devastating chronic inflammatory conditions such as IBD. It has been shown that the microenvironment in the mucosa and submucosa plays a critical role in maintaining this equilibrium. Several factors, such as transforming growth factor β (TGF-β), interleukin-10 (IL-10), retinoic acid and short chain fatty acids, have already been described in depth for their immunomodulatory effects in the intestine (Veldhoen & Brucklacher-Waldert, 2012; Thorburn *et al.* 2014). Lately, experimental and clinical evidence suggest that an additional actor, the parasympathetic nervous system via the vagus nerve, is playing a crucial role in modulating the intestinal microenvironment, preserving tissue homeostasis and immune tolerance (Fig. 1 and Table 1).

In contrast to the spleen, a direct vagal communication between the gut wall and the CNS has been experimentally proven in the gastrointestinal tract. Using anterograde tracers injected into the dorsal motor nucleus of the vagus (DMV), efferent vagal nerve terminals were shown to directly synapse with postganglionic neurons located in the enteric nervous system, rather than interacting with neurons in the prevertebral ganglia (Berthoud *et al.* 1990, 1991). Using the same experimental approach, a typical rostro-caudal gradient of vagal preganglionic innervation has been reported with the highest density of vagal fibres observed in the stomach followed by a subsequent decrease in the small bowel and colon (Berthoud *et al.* 1990).

Vagal innervation appears to be vital for maintaining functional and anatomical intestinal homeostasis and informing the CNS concerning the immunological and nutritional status of the gastrointestinal tract. Fascinatingly, the vagus nerve mediates signalling from the gut microbiota linking emotional and cognitive centres of the brain with peripheral intestinal functions, resulting during dysbiosis in central nervous disorders such as autism and anxiety-depressive behaviours (Carabotti *et al.* 2015; Mayer *et al.* 2015). Similarly, during local intestinal inflammation, direct activation of vagal nuclei such as the nucleus of the solitary tract has been reported by our group in a murine model of postoperative ileus (POI) (Cailotto *et al.* 2011). Interestingly, motor neurons of the dorsal nucleus of the vagus nerve, directly connected to the inflamed area, were also activated, which is compatible with the existence of a hard-wired 'inflammatory reflex' (Cailotto *et al.* 2011).

Although many intestinal immune cells express nicotinic receptors to react on the CAIP (de Jonge *et al.* 2005), macrophages and dendritic cells are described as the main effectors of this pathway. In line with this, it has been demonstrated that vagotomized mice developed more severe dextran sulfate sodium (DSS)-induced colitis as compared with control mice. Moreover, the same group showed that vagotomy had no effect in

Table 1. Overview of studies investigating the role of the CAIP in experimental models of gastrointestinal disorders

Disease	Model/treatment	Effect	Authors
Colitis	α7nAChR$^{-/-}$ mice	Increased colitis severity	Ghia *et al.* 2009
	Vagotomy and α7nAChr antagonist	Increased colitis severity	Ghia *et al.* 2006, 2008, 2011; Bianchi, 2007; O'Mahony *et al.* 2009; Ji *et al.* 2014
	Adoptive transfer of macrophages from vagotomized mice	Increased colitis severity	Ghia *et al.* 2011
	VNS	Decreased colitis severity	Sun *et al.* 2013
	α7nAChR agonist	Decreased colitis severity	Bianchi, 2007; Ghia *et al.* 2007, 2009, 2011; Snoek *et al.* 2010
	AChE inhibitors	Decreased colitis severity	Miceli & Jacobson, 2003; Ji *et al.* 2014
	Nicotine	Decreased colitis severity	Galitovskiy *et al.* 2011; Hayashi *et al.* 2014
Ileus	Vagotomy	Increased ileus severity	de Jonge *et al.* 2005
	α7nAChR agonist	Reduced intestinal inflammation and improved postoperative ileus	de Jonge *et al.* 2005
	Activation of cholinergic enteric neurons	Reduced intestinal inflammation and improved postoperative ileus	Tsuchida *et al.* 2011; Gomez-Pinilla *et al.* 2014
	VNS	Reduced intestinal inflammation and improved postoperative ileus	Matteoli *et al.* 2014; The *et al.* 2007
	VNS in α7nAChR$^{-/-}$ mice	VNS is ineffective in α7nAChr$^{-/-}$ mice during post-operative ileus	Matteoli *et al.* 2014
Pancreatitis	α7nAChr agonist GTS-21	Decreased pancreatitis severity	van Westerloo *et al.* 2006
	Vagotomy and α7nAChr antagonist	Increased pancreatitis severity	van Westerloo *et al.* 2006
	Nicotine treatment	Decreased pancreatitis severity	Schneider *et al.* 2014

