Brain, Behavior, & Immunity - Health 19 (2022) 100401

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Cholinergic immunomodulation in inflammatory bowel diseases

Michele A. Serafini [a,c], Ana H. Paz [b,c], Natalia S. Nunes [d,*]

[a] Biological Sciences, Physiology Graduate Program, Federal University of Rio Grande do Sul, 90050170, Porto Alegre, Brazil
[b] Morphological Sciences Department, Basic Health Sciences Institute, Federal University of Rio Grande do Sul, 90050170, Porto Alegre, Brazil
[c] Cells, Tissue and Genes Laboratory, Experimental Research Center, Hospital de Clinicas de Porto Alegre, 90035903, Porto Alegre, Brazil
[d] Experimental Transplantation Immunotherapy Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 20852, Bethesda, MD, USA

ARTICLE INFO

Keywords:
Cholinergic signaling
Inflammatory bowel diseases
Macrophage
T cell
Immunomodulation
Acetylcholine
Vagus nerve
Intestinal immunity
α-7-Nicotinic-acetylcholine receptor

ABSTRACT

Inflammatory bowel diseases (IBD) are chronic intestinal disorders characterized by dysregulated immune responses to resident microbiota in genetically susceptible hosts. The activation of the cholinergic system has been proposed for the treatment of IBD patients according to its potential anti-inflammatory effect in vivo. The α-7-nicotinic-acetylcholine receptor (α7nAChR) is involved in the inhibition of inflammatory processes, modulating the production of cytokines, suppressing dendritic cells and macrophage activity, leading to the suppression of T cells. In this review, we address the most recent studies and clinical trials concerning cholinergic signaling and its therapeutic potential for inflammatory bowel diseases.

1. Introduction

Inflammatory bowel diseases (IBD) are chronic intestinal disorders of unknown etiology, with rising incidence and prevalence, especially in industrialized countries. Current treatments for IBD fail to promote sustained disease remission, rendering the development of new therapeutic strategies a priority. In this scenario, recent research on the cholinergic signaling in inflammatory diseases has shed light onto new therapeutic possibilities. Vagal nerve stimulation (VNS) has been reported to lower disease activity in diverse inflammatory diseases in vivo. The main mechanism of action is likely the activation of α-7-nicotinic-acetylcholine receptor (α7nAChR) on immune cells by acetylcholine (Ach). Thus, the activation of cholinergic signaling has been proposed for the treatment of IBD.

In the present issue, we address the most recent work concerning cholinergic signaling as a potential therapy for inflammatory diseases, emphasizing intestinal immunity and clinical studies in IBD patients.

2. Inflammatory bowel diseases

Inflammatory bowel diseases (IBD) are chronic intestinal inflammatory disorders characterized by dysregulated immune responses to enteric resident microbiota in genetically susceptible hosts, and affect approximately 3 million people in the United States (Dahlhamer et al., 2016; Oka et al., 2020). Inflammation in IBD is presented in a remitting and relapsing pattern, where the major forms are known as ulcerative colitis (UC) and Crohn's disease (CD).

The incidence and prevalence of IBD have increased in recent years, especially in industrialized countries (Moum et al., 2014). Differences in diagnostic resources and investments into healthcare indubitably affect the diagnosis of IBD, which may explain why low income countries report lower IBD incidence rates. Interestingly, a global study observed an association between increasing latitude and IBD incidence, compatible with the previously reported relationship between increased latitude and decreased levels of serum D vitamin induced by UV radiation (Piovani et al., 2019). Since individuals with low levels of D vitamin show an increased risk of developing IBD, and D vitamin synthesis is consequent to UV-B exposure (which is highly sensitive to the scattering caused by air particulate and pollutants), this might justify the increased risk of IBD in individuals living in urban settings in highly industrialized countries (Ananthakrishnan et al., 2012; Kimlin et al., 2007; MacLaughlin et al., 1982; Ng et al., 2019; Piovani et al., 2019). Accordingly, epidemiologic studies in Brazil reported that the most developed, higher economy activity state also presented the highest IBD incidence rate, comparable to European countries such as Portugal and Ireland (Gasparini et al., 2018; Zaltman et al., 2021). Nevertheless, the incidence of IBD is increasing in

* Corresponding author.
E-mail addresses: serafini.mich@gmail.com (M.A. Serafini), anahpaz@gmail.com (A.H. Paz), natalia.schneidernunes@nih.gov (N.S. Nunes).

https://doi.org/10.1016/j.bbih.2021.100401
Received 13 August 2021; Received in revised form 29 November 2021; Accepted 4 December 2021
Available online 11 December 2021

several low income countries throughout Latin America and the Caribbean (Kotze et al., 2020).

Although the etiology of IBD is largely unknown, recent evidence suggests the role of genetic factors, gut microbiome and immune dysregulation in triggering and sustaining the chronic inflammatory response in these diseases (Ghouri et al., 2020; Longo et al., 2020). The combination of genetic and environmental factors in the context of host–microbe interactions appears to trigger IBD-initiating events (Amoroso et al., 2020). Diet alters the microbiome and modifies intestinal immune response, thus, it could play a role in the pathogenesis of IBD. The adoption of a western diet, namely increased intake of ultra-processed food, refined sugars and dietary fats and decreased intake of fibers, have been suggested as potential risk factors for IBD (Narula et al., 2021). Accordingly, the adherence to this diet is associated to higher levels of interleukin (IL) 6, C-reactive protein and TNF-α (Bujtor et al., 2021). Food allergies may also contribute to the development of bowel disorders. An abnormal immune response to food allergens may promote the activation of the gastrointestinal mucosal immune system, leading to increased intestinal permeability and several disorders, such as irritable bowel syndrome and eosinophilic colitis (Bobrus-Chociej et al., 2018; Kuźmiński et al., 2020; Park et al., 2006). Current treatments for IBD mainly aim to suppress the enhanced immune response by the use of steroids, thiopurines, biologic drugs and molecules blocking the homing of immune cells in the inflamed gut (Argollo et al., 2017; Lim et al., 2018). Nevertheless, these treatments may cause adverse effects, besides not being able to promote sustained disease remission. Additionally, several patients do not respond to the existing treatments, making it imperative to develop new therapeutic strategies (Peyrin-Biroulet et al., 2015).

2.1. Ulcerative colitis

Ulcerative colitis (UC) is a lifelong disease arising from an interaction between genetic and environmental factors. The initial presentation of UC is characterized by an inflamed rectum and the condition may be presented at any time and at all ages (Rubin et al., 2019). Multiple factors, such as genetic background, environmental and luminal factors, and mucosal immune dysregulation, have been suggested to contribute to UC pathogenesis (Kobayashi et al., 2020).

The mucosal immune system in the gut is tolerant towards symbiotic bacteria and defends against pathogen invasion by initiating immune responses against exogenous antigens. The innate immune system in the gut includes the intestinal epithelial cells (IECs) that express Toll-like receptors (TLRs) with dual-immune functions, macrophages, and dendritic cells (DCs) in the intestinal lamina propria, exerting immunomodulatory effects during inflammatory responses (Zhang et al., 2020). In UC, aberrant immunological responses in the gut can affect the epithelial barrier and increase intestinal tissue permeability for novel antigens, further, leading to chronic inflammation (Wéra et al., 2016). Both innate and adaptive immunity have been suggested in maintaining intestinal homeostasis and various inflammatory immune cells are involved in this process, such as neutrophils, cluster of differentiation (CD)4+ T cells, and macrophages. For instance, neutrophil migration into the colonic mucosa is a hallmark of inflammation, being the extent of neutrophil infiltration correlated with disease severity (Bressenot et al., 2015).

Macrophages are an important component of the innate immune systems, maintaining tissue homeostasis by regulating apoptosis and the production of growth factors. These cells exhibit plasticity and functional diversity; being able to differentiate into M1 or M2 subtypes. Under the conditions of infection or tissue damage, including UC, the circulating monocytes are recruited into the inflamed sites and differentiate into macrophages. If these classical activated macrophages (M1 subtype) cannot be controlled effectively, they aggravate the disease progression promoting inflammation and anti-microbial activity. Differently, M2 macrophages exert anti-inflammatory activities and play a role in wound healing and fibrosis. Several signaling pathways are involved in regulating the secretion of inflammatory cytokines and inflammatory mediators by macrophages, interacting amongst themselves to regulate inflammatory microenvironments (Zhang et al., 2020).

Adaptive immune cells are also involved in UC disease pathophysiology, specifically T-helper (Th)2 $CD4^+$ cells. Th2 cells are immune effectors that play a crucial role in the defense against parasites and in modulating allergic reactions through the secretion of IL4, IL5, and IL13 (Giuffrida et al., 2018). The transcription factor GATA binding protein 3 (GATA-3) is the most dominant regulator of Th2 cell differentiation (Zheng et al., 1997), and its expression is seen in higher levels in colonic tissue from UC patients compared to ileal CD patients or healthy individuals, as well as in colonic tissue from UC patients with active disease compared to UC patients with inactive disease (Popp et al., 2017). In UC, large cohort studies revealed that mRNA levels of IL5 and IL13 are particularly increased in macroscopically inflamed tissue compared to uninflamed tissue (Nemeth et al., 2017; Rosen et al., 2017). Additionally, IL13 has been reported to deregulate epithelial tight junctions and possibly to promote apoptosis (Heller et al., 2005). Excessive production of IL13 in UC could lead to a toxic effect on colonic epithelial cells and the epithelial barrier, increasing disease activity.

Furthermore, imbalance between Treg and Th17 cells are implicated in both CD and UC disease progressions. Tregs are $CD4^+$ T cells that express the high-affinity IL2 receptor α-chain (CD25) and transcription factor Forkhead box P-3 (Foxp3), presenting a crucial anti-inflammatory and immunoregulatory role in IBD (Clough et al., 2020). On the other hand, Th17 cells are $CD4^+$ pro-inflammatory T cells that play a pathogenic role in both UC and CD, being able to produce multiple cytokines, including IL17, IL21, and IL22. The increased prevalence of Th17 cells has been reported in the colon of IBD patients, when compared to healthy individuals, as well as increased IL17A levels in serum and mucosa (Fujino et al., 2003). In addition, dextran sodium sulphate (DSS) and 2,4,6-trinitrobenzenesulfonic acid (TNBS) animal models of colitis have shown elevation of IL 21 production and protection from colitis in Il21−/− mice (De Souza et al., 2016), suggesting Th17 cells play an important role in the disease onset.

