

REVIEW

Neuronal regulation of the gut immune system and neuromodulation for treating inflammatory bowel disease

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Abstract

The gut immune system in the healthy intestine is anti-inflammatory, but can move to a pro-inflammatory state when the gut is challenged by pathogens or in disease. The nervous system influences the level of inflammation through enteric neurons and extrinsic neural connections, particularly vagal and sympathetic innervation of the gastrointestinal tract, each of which exerts anti-inflammatory effects. Within the enteric nervous system (ENS), three neuron types that influence gut immune cells have been identified, intrinsic primary afferent neurons (IPANs), vasoactive intestinal peptide (VIP) neurons that project to the mucosa, and cholinergic neurons that influence macrophages in the external muscle layers. The enteric neuropeptides, calcitonin gene-related peptide (CGRP), tachykinins, and neuromedin U (NMU), which are contained in IPANs, and VIP produced by the mucosa innervating neurons, all influence immune cells, notably innate lymphoid cells (ILCs). ILC2 are stimulated by VIP to release IL-22, which promotes microbial defense and tissue repair. Enteric neurons are innervated by the vagus, and, in the large intestine, by the pelvic nerves. Vagal nerve stimulation reduces gut inflammation, which may be both by stimulation of efferent (motor) pathways to the ENS, and stimulation of afferent pathways that connect to integrating centers in the CNS. Efferent pathways from the CNS have their anti-inflammatory effects through either or both vagal efferent neurons and sympathetic pathways. The final neurons in sympathetic pathways reduce gut inflammation by the action of noradrenaline on β_2 adrenergic receptors expressed by immune cells. Activation of neural anti-inflammatory pathways is an attractive option to treat inflammatory bowel disease that is refractory to other treatments.

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); APC, antigen presenting cell; CD, Crohn's disease; CGRP, calcitonin gene-related peptide; CNS, central nervous system; CRLR, calcitonin receptor like receptor; DC, dendritic cell; DSS, dextran sodium sulfate; EEC, enteroendocrine cell; ENS, enteric nervous system; GI, gastrointestinal; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IEL, intraepithelial lymphocyte; IL, interleukin; ILC, innate lymphoid cell; IPAN, intrinsic primary afferent neuron; M, microfold; M1, muscarinic 1 receptor; MC, microscopic colitis; M Φ , macrophage; nAChR, nicotinic acetylcholine receptor; NMU, neuromedin U; RAMP, receptor activity modifying protein; SNS, sympathetic nerve stimulation; TNBS, trinitrobenzene sulfonate; UC, ulcerative colitis; VIP, vasoactive intestinal peptide; VNS, vagal nerve stimulation.

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Further investigation of the ways in which enteric reflexes, vagal pathways and sympathetic pathways integrate their effects to modulate the gut immune system and gut inflammation is needed to optimize neuromodulation therapy.

KEYWORDS

enteric nervous system, inflammatory bowel disease, innate lymphoid cells, neuropeptides, sympathetic nerves, vagus nerve

1 | INTRODUCTION

The gut, especially the small intestine and colon, is always in a state of readiness to defend against pathogens. This is because the gut is always challenged by its contents, that includes vast microbial populations, within which are pathogenic bacteria, viruses and fungi, and challenging components of foods, such as toxins from food spoilage and plant alkaloids.¹⁻³ To manage threats to the gut, the level of inflammation is finely tuned. An important part of this control is neural: a role of the nervous system is to limit inflammation so that its level is appropriate for tissue defense but does not itself damage the tissue.⁴ Because the medical treatment of inflammatory bowel disease (IBD) commonly proves inadequate, for example targeted biologic therapies can be effective, but up to 30% of patients do not respond to initial treatment and up to 50% lose responsiveness over time,⁵ treatment by direct electrical stimulation of anti-inflammatory nerve pathways is an attractive option.⁶ Although stimulation of vagal, sympathetic or pelvic nerve pathways that control inflammation of the gastrointestinal tract has been shown to reduce the inflammatory states in animal models, only vagal nerve stimulation (VNS) has been tested in IBD patients.^{6,7} Stimulation reduced disease activity in some patients, but only a small number of patients was tested. It is not clear whether the sites or parameters of stimulation were optimal in either animal or patient tests. One of the complicating factors for neuromodulation for gastrointestinal disorders is that interactions between the central nervous system (CNS) and the enteric nervous system (ENS) are essential for the adequate control of gastrointestinal function,⁸ meaning that it is unknown which are optimal sites for stimulation. This review provides an overview, which aims to integrate knowledge of the interactions between the nervous system, gut endocrine system and gut immune system that are relevant to inflammatory bowel disease.

2 | INFLAMMATORY BOWEL DISEASE(S)

Inflammatory bowel disease, including Crohn's Disease (CD), Ulcerative Colitis (UC) and Microscopic Colitis

(MC), is disturbingly common, affecting about 1% of people worldwide and having an increasing incidence.⁹ IBD also has significant impact on psychological well-being and social functioning.¹⁰ CD and UC both involve severe inflammation with disturbance of the gut immune system.⁵ CD can affect any region of the gastrointestinal tract and may be present at several sites. Inflammation characteristically involves all gut layers, which can lead to abscesses, fistulas and perforation. UC is characterized by a contiguous severe diffuse mucosal inflammation and ulceration, not penetrating into the muscle layers. UC usually presents as diarrhea with or without passage of blood and can in its most severe form present as fulminant colitis. In fact, diarrhea is a hallmark symptom of CD and UC, and affects about 80% of patients.^{11,12} The chronic relapsing and remitting nature of CD and UC, along with the expense of treatments, imposes a huge financial burden; in the US, this amounts to around \$30 billion p.a., \$14.9 billion for UC and \$15.5 billion for CD, in annual combined direct and indirect costs.^{13,14} MC is a chronic inflammation of the large intestine that predominantly affects the elderly, in which the colonic mucosa has a nearly normal endoscopic appearance, but histology reveals lymphocytic infiltration of the mucosa; it appears to have shared pathogenic mechanisms with CD and UC.¹⁵

