Bioelectronics in the brain–gut axis: focus on inflammatory bowel disease (IBD)

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Abstract

Accumulating evidence shows that intestinal homeostasis is mediated by cross-talk between the nervous system, enteric neurons and immune cells, together forming specialized neuroimmune units at distinct anatomical locations within the gut. In this review, we will particularly discuss how the intrinsic and extrinsic neuronal circuitry regulates macrophage function and phenotype in the gut during homeostasis and aberrant inflammation, such as observed in inflammatory bowel disease (IBD). Furthermore, we will provide an overview of basic and translational IBD research using these neuronal circuits as a novel therapeutic tool. Finally, we will highlight the different challenges ahead to make bioelectronic neuromodulation a standard treatment for intestinal immune-mediated diseases.

Keywords: enteric nervous system, macrophages, vagus nerve

Introduction

Bioelectronic medicine combines the worlds of neuroscience, engineering and computational science to provide a powerful tool for the diagnosis and treatment of a range of neurological and inflammatory diseases using state-of-the-art medical devices. This multidisciplinary field emerged from the revolutionary discovery that the nervous system regulates biological processes underlying health and disease using closed-loop mechanisms called neural reflexes. These include sensing, integration and effector responses [1, 2]. Since most organs are neurally innervated, sensory signals could be collected throughout the body by recording nerve activity and subsequently integrated by decoding nerve activity patterns. Conversely, the function of a variety of organs can be modulated by electrically stimulating their innervation, clearly illustrating the potential of bioelectronic medicine with respect to both diagnostic and therapeutic applications. Bioelectronic neuromodulation offers clear advantages compared to standard pharmacological treatments by providing real-time and personalized therapy with a higher anatomical specificity and fewer adverse effects than conventional drug treatments [2, 3].

Since the group of Tracey showed that vagus nerve stimulation (VNS) potently attenuated cytokine production in a model of sepsis [1], the vagus nerve, the main parasympathetic nerve, became an interesting target for neuromodulation. This groundbreaking research led to the introduction of the concept of the cholinergic anti-inflammatory pathway (CAP),

a hard-wired connection between the nervous system and the immune system closely interacting to control inflammation [1]. This loop consists of sensory neurons detecting inflammation and transmitting neural signals to the central nervous system, which ultimately modulates peripheral inflammation locally via the release of neuronal mediators from efferent nerves [4]. In 2005, we and others extended the CAP to the gut revealing anti-inflammatory properties of VNS in different models of intestinal inflammation [5–8], including inflammatory bowel disease (IBD) [9–12].

In the present review, we will discuss the current knowledge and the clinical implications of intestinal neuromodulation with a focus on IBD. In addition, we will describe the different challenges ahead to make bioelectronic medicine a potential treatment for intestinal immune-mediated diseases.

Innervation of the gastrointestinal tract

The gastrointestinal (GI) tract itself is under the intrinsic control of the enteric nervous system (ENS), also referred to as the 'little brain of the gut'. It is a complex, extensive network of interconnected neurons and glial cells arranged in the sub-mucosal plexus and myenteric plexus, capable of functioning autonomously from the central nervous system [13]. The sub-mucosal plexus regulates fluid secretion and nutrient absorption, whereas the myenteric plexus mainly coordinates gut muscle contractility. Both plexi are

composed of intrinsic primary afferent neurons (IPANs), interneurons and motor neurons. In particular, IPANs sense mechanical and molecular alterations in the GI tract, information that is relayed to intrinsic motor neurons via other IPANs and interneurons (13). Although the majority of enteric neurons are cholinergic in origin, the ENS communicates via a variety of neurotransmitters and neuropeptides including nitric oxide, adenosine triphosphate, vasoactive intestinal peptide and calcitonin gene-related peptide (CGRP) (4).

The extrinsic neural control of the gut is composed of both sympathetic and parasympathetic nerve fibers, which regulate vital functions such as motility, blood flow and fluid secretion (4). The vagus nerve, representing the principal component of the parasympathetic nervous system, innervates the GI tract from the esophagus to the descending colon. It comprises two major branches, i.e. the left (or anterior) and right (or posterior) vagus nerves. The left branch innervates the GI tract until the duodenum, whereas the right vagus nerve reaches until the colonic splenic flexure (14, 15). To what extent the distal intestine receives vagal input in human is under debate by anatomists (16).

The vagus nerve itself is a mixed nerve composed of 80% afferent and 20% efferent fibers. Vagal afferents relay information about GI function including inflammation centrally to the nucleus tractus solitarius (NTS). In turn, GI function is modulated via efferent vagal fibers originating from two nuclei located in the brain stem, i.e. the nucleus ambiguous (NA) and the dorsal motor nuclei of the vagus nerve (DMV). Of note, gut vagal efferents synapse with neurons located in the ENS (Fig. 1) (17, 18).

Besides its parasympathetic input, the GI tract is also innervated by the sympathetic nervous system (50% efferent and 50% afferent). Several brain centers such as paraventricular nucleus of the hypothalamus, locus coeruleus, the A5 noradrenergic group and the C1 adrenergic group activate efferent sympathetic pathways (19, 20), which pass through the intermediolateral column of the spinal cord to reach the abdominal pre-vertebral ganglia innervating the GI tract (Fig. 1), i.e. the stomach receives innervation from the celiac ganglion, whereas the small intestine and colon are innervated by the celiac ganglion, superior or inferior mesenteric ganglion or pelvic ganglion. From the pre-vertebral ganglia, postganglionic sympathetic noradrenergic fibers arise, which together with arteries enter the gut via the mesenteric serosal surface (Fig. 1). There, they terminate in the ENS and near blood vessels in (sub)mucosa to regulate secretion, motility and vascular tone as well as inflammation (21).