2.2. Crohn's disease

Crohn's disease (CD) is a chronic, progressive, and transmural granulomatous inflammatory disorder that can involve any part of the gastrointestinal tract from mouth to anus, predominantly the terminal ileum, ileocecal region, colon, and perianal region (Farraye et al., 2017). Patients with CD experience periods of flare and remission during their disease course. Disease severity and location dictate the associated symptoms, leading to a wide spectrum of clinical presentations. Usually, it is manifested as intermittent abdominal pain, diarrhea, hematochezia, fever, and weight loss. In CD patients, the lifetime risk of surgery still approaches 80% in spite of the increasing use of anti-TNF and immunosuppressive therapies (Wolford et al., 2020). On pathology examination, granulomas may be identified on biopsies, with a discontinuous distribution along the longitudinal axis. This inflammatory process often leads to irreversible tissue damage in the form of intestinal stenosis or fistulas, inflammatory masses, or intra-abdominal abscesses.

Apart from pathophysiological similarities to UC disease, such as the role of macrophages, Th17 and Treg cells in tissue inflammation, a dysregulation of various components of the immune system can be observed in CD patients' mucosa. The most pronounced alteration appears to be the hyperactivity of T cells with excessive production of cytokines IL12 and IFNγ, promoting a Th1 lymphocytic phenotype. Further analysis of T cell subsets has revealed the presence of Th1 and Th17 cells in CD, whereas the cytokines considered more involved are TNF, IL12, and IL23. Accordingly, CD is thought to be a consequence of an immune imbalance towards Th1 or Th17 versus and Treg cells, resulting in increased levels of pro-inflammatory cytokines IL17, TNFα, and IFNγ due an environmental trigger in a genetically predisposed patient (Gomes et al., 2018).

3. Vagus nerve and cholinergic immunomodulation

The vagus nerve (VN) is the longest cranial nerve in the parasympathetic nervous system and links the central nervous system (CNS) to the body. Through innervation of major visceral organs, the VN maintains several physiological mechanisms, including digestion, immune responses, hormone secretion, and respiratory function (Bonaz et al., 2017; Murray et al., 2018). The importance of the VN in regulating anti-inflammatory responses has been experimentally demonstrated utilizing vagotomy and vagal nerve stimulation (VNS) techniques during acute inflammation. Research on the cholinergic anti-inflammatory pathway (CAIP) has shown macrophages are effectively deactivated when exposed to acetylcholine (ACh) through significant reduction of cytokines, such as tumor necrosis factor (TNF), IL1β, IL6, and IL18 in lipopolysaccharide (LPS)-stimulated human macrophage in vitro (Borovikova et al., 2000; Tracey, 2002). Borovikova and collaborators (2000) also demonstrated that VNS significantly reduced TNFα production by splenic macrophages and diminished mortality in a mouse model of septic shock.

The effects of Ach are mediated by two pharmacologically distinct families of receptors: nicotinic and muscarinic Ach receptors (nAChRs and mAChRs). The mAChRs comprise a family of five related G protein-coupled receptors expressed by immune cells, in which selective activation modulates the production of pro-inflammatory cytokines (Fujii et al., 2003; Kruse et al., 2014). In contrast, nAChRs are classical ionotropic receptors which subunits are differently expressed in the immune cells; in some cases, correlating to their differentiation state (Gatta et al., 2020). The α7nAChR is the best characterized nicotinic receptor subtype in the immune system and is mainly involved in the inhibition of inflammation, modulating the production of anti-inflammatory cytokines, suppressing dendritic cells and macrophage activity and T cell differentiation (Kawashima et al., 2015).

Furthermore, CAIP experimental activation by direct electrical stimulation of the efferent VN inhibits TNF synthesis in liver, spleen, and heart, attenuating TNF serum concentrations during endotoxemia by LPS, whereas surgical or chemical vagotomy rendered animals sensitive to TNF release and shock (Bernik et al., 2002). Additionally, VNS has been demonstrated to significantly reduce inflammation in a series of conditions, including rheumatoid arthritis (Koopman et al., 2016), ischemia reperfusion injury (Inoue et al., 2016) and IBD (Ji et al., 2014).

Despite these advances, the precise neural circuitry and mechanisms of action responsible for immune inhibition remain contested. Macrophages presenting with α7nAChR deficiency have rendered VN ineffective as a physiological pathway to inhibit TNFα release, indicating that α7nAChR is essential for VN regulation of acute TNFα release during systemic inflammatory response to endotoxemia (Wang et al., 2003). Thus, ACh released from VN endings, or perhaps from other sources, can specifically inhibit macrophage activation.

Although VNS can modulate the peripheral inflammation, it may control ACh production directly by the immune cells in the spleen or in other lymphoid organs through adrenergic stimulation (Carnevale et al., 2016; Rosas-Ballina et al., 2011). Additionally, considering that ACh has an extremely short half-life in vivo as a consequence of the ubiquitous distribution of its hydrolytic enzymes (Soreq, 2001), it is plausible to conclude that the cholinergic source of ACh must be very close to the site of action (paracrine function).

It has been demonstrated that immune cells are able to produce ACh and respond to cholinergic stimuli. In 1982, Tucek (Tuček, 1982) reported that ACh is synthesized by choline acetyltransferase (ChAT) in the peripheral tissues of the rat. The expression of ChAT mRNA was detected only in T cell lines, and the ACh content correlated well with ChAT activity, suggesting ChAT is responsible for ACh synthesis in lymphocytes (Kawashima et al., 2015). Moreover, experiments have suggested that VN directly interacts with the sympathetic neurons innervating the spleen in prevertebral ganglia, including the celiac ganglia (Berthoud et al., 1996). VN fibers terminate in the celiac ganglia, where neural cell bodies project axons to the splenic nerve. Therefore, activation of vagal efferent signaling by VNS results in activation of the sympathetic innervation and release of norepinephrine (NE) in the spleen. Accordingly, splenectomy reverses VNS protective effects in models of endotoxemia (Huston et al., 2006), nephropathy (Uni et al., 2020), kidney ischemia-reperfusion injury (Inoue et al., 2016) and colitis (Ji et al., 2014) (Fig. 1a).

Protection afforded during septic shock by VNS requires activation of a specialized subset of splenic $CD4^+$ T-cells that express ChAT (ChAT + T cells), synthesize Ach, and serve as a non-neuronal source of ACh in the spleen (Rosas-Ballina et al., 2011). The release of ACh from ChAT + T-cells is a critical step in regulating inflammation and occurs following the activation of β2 adrenergic receptors on ChAT + $CD4^+$ T-cells by NE. ACh released from these T-cells binds to α7nAChR expressed on macrophages, resulting in inhibition of activation and TNFα production (Koopman et al., 2016) (Fig. 1b).

4. Cholinergic signaling and intestinal immunity

Gastrointestinal major functions, such as motility, secretion, and vasoregulation, are controlled both by the CNS through vagal afferent and efferent neurons, and by diverse peripheral nervous system (PNS) neurons located either outside of the gut or within the gut tissue, where they form the enteric nervous system (ENS) (Kulkarni et al., 2021). The ENS consists of small aggregations of nerve cells (enteric ganglia) that innervate effector tissues, such as the intestinal muscular layer, blood vessels, and endocrine cells (Brinkman et al., 2019).

Neuromodulation of intestinal immunity is regulated both by intrinsic (ENS) and extrinsic (CNS and PNS) innervation. Although the ENS receives ample input by the CNS by means of sympathetic and parasympathetic innervation, it can operate autonomously. Enteric neurons, in particular those residing in the myenteric plexus (MP), are 70% cholinergic and have recently been identified as important gatekeepers of immune homeostasis in the GI tract (Verheijden et al., 2018). The ENS seems to rely on resident immune cells, mostly muscularis macrophages (MMØs), to efficiently execute its function, via a reciprocal interaction. Under physiological conditions, myenteric neurons provide colony-stimulating factor-1 (CSF-1) to support maintenance of MMØs, which in return produce bone-morphogenic protein 2 (BMP2), involved in fine-tuning peristalsis, supposedly by modulating enteric neuron activity (Muller et al., 2014). Furthermore, under inflammatory conditions in mouse models of inflammation and postoperative ileus, this interaction seems to be crucial for VNS therapeutic effects. When activated through VNS, these neurons release ACh, dampening inflammatory activation of MMØs and leading to a reduced secretion of TNFα and IL6 (de Jonge et al., 2005) (Fig. 1c).

Although intrinsic enteric neural networks allow a substantial degree of autonomy over GI functions, extrinsic neural inputs provided by both sympathetic and parasympathetic nervous fibers are essential to integrate, regulate, and modulate these functions. Noteworthy, VNS most likely dampens the activation of MMØs by an indirect mechanism, since vagal efferent nerves present in the MP do not interact directly with resident macrophages in the gut or spleen. Instead, the VN interacts with cholinergic myenteric neurons with nerve endings in close proximity of resident MMØs, suggesting that intestinal MMØs expressing α7nAChR are most likely the ultimate target of the gastrointestinal CAIP (Cailotto et al., 2014).