Inflammatory bowel disease is difficult to treat.¹⁶⁻¹⁸ The major forms of IBD, CD and UC, are currently treated with anti-inflammatory and immunosuppressive agents, that include thiopurines, which inhibit clonal expansion of lymphocytes, steroidal and nonsteroidal anti-inflammatory drugs and biological therapies, such as anti-TNF, anti-IL12 or anti-IL23, and anti- α 4-integrin antibodies.^{5,19} Antibiotics are also used to decrease intestinal bacterial load. Major drawbacks of these treatments include adverse effects, such as systemic immunosuppression, a lack of effectiveness in certain patients and development of refractory disease with continued treatment.^{19,20} Other serious safety concerns with the long-term use of biological therapies, such as anti-TNF, include development of opportunistic infections, reactivation of latent tuberculosis and development of hematological malignancies. Despite the common use of expensive biological treatments for IBD, the disease becomes refractory

to treatment in many patients and surgical removal of the inflamed gut is then their only treatment option.^{19,21,22} Furthermore, in a substantial proportion of Crohn's patients the disease recurs after surgery, despite ongoing biological or pharmacological preventive treatment.²³ Thus, new therapies that are more effective and have fewer adverse effects than current treatments are needed.

3 | THE GUT IMMUNE SYSTEM AND IMMUNE HOMEOSTASIS

The gut is protected from potential pathogens in the lumen by a single layer of vulnerable epithelium, by protective mucins produced by goblet cells, through the production of antimicrobial products by Paneth cells as well as by a network of immune cells.² Behind the epithelium is a layer of connective tissue, the lamina propria, within which are dispersed large numbers of specialized immune cells, including cells of the innate immune system (dendritic cells (DC), macrophages (M ϕ), innate lymphoid cells (ILC), eosinophils, mast cells and neutrophils) and adaptive immune cells (effector, regulatory and memory T cells and B cells). Organized lymphoid aggregations, in the form of lymphoid follicles that include the Peyer's patches, also occur in the gut wall (Figure 1). Some T cells are located within the lining epithelium. These intraepithelial lymphocytes (IELs) are very frequent, there being about 10–15 IELs per 100 epithelial cells in the small intestine. The gut mucosal immune system interacts with neurons of the ENS (Figure 1) and with gut enteroendocrine cells.^{24,25}

Microfold cells (M cells) and goblet cells transfer small antigen molecules from the lumen and present them to the DC^{26,27} (Figure 1). In addition, some DCs sample antigen directly from the intestinal lumen by projecting processes between epithelial cells, although most dendritic cells (DC) fully reside in the lamina propria. At steady-state, the gut environment is considered overall anti-inflammatory.⁵ Consistent with this, macrophages in the healthy intestinal mucosa do not produce inflammatory cytokines, but produce large amounts of anti-inflammatory interleukin (IL)-10.⁵ Moreover, DCs in the gut facilitate the generation of regulatory T cells (Tregs) that produce IL-10 and TGF β . ILCs have also been implicated in the immune homeostasis of the gut, in particular the group 3 ILCs (ILC3) which constitutively express IL-22 which promotes mucus secretion, antimicrobial products, and epithelial regeneration to impede tissue damage.^{28–30} ILC2 and ILC3, in particular, respond to enteric neuropeptides.³¹ DCs and ILC3 also support the production of IgA by plasma cells in the Peyer's patches and the mucosa in response to antigen exposure. Secreted IgA is transported

into the lumen and neutralizes pathogenic entities, but its lack of complement binding means it does not contribute to cell lysis, tissue damage or inflammation. The normal healthy anti-inflammatory environment can switch to a pro-inflammatory state when the gut is overtly challenged by pathogens or in disease states, such as in IBD.

In CD and UC, the epithelial barrier is breached, allowing the entry of luminal microflora that stimulate a proinflammatory immune response, including the activation of macrophages, mast cells and ILCs to release pro-inflammatory cytokines.^{5,32,33} The mucosal injury, entry of luminal factors, dysbiosis and cytokine release overwhelms tissue protection and repair.

4 | NEURAL INFLUENCES ON INFLAMMATION OF THE DIGESTIVE TRACT

The existence of neural influences on the inflammatory state of the gastrointestinal tract is very clear from experiments in which nerves were lesioned or stimulated, or where neurotransmission has been manipulated with drugs. Neural influences on inflammation come from the vagus nerve, the pelvic nerves and the sympathetic innervation of the gut, and from intrinsic neurons of the enteric nervous system (ENS) (Figure 2). Moreover, immune cells express receptors for a range of neurotransmitters, including receptors for ENS peptide neurotransmitters (neurokinin receptors, CGRP receptors, VIP receptors and NMU receptors), glycine receptors, muscarinic acetylcholine receptors, $\alpha 7$ and $\beta 2$ nicotinic acetylcholine receptors (nAChR), adrenergic receptors, including $\beta 2$ receptors ($\beta 2$ -AR) and $\alpha 2$ receptors, and P2 purine receptors.^{34–37} Each of the innervation pathways carries anti-inflammatory signals but the integration between the vagal, sympathetic, pelvic and enteric neural influences is not yet clarified.