Sympathetic spinal afferents from the GI tract whose cell bodies are located in the dorsal root ganglion synapse in the dorsal horn of the thoracolumbar spinal cord (Fig. 1). These spinal afferents relay information on pain, inflammation and discomfort to the central nervous system.

The sympathetic nervous system also branches to the gut-associated lymphoid tissue (GALT). Interestingly, within the GALT, sympathetic varicosities are found in close approximation to parenchymal immune cells including T cells, macrophages and plasma cells (22). Notably, these sympathetic varicosities release norepinephrine (NE) 'en passage' allowing diffusion up to 1 μm before interacting with immune cells (23).

Neuroimmune cross-talk in the gut and beyond

Cholinergic modulation in the intestine

The GI tract is continuously exposed to the lumen harboring commensals, pathogens and dietary food antigens. The gut is therefore equipped with a highly diverse population of innate and adaptive immune cells strategically positioned in the gut wall to protect our body against invading pathogens, while also remaining tolerant towards trillions of commensal bacteria (24, 25). Particularly, macrophages (Mϕ) are historically known for their role in host defense and pathogen clearance, but are now increasingly recognized as a heterogeneous population of tissue-supportive cells, exemplified by their distinct gene expression profile (26). Their function is mainly driven by intrinsic features such as origin, and by environmental factors produced in the niche in which they reside (Fig. 2).

In the lamina propria, CX3CR1^{hi} Mϕ are the first defenders against organisms invading from the intestinal lumen, a task which they accomplish by constantly surveilling the environment, phagocytosing harmful antigens and promoting epithelial cell renewal. In addition, lamina propria Mϕ prevent excessive inflammation in response to harmless commensals and promote tolerance by favoring the expansion of antigen-specific CD4$^+$CD25$^+$ regulatory T cells (T$_{regs}$), mainly via the production of IL-10, retinoic acid and transforming growth factor β (TGF-β) (Fig. 3a) (27, 28). Thus, lamina propria Mϕ are considered key players in the establishment and maintenance of mucosal homeostasis (27, 29). On the other hand, in the *muscularis externa*, Mϕ are found in close association with the ENS to maintain neuronal health (30) and promote peristalsis using signaling via bone morphogenetic protein 2 (BMP2) and its receptor (BMP2R) (Fig. 3b) (31). Interestingly, these different populations of intestinal Mϕ express receptors for neuropeptides and neurotransmitters including neurokinin receptors, glycine receptors, α7 and β2 nicotinic acetylcholine receptors (nAChR), β2 adrenergic receptor (β2-AR) and P2 purine receptors (26, 32–35). Moreover, enteric neurons were found to produce CSF1, crucial for the maintenance of the Mϕ compartment (31), indicating that neuronally derived mediators modulate Mϕ function in the gut (Fig. 3b).

The concept that the nervous system modulates Mϕ was first introduced in 2000 by the group of Tracey (1). In a model of sepsis, they showed that electrical VNS reduced TNF-α release from splenic α7nAChR$^+$ Mϕ resulting in increased survival (36, 37). This splenic CAP is supposed to be triggered by a direct connection between the efferent vagus nerve and the sympathetic splenic nerve at the level of the celiac ganglion, causing the release of NE within the splenic white pulp (Fig. 1). NE promoted ACh secretion by β2-AR$^+$ ChAT$^+$ T cells interacting with α7nAChR located on adjacent Mϕ (38, 39). Notably, the exact neuronal circuitry of this splenic CAP is still under debate, since some authors argue that the anti-inflammatory properties of VNS is exerted via vago-sympathetic or vago-vagal pathways [for excellent reviews on this topic, we refer to references (40–42)].
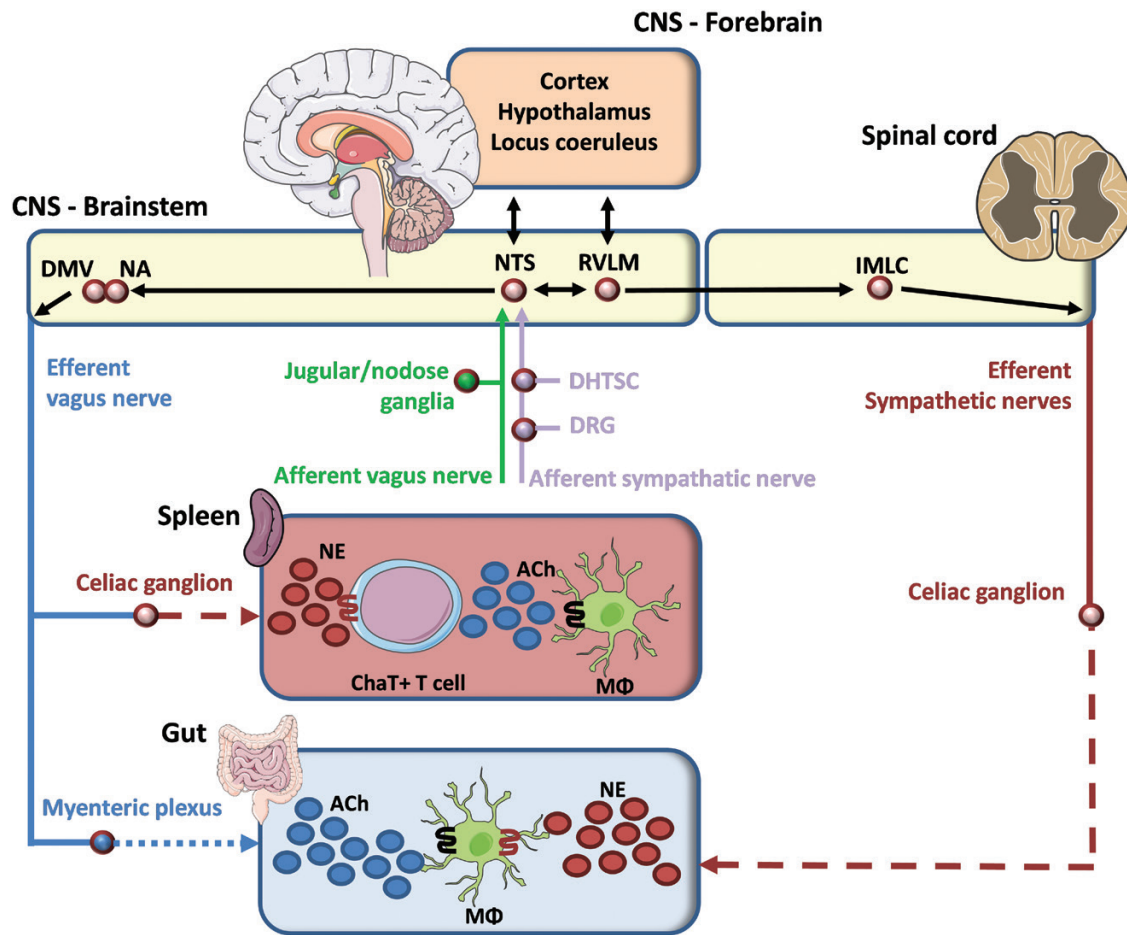
Fig. 1. Brain–gut signaling pathways. Gut afferents from the vagus and sympathetic nerves are transmitted to the NTS interacting with the DMV and NA, the origin of vagal efferents. In the case of overt inflammation, the vagal efferent arm modulates the immune response in the spleen via the celiac ganglion. Particularly, norepinephrine (NE) binds to a subset of ChAT$^+$ T cells leading to the release of ACh interacting with α7nAChR$^+$ Mϕ. During localized intestinal inflammation, the central nervous system (CNS) is alarmed by gut afferents which will lead to the activation of vagal efferents synapsing on myenteric neurons. The latter release ACh modulating intestinal inflammation via the interaction between ACh and α7nAChR$^+$ Mϕ. Notably, intestinal infection promotes the tissue-protective phenotype of Mϕ, an effect mediated by the interaction between NE and Mϕ that express β2 adrenergic receptor. DHTSC, dorsal horn of the thoracolumbar spinal cord; DRG, dorsal root ganglion; IMLC, intermediolateral cell column of spinal cord; RVLM, rostral ventrolateral medulla.