macrophage-deficient mice, indicating a critical role for the presence of macrophages (Ghia *et al.* 2006). In addition, they have shown that adoptive transfer of macrophages isolated from vagotomized wild-type mice into colony stimulating factor 1 deficient (CSF1$^{-/-}$) mice resulted in severe colitis, suggesting again a central role of macrophages in the vagal anti-inflammatory effect (Ghia *et al.* 2011). In a more recent study, the pro-inflammatory effect of vagotomy during colitis was correlated with a reduction of splenic CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells (O'Mahony *et al.* 2009). Interestingly, in the same study adoptive transfer of splenic CD4$^+$CD25$^-$ T lymphocytes isolated from vagotomized mice increased the severity of colitis in DSS-treated recipients (O'Mahony *et al.* 2009).

In support of these findings, two recent studies by Ghia's research group elegantly showed that also central activation of the cholinergic pathway, via the administration of the acetylcholinesterase inhibitor galantamine or treatment with muscarinic acetylcholine receptor agonist, reduced mucosal inflammation both in DSS and in 2,4-dinitrobenzenesulfonic acid (DNBS)-induced colitis (Ji *et al.* 2014; Munyaka *et al.* 2014). This effect was the direct consequence of reduced pro-inflammatory cytokine secretion and maturation of splenic dendritic cells in an α7nAChR-dependent fashion. Of note, the anti-inflammatory effect was abolished in mice with vagotomy, splenic neurectomy, or splenectomy, indicating that central cholinergic activation of a vagus nerve-to-spleen circuit controls intestinal inflammation and this regulation can be explored to develop novel therapeutic strategies (Ji *et al.* 2014; Munyaka *et al.* 2014).

In line with these data, systemic nicotine treatment can suppress acute DSS-induced colitis by down-regulation of pro-inflammatory cytokines interleukin-6 (IL-6) and TNF-α (Ghia *et al.* 2006; Hayashi *et al.* 2014). Moreover, the acetylcholine esterase inhibitors neostigmine and physostigmine were able to significantly attenuate macroscopic damage, influx of myeloperoxidase positive cells and smooth muscle thickness in a rodent DNBS model of colitis (Miceli & Jacobson, 2003).

Contrary to sepsis, the crucial role of the α7nAChR still remains ambiguous in colitis. In a model of depression-induced colitis, α7nAChR$^{-/-}$ mice had a higher severity of acute DSS-induced colitis, which was reduced after treatment with choline chloride (an α7nAChR specific agonist) (Ghia *et al.* 2009). On the other hand, Snoek *et al.* described that treatment with specific α7nAChR agonists (AR-R17779 and GSK1345038A) reduced inflammation, inhibiting NF-κB activity and cytokine expression without improving the clinical signs of colitis (Snoek *et al.* 2010). In Table 1 an overview of the role of the CAIP in the gastrointestinal tract is shown. So far, no conclusive data support the involvement of the α7nAChR in the vagal anti-inflammatory effect in colitis. Thus, possible therapeutic approaches based on

the activation of α7nAChR for the treatment of colitis should be evaluated with caution.