In experimental colitis, sub-diaphragmatic vagotomy³⁸ or selective destruction of vagal afferents with capsaicin treatment both worsen disease severity³⁹ and increase mortality,⁴⁰ implying that nerve pathways that hold inflammation in check are regulated by the vagus. Interestingly, vagotomized mice, but not $\alpha 7$ nAChR^{-/-} mice, developed a more severe DSS colitis than control mice treated with DSS, implying that a cholinergic pathway that is independent of $\alpha 7$ nAChR is involved in holding intestinal inflammation in check.⁴¹ Moreover, stimulation of the vagus exerts anti-inflammatory effects in animal models^{42–44} and in humans.^{7,45} Inflammation in the distal gut can be suppressed by stimulation of the pelvic nerves⁴⁶ and inflammation is also reduced by stimulation of sympathetic nerves⁴⁷ or by activation of enteric nerve pathways.³⁵

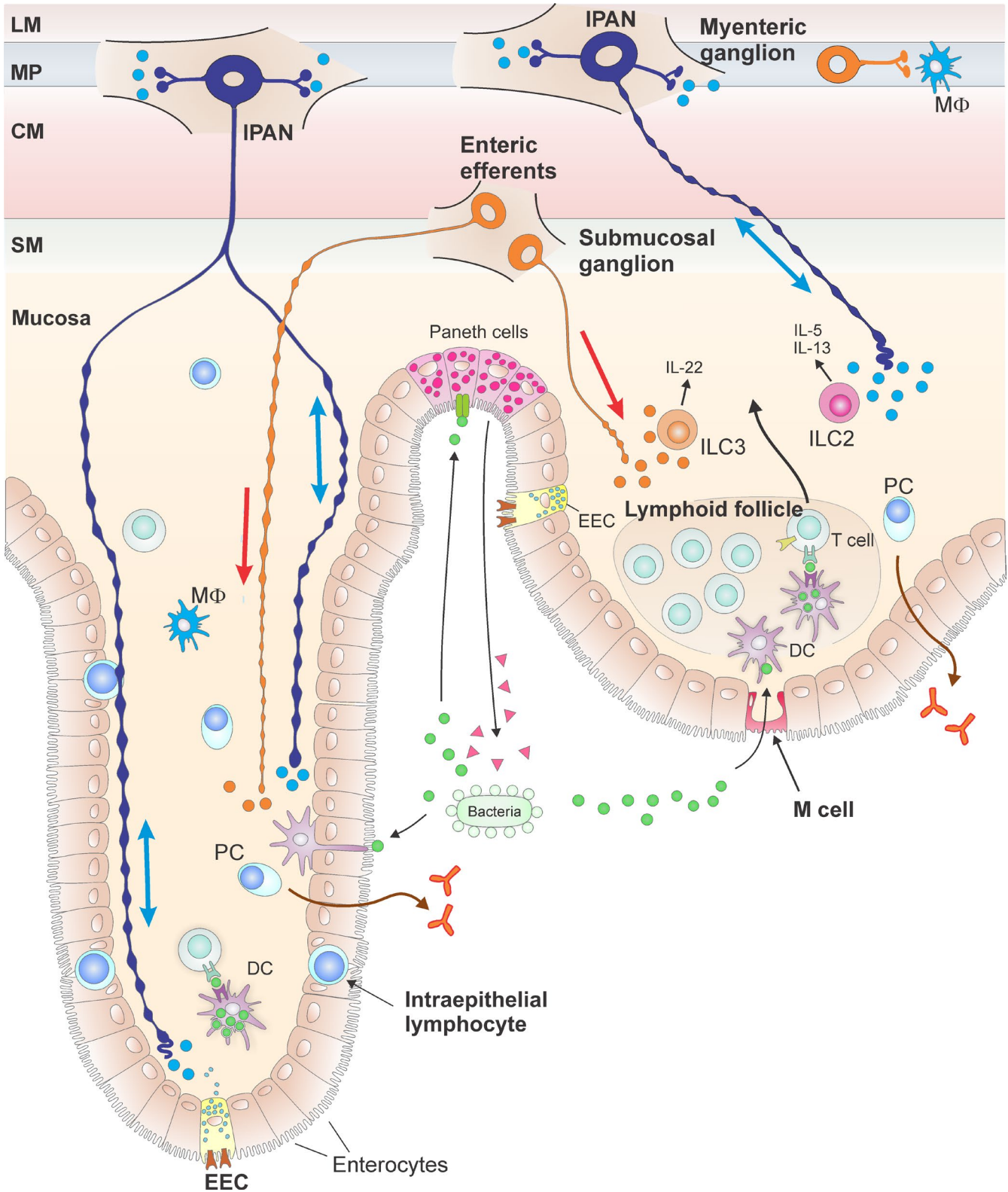


FIGURE 1 The interacting gut immune and enteric nervous systems. The immune system in the intestinal mucosa has a two-way interaction with enteric neurons, particularly with the sensory neurons within the gut wall (intrinsic primary afferent neurons; IPANs) that both respond to cytokines and release neuropeptides (double ended arrow) that interact with immune cells, notably ILC2 and ILC3, from their endings in the mucosa. Efferent neurons that project to gut immune tissues (single ended arrow) have cell bodies in submucosal and myenteric ganglia. Products of gut enteroendocrine cells (EEC), notably 5-HT, interact with both the gut immune system and enteric neurons.