In the gut, we and others discovered that the enteric neurons and Mϕ share anatomical niches and interact functionally in certain regions of the intestine to maintain homeostasis, but also modulate inflammation (24, 30, 43, 44). Inflammatory mediators released during a localized inflammation are sensed by gut vagal afferents via binding of cytokines and chemokines such as IL-1 and mast cell mediators (45). For instance, intestinal inflammation in response to *Campylobacter jejuni* infection or intestinal manipulation increased the expression of c-Fos, a marker of neuronal activity, in the NTS (43, 46) and subsequently the DMV (43). Using antegrade and retrograde tracing experiments, we previously showed that the activated neurons in the DMV are connected to the site of inflammation, supporting the concept of a hard-wired neuronal circuitry able to modify intestinal inflammation (43).

The therapeutic properties of this neuronal circuitry in the gut were clearly demonstrated in a model of postoperative ileus, a condition characterized by an impaired GI motility due to a subtle inflammation of the *muscularis externa* in response to surgical handling. The key orchestrators of this inflammatory process are the muscularis Mϕ (mMϕ). Interestingly, electrical stimulation of the efferent cervical and abdominal vagus nerve attenuated inflammation in the *muscularis externa* by activation of cholinergic enteric neurons in close approximation to α7nAChR$^+$ mMϕ (Fig. 3b) (6, 44, 47).

This inhibitory effect on mMϕ is, however, most likely mediated by enteric neurons, as anterograde tracer studies showed that vagal efferents only communicate with cholinergic enteric neurons but not directly with mMϕ (43). In line with this, stimulation of cholinergic enteric neurons using specific 5-hydroxytryptamine receptor 4 agonists dampened the inflammatory response and significantly improved clinical recovery in a model of surgery-induced inflammation, an observation that was confirmed in patients undergoing colonic resection (44, 48, 49).

Whether electrical stimulation of the vagus nerve also has a similar anti-inflammatory effect in the lamina propria remains
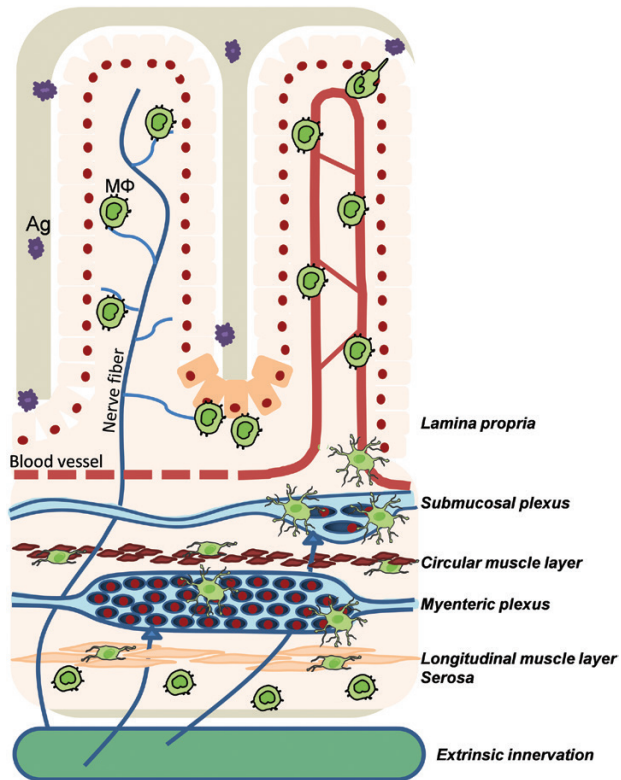
Fig. 2. Anatomical distribution of intestinal Mφ. Gut Mφ reside within different layers of the GI tract. Mφ (green) are mostly located randomly in the villi of the lamina propria, but are also located in close association with Paneth cells (orange), epithelium, blood vessels (red) and nerve fibers (blue). More distal from the lumen, sub-mucosal Mφ are found nearby sub-mucosal neurons and blood vessels (red). Within the muscularis externa, stellate Mφ can be located in the myenteric plexus, and bipolar Mφ are interspaced between circular and the longitudinal muscle fibers. Finally, a sub-population of intestinal Mφ resides in the serosa.