Another subset of intestinal resident macrophages, which are located between the muscle layers of the intestinal wall at the level of the myenteric plexus, is also regulated by the CAIP. Since these macrophages are located in a highly innervated area of the gut, they might therefore be strongly affected by secreted neuronal factors. By means of a murine model of POI, a disease characterized by localized muscularis externa inflammation, it has been shown that electrical stimulation of the vagus nerve lowered intestinal muscular inflammation, reduced cytokine production and decreased recruitment of inflammatory immune cells (de Jonge *et al.* 2005). This protective effect was later demonstrated to be independent of the spleen but mediated by local release of ACh inhibiting activation of resident macrophages expressing α7nAChR (Matteoli *et al.* 2014). Indeed, direct pharmacological engagement of α7nAChR on these resident macrophages reduces ATP-induced Ca^{2+} increase *in situ*, pointing towards an anti-inflammatory effect of cholinergic neurotransmitters (Matteoli *et al.* 2014).

In the intestinal wall, the vagus nerve contacts immune cells only indirectly, since vagal efferents solely synapse with enteric neurons. However, recent anatomical evidence revealed a close proximity between cholinergic neuronal fibres and intestinal resident macrophages both at the level of the myenteric plexus and in the lamina propria (Cailotto *et al.* 2011; Nemethova *et al.* 2013; Matteoli *et al.* 2014). This suggests that stimulation of the vagus nerve leads to the activation of enteric neurons that subsequently release factors, such as ACh, that might affect the local immune system.

Interestingly, two independent studies have recently demonstrated that activation of cholinergic enteric neurons via specific chemical compounds lowered intestinal inflammation and restored gastrointestinal motility in POI (Gomez-Pinilla *et al.* 2014; Tsuchida *et al.* 2011). In both studies, 5-hydroxytryptamine 4 receptor (5-HT_4R) agonists were used to increase ACh release from cholinergic enteric neurons, which subsequently was able to inhibit monocyte and macrophage activation in an α7nAChR-dependent manner (Tsuchida *et al.* 2011; Gomez-Pinilla *et al.* 2014). Altogether, these data strongly indicate that pharmacological engagement of cholinergic enteric neurons by means of 5-HT_4R agonists might represent a novel therapeutic approach to treat intestinal immune-mediated diseases.

Clinical significance of the intestinal cholinergic anti-inflammatory pathway

Inflammatory bowel disease

Patients with IBD suffer from chronic inflammation in the gastrointestinal tract that can be divided into two major forms based on their clinical presentation (Rutgeerts *et al.* 2009). Although the inflammation in UC is mainly superficial and found in the colon, patients with CD have a transmural inflammation throughout the whole gastrointestinal tract. Despite the fact that treatment of IBD patients has been improved in the last decade with the introduction of, for example, anti-TNF antibodies (Rutgeerts *et al.* 2009; Van Assche *et al.* 2011), there is still much room for improvement as patients still often have complications or need to undergo intestinal resection.

Various studies have tried to correlate autonomic dysfunction, such as alteration of the vagal tone, with clinical outcome in IBD patients (Jerndal *et al.* 2010; Rubio *et al.* 2014). Bonaz and colleagues reported a negative correlation between low vagal tone and increased plasma levels of TNF-α, suggesting that the CAIP may be altered in these patients (Pellissier *et al.* 2014). However, a clear correlation between IBD and vagal tone has still not been convincingly verified. Recently, Clarencon *et al.* (2014) described the first attempt of VNS in a patient with CD. The patient, subjected to long-term low frequency VNS, showed significant improvement with reduced clinical disease activity index and endoscopic remission (Clarencon *et al.* 2014). This beneficial effect was correlated with an increased parasympathetic tone. Even though a therapeutic role for VNS in IBD is proposed in this report, results should be taken with caution considering the size of the study and the fact that a placebo effect could not be ruled out using this experimental approach.