Vagal nerve stimulation can contribute to reduction of inflammation in the gut in at least three ways: by stimulating afferent neurons that signal to the brain to activate efferent pathways, that may include sympathetic outputs from the central nervous system⁴⁷; by activating vagal efferent pathways; and by stimulating vagal afferents to release transmitters from their peripheral ends (Figure 1). Evidence for effects of the vagal efferents acting on enteric neurons includes that intestinal inflammation triggers a vagally mediated circuit leading to activation of vagal motor neurons connected to the inflamed intestine.^{35,48}

4.1 | Transmitter release from the peripheral ends of afferent neurons

Antidromic action potentials, that is action potentials that travel opposite to their conventionally described direction, towards rather than away from sensory endings, of neurons innervating the gastrointestinal tract, and other tissues such as the skin, result in peripheral release of neuropeptides, including tachykinins and CGRP.⁴⁹ These peptides cause vasodilatation, facilitate tissue repair and have anti-inflammatory effects (see below). The CGRP-containing afferents are sensitive to capsaicin, which causes their degeneration over periods of hours or days.⁴⁹ In the gastrointestinal tract, capsaicin-sensitive afferent fibers exert protective anti-inflammatory effects, reducing mucosal damage via peptide (primarily CGRP) release from their peripheral endings.⁵⁰⁻⁵²

The protective anti-inflammatory effects of afferents are observed in rat and rabbit models of colitis. Following the degeneration of capsaicin-sensitive afferent fibers, the severity of trinitrobenzene sulfonate (TNBS)-induced colitis was increased, but colonic transit time did not change, indicating that gut motility was not affected.^{39,53,54}

4.2 | Vagal afferent signaling of inflammatory states to the brain

Despite the general belief that the vagal afferents are concerned with non-noxious signaling, the vagus responds to the presence of toxins that induce feelings of nausea from the stomach and intestines,⁵⁵ potentially injurious helminths⁵⁶ and the sensory endings of vagal afferents are stimulated by inflammatory cytokines.^{57,58} Consistent with these observations, intestinal inflammation in response to *Campylobacter jejuni* infection, or inflammation-inducing intestinal manipulation, increased the expression of c-Fos, a marker of neuronal activity, in the nucleus tractus solitarius of the lower brain stem.^{48,59} Furthermore, infection of the small intestine with *T. spiralis* resulted in 5-HT

release (almost certainly from EEC), which acted through 5-HT₃ receptors to increase the excitability of neurons with cell bodies in the nodose ganglia.⁵⁶ Included amongst the vagal afferents are neurons whose reflex activation reduces inflammation via increased activity in sympathetic pathways to the abdominal viscera.⁶⁰ Hepatic afferents are likely to be involved in signaling the inflammatory state of the intestine. Almost all venous drainage of the gastrointestinal tract passes via the portal vascular system to the liver. Thus, hepatic afferents are positioned to provide an integrated signal of the inflammatory state of the whole gastrointestinal tract. Direct recordings from hepatic afferents and induction of c-Fos demonstrate that these neurons are responsive to inflammatory mediators.^{35,57} Other data implicates hepatic afferents in the initiation of an anti-inflammatory effect mediated through vagal efferents that synapse with enteric neurons.³⁵

4.3 | Involvement of enteric neurons in control of inflammation

Any effect of the efferent vagal nerves that innervate the gut must be via enteric neurons, because efferent pathways in the vagus do not directly innervate effector tissue in the gastrointestinal tract, they act via enteric neurons that are also component neurons of enteric reflex pathways.⁸ Sympathetic post-ganglionic (noradrenergic) neurons provide a dense innervation of enteric ganglia and influence gastrointestinal functions through effects on enteric reflex pathways.⁸ They also directly innervate some effectors in the gut wall.

Within the enteric nervous system, intrinsic primary afferent (sensory) neurons (IPANs) are excited by inflammation and release peptides that act on gut immune cells (Figure 3). A role of IPANs in response to inflammation is strongly suggested by the long-lasting hyper-excitability of these neurons that occurs after inflammation of the gut is induced.⁶¹⁻⁶³ The IPANs are directly excited by inflammatory mediators, including histamine, prostaglandins, leukotrienes, interleukins, activation of proteinase-activated receptors and by 5-HT.⁶⁴ A variety of receptors for transmitters of enteric neurons, including vasoactive intestinal peptide receptors,^{37,65} neuromedin U receptor 1,⁶⁶ CGRP receptors,⁶⁷ tachykinin receptors^{68,69} and acetylcholine (muscarinic and nicotinic) receptors,^{35,70} are expressed by immune cells in the intestine. Activation of VIP, CGRP, tachykinin and muscarinic cholinergic receptors is anti-inflammatory, and activation of the neuromedin U receptor causes protective responses.^{66,71} Immune cells also express receptors for 5-HT, a pro-inflammatory hormone.²⁴ The major source of 5-HT in the gut is enteroendocrine cells (EEC), although there is also a small population of enteric