to be further elucidated. Of interest though, abdominal vagotomy was reported to elevate the basal expression of NF-κB and the production of pro-inflammatory cytokines in the lamina propria (50, 51). In addition, oral tolerance towards commensals and dietary antigens is impaired in vagotomized mice, an observation that is closely related with a reduced induction and expansion of T_{regs} in the lamina propria (52). Since Hadis *et al.* revealed that lamina propria CX3CR1+ Mφ are the key producers of IL-10, crucial to maintain intestinal homeostasis (27), one can argue that vagal input is involved in driving IL-10 production in these immune cells. This hypothesis is indirectly supported by the fact that cholinergic fibers have been observed in close approximation to intestinal monocytes and Mφ at the level of the sub-mucosal plexus and lamina propria (6, 43).

Interestingly, Teratani *et al.* recently discovered that the hepatic vagal afferents are also able to indirectly sense the gut microenvironment and relay information to the NTS, and ultimately to the efferent vagus nerve and enteric neurons (53). In particular, surgical or chemical disruption of hepatic vagal afferents significantly decreased the number of colonic T_{regs}, the T-cell sub-population crucial to maintain homeostasis

in the gut lamina propria, because of reduced levels of aldehyde dehydrogenase (ALDH; gene encoding the retinoic acid-synthesizing enzymes RALDH1 and RALDH) and retinoic acid in intestinal antigen-presenting cells including Mφ. Reciprocally, activation of muscarinic acetylcholine receptors (mAChR) on colonic antigen-presenting cells increased ALDH expression. Notably, disruption of the left hepatic branch also resulted in increased susceptibility to colitis most likely caused by the reduction of the colonic pool of T_{regs}, thus demonstrating the existence of a novel vago-vagal liver–brain–gut reflex.

Interestingly, capsaicin-sensitive afferent nerves also possess anti-inflammatory properties in the intestine. Indeed, previous studies have showed that electrical and physiological stimulation of afferents causes the release of neurotransmitters such as tachykinins and CGRP (54), which reduced mucosal damage in the gut (55–58). On the other hand, ChAT+ T_h17 cells, the cell population previously identified as the effector cells of the splenic CAP, were also discovered in the GALT and lamina propria of the gut. These ChAT+ T_h17 cells promoted epithelial AMP production via β2-AR stimulation on dendritic cells in the healthy intestine, potentially protecting the gut from mucosal damage during inflammation (59). Indeed, during the resolution of dextran sulfate sodium (DSS)-induced colitis, ChAT+ T_h17 cells supported mucosal healing (60). Hence, both neural and non-neural targets could be used to modulate immune responses during mucosal inflammation.

Sympathetic modulation of the intestine

Besides the parasympathetic nervous system, the GI tract is also densely innervated by the sympathetic nervous system. Here, its main functions are the regulation of blood flow, secretion and motility. The sympathetic fibers mostly innervate the sub-mucosal plexus and myenteric plexus as well as the intestinal mucosa (61). In contrast to the vagus nerve, the sympathetic nervous system was observed to be in direct communication with immune cells especially in the GALT (23, 62). Interestingly, intestinal Mφ express various (nor)adrenergic receptors, supporting the hypothesis that sympathetic fibers may control the intestinal immune response (38, 63, 64).

Notably, adrenergic receptors were shown to have different thresholds of activation depending on the catecholamine concentration: high concentrations activate β-adrenoreceptors having anti-inflammatory effects, whereas low concentrations preferentially activate α-adrenoreceptors leading to pro-inflammatory mediator release (65). This difference may explain the contrasting data obtained in animal and clinical studies investigating the pro- and anti-inflammatory effects of the sympathetic nervous system during local inflammation. Indeed, chemical sympathectomy using 6-hydroxydopamine treatment was shown to decrease the severity of acute DSS-induced and 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis (66, 67), whereas it worsened the disease course in chronic DSS-induced colitis (68).