Another strong suggestion that the CAIP could have a significant impact on the pathogenesis of IBD comes from the effect of smoking in UC and CD patients. Whereas UC patients have beneficial effects of smoking with a reduced disease severity (Gheorghe *et al.* 2004; Hoie *et al.* 2007; Bastida & Beltran, 2011), CD patients will increase their risk of relapses, repeat surgeries and the need for more aggressive immunosuppressive treatment after smoking (Cosnes *et al.* 1996; Yamamoto, 2005; Nos & Domenech, 2011). Although the exact mechanisms of these differences in outcome are not known yet, Galitovskiy *et al.* (2011) have recently tried to clarify this discrepancy in mice. Within this study they have examined the different effects of nicotine in two different mouse models of colitis mimicking a Th1 or Th2 type of inflammation. Within the Th2 model of inflammation, the expression of α7nAChR was induced on $CD4^+$ T cells after nicotine treatment, leading to increased regulatory T cells and reduced inflammation. On the contrary, the mice with a Th1 inflammation demonstrated no increase in expression of this receptor or diminished inflammation (Galitovskiy *et al.* 2011). These findings may explain the differential effect of smoking in UC and CD patients mediated via the expression of α7nAChR.

Table 2. Overview of clinical trials investigating the CAIP in gastrointestinal disorders

Clinical Trial Identifier:	Official title	Recruitment status	Principle investigator
NCT01569503	Vagus Nerve Stimulation a New Approach in the Treatment of Crohn's Disease	Recruiting	Bruno Bonaz
NCT01572155	Pilot Study: Anti-inflammatory Effect of Peroperative Stimulation of the Vagus Nerve	Completed	Guy Boeckxstaens
NCT02524626	Anti-inflammatory Effect of Peroperative Stimulation of the Vagus Nerve	Recruiting	Guy Boeckxstaens
NCT02311660	Vagus Nerve Stimulation in Crohn's Disease	Recruiting	Geert D'Haens
NCT02620176	The Effect of Transcutaneous Vagal Nerve Stimulation on Reducing Oesophageal Pain Hypersensitivity	Recruiting	Adam Farmer
NCT02388269	Non-invasive Vagus Nerve Stimulator Device for Treatment of Functional Dyspepsia or Irritable Bowel Syndrome	Completed	Owen Epstein
NCT02359188	Transcutaneous Vagal Nerve Stimulation: Influence on miRNA, Inflammation, Cerebral Resting State and Gastric Motility	Not yet recruiting	Felix Rosenow
NCT02420158	Trans-cutaneous Vagal Nerve Electrical Stimulation and Irritable Bowel Syndrome	Recruiting	François Mion
NCT02425774	Anti-inflammatory Effect of Pre-operative Stimulation of the Cholinergic Anti-Inflammatory Pathway	Recruiting	Guy Boeckxstaens
NCT02367729	Efficacy of Ear Neurostimulation for Adolescents With Functional Abdominal Pain	Recruiting	Katja Kovacic
NCT02648191	Preoperative Treatment With Noninvasive Intra-auricular Vagus Nerve Stimulation Pending Bariatric Surgery	Not yet recruiting	Radwan Kassir

Selective α7nAChR agonists are currently being evaluated as potential therapeutic drugs for the treatment of cognitive impairments in schizophrenia and Alzheimer's disease. Only a very limited number of studies have been performed to evaluate their anti-inflammatory potential in humans. Based on the observation that the selective α7nAChR agonist GTS-21 attenuated cytokine production from whole blood and human monocytes more potently than nicotine (Kox *et al.* 2009; Rosas-Ballina *et al.* 2009), its effect was tested in a human endotoxin model. However, no significant reduction in cytokine response to endotoxin injection in healthy subjects was observed between GTS-21 and placebo treated subjects (Kox *et al.* 2011). Clinical studies evaluating the specific effect of α7nAChR activation are therefore awaited with great interest.