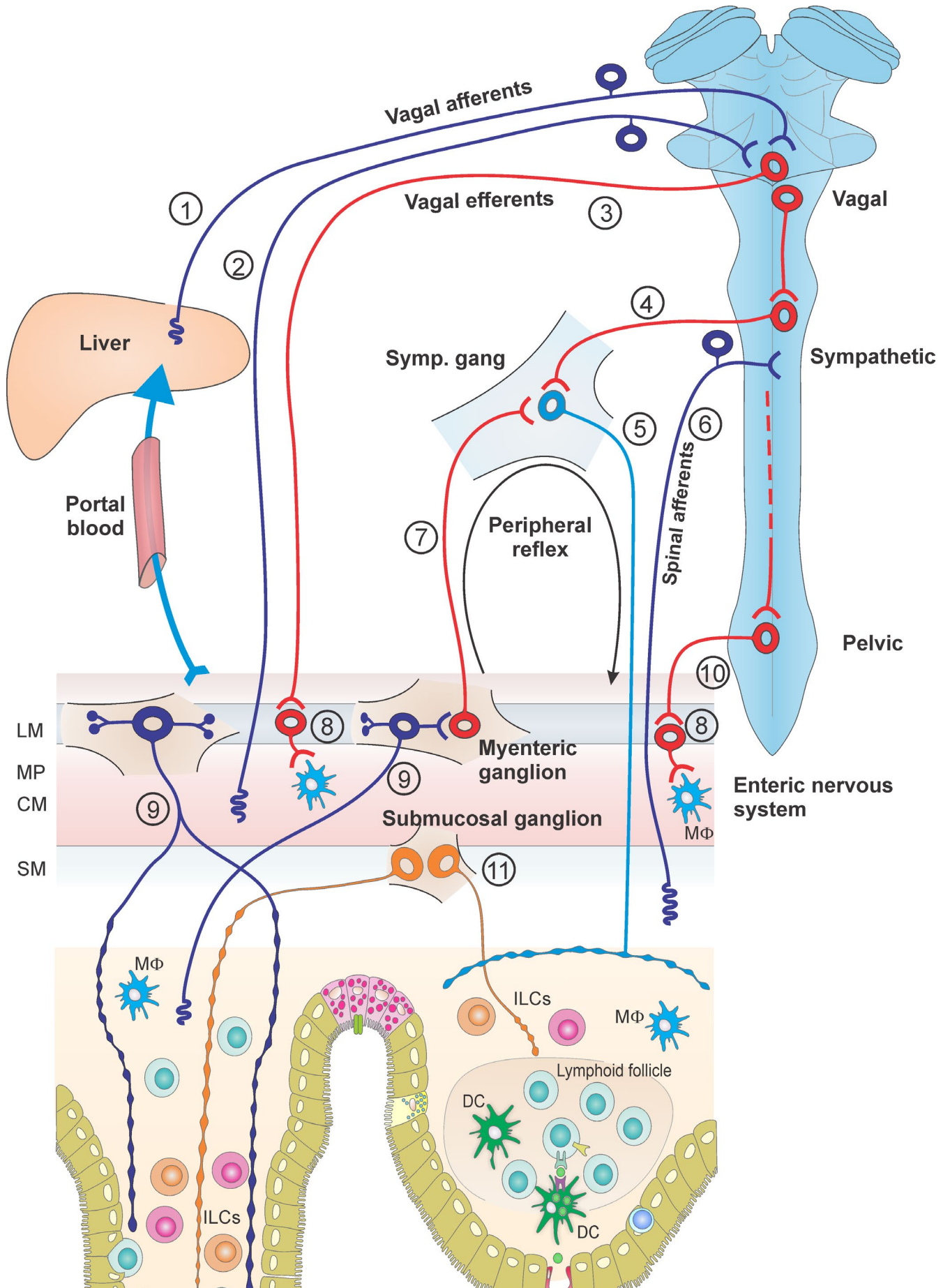


FIGURE 2 Nerve pathways that modulate intestinal inflammation. The gut immune system is maintained in a state of activity due to the exposure of the mucosa to gut contents, including the microbiota.¹⁰⁶ In order that inflammation is not so severe as to induce tissue damage, the nervous system restricts inflammation to levels that are appropriate to protect the gut.⁴⁰ The efferent anti-inflammatory pathways are vagal, sympathetic and pelvic outflows from the CNS and through transmitters released from neurons of the enteric nervous system. The degree of inflammation is signaled by vagal afferents that detect inflammatory mediators reaching the liver in the portal blood (1) or detect inflammation in the gut wall (2). Sensory information is also relayed by spinal afferent neurons (6). Outputs from the CNS travel to the gut in the vagus (3), sympathetic (4,5) and pelvic (10) nerves. The enteric nervous system is involved on the control of inflammation through intrinsic primary afferent neurons (IPANs; 9) and motor neurons (8,11). Sympathetic pathways can be activated from the CNS (4) or through peripheral reflexes involving intestinofugal neurons (7).

neurons that produce 5-HT. Reduction of 5-HT stores in EEC by inhibition of its synthesis decreases the severity of inflammation induced by TNBS in mice.⁷² Moreover, the interleukin, IL1 β , and bacterial lipopolysaccharide both stimulate release of 5-HT from EEC cells isolated from the human intestine,⁷³ to a greater extent for 5-HT cells from individuals with Crohn's disease than those from control subjects.