These controversial results suggest that sympathetic nerve fibers may have a dual role: they promote inflammation during acute disease, but confer resolution during
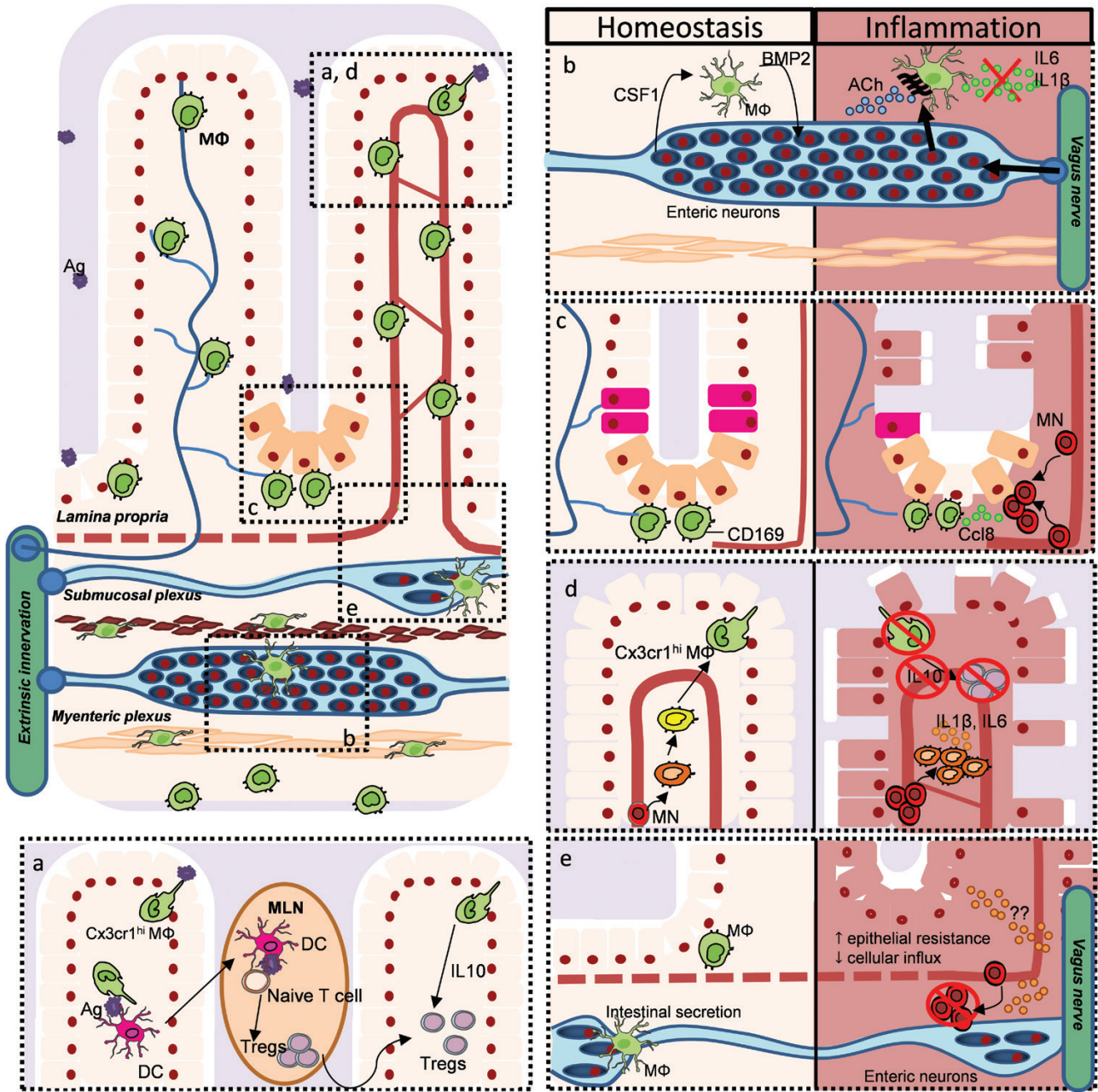
Fig. 3. Neuroimmune interactions in the gut during health and disease. Areas designated (a–e) in the main part of the figure (upper left) are expanded and adapted in the rest of the figure. For example, in the lower left, (a) lamina propria (LP) CX3CR1^{hi} Mφ (green) promote oral tolerance via sampling the lumen and transferring trapped antigens (purple) to dendritic cells (DC; pink). The latter cells migrate to mesenteric lymph nodes (MLNs) to induce naive T cells to become FoxP3$^+$ T$_{regs}$, which then migrate back to the LP. LP CX3CR1^{hi} Mφ maintain the T$_{reg}$ population by their IL-10 production. (b) Within the muscular externa, Mφ are maintained by CSF1 produced by enteric neurons. Reciprocally, Mφ produce BMP2 regulating peristalsis. During DSS-induced colitis, VNS dampens Mφ activation via ACh binding to α7nACh$^+$ Mφ. (c) CD169$^+$ Mφ are found close to the intestinal crypt where they promote epithelial proliferation (pink) and Paneth cell (light orange) differentiation. During DSS-induced colitis, CD169$^+$ Mφ release CCL8, thus promoting the influx of blood leukocytes. (d) The LP Mφ pool is constantly replenished by incoming Ly6C^{hi} monocytes (red) that differentiate into mature CX3CR1^{hi} Mφ. During intestinal inflammation, the differentiation of Ly6C^{hi} monocytes into CX3CR1^{hi} Mφ is arrested. As such, immature CX3CR1^{int} Mφ (orange) are retained in the LP, where they fail to release IL-10, thus reducing the T$_{reg}$ pool. (e) Within the sub-mucosa, Mφ support neuronal and vascular health. During DSS-induced colitis, VNS reduces the influx of pro-inflammatory leukocytes, but also increases epithelial resistance (N. Stakenborg and G. E. Boeckxstaens, unpublished results).

the chronic phase of inflammation (69). Yet again, the method of sympathectomy may also affect results, since surgical sympathectomy, in contrast to chemical sympathectomy, exacerbated colitis severity during acute

disease (70). Nevertheless, intermittent electrical stimulation of sympathetic fibers at the level of the superior mesenteric nerve improved disease severity in a DSS colitis model (70). Furthermore, a recent retrospective study

showed that β-blockers exacerbated inflammation in IBD patients, increasing relapse risk (71). In line with this, *ex vivo* catecholaminergic treatment of GALT led to lower rates of *Salmonella* translocation (72), potentially mitigating infection. Further evidence for sympathetic neuroimmune interaction in the gut is provided by Gabanyi *et al.* (26). They showed that an enteric infection with *Salmonella typhimurium* augmented the tissue-protective phenotype of mMφ, an effect possibly mediated by interaction between extrinsic sympathetic fibers and mMφ expressing β2-AR (26).

Together, these data suggest that sympathetic anti-inflammatory pathways are activated during gut inflammation, an effect that is most likely mediated via β-AR.