Sacral nerve stimulation (SNS) is currently used to treat constipation and fecal incontinence, among other disorders (Carrington et al., 2014). Recent studies suggest that SNS presents similar anti-inflammatory effects to VNS, by inhibiting proinflammatory and increasing anti-inflammatory cytokines via the autonomic pathway. In a 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis model, SNS significantly reduced colon inflammation, demonstrated by measure of disease activity index (DAI), weight loss, histological score, myeloperoxidase (MPO) activity and TNFα and IL6 levels. SNS also increased ACh in colon tissue, suggesting SNS therapeutic effects are due to CAIP activation (Tu

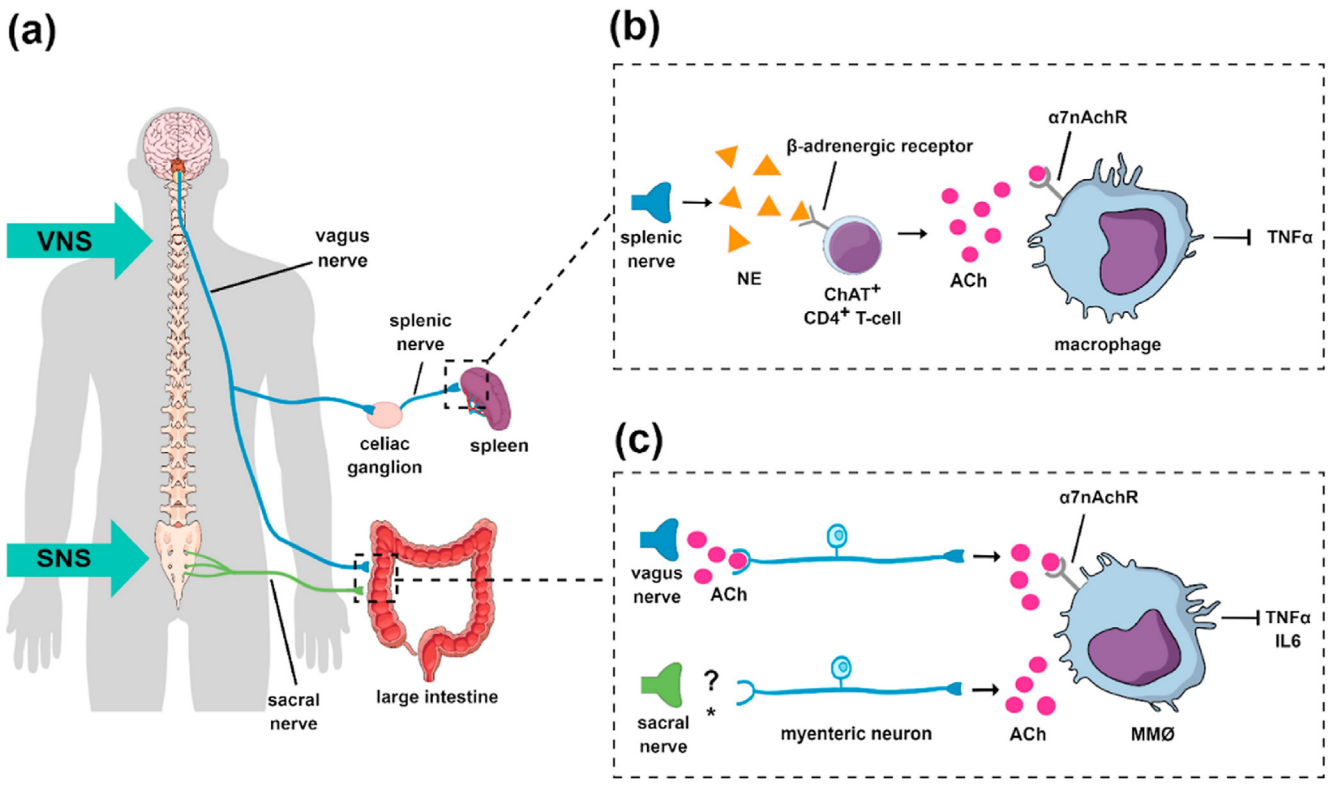

Fig. 1. Vagus nerve stimulation and sacral nerve stimulation suppress inflammation in the spleen and in the colon via the cholinergic anti-inflammatory pathway. (**a**) Splenic and intestinal innervation by splenic nerve, vagus nerve and sacral nerve. (**b**) In the spleen, norepinephrine released by sympathetic splenic nerves binds to β2 adrenergic receptors on choline acetyltransferase (ChAT)+ $CD4^+$ T-cells, which then synthetize and release acetylcholine (Ach). Thereafter, ACh binds to α7nAChR in splenic macrophages, inhibiting its activation and TNFα production. (**c**) In the large intestine, SNS and VNS activate myenteric neurons, which release ACh. In turn, ACh binds to α7nAChR in muscularis macrophages, inhibiting its activation and TNFα and interleukin (IL)-6 production. *The mechanisms of SNS circuit are still not clear; however, it has been observed that SNS does increase ACh tissue levels in the colon (Pasricha et al., 2020; Tu et al., 2020a). Figure Abbreviations: α7nAChR: α7-nicotinic-acetylcholine-receptor. ACh: acetylcholine. MMØ: muscularis macrophage. NE: norepinephrine. SNS: sacral nerve stimulation. TNFα: Tumor necrosis factor α. VNS: vagal nerve stimulation.

et al., 2020b). A recent study compared SNS and VNS effects on a TNBS-colitis model and reported both treatments significantly increased vagal activity and decreased sympathetic activity, as well as DAI, macroscopic scores, MPO activity, and IL6, IL17A, and TNFα. Similar SNS effects were noted in dextran sodium sulphate (DSS)-induced colitis (Guo et al., 2019). Additionally, it has been demonstrated that SNS decreases DAI, MPO activity, colonic TNFα and histological scores in a 5% DSS-colitis model, while increasing colonic ACh levels and vagal activity. Moreover, tissue ACh levels were positively correlated to vagal activity, indicating the increase in ACh was induced by SNS (Pasricha et al., 2020). These results suggest SNS, similar to VNS, may also exert a therapeutic potential for IBD (Fig. 1a,c).

Several intestinal immune cells express nicotinic receptors to respond to CAIP; nevertheless, macrophages and dendritic cells are described as the main effectors (Goverse et al., 2016). In a DSS colitis model, vagotomy significantly worsened colitis, as evidenced by increased DAI, greater loss of colonic architecture and increased colonic inflammation (Ghia et al., 2006). Furthermore, the same group reported vagotomy had no effect in macrophage-deficient mice, indicating a critical role of macrophages in regulating inflammation. Remarkably, mice treated with nicotine did not develop exacerbated colitis after vagotomy, suggesting VN affects intestinal macrophage activation by cholinergic modulation.

Central CAIP activation by VNS reduces mucosal inflammation both in DSS and in 2,4-dinitrobenzenesulfonic acid (DNBS)-induced colitis as a direct consequence of reduced pro-inflammatory cytokine secretion and maturation of splenic dendritic cells in an α7nAChR-dependent fashion (Ji et al., 2014; Munyaka et al., 2014). However, this 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. This regulation can be explored for the development of therapeutic strategies for IBD.

Rather interestingly, nicotine absorption via cigarette smoking affects both UC and CD but with opposite effects, ameliorating UC and worsening CD. Although the exact mechanisms are not known yet, Galitovskiy and collaborators (Galitovskiy et al., 2011) examined the different effects of nicotine in two different colitis models, mimicking a Th1 or Th2 type of inflammation. Within the Th2 model, the expression of α7nAChR was induced on $CD4^+$ T cells after nicotine treatment, leading to increased regulatory T cells and reduced inflammation. However, mice with a Th1 inflammation demonstrated no increase in expression of this receptor or diminished inflammation.

Accordingly, since immunologically CD is considered to be a Th1-predominant disease and UC Th2-predominant, VN immunological effects may be exerted via different mechanisms. Subclinical autonomic changes in IBD have been shown to predominantly affect patients with UC rather than CD, yet VNS has shown promising early clinical efficacy in CD. These observations might suggest that increasing the vagal tone in CD may promote a Th2-favorable environment, whilst restoring the sympatho-vagal balance may be of greater importance for patients with UC. Further research in this field is required for clearer delineation of these mechanisms (Mogilevski et al., 2019).

As previously mentioned, VNS activates the CAIP, which induces production and release of ACh by splenic ChAT + T cells. Subsequently, ACh binds to α7nAChR in splenic macrophages, inhibiting its activation. Remarkably, it is likely that the regulatory effect of ChAT + T cells is not restricted to the spleen, since these cells are found in mice lymph nodes

and Peyer's patches, which are similarly regulated by adrenergic neurons (Rosas-Ballina et al., 2011). Acetylcholine released by these ChAT + T cells acts on circulating and tissue cells that possess the α7nAChR, thus completing CAIP efferent arm (Mogilevski et al., 2019). Accordingly, in a DSS-colitis model, VNS increased both ACh colon levels and colonic M2 macrophage population, suggesting an anti-inflammatory polarization of lamina propria macrophages in response to VNS (Pasricha et al., 2020).

In addition to macrophages, dendritic cells and ChAT + T cells, other cell types also present an important role in intestinal immunity and can be influenced by cholinergic signaling. When activated by an inflammatory environment, mast cells release mediators, such as tryptase and prostaglandins E2 (PGE2), which increase the colonic mucosal layer permeability and allow further access of pathogens to the mucosa, provoking further inflammatory response. In a TNBS-colitis model, tryptase and PGE2 expression were increased when compared to healthy animals, which was normalized by SNS, suggesting cholinergic signaling could prevent mast cell activation (Tu et al., 2020a). Tuft cells are chemosensory epithelial cells present in most of the GI tract. It has been suggested that these cells participate in the induction of protective reflexes and inflammatory events by cholinergic signaling (Schütz et al., 2015). They express choline acetyltransferase (ChAT) (Schneider et al., 2019), which is necessary for ACh synthesis, suggesting these cells can be a non-neuronal source of Ach in the intestine.

Finally, there is evidence to suggest that up-regulation of Ach signaling through VNS/SNS and the neuronal amplification of this signal in the enteric nervous system acts directly on dendritic cells, muscularis macrophages and intestinal mast cells. Modulation of parasympathetic tone via the up-regulation of acetylcholine signaling at a local intestinal level represents, therefore, a novel and potentially important therapeutic target in downregulating intestinal inflammation (Mogilevski et al., 2019).

5. Cholinergic signaling in IBD: patient studies and clinical trials

During the last decade, CAIP activation has been proposed for the treatment of IBD patients according to its potential anti-inflammatory effect on immune cells in animal models (Ghia et al., 2006). Furlan and collaborators (Furlan et al., 2006) examined 23 active UC patients and 20 healthy individuals to investigate whether an exaggerated sympathetic activity characterizes active UC, and observed a higher heart rate in subjects with UC. Subsequently, 16 from these original 23 active UC patients were randomly assigned to a protocol of transdermal clonidine or placebo, to investigate whether a reduction of sympathetic activity by clonidine would be associated with clinical changes of UC. Clonidine, an adrenergic receptor agonist, reduced systemic neural sympathetic activity and increased vagal cardiac modulation, which was associated to decreased DAI, indicated by amelioration of clinical symptoms and colon endoscopic pattern. However, these results should be interpreted with caution, since important factors, such as smoking status, previous UC therapy, and gender, were registered but not separately analyzed, possibly due to the small sample size. Furthermore, the researchers do not specify whether a post-hoc test was performed in the statistical analysis, which is an important analysis factor to be considered.