An anti-inflammatory effect of VNS mediated through enteric neurons was shown in experiments in which inflammation was induced using mechanical stimuli applied to the serosal surface of the intestines to cause an ileus (modelling postoperative ileus, a stasis of the intestine that is observed after surgery) that activates macrophages within the muscularis externa^{70,74} This vago-enteric pathway is independent of the vagal influences on the spleen, because splenic denervation does not prevent the anti-inflammatory effect, but it does involve vagal stimulation of acetylcholine release from enteric neurons, which then acts on intestinal macrophages.⁷⁰ In these experiments, VNS reduced the inflammation of the small intestine, produced by mechanical irritation, in normal mice, in mice with the spleen denervated, and in T-cell-deficient mice. VNS was ineffective in α 7nAChR knockout mice. Enteric neurons innervated macrophages that expressed the α 7nAChR and activation of α 7nAChR reduced their excitability. The data suggest that resident macrophages are a target cell through which VNS mediates this anti-inflammatory effect.

Inflammation of the intestine activates a vago-vagal anti-inflammatory reflex that acts back on the intestine (a negative feedback). As mentioned above, all inflammatory mediators that are swept up into the venous drainage from the intestines are transported through the portal veins to the liver (Figure 2). Teratani et al.³⁵ showed that these inflammatory mediators excite vagal hepatic afferents that relay the inflammatory signals to the brain stem. From here, vagal efferent pathways signal to the gut, attenuating inflammatory responses by inhibition of antigen presenting cells (APCs) and regulatory T cells in the gut mucosa. Evidence is convincing that the inhibition is mediated through M1 muscarinic ACh receptors on APCs, although it is possible that M1 receptors

on IPANs also contribute, because these neurons express *Chrm1*⁷⁵ and are excited by muscarinic agonists acting at M1 receptors.⁷⁶ The anti-inflammatory effect was abolished if the left vagus (which receives the hepatic connection) was cut or muscarinic receptors were ablated. This is consistent with our data; we have stimulated the left vagus which would be predicted to activate this vago-vagal anti-inflammatory reflex, and found substantial suppression of inflammation in the small intestine.⁴⁴ Enhancement of muscarinic transmission at M1AChRs would be expected to enhance the reflex and improve the effectiveness of VNS applied to the left vagus nerve. Thus, M1 positive allosteric modulators (M1PAMs), which potentiate propulsive reflexes in the gut,^{77,78} may enhance anti-inflammatory effects. Enhancing propulsion and reducing inflammation may be synergistic in removing colonic content that is potentially deleterious to the large intestine.

4.4 | Enteric neuropeptides that influence inflammation

Several studies have suggested that there is local neural regulation of ILCs by the peptide neuromedin U (NMU). NMU is contained in IPANs (neurons with Dogiel type II morphology) in the small intestine.⁷⁹ IPANs provide a rich innervation of the mucosa,⁶⁴ and as mentioned above, are excited by inflammatory mediators. Recent expression analysis suggests that *Nmu* expression provides a unique marker of IPANs.^{75,80} Group 2 ILCs (ILC2) in the mucosa express the NMU receptor 1.⁶⁶ In nematode infected mice, NMU causes ILC2 proliferation, release of type 2 cytokines and worm expulsion.^{66,71} This is consistent with earlier indications that IPANs are involved in anti-nociceptive reflexes, including propulsive expulsion of toxic materials from the large intestine.¹ For expulsion to be effective, there needs to be a coordinated activity of enteric neurons and the immune system, implying communication from IPANs to the immune system (through NMU and other IPAN products acting on immune cells) and from the immune system to IPANs, for example through cytokines acting on IPANs^{1,64} (Figure 3).