As the sub-mucosal region is highly vascularized and sympathetically innervated, NE could also act as a chemoattractant to recruit circulating immune cells into the lamina propria during inflammation by guiding them to the sympathetic terminals (73). Indeed, Asano *et al.* have discovered a lamina propria CD169^{+} Mφ population that is enriched near the crypt base close to GALT and contributes to disease severity during mucosal inflammation by attracting circulating monocytes through the release of chemoattractant CCL8 (Fig. 3c) (74). As such, neuromodulation of the mucosal sympathetic fibers may potentially halt the influx of inflammatory monocytes during gut inflammation.

Neuromodulation of IBD using bioelectronics

Epidemiology and current treatment strategies

IBD is a debilitating chronic inflammatory disease of the gut with Crohn's disease (CD) and ulcerative colitis (UC) as its two main clinical presentations (75). Whereas UC is restricted to the mucosal layers of the large intestine, CD is characterized by transmural inflammation involving the entire gut. Although the exact etiology of IBD remains to be elucidated, it is evident that genetic predisposition, environmental factors (e.g. stress, diet, etc.), immunity and the intestinal microbiome are implicated in disease development and its progression (76). In particular, IBD patients are known to have an altered immune response to their microbiome. Moreover, it has become apparent that gut commensals promote immune development and homeostasis in the lamina propria. As such, a disturbed gut microbiota is strongly associated with acute and chronic infections (8). Interestingly, metagenomic studies have found that IBD patients have a decreased intestinal microbiota diversity and stability (77).

In addition, aberrant Mφ immune responses towards commensals generally lead to a loss of tolerance, which is believed to underlie the inflammation observed in IBD (78). Pre-clinical evidence shows that in DSS colitis models, Ly6C^{hi} monocytes entering the lamina propria rapidly differentiate into pro-inflammatory effector cells (75, 79–81), a finding that was confirmed in inflamed lamina propria tissue of IBD patients (82–85). Of interest, under inflammatory conditions, the differentiation of these incoming Ly6C^{hi} CX3CR1[lo] monocytes is halted, thus preventing them from fully differentiating into mature CX3CR1[hi] Mφ (79, 82). As such, immature CX3CR1[int] Mφ are retained in the lamina propria where they fail to release

IL-10. Instead, they produce pro-inflammatory mediators and display increased respiratory burst activity, thereby recruiting additional innate and adaptive immune cells including neutrophils, T$_h$1 and T$_h$17 cells (Fig. 3d) (76, 79, 82, 84, 86).

It has been proposed that these recruited inflammatory cells play a pathological role in DSS-induced colitis. Indeed, genetic depletion of CCR2 ameliorates inflammation in acute DSS-induced colitis (87, 88). However, the mechanisms underlying the halted differentiation of recruited Ly6C^{hi} monocytes to mature CX3CR1[hi] Mφ in the context of intestinal inflammation remains to be further elucidated (Fig. 3d). Yet, a reduction of essential mediators promoting differentiation including retinoic acid, IL-10 and TGF-β as well as the increased pro-inflammatory milieu in the lamina propria may inhibit proper Mφ differentiation in the gut.

The phase of intestinal inflammation is followed by a resolution phase. This repeated cycle of inflammation and resolution eventually leads to complications such as intestinal stenosis and fibrosis, bleeding and fistula formation (89). Given the above findings that monocytes and Mφ appear to be the main orchestrators of the inflammatory cascade during IBD flares, there is renewed interest in targeting the monocyte/macrophage lineage for prophylactic or therapeutic purposes in IBD. Especially as inhibition or even prevention of activation of Mφ may prove effective in treating/preventing IBD flares.

Current lines of IBD treatments consist of anti-inflammatory, immunosuppressive and biological therapeutics [for excellent review, we refer to references (29, 90)]. Major limitations of these therapies are their side-effects including systemic immunosuppression, loss of effectiveness over time in a certain percentage of patients and development of refractory disease because of chronic treatment (76). In the latter case, surgical resection of the involved gut is inevitable. Unfortunately, this surgical intervention does not prevent recurrence of the disease in a significant proportion of IBD patients despite the patients continuing their biological or pharmacological treatment (76). Therefore, new treatments such as bioelectronic therapy that are more targeted and give fewer side-effects are necessary.

Rationale of targeting the autonomic nervous system in IBD

IBD patients readily present with a blunted vagal tone and a sympathetic overbalance (91, 92). Several studies have demonstrated its predictive value in disease progression of chronic inflammatory disorders including rheumatoid arthritis and IBD (9, 11, 93). Indeed, Pellissier *et al.* showed that impaired vagal activity is associated with a pro-inflammatory profile in IBD patients (i.e. high levels of serum TNF-α and salivary cortisol levels) (93, 94).

These findings support the hypothesis that autonomic dysfunction could interfere with the anti-inflammatory properties of the CAP, causing increased levels of pro-inflammatory cytokines (95), a finding that was confirmed in pre-clinical studies. In particular, reduced vagal input induced by abdominal vagotomy aggravated the severity of DSS-induced colitis and arrested the expansion of T$_{regs}$ in the lamina propria following DSS administration (51, 52, 96–98). Interestingly,

vagotomized mice deficient in Mφ, i.e. M-CSF[op/op] mice, did not develop colitis, underlying a key role for Mφ in the CAP (96). Therefore, restoring the vago-sympathetic balance through VNS may be crucial to reduce the recurrence of IBD via modulation of gut Mφ.

Pharmacological modulation of anti-inflammatory pathways in IBD: a tool to unravel the underlying mechanisms of CAP in IBD

The concept of exploiting the autonomic nervous system, especially the CAP, has become appealing and may be a promising approach to treat IBD. Given the potent anti-inflammatory properties of the cholinergic network, one might assume that increased cholinergic activity in the sub-mucosal plexus could impact on mucosal immune homeostasis (69). This can be achieved through acetylcholinesterase (AChE) inhibitors. AChE is an enzyme that rapidly hydrolyzes ACh to terminate synaptic transmission in both central and peripheral pathways.