Pellissier and collaborators (Pellissier et al., 2014) first investigated the association between vagal tone and inflammation markers in patients with CD ($n = 21$) compared to healthy subjects ($n = 26$). No difference was observed in heart rate between CD patients and healthy individuals. However, when CD patients were separated between high and low vagal tone, an inverse association ($r = 20.48$; $p < 0.05$) was observed between the vagal tone and TNFα level in CD patients, suggesting that the CAIP may be blunted in CD patients with low vagal tone. Patients inclusion criteria were specific regarding disease treatment, rendering study population nearly homogeneous; nevertheless, the study analyzed only CD patients in disease remission. Further investigation on vagal tone and inflammation markers in active CD patients is warranted.

Subsequently, a pilot study (Bonaz et al., 2016) aimed to evaluate VNS as a therapy in CD patients. In seven active CD patients, VNS was performed by an implanted device (Model 302, Cyberonics, Houston, TX, USA) wrapped around the left VN in the neck, continuously for over 6 months. No major side-effects were observed. All patients reported a decreased digestive pain after VNS treatment. However, two patients, both untreated prior to the experiment, were removed from the study at 3 months due to clinical worsening after experimental treatment. The five remaining patients evolved towards clinical and endoscopic remission with a restored vagal tone, measured via heart rate variability (HRV). These findings suggest VNS is feasible and safe, and its efficacy may be explored in future studies with larger groups of patients. After this pilot study, Sinniger and collaborators (Sinniger et al., 2020) investigated VNS in nine Crohn's disease patients through an implanted device (an electrode Model 302; Cyberonics wrapped around the left cervical VN) continuously for 12 months. After the intervention, five of nine patients were in clinical remission (DAI<150) and two patients had only slight disease activity (DAI = 171 and 180, respectively). Two patients were removed from the study after a 3-month follow-up, due to clinical worsening. Among the remaining seven patients, median DAI went from 264 (range: 175–358) to 88 (range: 0–180). After 12 months of VNS, endoscopic index of severity scores were reduced by 60–100% in 5 out of 7 patients, and the median digestive pain score reduced from 4 to 1. Further, serum IL6, IL12, TNFα, IL23, and IFNγ were also decreased when compared to before intervention. However, these results only suggest VNS efficacy on moderate CD therapy, since no statistical analysis was possible due to study population size. Moreover, DAI range of variation was somewhat large, suggesting a variability in immunomodulation in response to VNS treatment among patients, which should be considered with caution.

Controversially, a larger case-control study (Shao et al., 2020) investigated whether serum cholinesterase (ChE) levels were associated to IBD. A total of 60 CD and 142 UC patients were included retrospectively in the study and compared to 264 healthy individuals. CD patients displayed significantly lower serum ChE levels than patients with UC (5181U/L versus 6376U/L, $p < 0.01$), and both CD and UC patients presented substantially lower serum ChE levels when compared to healthy controls (8418U/L, $p < 0.001$). A negative association between serum ChE levels and the Crohn's Disease Activity Index (CDAI) score of patients with CD ($p = 0.011$) and the Simple Clinical Colitis Activity Index score of patients with UC ($p = 0.018$) was also observed. Together, these findings suggest serum ChE levels have important clinical significance in the diagnosis and assessment of clinical activity in patients with IBD. It is already known that ChE degrades ACh, thus, lower serum ChE levels suggest higher serum ACh levels and, according to the CAIP mechanism, lower inflammation markers levels. However, the study didn't investigate ACh levels nor inflammatory markers. Therefore, the conclusion that the reduction in ChE levels in IBD patients reflects a reduced sympathetic activity should be taken with caution. The authors state that ChE is a common serum marker that reflects the nutritional status of patients, and also that CD is frequently associated with malabsorption and malnutrition. This is more likely to be the cause of the difference in ChE levels observed in the study. Additional studies would provide further insight into the role of CAIP in IBD.

Although several pre-clinical (Hayashi et al., 2014; Pelissier-Rota et al., 2015; Yoshikawa et al., 2006) and clinical studies (McGilligan et al., 2007; Pullan et al., 1994) reported a protective role of nicotine in UC via α7nAChR modulation, these results are conflicted by side effects caused by the high systemic nicotine concentration needed for UC therapy (Cosnes, 2004). Furthermore, patients are encouraged to quit smoking to reduce the risk of cardiopulmonary diseases. Nevertheless, pilot clinical studies performed in the 90s demonstrated interesting results. Treatment with 6 mg nicotine/100 mL enema every night for 4 weeks, concomitantly to conventional treatment (either with mesalazine, prednisolone, cyclosporin, or azathioprine), improved UC symptoms in twelve of the seventeen patients within the study, with full remission in

Table 1
Published studies on cholinergic immunomodulation in inflammatory bowel diseases patients.

Author, Year	Number of Patients	Intervention	Major Findings
Pullan, (1994)	77 UC patients	Transdermal nicotine patches (35 nicotine + 37 placebo)	Complete symptomatic relief and global clinical grade of 0 in 17/35 patients in nicotine group and only 9/37 in placebo group; no stool mucus in 20/35 patients in nicotine group and 8/37 in placebo group; 3 patients withdrew because of side effects (nausea, lightheadedness, headache, sleep disturbance, dizziness, tremor)
Green, (1997)	17 UC patients, non-smokers	Nicotine enema	12 patients reduced urgency and stool frequency; 3 patients had a full remission after 4 weeks and another 2 after 8 weeks; enema was well-tolerated; still, 1 patient was withdrawn due to side effects (a 22-year-old who experienced vasovagal symptoms shortly after enema)
Sandborn, (1997)	10 UC patients, non-smokers	Nicotine enema	Clinical improvement in 5 of 7 patients; 3 patients discontinued therapy within 7 days due to inability to retain the liquid enemas; mild adverse events occurred in 4/10 patients
Furlan, (2006)	23 UC patients 20 healthy controls	Clonidine	Treatment with clonidine increased vagal cardiac modulation, which was associated to reduced disease activity
Pellissier, (2014)	21 CD patients 26 healthy controls	None (observational case-control)	TNFα plasma levels in CD patients is negatively correlated to vagal tone
Bonaz, (2016)	7 CD patients	VNS (implanted device Model 302, Cyberonics)	Five patients evolved towards clinical and endoscopic remission with a restored vagal tone; all patients reported a decreased digestive pain after VNS treatment; no major side-effects were observed and the device was well-tolerated in all patients
Shao, (2020)	141 UC patients 60 CD patients 264 healthy controls	None (retrospective case-control)	CD and UC patients both presented substantially lower serum ChE levels when compared to healthy controls (8418 U/L, $p < 0.001$); a negative association between serum ChE levels and both CD and UC patients' disease activity index
Sinniger, (2020)	9 CD patients	VNS (implanted device Model 302; Cyberonics)	Five patients were in clinical remission and two patients had only slight disease activity; median disease activity went from 264 to 88; median digestive pain went from 4 to 1; IL6, IL12, TNFα, IL23, and IFNγ serum levels were decreased

five patients and only few side effects (Green et al., 1997). Similarly, treatment with 3 mg/day for 1 week and then 6 mg/day for 3 weeks nicotine enemas reported clinical and sigmoidoscopic improvement in five of seven patients. Three patients discontinued therapy within 7 days due to inability to retain the liquid enemas. Only mild adverse events occurred in 4/10 patients (nausea, lightheadedness, tremor, or sleep disturbance) (Sandborn et al., 1997). However, controlled randomized studies are still needed to further investigate these therapeutic effects. There are no current clinical trials on nicotine immunomodulation in IBD.

Published studies regarding cholinergic immunomodulation in IBD patients are summarized in Table 1.

Although early studies on cholinergic modulation for the treatment of IBD present encouraging results, further investigation is necessary to obtain a deeper understanding of the therapeutic mechanisms. Thereby, six different research groups are presently conducting clinical studies with VNS for cholinergic immunomodulation signaling in IBD (https://clinicaltrials.gov/: NCT03953768, NCT03863704, NCT02311660, NCT02951650, NCT03908073, and NCT00734331; accessed on: Feb 4, 2021) (Table 2).

VNS is a highly invasive neurosurgical procedure that requires extreme caution due to VN proximity to the jugular vein and external carotid artery. Therefore, the development of non-invasive VNS techniques is warranted. Responses to transcutaneous vagal nerve stimulation (tVNS) have been studied in healthy human volunteers. Clancy and collaborators (Clancy et al., 2014) investigated tVNS via electrical stimulation of the auricular branch of VN distributed throughout the skin of the ear. Active tVNS significantly increased HRV in healthy participants, indicating a shift in cardiac autonomic function towards parasympathetic predominance. Accordingly, tVNS can reduce sympathetic nerve outflow, providing a simple and inexpensive alternative to invasive VNS. However, these patients received a single dose of tVNS, and a longer tVNS treatment protocol may be further investigated. Similarly, tVNS applied for 2 min to the right antero-lateral surface of the neck in thirteen healthy individuals significantly activated primary vagal projections including the nucleus of the solitary tract (primary central relay of vagal afferents), the parabrachial area, the primary sensory cortex, and the insula (Frangos et al., 2017). Such effects indicate that cervical vagal afferents can be accessed non-invasively via transcutaneous electrical stimulation of the antero-lateral surface of the neck, which overlies the course of the nerve, suggesting an alternative and feasible method of VNS. Additionally, these data suggest that the levels of VN activation achieved via transcutaneous stimulation are similar to those achieved by implantable VNS devices. Although these results are encouraging, further studies are necessary to evaluate long-term non-invasive VNS techniques in patients under inflammatory conditions.