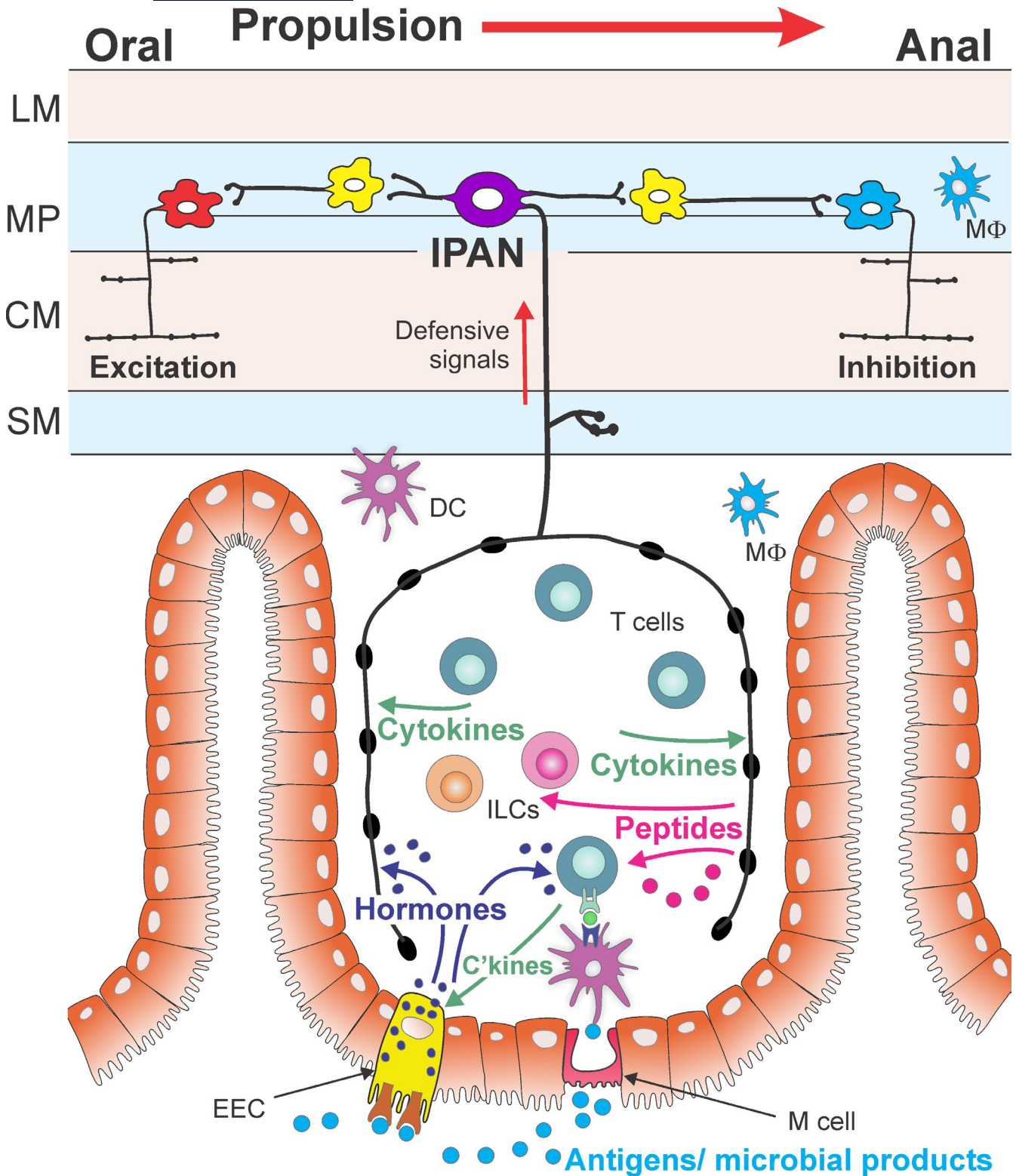


FIGURE 3 Interactions between enteric neurons, the gut immune system and the gut endocrine system to defend the gut. Interaction of the gut with pathogens is signaled by antigens being presented to immune cells and activating cytokine cascades, and by stimulating release of 5-HT from enteroendocrine cells (EEC). Intrinsic primary afferent neurons (IPANs) express excitatory receptors for cytokines and for 5-HT. Activation of IPANs engages the enteric nerve circuitry, propelling the contents which leaves the body with its cargo of pathogens.

Calcitonin gene-related peptide (CGRP) has protective effects when released by the peripheral ends of vagal

afferents (see above). However, its role when released from sources within the intestine is less clear. It is present within

IPANs, as indicated by immunohistochemistry⁶⁴ and expression analysis,⁷⁵ which shows that the form in IPANs is CGRP β . Immune cells in the intestine express the gene for CGRP α , *Calca*, and are presumed to produce and release CGRP α .⁶⁷ It is noted that *Calca* also codes for calcitonin, but this peptide does not appear to be produced by gut immune cells.⁶⁷ The calcitonin receptor occurs transiently in enteric neurons during early development.⁸¹ Genes for the molecular components of the CGRP receptor (CRLR and RAMP1) were identified in ILC2, and CGRP was found to reduce activation of ILC2.⁶⁷ IPANs, identified by their expression of *Nmu*, express the CGRP receptor genes, *Calcr1* and *Ramp1*.^{67,75} An anti-inflammatory feed-back loop involving CGRP and its receptor, similar to that illustrated for NMU/NMUR in Figure 3, may occur. A further complication is that CGRP is an agonist at other calcitonin family receptors, such as the amylin1 (AMY1) receptor.⁸²

Recent evidence indicates that VIP and the VIP2 receptor (VIPR2, also known as the VPAC2 receptor, because both VIP and PACAP are agonists) are involved in immune regulation in the gastrointestinal tract. Through the VIPR2, VIP is a potent inducer of cytokines IL-5 and IL-22 in ILC2s and ILC3s respectively.^{37,65} ILC3s that expressed *Il22* in the small intestine and colon had high expression of *Vipr2*, and exposure of isolated ILC3s to VIP increased the production of IL-22. Knockout of *Vipr2* increased the severity of colitis induced by dextran sodium sulfate (DSS). In DCs and macrophages, VIP inhibits the expression of CXCL10 and IL-12 that are known to promote Th1 responses.^{83,84} In contrast, VIP induces type 2 responses by increasing CCL22 production by DCs and IL-10 expression in macrophages.^{83,85} VIP is found in a number of neuron types in the intestine, including a prominent group of neurons with cell bodies in submucosal ganglia that supply a dense innervation of the mucosa. These neurons have been identified as secretomotor/ vasodilator neurons,⁸⁶ but it is feasible that there are actually subgroups of submucosal VIP neurons and that a subgroup, separate from, or part of the secretomotor/ vasodilator neuron group, is involved in immune regulation. Consistent with this hypothesis, a recent expression study indicates that the “secretomotor/ vasodilator” neurons fall into two clusters.⁸⁷ Some submucosal neurons project to lymphoid tissue in the gut wall,⁸⁸ as illustrated in Figure 1.