Inactivation of AChE maximizes the half-life of ACh, increasing the stimulation of muscarinic and nicotinic ACh receptors. For instance, pre-treatment with physostigmine, an AChE inhibitor able to cross the blood–brain barrier (BBB), attenuated the severity of dinitrobenzene sulfonic acid (DNBS)-induced colitis to a greater extent than neostigmine, an AChE inhibitor unable to cross the BBB, suggesting central cholinergic pathways exert a greater protective effect than peripheral pathways (99). Also in DSS-induced colitis, the AChE inhibitors pyridostigmine and rivastigmine ameliorated the disease course evidenced by decreased macroscopic damage, reduced myeloperoxidase (MPO) infiltration and inflammatory mediators (100, 101).

Interestingly, more mechanistic studies using galantamine, yet another AChE inhibitor able to cross the BBB, revealed anti-inflammatory effects in the intestine via interaction with central mAChR (102), provoking increased efferent vagal activity (103). Accordingly, administration of galantamine alleviated mucosal inflammation in DNBS-induced and DSS-induced colitis as observed by reduced major histocompatibility complex class II (MHCII) levels and reduced pro-inflammatory mediators (i.e. IL1β, IL6 and TNF-α) released by splenic CD11c^{+} cells (104). Similarly, galantamine reduced TNBS-induced ulcers, neutrophil adhesion, MPO infiltration and pro-inflammatory mediators, while increasing anti-apoptotic signaling pathways and IL-10 production (105).

Central activation of the CAP was also effective in alleviating DNBS-induced colitis using the M1 mAChR agonist, McN-A343. Indeed, intra-cerebroventricular injection of this compound decreased colonic inflammation as shown by a reduced pro-inflammatory T_h1/T_h17 colonic and splenic cytokine release, an effect mediated by modulation of interaction between splenic dendritic cells and CD4^{+}CD25^{-} T cells via α7nAChR and NF-κB signaling pathways. Of note, this anti-inflammatory effect was abrogated in vagotomized and splenectomized mice, indicating that gut inflammation is controlled via a vagus nerve-to-spleen neural connection (50, 104)

Another approach to pharmacologically mimic the anti-inflammatory properties of the CAP is the use of α7nAChR agonists. This strategy is based on the knowledge that the splenic and intestinal CAP is mediated by α7nAChR located on Mφ. However, the true involvement of this receptor remains ambiguous in colitis and by extension the lamina propria. For example, administration of nicotine, a non-selective agonist for nAChR, consistently improves colitis severity and inflammation in DSS-induced colitis (96, 106, 107). Other studies, however, showed that male α7nAChR^{-/-} mice had a more severe disease course of DSS colitis than their littermate controls (104, 108, 109). In line with this, administration of (partial) α7nAChR agonists (i.e. choline, PHA-543613, GTS21) alleviated the disease activity index (DAI) score and colonic inflammation in DSS-induced colitis (106, 108), whereas other α7nAChR agonists, i.e. AR-R17779 and GSK1345038A, worsened disease activity (110). Thus, to what extent α7nAChR agonists are potential targets to treat IBD require further investigation.

Bioelectronic medicine in IBD

Pre-clinical studies

Animal studies provided initial evidence that chronic neuromodulation has therapeutic properties in TNBS-induced colitis. Using a chronically implanted electrode, the vagus nerve was stimulated electrically 3 h per day for 5 days [Meregnani *et al.*: 5 Hz, 1 mA, 500 μs, 10 s ON, 90 s OFF; continuous cycle (111); Sun *et al.*: 0.25 mA, 20 Hz, 500 ms, 30 s ON, 5 min OFF continuously (112)]. This bioelectronic treatment alleviated the disease severity of TNBS colitis, such as body weight loss, bleeding diarrhea and DAI, an effect mediated by the inhibition of NF-κB and mitogen-activated protein kinase (MAPK) nuclear translocation (111–113). Moreover, VNS treatment led to a marked reduction in colonic damage following decreased inflammatory infiltration and improved ulcer healing in comparison to sham-stimulated rats, an effect mostly likely related to decreased IL-6 and TNF-α release.

Moreover, Jin *et al.* found that VNS decreased the DAI and pro-inflammatory mediators such as TNF-α, IL-1β, IL-6 and MPO in rats with TNBS-induced colitis via the autonomic pathways (113, 114). Interestingly, another study showed that chronic abdominal VNS possessed anti-inflammatory properties similar to chronic cervical VNS in TNBS-induced colitis. In particular, the authors demonstrated that abdominal VNS-treated rats had improved stool quality, decreased pro-inflammatory mediators in the blood and reduced resident inflammatory cell populations within the gut (115).

In an oxazolone colitis model, Meroni *et al.* recently demonstrated that even a single application of cervical VNS (5 Hz, 1 mA, 1 ms for 5 min) improved intestinal inflammation (i.e. TNF-α, IL-6 and CXCL1) and survival (Fig. 3e) (8). Similarly, we showed that therapeutic treatment with VNS (5 Hz, 1 mA, 1 ms for 5 min) reduced TNF-α and CXCL1 expression in immature intestinal Mφ and contributed to improved DAI scores (N. Stakenborg and G. E. Boeckxstaens, unpublished results). Interestingly, indirect activation of the vagus nerve via low power therapeutic ultrasound, in which ultrasound oscillation and pressure are capable of inducing biological effects through heating, radiation forces and other mechanotransducive effects, was also shown to alleviate

DSS-induced colitis severity (116). Taken together, these experimental data support the concept that bioelectronic medicine could be used as a novel approach to treat IBD in humans.