Nunes and collaborators (Nunes et al., 2019) proposed the use of therapeutic ultrasound (TUS) in order to activate CAIP in a DSS-colitis model. TUS, applied to the left side of mice abdomen, aiming at the spleen, attenuated DAI by reducing clinical scores, colon shortening and histological damage, besides inducing proteomic tolerogenic responses in the gut during the injury phase and early recovery of experimental colitis. Noteworthy, TUS did not improve clinical and pathological outcomes in splenectomized mice, while α7nAChR knockout animals presented disease worsening, suggesting that the therapeutic use of ultrasound acts through the splenic nerve and possibly the VN, with CAIP activation in the DSS-induced colitis model. Accordingly, Benjamin Sahn (clinicaltrials.gov: NCT03863704) and Qasim Qziz (clinicaltrials.gov: NCT03908073) groups are both evaluating the use of non-invasive VNS via a transcutaneous device as a potential therapy in IBD.

6. Pre-clinical studies: promising therapies under investigation

In addition to the above-mentioned drugs and interventions for IBD, novel therapeutics exploring CAIP are being investigated in pre-clinical studies, in particular, selective α7nAChRs agonists and acetylcholinesterase inhibitors.

Administration of tropisetron, a partial agonist of α7nAChRs reported to have anti-inflammatory effects, ameliorated the development of DSS-induced colitis in a dose-dependent manner (Tasaka et al., 2015). Additionally, stimulation of α7nAChRs by PNU282987, a selective α7nAChRs agonist, decreased macrophage infiltration into the colonic mucosa caused by DSS administration. These findings suggest tropisetron could be a candidate therapeutic agent for UC. Treatment with

Table 2
Clinical trials in cholinergic modulation for inflammatory bowel diseases.

ClinicalTrials.gov Identifier, Principal Investigator	Official Title	Intervention	Study Status
NCT03953768 Ian S Mutchnick	"VNS Prospective Neuromodulation of Autonomic, Immune and Gastrointestinal Systems (VNSAIG)"	Device: Vagal nerve stimulation (VNS)	Recruiting
NCT03863704 Benjamin Sahn	"Transcutaneous VNS to Treat Pediatric IBD (STIMIBD)"	Device: Transcutaneous Electrical Nerve Stimulation (tENS)	Recruiting
NCT02311660 Geert D'Haens	"Vagus Nerve Stimulation in Crohn's Disease"	Device: Vagus Nerve Stimulation Device	Unknown. Recruitment status was: Active, not recruiting
NCT02951650 Geert D'Haens	"Long Term Observational Study of a Vagal Nerve Stimulation Device in Crohn's Disease"	Device: Cyberonics VNS	Unknown. Recruitment status was: Active, not recruiting
NCT03908073 Qasim Qziz Tamara Mogilevski	"Electrical Vagal Nerve Stimulation in Ulcerative Colitis (EVASION-UC)"	Device: Transcutaneous vagal nerve stimulation	Unknown. Recruitment status was: Recruiting
NCT00734331 Michal Roll	"Micro Ribonucleic Acid (RNA) as Cholinergic Tone and Inflammatory Regulator in Inflammatory Bowel Disease"	None (Observational)	Completed (no results posted)

PNU282987 ameliorates DSS-induced colitis, reflected by decreased weight loss, histological score and IL1β, IL6, TNFα, IL12, and IL23 colonic concentration, including a significant increase in IL10 (Xiao et al., 2020). However, further investigation is needed to demonstrate tropisetron's potential as a therapy for IBD, prior to evaluating this molecule's effect in clinical studies. Moreover, pyridostigmine bromide, an acetylcholinesterase inhibitor, attenuated DSS-induced colitis, by increasing Ach tissue levels, reducing colon eosinophilic infiltration and Th2 pro-inflammatory factors and promoting MUC2 synthesis, a mucin that plays a critical role in gut homeostasis (Singh et al., 2020). Nevertheless, the study did not evaluate whether said increase in ACh tissue levels was followed by a decrease in macrophage activation and subsequent decreased secretion of TNF-α. Additionally, prokinetic 5-hydroxytryptamine 4 receptor (5-HT4R) agonists are potential therapeutic agents for directly ameliorating motility disorders associated with postoperative ileus (POI). Stimulating the 5-HT4R accelerates ACh release from cholinergic myenteric neurons, which subsequently binds to α7nAChR on activated monocytes or macrophages to inhibit their inflammatory reactions in the muscle layer (Tsuchida et al., 2011). In a POI model, 5-HT4R agonists mosapride citrate (MOS) and CJ-033466 attenuated intestinal motility dysfunction and leucocyte infiltration, besides reducing the expression of inflammatory mediators IL1β, IL6, TNFα and inducible nitric oxide synthase. In addition, the autonomic ganglionic blocker hexamethonium and the α7nAChR antagonist methyl lycaconitine citrate blocked MOS-mediated ameliorative actions, suggesting that MOS anti-inflammatory mechanism is α7nAChR dependent.

Finally, treatment with encenicline, a partial agonist specific for α7nAChR, attenuated DAI in both DSS and TNBS induced models, as indicated by significantly reduced macroscopic parameters and MPO activity (Salaga et al., 2015). In the TNBS model, encenicline reduced macrophages, neutrophils and B cells infiltration in the colon, whereas in the DSS model it increased the frequency of FoxP3+ T cells and reduced IL17A + T cells. These results suggest stimulation of α7nAChR with partial agonist encenicline alleviates colitis via immunomodulation in the gut, emphasizing a potential role of α7nAChRs as a target for anti-colitis drugs.

7. Conclusions

The cholinergic anti-inflammatory pathway (CAIP) seems to be activated both by VNS and SNS, and is reported to have a pronounced effect in decreasing disease activity in diverse inflammatory conditions in vivo. The main mechanism of action appears to be the activation of α-7-nicotinic-acetylcholine receptor (α7nAChR) on immune cells by acetylcholine (ACh). In the present issue, we addressed the most recent work concerning cholinergic signaling as a potential therapy for inflammatory diseases.

Cholinergic immunomodulation in IBD seems to occur globally, via VNS and ChAT + T cells through Ach production in the spleen, which inhibits macrophage activation. Locally, VNS or SNS may directly stimulate enteric neurons, which in turn secrete Ach and also inhibit MMØ activation in the gut. Preliminary clinical studies with VNS reported lower IBD disease indexes and reduced inflammatory cytokine mediators in patients' serum and colonic tissue. However, both VNS and SNS are invasive procedures and reaffirm the need for non-invasive VNS techniques. In spite of promising preclinical and clinical studies, further research in the field is warranted aiming an alternative or combined therapy for future clinical practice in IBD.

Funding

This work was supported by CNPq (National Council for Scientific and Technological Development). The funding source had no role in the writing of the manuscript or the decision to submit it for publication.

Declaration of competing interest

The authors declare no conflict of interest.

References

Amoroso, C., Perillo, F., Strati, F., Fantini, M., Caprioli, F., Facciotti, F., 2020. The role of gut microbiota biomodulators on mucosal immunity and intestinal inflammation. Cells 9 (5), 1–24. https://doi.org/10.3390/cells9051234.

Ananthakrishnan, A.N., Khalili, H., Higuchi, L.M., Bao, Y., Korzenik, J.R., Giovannucci, E.L., Richter, J.M., Fuchs, C.S., Chan, A.T., 2012. Higher predicted vitamin D status is associated with reduced risk of crohn's disease. Gastroenterology 142 (3), 482–489. https://doi.org/10.1053/j.gastro.2011.11.040.

Argollo, M., Fiorino, G., Hindryckx, P., Peyrin-Biroulet, L., Danese, S., 2017. Novel therapeutic targets for inflammatory bowel disease. In: Journal of Autoimmunity, vol. 85. Academic Press, pp. 103–116. https://doi.org/10.1016/j.jaut.2017.07.004.

Bernik, T.R., Friedman, S.G., Ochani, M., Diraimo, R., Ulloa, L., Yang, H., Sudan, S., Czura, C.J., Ivanova, S.M., Tracey, K.J., 2002. Pharmacological stimulation of the cholinergic antiinflammatory pathway. Issue 6. In: J. Exp. Med. the, vol. 195. Rockefeller University Press. Retrieved from. http://www.jem.org/cgi/content/full/195/6/781.

Berthoud, H.R., Powley, T.L., 1996. Interaction between parasympathetic and sympathetic nerves in prevertebral ganglia: morphological evidence for vagal efferent innervation of ganglion cells in the rat. Microsc. Res. Tech. 35 (1), 80–86. https://doi.org/10.1002/(SICI)1097-0029(19960901)35:1<80::AID-JEMT7>3.0.CO;2-W.

Bobrus-Chociej, A., Daniluk, U., Alifier, M., Stasiak-Barmuta, A., Kaczmarski, M.G., 2018. Alterations of lymphocyte subpopulations and TGF-β in children with transient or persistent cow's milk allergy. Food Agric. Immunol. 29 (1), 400–411. https://doi.org/10.1080/09540105.2017.1387234.

Bonaz, B., Sinniger, V., Hoffmann, D., Clarençon, D., Mathieu, N., Dantzer, C., Vercueil, L., Picq, C., Trocmé, C., Faure, P., Cracowski, J.L., Pellissier, S., 2016. Chronic vagus nerve stimulation in Crohn's disease: a 6-month follow-up pilot study. Neuro Gastroenterol. Motil. 28 (6), 948–953. https://doi.org/10.1111/nmo.12792.