Postoperative ileus and other GI disorders

Despite numerous advances in surgical techniques and perioperative care, POI remains one of the most common side-effects of abdominal surgery. It is associated with an abnormal pattern of gastrointestinal motility with additional symptoms such as nausea, vomiting, abdominal distension, intolerance to food and constipation. Many articles propose that POI is an essential phase of recovery after any abdominal procedure. However, POI has been shown to slow patient recovery and imparts a substantial financial burden on the health care system.

The pathophysiology of POI is not yet fully understood. However, it is accepted that activation of tissue resident macrophages in the myenteric plexus results in microscopic inflammation of the intestinal muscularis followed by influx of inflammatory cells such as neutrophils and monocytes (Eskandari *et al.* 1997; Kalff *et al.* 1998, 1999). The modulation of this inflammatory response by CAIP might represent an effective strategy to prevent POI (The *et al.* 2007). Previous preclinical studies support this hypothesis, as stimulation of the vagus nerve via electrical current (The *et al.* 2007), high-fat enteral feeding (Lubbers *et al.* 2009) or peripheral α7nAChR activation (The *et al.* 2007) reduces cytokine production and infiltration of immune cells into the muscularis externa and ameliorates gastrointestinal motility.

In addition to a direct effect on immune cells, VNS also has a beneficial effect on preserving the intestinal epithelial barrier during inflammation via the release of local ACh (MacFie *et al.* 1999; Clark & Coopersmith, 2007). This effect is crucial in the resolution of various intestinal pathologies such as ischaemia–reperfusion or severe burn injury where a 'leaky gut' is the major effector in the pathogenesis of the disease. The vagal tone has been shown to have a marked anti-inflammatory effect in several other gastrointestinal conditions as well, including pancreatitis (van Westerloo *et al.* 2006; Schneider *et al.* 2014) and leaking gut syndrome (Costantini *et al.* 2012). For instance, electrical stimulation of the vagus nerve was able to prevent loss of intestinal barrier function

after burn injury mainly via sustaining epithelial tight junction protein expression, which is essential to prevent the translocation of microbial products into the systemic circulation (Costantini *et al.* 2012).

Conclusion and perspective

Increasing evidence has demonstrated that the intestinal microenvironment dictates the phenotype of mucosal and circulating immune cells at steady state and during inflammation. Nowadays, it is clear that the interaction between the nervous system and the intestinal immune cells is of great importance in maintaining tissue homeostasis and in regulating mucosal inflammatory responses. In the gastrointestinal tract, the vagus nerve seems to play a major role in setting the anti-inflammatory cholinergic tone, via both a direct modulation of enteric neurons and an indirect interaction with splenic immune cells.

Despite the fact that the exact mechanisms and neuronal circuits of the intestinal CAIP are not yet completely understood, solid preclinical studies have demonstrated that an increased cholinergic tone (via electrical or chemical vagal nerve stimulation) reduces the inflammatory response, while alterations in this pathway result in increased disease severity and intestinal inflammation. Although data from animal studies are of great promise, so far there is no conclusive evidence that the vagus nerve plays a crucial role in controlling the human intestinal immune system. Proof of the existence of the vagal anti-inflammatory pathway in humans will be a significant breakthrough for the clinical management of intestinal immune-mediated inflammatory diseases. The fact that electrical stimulation of the vagus nerve is already a well-established therapeutic option for intractable epilepsy and treatment-resistant depression may anticipate possible clinical applications in intestinal disorders (Table 2).

Of note, several ongoing clinical studies are evaluating the effect of vagal nerve stimulation in patients suffering from intestinal inflammatory diseases such as CD and POI (Table 2). Results from these studies may represent the final proof that activation of the CAIP is a powerful tool to be included in the therapeutic armamentarium against chronic intestinal immune-mediated diseases.

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Additional information

Competing interests

The authors declare no competing financial interests.

Author contributions

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