Human studies

Small open-label pilot studies have also assessed the potential of VNS in patients with ileocolonic CD (11, 12, 93). The group of Bonaz implanted VNS devices in nine patients with moderately active disease (Cyberonics, model 302) who were treated for 1 year (10 Hz, 0.75–1.25 mA and 250–500 μs; duty cycle: 30 s ON/5 min OFF) (93). The device itself was safe and well tolerated. The most common adverse events included voice alteration, cough, dyspnea, nausea and headache, which were well controlled by reducing stimulation intensity. Two patients with more severe disease withdrew from the study after 3 months because of a worsening of the disease, suggesting that VNS is better indicated for mildly to moderately active CD. Notably, among the remaining seven patients, five were still in endoscopic and clinical remission after 12 months and their vagal tone was restored (93).

These results are in line with the preliminary results of D'Haens *et al.* (117), showing clinical, biomarker and endoscopic improvement in 8 of 16 CD disease patients following 4 months of VNS therapy. Kibleur *et al*. also evaluated the effects of VNS on inflammation and brain activity in nine CD patients. After 12 months of chronic VNS, CD DAI, fecal calprotectin, anxiety state and vagal tone were improved, which correlated with a decreased power in the alpha frequency band of the electroencephalogram (118). Electrical stimulation of the sacral nerve was also shown to improve mucosal integrity and DAI scores over an 18-month period in a single patient with proctitis (i.e. a condition in which the rectal mucosa is inflamed) (119), a finding which was confirmed in a rat model of TNBS colitis (120–123).

Despite the promising results of these studies, larger randomized double-blinded control studies and a long-lasting follow-up of the patients are required to confirm the current results.

Challenges in performing bioelectronic medicine in humans

Despite the experimental and clinical use of VNS is challenging, several challenges remain before successful translation of bioelectronic neuromodulation to the clinic.

Achieving the optimum stimulation parameters of VNS is challenging, mainly as it remains to be fully elucidated which vagal fibers are involved in the intestinal CAP (124). Especially as the stimulation threshold varies significantly according to the type of nerve fiber targeted (i.e. afferent, efferent, etc.), knowledge about the exact mechanisms underlying the CAP is of great importance in view of the optimal stimulation parameters to be used. Especially as many variations can be applied because of the large combinations with all the possible parameters (frequency, pulse width, current, pulse shape, duty cycle, etc.), so the optimum stimulation

parameters in the clinic remain an educated guess based on animal studies, human *ex vivo* studies and small pilot studies.

Notably, caution is warranted when translating pre-clinical data to humans, since we observed morphological differences between the vagus nerve of small animals (i.e. mice) and humans. Therefore, larger animals (i.e. sheep, pigs, etc.) should be used to optimize stimulation parameters to be used in clinical trials (124). Moreover, the addition of recording electrodes to human VNS devices would enable future clinical trials to study which nerve fibers type are activated with different parameter combinations.

Choosing the best stimulation location for GI disorders is another parameter under debate. Most studies so far have chronically stimulated the left cervical vagus nerve (Cyberonics, model 302) to treat IBD. Yet, this position of the stimulation electrode activates vagal fibers to the larynx, lungs and heart, leading to adverse effects such as cough, hoarseness, voice alteration, paresthesias and rarely bradycardia. Stimulation of the abdominal vagus nerve circumvents the activation of these nerve fibers. In particular, it avoids stimulation of the larynx and minimizes the risk of interfering with cardiopulmonary function in humans (44, 47, 125).

In recent years, the development of non-invasive VNS techniques, i.e. approaches that do not require surgical implantation of the electrode and neurostimulator, has become of interest. These applications can improve the safety and tolerance of VNS, since implanted devices remain a potential source of infection. Moreover, the implanted stimulation electrode may become less effective over time because of fibrosis around the electrode or device failure (e.g. empty battery, leak breakage etc.).

Non-invasive VNS techniques include transcutaneous activation of the vagus nerve via the auricular concha innervated by vagal afferents (126). Some studies have demonstrated that transcutaneous VNS promotes a higher vagal tone (127–130) and reduces inflammatory mediator release in human whole blood (130–132). However, to date, no clinical results have been published about the use of transcutaneous VNS devices in IBD. Currently, 2 transcutaneous VNS devices are commercially available: (i) NEMOS (Cerbomed) is an external device providing transcutaneous stimulation of the auricular branch of the VN and (ii) Gammacore (electroCore LLC,) is another external model consisting of two stainless steel discs delivering transcutaneous low-voltage electrical signal to the cervical vagus nerve (120 s, 25 Hz).

Bioelectronic medicine also faces some ethical issues that undermine researchers and clinicians in conducting well-designed clinical trials. For instance, chronically relapsing and remitting patients cannot be withdrawn for standard care to treat them with VNS, since it would put them at risk. As a result, the true clinical benefit of VNS in inflammatory disorders will be difficult to determine. In addition, sham-stimulated patients cannot be included in a clinical trial as it is unethical to surgically implant a VNS device without switching the stimulator on. As such, clinical studies using implantable VNS devices consistently lack non-stimulated controls and the placebo effect of VNS cannot be accurately measured in such IBD clinical trials.

Conclusion

There has been a rapid expansion of our knowledge regarding the existence and function of neuroimmune units in the intestine and their cross-talk with the brain. Yet, many questions remain unanswered. In particular, discovering the cell types and mediators involved in the neuroimmune signaling in the intestinal mucosa has proven to be difficult due to its complexity and diversity. Future studies should focus on elucidating these mechanisms to better comprehend how they support homeostasis, but also what their contribution is to the intestinal pathology of IBD. Indeed, a clear understanding of all neuroimmune interactions in the gut may enable researchers to discover better targets and further optimize stimulation parameters and location for bioelectronic neuromodulation, as until now, peripheral nerve stimulation has shown only variable success in IBD.

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