Bonaz, B., Sinniger, V., Pellissier, S., 2017. Vagus nerve stimulation: a new promising

therapeutic tool in inflammatory bowel disease. J. Intern. Med. 282 (1), 46–63. https://doi.org/10.1111/joim.12611.
Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Wang, H., Abumrad, N., Eaton, J.W., Tracey, K.J., 2000. Vagus nerve stimulation attenuates the systemic inammatory response to endotoxin. Retrieved from. www.nature.com.
Bressenot, A., Salleron, J., Bastien, C., Danese, S., Boulagnon-Rombi, C., Peyrin-Biroulet, L., 2015. Comparing histological activity indexes in UC. Gut 64 (9), 1412–1418. https://doi.org/10.1136/gutjnl-2014-307477.
Brinkman, D.J., ten Hove, A.S., Vervoordeldonk, M.J., Luyer, M.D., de Jonge, W.J., 2019. Neuroimmune interactions in the gut and their significance for intestinal immunity. Cells 8 (7), 670. https://doi.org/10.3390/cells8070670.
Bujtor, M., Turner, A.I., Torres, S.J., Esteban-Gonzalo, L., Pariante, C.M., Borsini, A., 2021. Associations of dietary intake on biological markers of inflammation in children and adolescents: a systematic review. Nutrients 13 (2), 1–29. https://doi.org/10.3390/nu13020356.
Cailotto, C., Gomez-Pinilla, P.J., Costes, L.M., Van Der Vliet, J., Di Giovangiulio, M., Némethova, A., Matteoli, G., Boeckxstaens, G.E., 2014. Neuro-anatomical evidence indicating indirect modulation of macrophages by vagal efferents in the intestine but not in the spleen. PLoS One 9 (1). https://doi.org/10.1371/journal.pone.0087785.
Carnevale, D., Perrotta, M., Pallante, F., Fardella, V., Iacobucci, R., Fardella, S., Carnevale, L., Carnevale, R., De Lucia, M., Cifelli, G., Lembo, G., 2016. A cholinergic-sympathetic pathway primes immunity in hypertension and mediates brain-to-spleen communication. Nat. Commun. 7, 1–13. https://doi.org/10.1038/ncomms13035.
Carrington, E.V., Evers, J., Grossi, U., Dinning, P.G., Scott, S.M., O'Connell, P.R., Jones, J.F.X., Knowles, C.H., 2014. A systematic review of sacral nerve stimulation mechanisms in the treatment of fecal incontinence and constipation. Neuro Gastroenterol. Motil. 26 (9), 1222–1237. https://doi.org/10.1111/nmo.12388.
Clancy, J.A., Mary, D.A., Witte, K.K., Greenwood, J.P., Deuchars, S.A., Deuchars, J., 2014. Non-invasive Vagus nerve stimulation in healthy humans reduces sympathetic nerve activity. Brain Stimulation 7 (6), 871–877. https://doi.org/10.1016/j.brs.2014.07.031.
Clough, J.N., Omer, O.S., Tasker, S., Lord, G.M., Irving, P.M., 2020. Regulatory T-cell therapy in Crohn's disease: challenges and advances. Gut 69 (5), 942–952. https://doi.org/10.1136/gutjnl-2019-319850.
Cosnes, J., 2004. Tobacco and IBD: relevance in the understanding of disease mechanisms and clinical practice. Best Pract. Res. Clin. Gastroenterol. 18 (3), 481–496. https://doi.org/10.1016/j.bpg.2003.12.003.
Dahlhamer, J.M., Zammitti, E.P., Ward, B.W., Wheaton, A.G., Croft, J.B., 2016. Prevalence of inflammatory bowel disease among adults aged ≥18 Years — United States, 2015. MMWR. Morbidity and Mortality Weekly Report 65 (42), 1166–1169. https://doi.org/10.15585/mmwr.mm6542a3.
de Jonge, W.J., van der Zanden, E.P., The, F.O., Bijlsma, M.F., van Westerloo, D.J., Bennink, R.J., Berthoud, H.R., Uematsu, S., Akira, S., van den Wijngaard, R.M., Boeckxstaens, G.E., 2005. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. Nat. Immunol. 6 (8), 844–851. https://doi.org/10.1038/ni1229.
De Souza, H.S.P., Fiocchi, C., 2016. Immunopathogenesis of IBD: current state of the art. Nat. Rev. Gastroenterol. Hepatol. 13 (1), 13–27. https://doi.org/10.1038/nrgastro.2015.186.
Farraye, F.A., Melmed, G.Y., Lichtenstein, G.R., Kane, S.V., 2017. ACG clinical guideline: preventive care in inflammatory bowel disease. Am. J. Gastroenterol. 112 (2), 241–258. https://doi.org/10.1038/ajg.2016.537.
Frangos, E., Komisaruk, B.R., 2017. Access to vagal projections via cutaneous electrical stimulation of the neck: fMRI evidence in healthy humans. Brain Stimulation 10 (1), 19–27. https://doi.org/10.1016/j.brs.2016.10.008.
Fujii, T., Watanabe, Y., Inoue, T., Kawashima, K., 2003. Upregulation of mRNA encoding the M5 muscarinic acetylcholine receptor in human T- and B-lymphocytes during immunological responses. Neurochem. Res. 28 (3–4), 423–429. https://doi.org/10.1023/A:1022840416292.
Fujino, S., Andoh, A., Bamba, S., Ogawa, A., Hata, K., Araki, Y., Bamba, T., Fujiyama, Y., 2003. Increased expression of interleukin 17 in inflammatory bowel disease. Gut 52 (1), 65–70. https://doi.org/10.1136/gut.52.1.65.
Furlan, R., Ardizzone, S., Palazzolo, L., Rimoldi, A., Perego, F., Barbic, F., Bevilacqua, M., Vago, L., Porro, G.B., Malliani, A., 2006. Sympathetic overactivity in active ulcerative colitis: effects of clonidine. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290 (1), 224–233. https://doi.org/10.1152/ajpregu.00442.2005.
Galitovskiy, V., Qian, J., Chernyavsky, A.I., Marchenko, S., Gindi, V., Edwards, R.A., Grando, S.A., 2011. Cytokine-induced alterations of alpha-7-nicotinic-receptor in colonic Cd4 T cells mediate dichotomous response to nicotine in murine models. J. Immunol. 187 (5), 2677–2687. https://doi.org/10.4049/jimmunol.1002711.CYTOKINE-INDUCED.
Gasparini, R.G., Sassaki, L.Y., Saad-Hossne, R., 2018. Inflammatory bowel disease epidemiology in São Paulo State, Brazil. Clin. Exp. Gastroenterol. 11, 423–429. https://doi.org/10.2147/CEG.S176583.
Gatta, V., Mengod, G., Reale, M., Tata, A.M., 2020. Possible correlation between cholinergic system Alterations and neuro/inflammation in multiple sclerosis. Biomedicines. https://doi.org/10.3390/biomedicines8060153.
Ghia, J.E., Blennerhassett, P., Kumar-Ondiveeran, H., Verdu, E.F., Collins, S.M., 2006. The vagus nerve: a tonic inhibitory influence associated with inflammatory bowel disease in a murine model. Gastroenterology 131 (4), 1122–1130. https://doi.org/10.1053/j.gastro.2006.08.016.
Ghouri, Y.A., Tahan, V., Shen, B., 2020. Secondary causes of inflammatory bowel diseases. World J. Gastroenterol. 26 (28), 3998–4017. https://doi.org/10.3748/wjg.v26.i28.3998.
Giuffrida, P., Corazza, G.R., Di Sabatino, A., 2018. Old and new lymphocyte players in inflammatory bowel disease. Dig. Dis. Sci. 63 (2), 277–288. https://doi.org/10.1007/s10620-017-4892-4.
Gomes, J.P., Watad, A., Shoenfeld, Y., 2018. Nicotine and autoimmunity: the lotus' flower in tobacco. Pharmacol. Res. 128, 101–109. https://doi.org/10.1016/j.phrs.2017.10.005.
Goverse, G., Stakenborg, M., Matteoli, G., 2016. The intestinal cholinergic anti-inflammatory pathway. J. Physiol. 594 (20), 5771–5780. https://doi.org/10.1113/JP271537.
Green, J.T., Thomas, G.A.O., Rhodes, J., Williams, G.T., Evans, B.K., Russell, M.A.H., Feyerabend, C., Rhodes, P., Sandborn, W.J., 1997. Nicotine enemas for active ulcerative colitis - a pilot study. Aliment Pharmacol. Therapeut. 11 (5), 859–863. https://doi.org/10.1046/j.1365-2036.1997.00220.x.
Guo, J., Jin, H., Shi, Z., Yin, J., Pasricha, T., Chen, J.D.Z., 2019. Sacral nerve stimulation improves colonic inflammation mediated by autonomic-inflammatory cytokine mechanism in rats. Neuro Gastroenterol. Motil. 31 (10). https://doi.org/10.1111/nmo.13676.
Hayashi, S., Hamada, T., Zaidi, S.F., Oshiro, M., Lee, J., Yamamoto, T., Ishii, Y., Sasahara, M., Kadowaki, M., 2014. Nicotine suppresses acute colitis and colonic tumorigenesis associated with chronic colitis in mice. Am. J. Physiol. Gastrointest. Liver Physiol. 307 (10), G968–G978. https://doi.org/10.1152/ajpgi.00346.2013.
Heller, F., Florian, P., Bojarski, C., Richter, J., Christ, M., Hillenbrand, B., Mankertz, J., Gitter, A.H., Bürgel, N., Fromm, M., Zeitz, M., Fuss, I., Strober, W., Schulzke, J.D., 2005. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology 129 (2), 550–564. https://doi.org/10.1053/j.gastro.2005.05.002.
Huston, J.M., Ochani, M., Rosas-Ballina, M., Liao, H., Ochani, K., Pavlov, V.A., Gallowitsch-Puerta, M., Ashok, M., Czura, C.J., Foxwell, B., Tracey, K.J., Ulloa, L., 2006. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. J. Exp. Med. 203 (7), 1623–1629. https://doi.org/10.1084/jem.20052362.
Inoue, T., Abe, C., Sung, S., Moscalu, S., Kankowski, J., Huang, L., Ye, H., Guyenet, P., Okusa, M., 2016. Vagus nerve stimulation mediates protection from kidney ischemia-reperfusion injury through α7nAChR+ splenocytes. J. Clin. Invest. 126 (5), 1939–1952. https://doi.org/10.1172/JCI83658.
Ji, H., Rabbi, M.F., Labis, B., Pavlov, V.A., Tracey, K.J., Ghia, J.E., 2014. Central cholinergic activation of a vagus nerve-to-spleen circuit alleviates experimental colitis. Mucosal Immunol. 7 (2), 335–347. https://doi.org/10.1038/mi.2013.52.
Kawashima, K., Fujii, T., Moriwaki, Y., Misawa, H., Horiguchi, K., 2015. Non-neuronal cholinergic system in regulation of immune function with a focus on α7 nAChRs. Int. Immunopharm. 29 (1), 127–134. https://doi.org/10.1016/j.intimp.2015.04.015.
Kimlin, M.G., Olds, W.J., Moore, M.R., 2007. Location and Vitamin D Synthesis: Is the Hypothesis Validated by Geophysical Data? Journal of Photochemistry and Photobiology B: Biology. Retrieved from. https://sci hub.st/10.1016/j.jphotobiol.2006.10.004.
Kobayashi, T., Siegmund, B., Le Berre, C., Wei, S.C., Ferrante, M., Shen, B., Bernstein, C.N., Danese, S., Peyrin-Biroulet, L., Hibi, T., 2020. Ulcerative colitis. Nat. Rev. Dis. Primers 6 (1), 74. https://doi.org/10.1038/s41572-020-0205-x.
Koopman, F.A., Chavan, S.S., Miljko, S., Grazio, S., Sokolovic, S., Schuurman, P.R., Mehta, A.D., Levine, Y.A., Faltys, M., Zitnik, R., Tracey, K.J., Tak, P.P., 2016. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in Rheumatoid arthritis. Proc. Natl. Acad. Sci. U.S.A. 113 (29), 8284–8289. https://doi.org/10.1073/pnas.1605635113.
Kotze, P.G., Underwood, F.E., Damião, A.O.M.C., Ferraz, J.G.P., Saad-Hossne, R., Toro, M., Iade, B., Bosques-Padilla, F., Teixeira, F.V., Juliao-Banos, F., Simian, D., Ghosh, S., Panaccione, R., Ng, S.C., Kaplan, G.G., 2020. Progression of inflammatory bowel diseases throughout Latin America and the caribbean: a systematic review. Clin. Gastroenterol. Hepatol. 18 (2), 304–312. https://doi.org/10.1016/j.cgh.2019.06.030.
Kruse, A.C., Kobilka, B.K., Gautam, D., Sexton, P.M., Physiology, C., Section, S., Diseases, K., 2014. Muscarinic Acetylcholine Receptors: Novel Opportunities for Drug Development, vol. 13, pp. 549–560. https://doi.org/10.1038/nrd4295.Muscarinic, 7.
Kulkarni, S., Kurapati, S., Bogunovic, M., 2021. Neuro-innate immune interactions in gut mucosal immunity. Curr. Opin. Immunol. 68, 64–71. https://doi.org/10.1016/j.coi.2020.09.007.
Kuźmiński, A., Przybyszewski, M., Graczyk, M., Żbikowska-Gotz, M., Sokołowska-Ukleja, N., Tomaszewska, A., Bartuzi, Z., 2020. Selected allergic diseases of the gastrointestinal tract. Przegląd Gastroenterol. 15 (3), 194–199. https://doi.org/10.5114/pg.2019.87681.
Lim, S.Z., Chua, E.W., 2018. Revisiting the role of thiopurines in inflammatory bowel disease through pharmacogenomics and use of novel methods for therapeutic drug monitoring. Front. Pharmacol. 9 (OCT), 1–16. https://doi.org/10.3389/fphar.2018.01107.
Longo, S., Chieppa, M., Cossa, L.G., Spinelli, C.C., Greco, M., Maffia, M., Giudetti, A.M., 2020. New insights into inflammatory bowel diseases from proteomic and lipidomic studies. In: Proteomes, vol. 8. MDPI AG. https://doi.org/10.3390/PROTEOMES8030018. Issue 3.
MacLaughlin, J.A., Anderson, R.R., Holick, M.F., 1982. Spectral character of sunlight modulates photosynthesis of. Science 216, 1001–1003. May.
McGilligan, V.E., Wallace, J.M.W., Heavey, P.M., Ridley, D.L., Rowland, I.R., 2007. Hypothesis about mechanisms through which nicotine might exert its effect on the interdependence of inflammation and gut barrier function in ulcerative colitis. Inflamm. Bowel Dis. 13 (1), 108–115. https://doi.org/10.1002/ibd.20020.
Mogilevski, T., Burgell, R., Aziz, Q., Gibson, P.R., 2019. Review article: the role of the autonomic nervous system in the pathogenesis and therapy of IBD. Aliment Pharmacol. Therapeut. 50 (7), 720–737. https://doi.org/10.1111/apt.15433.

Moum, B., Hovde, Ø., Høivik, M.L., 2014. What have we learnt about the role of the environment and natural course of IBD in the new millennium? 20-year follow-up of the IBSEN cohort. Dig. Dis. 32 (s1), 2–9. https://doi.org/10.1159/000367818.

Muller, P.A., Koscsó, B., Rajani, G.M., Stevanovic, K., Berres, M.L., Hashimoto, D., Mortha, A., Leboeuf, M., Li, X.M., Mucida, D., Stanley, E.R., Dahan, S., Margolis, K.G., Gershon, M.D., Merad, M., Bogunovic, M., 2014. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. Cell 158 (2), 300–313. https://doi.org/10.1016/j.cell.2014.04.050.

Munyaka, P., Rabbi, M.F., Pavlov, V.A., Tracey, K.J., Khafipour, E., Ghia, J.E., 2014. Central muscarinic cholinergic activation alters interaction between splenic dendritic cell and CD4+CD25- T cells in experimental colitis. PLoS One 9 (10), 1–12. https://doi.org/10.1371/journal.pone.0109272.

Murray, K., Reardon, C., 2018. The cholinergic anti-inflammatory pathway revisited. Neuro Gastroenterol. Motil. 30 (3). https://doi.org/10.1111/nmo.13288, 0–3.

Narula, N., Wong, E.C.L., Dehghan, M., Mente, A., Rangarajan, S., Lanas, F., Lopez-Jaramillo, P., Rohatgi, P., Lakshmi, P.V.M., Varma, R.P., Orlandini, A., Avezum, A., Wielgosz, A., Poirier, P., Almadi, M.A., Altuntas, Y., Ng, K.K., Chifamba, J., Yeates, K., et al., 2021. Association of ultra-processed food intake with risk of inflammatory bowel disease: prospective cohort study. BMJ 374. https://doi.org/10.1136/bmj.n1554.

Nemeth, Z.H., Bogdanovski, D.A., Barratt-Stopper, P., Paglinco, S.R., Antonioli, L., Rolandelli, R.H., 2017. Crohn's disease and ulcerative colitis show unique cytokine profiles. Cureus 9 (4). https://doi.org/10.7759/cureus.1177.

Ng, S.C., Kaplan, G.G., Tang, W., Banerjee, R., Adigopula, B., Underwood, F.E., Tanyingoh, D., Wei, S.C., Lin, W.C., Lin, H.H., Li, J., Bell, S., Niewiadomski, O., Kamm, M.A., Zeng, Z., Chen, M., Hu, P., Ong, D., Ooi, C.J., et al., 2019. Population density and risk of inflammatory bowel disease: a prospective population-based study in 13 countries or regions in asia-pacific. Am. J. Gastroenterol. 114 (1), 107–115. https://doi.org/10.1038/s41395-018-0233-2.

Nunes, N.S., Chandran, P., Sundby, M., Visioli, F., da Costa Gonçalves, F., Burks, S.R., Paz, A.H., Frank, J.A., 2019. Therapeutic ultrasound attenuates DSS-induced colitis through the cholinergic anti-inflammatory pathway. EBioMedicine 45, 495–510. https://doi.org/10.1016/j.ebiom.2019.06.033.

Oka, A., Sartor, R.B., 2020. Microbial-Based and microbial-targeted therapies for inflammatory bowel diseases. Dig. Dis. Sci. 65 (3), 757–788. https://doi.org/10.1007/s10620-020-06090-z.Microbial-Based.

Park, M.I., Camilleri, M., 2006. Is there a role of food allergy in irritable bowel syndrome and functional dyspepsia? A systematic review. Neuro Gastroenterol. Motil. 18 (8), 595–607. https://doi.org/10.1111/j.1365-2982.2005.00745.x.

Pasricha, T.S., Zhang, H., Zhang, N., Chen, J.D.Z., 2020. Sacral nerve stimulation prompts vagally-mediated amelioration of rodent colitis. Physiol. Rep. 8 (1), 1–7. https://doi.org/10.14814/phy2.14294.

Pelissier-Rota, M.A., Pelosi, L., Meresse, P., Jacquier-Sarlin, M.R., 2015. Nicotine-induced cellular stresses and autophagy in human cancer colon cells: a supportive effect on cell homeostasis via up-regulation of Cox-2 and PGE2 production. Int. J. Biochem. Cell Biol. 65, 239–256. https://doi.org/10.1016/j.biocel.2015.06.013.

Pellissier, S., Dantzer, C., Mondillon, L., Trocme, C., Gauchez, A.S., Ducros, V., Mathieu, N., Toussaint, B., Fournier, A., Canini, F., Bonaz, B., 2014. Relationship between Vagal Tone, Cortisol, TNF-alpha, epinephrine and negative affects in Crohn's disease and irritable bowel syndrome. PLoS One 9 (9), 1–9. https://doi.org/10.1371/journal.pone.0105328.

Peyrin-Biroulet, L., Sandborn, W., Sands, B.E., Reinisch, W., Bemelman, W., Bryant, R.V., D'Haens, G., Dotan, I., Dubinsky, M., Feagan, B., Fiorino, G., Gearry, R., Krishnareddy, S., Lakatos, P.L., Loftus, E.V., Marteau, P., Munkholm, P., Murdoch, T.B., Ordás, I., et al., 2015. Selecting therapeutic targets in inflammatory bowel disease (STRIDE): determining therapeutic goals for treat-to-target. Am. J. Gastroenterol. 110 (9), 1324–1338. https://doi.org/10.1038/ajg.2015.233.

Piovani, D., Danese, S., Peyrin-Biroulet, L., Nikolopoulos, G.K., Lytras, T., Bonovas, S., 2019. Environmental risk factors for inflammatory bowel diseases: an umbrella review of meta-analyses. Gastroenterology 157 (3), 647–659. https://doi.org/10.1053/j.gastro.2019.04.016 e4.

Popp, V., Gerlach, K., Mott, S., Turowska, A., Garn, H., Atreya, R., Lehr, H.A., Ho, I.C., Renz, H., Weigmann, B., Neurath, M.F., 2017. Rectal delivery of a DNAzyme that specifically blocks the transcription factor GATA3 and reduces colitis in mice. Gastroenterology 152 (1), 176–192. https://doi.org/10.1053/j.gastro.2016.09.005 e5.

Pullan, R., Rhodes, J., Ganesh, S., Mani, V., Morris, J., Williams, G., Sawe, U., 1994. Transdermal nicotine for active ulcerative colitis. N. Engl. J. Med. 330 (12), 811–815. https://doi.org/10.1056/nejm199403243301202.

Rosas-Ballina, M., Olofsson, P.S., Ochani, M., Sergio, I., Valdés- Ferrer, Y.A.L., Reardon, C., Tusche, M.W., Pavlov, V.A., Andersson, U., Chavan, S., Mak, T.W., Tracey, K.J., Kevin, 2011. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science 334 (6052), 98–101. https://doi.org/10.1126/science.1209985.Acetylcholine-Synthesizing.

Rosen, M.J., Karns, R., Vallance, J.E., Bezold, R., Waddell, A., Collins, M.H., Haberman, Y., Minar, P., Baldassano, R.N., Hyams, J.S., Baker, S.S., Kellermayer, R., Noe, J.D., Griffiths, A.M., Rosh, J.R., Crandall, W.V., Heyman, M.B., Mack, D.R., Kappelman, M.D., et al., 2017. Mucosal expression of type 2 and type 17 immune response genes distinguishes ulcerative colitis from colon-only crohn's disease in treatment-naive pediatric patients. Gastroenterology 152 (6), 1345–1357. https://doi.org/10.1053/j.gastro.2017.01.016 e7.

Rubin, D.T., Ananthakrishnan, A.N., Siegel, C.A., Sauer, B.G., Long, M.D., 2019. ACG clinical guideline: ulcerative colitis in adults. Am. J. Gastroenterol. 114 (3), 384–413. https://doi.org/10.14309/ajg.0000000000000152.

Salaga, M., Blomster, L., Piechota-Polańczyk, A., Zielińska, M., Jacenik, D., Cygankiewicz, A., Krajewska, W., Mikkelsen, J., Fichna, J., 2015. Encenicline, an α7 nicotinic acetylcholine receptor partial agonist, reduces immune cell infiltration in the colon and improves experimental colitis in mice. J. Pharmacol. Exp. Therapeut. 356 (1), 157–169. https://doi.org/10.1124/jpet.115.228205.

Sandborn, W.J., Tremaine, W.J., Leighton, J.A., Lawson, G.M., Zins, B.J., Compton, R.F., Mays, D.C., Lipsky, J.J., Batts, K.P., Offord, K.P., Hurt, R.D., Green, J., 1997. Nicotine tartrate liquid enemas for mildly to moderately active left-sided ulcerative colitis unresponsive to first-line therapy: a pilot study. Aliment Pharmacol. Therapeut. 11 (4), 663–671. https://doi.org/10.1046/j.1365-2036.1997.00208.x.

Schneider, C., O'Leary, C.E., Locksley, R.M., 2019. Regulation of immune responses by tuft cells. Nat. Rev. Immunol. 19 (9), 584–593. https://doi.org/10.1038/s41577-019-0176-x.

Schütz, B., Jurastow, I., Bader, S., Ringer, C., von Engelhardt, J., Chubanov, V., Gudermann, T., Diener, M., Kummer, W., Krasteva-Christ, G., Weihe, E., 2015. Chemical coding and chemosensory properties of cholinergic brush cells in the mouse gastrointestinal and biliary tract. Front. Physiol. 6 (MAR), 1–14. https://doi.org/10.3389/fphys.2015.00087.

Shao, X., Yang, L., Hu, K., Shen, R., Ye, Q., Yuan, X., Zhao, Q., Shen, J., 2020. Serum Cholinesterases, a Novel Marker of Clinical Activity in Inflammatory Bowel Disease: A Retrospective Case-Control Study. Mediators of Inflammation, 2020. https://doi.org/10.1155/2020/4694090.

Singh, S.P., Chand, H.S., Banerjee, S., Agarwal, H., Raizada, V., Roy, S., Sopori, M., 2020. Acetylcholinesterase inhibitor pyridostigmine bromide attenuates gut pathology and bacterial dysbiosis in a murine model of ulcerative colitis. Dig. Dis. Sci. 65 (1), 141–149. https://doi.org/10.1007/s10620-019-05838-6.

Sinniger, V., Pellissier, S., Fauvelle, F., Trocmé, C., Hoffmann, D., Vercueil, L., Cracowski, J.L., David, O., Bonaz, B., 2020. A 12-month pilot study outcomes of vagus nerve stimulation in Crohn's disease. Neuro Gastroenterol. Motil. 32 (10), 1–16. https://doi.org/10.1111/nmo.13911.

Soreq, H., 2001. Acetylcholinesterase — new roles for an old actor. Nat. Rev. Neurosci. 2 (4), 294–302. https://doi.org/10.1038/35067589.

Tasaka, Y., Yasunaga, D., Kiyoi, T., Tanaka, M., Tanaka, A., Suemaru, K., Araki, H., 2015. Involvement of stimulation of α7 nicotinic acetylcholine receptors in the suppressive effect of tropisetron on dextran sulfate sodium-induced colitis in mice. J. Pharmacol. Sci. 127 (3), 275–283. https://doi.org/10.1016/j.jphs.2014.12.016.

Tracey, K.J., 2002. The inflammatory reflex. In: Nature, vol. 420, pp. 853–859. https://doi.org/10.1038/nature01321, 6917.

Tsuchida, Y., Hatao, F., Fujisawa, M., Murata, T., Kaminishi, M., Seto, Y., Hori, M., Ozaki, H., 2011. Neuronal stimulation with 5-hydroxytryptamine 4 receptor induces anti-inflammatory actions via α7nACh receptors on muscularis macrophages associated with postoperative ileus. Gut 60 (5), 638–647. https://doi.org/10.1136/gut.2010.227546.

Tu, L., Gharibani, P., Yin, J., Chen, J.D.Z., 2020a. Sacral nerve stimulation ameliorates colonic barrier functions in a rodent model of colitis. Neuro Gastroenterol. Motil. 32 (10), 1–13. https://doi.org/10.1111/nmo.13916.

Tu, L., Gharibani, P., Yin, J., Chen, J.D.Z., 2020b. Sacral nerve stimulation ameliorates colonic barrier functions in a rodent model of colitis. Neuro Gastroenterol. Motil. 32 (10). https://doi.org/10.1111/nmo.13916.

Tuček, S., 1982. The synthesis of acetylcholine in skeletal muscles of the rat. J. Physiol. 322 (1), 53–69. https://doi.org/10.1113/jphysiol.1982.sp014022.

Uni, R., Inoue, T., Nakamura, Y., Fukaya, D., Hasegawa, S., Wu, C.H., Fujii, R., Surattichaiyakul, B., Peerapanyasut, W., Ozeki, A., Akimitsu, N., Wada, Y., Nangaku, M., Inagi, R., 2020. Vagus nerve stimulation even after injury ameliorates cisplatin-induced nephropathy via reducing macrophage infiltration. Sci. Rep. 10 (1), 9472. https://doi.org/10.1038/s41598-020-66295-0.

Verheijden, S., Boeckxstaens, G.E., 2018. Neuroimmune interaction and the regulation of intestinal immune homeostasis. Am. J. Physiol. Gastrointest. Liver Physiol. 314 (1), G75–G80. https://doi.org/10.1152/ajpgi.00425.2016.

Wang, H., Yu, M., Ochani, M., Amelia, C.A., Tanovic, M., Susarla, S., Li, J.H., Wang, H., Yang, N., Ulloa, L., Al-Abed, Y., Czura, C.J., Tracey, K.J., 2003. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. Nature 421 (6921), 384–388. https://doi.org/10.1038/nature01339.

Wéra, O., Lancellotti, P., Oury, C., 2016. The dual role of neutrophils in inflammatory bowel diseases. J. Clin. Med. 5 (12), 118. https://doi.org/10.3390/jcm5120118.

Wolford, D.D., Fichera, A., 2020. Prophylaxis of Crohn's disease recurrence: a surgeon's perspective. Annals Gastroenterol. Surgery 4 (5), 514–520. https://doi.org/10.1002/ags3.12368.

Xiao, J., Zhang, G., Gao, S., Shen, J., Feng, H., He, Z., Xu, C., 2020. Combined administration of SHP2 inhibitor SHP099 and the α7nAChR agonist PNU282987 protect mice against DSS-induced colitis. Mol. Med. Rep. 22 (3), 2235–2244. https://doi.org/10.3892/mmr.2020.11324.

Yoshikawa, H., Kurokawa, M., Ozaki, N., Nara, K., Atou, K., Takada, E., Kamochi, H., Suzuki, N., 2006. Nicotine inhibits the production of proinflammatory mediators in human monocytes by suppression of I-κB phosphorylation and nuclear factor-κB

transcriptional activity through nicotinic acetylcholine receptor α7. Clin. Exp. Immunol. 146 (1), 116–123. https://doi.org/10.1111/j.1365-2249.2006.03169.x.

Zaltman, C., Parra, R.S., Sassaki, L.Y., Santana, G.O., de Lourdes Abreu Ferrari, M., Miszputen, S.J., Amarante, H.M.B.S., Kaiser, R.L., Flores, C., Catapani, W.R., Parente, J.M.L., Bafutto, M., Ramos, O., Gonçalves, C.D., Guimaraes, I.M., da Rocha, J.J.R., Feitosa, M.R., Feres, O., Saad-Hossne, R., Chebli, J.M., 2021. Real-world disease activity and sociodemographic, clinical and treatment characteristics of moderate-to-severe inflammatory bowel disease in Brazil. World J. Gastroenterol. 27 (2), 208–223. https://doi.org/10.3748/WJG.V27.I2.208.

Zhang, J., Zhao, Y., Hou, T., Zeng, H., Kalambhe, D., Wang, B., Shen, X., Huang, Y., 2020. Macrophage-based nanotherapeutic strategies in ulcerative colitis. J. Contr. Release 320, 363–380. https://doi.org/10.1016/j.jconrel.2020.01.047.

Zheng, W.-P., Flavell, R.A., 1997. The transcription factor GATA-3 is necessaryand sufficient for Th2 cytokine GeneExpression in CD4 T cells. Cell 89, 587–596. https://doi.org/10.1016/S0030-6657(08)70226-9.