Tachykinins may also be involved in neuro-immune interactions in the small intestine, because the peptides are found in a number of classes of enteric neuron⁸⁶ and their receptors are on both enteric neurons and gut immune cells, including T cells, macrophages and mast cells.⁶⁸ Interpretation of the roles of tachykinins and their receptors are considerably muddled by the production of the closely related hemokinins by immune cells in the gut wall and in the circulation.⁸⁹ Hemokinins are a subgroup

of tachykinins with which the earlier identified mammalian tachykinins (substance P, NKA, neuropeptide K and neuropeptide gamma) share a common C-terminal sequence -FXGLM-NH₂ (where X in mammals is F, Y, V) and they act at the same 3 tachykinin receptors, NK1, 2 and 3 as tachykinins. The lack of clarity concerning relationships between sources, sequences and sites of action of tachykinins to modify inflammation in the intestines means their physiological roles are unresolved, although tachykinins are generally pro-inflammatory.⁶⁸

4.5 | Anti-inflammatory effects mediated by sympathetic neurons that innervate the intestine

Sympathetic noradrenergic neurons innervate gut-associated lymphoid tissue⁹⁰ and evidence gathered over a long period of time indicates that catecholamines (epinephrine and norepinephrine) stimulate β 2-adrenoreceptor of immune cells to inhibit the production of proinflammatory cytokines, such as IL-12, TNF α and interferon- γ by immune cells, whereas they stimulate the production of anti-inflammatory cytokines such as IL-10 and TGF β .⁹¹ The anti-inflammatory actions of catecholamines have been demonstrated both *in vitro*⁹² and *in vivo*.⁹³ In macrophages, β 2-adrenergic signalling promotes differentiation into an M2 regulatory phenotype, that is associated with anti-inflammatory and wound healing properties. Stimulation of this pathway increases M2 macrophage capacity to secrete IL-10⁹⁴ whereas TNF- α expression is inhibited.^{95,96} Matheis and colleagues showed that β 2-AR signalling induces the expression of arginase 1 (Arg1) in intestinal macrophages, protecting enteric neurons from damage after bacterial infection.⁹⁷ Beta2 adrenergic receptor-mediated signaling suppresses ILC2 activity in intestinal infection models.^{98,99} Inflammation caused by DSS is inhibited by intermittent electrical stimulation of sympathetic post-ganglionic neurons of the superior mesenteric nerves that supply the gut, but not the spleen.⁴⁷ Moreover, beta blockers exacerbate inflammation in Crohn's disease in humans, increasing the incidence of relapse following surgery.¹⁰⁰ An interpretation of these data is that sympathetic anti-inflammatory pathways are activated in Crohn's disease and that their effect is mediated via beta receptors.

4.6 | Signaling through spinal afferent neurons

Evidence summarized above indicates that extrinsic anti-inflammatory reflexes can be initiated through vagal afferents that innervate the gut or liver. It is also

feasible that spinal (dorsal root) afferent neurons could feed into these reflex pathways. Application of inflammatory agents to the gut increases activity in spinal afferent neurons acutely, and if inflammation of the gut is maintained over days or weeks, the sensitivity of spinal afferent neurons to inflammatory agents in the gut, or to physiological stimuli, such as distension, increases.¹⁰¹ Visceral hypersensitivity outlasts the inflammation, and may contribute to ongoing disease, notably irritable bowel syndrome (IBS).¹⁰² The hypersensitive spinal afferent neurons signal pain and discomfort, but whether they also provide afferent input to reflex pathways through the CNS that regulate the degree of inflammation in the intestine is unknown. Spinal afferents modulate immune responses by release of CGRP from their terminals in the intestine,¹⁰³ an effect that is similar to the modulation caused by release of peptides from vagal afferents (see above). Infection with pathogenic *Salmonella* caused CGRP release for the ends of spinal afferents that suppressed M cell numbers and thus reduced trans-epithelial transfer of bacterial toxins.¹⁰³

5 | OVERVIEW OF NERVE MEDIATED EFFECTS ON GI INFLAMMATION

We have reviewed evidence that the nervous system influences the intensity of inflammation in the gastrointestinal tract. These influences come from the vagal and sympathetic pathways that supply the gastrointestinal tract and from the intrinsic nervous system of the digestive system, the ENS. Each of these interacts, such that the control of the gut immune system can be anticipated to be an integration of vagally-mediated, sympathetically-mediated and enteric reflex effects (Figure 1). A major challenge is to determine how activities in these pathways combine to finely tune inflammation.

Consistent data in animals, and limited patient data, indicates that VNS reduces gut inflammation. Animal studies that have investigated effects of VNS stimulation on colon inflammation^{42,43} have produced only mild improvement of the disease state. Inflammation of the small intestine was reduced to a greater degree⁴⁴ and the only human study that has been published provided evidence of remission of Crohn's disease, a predominantly small intestinal condition.^{6,7} This difference in effect on small and large intestine may be expected, as vagal innervation is denser in the small compared to the large intestine.¹⁰⁴ However, these comparisons are based on a limited number of studies, that utilised different models, and in particular different frequencies of stimulation and patterns of stimulus application. In a clinical study that reduced

inflammation of the small intestine,⁷ monophasic 0.5 ms stimuli were applied at 10 Hz, 30 min on, 5 min off, 24 hr a day, whereas in an animal study⁴⁴ biphasic pulses, 0.2 ms per phase, 10 Hz, 30 min on and 5 min off, for only 3 hr a day substantially reduced inflammation.

Evidence for sympathetic inhibition of GI inflammation, mediated through β_2 catecholamine receptors is strong, and sympathetic nerve stimulation (SNS) reduced inflammation in an animal model. Sympathetic nerves also carry vasoconstrictor and motility inhibiting signals and engagement of these modalities along with anti-inflammatory effects would be undesirable, as restriction of blood flow could further compromise IBD, and inhibition of motility could exacerbate dysbiosis. Willemze et al.⁴⁷ adjusted their stimulus parameters to avoid changes in blood flow. SNS was applied using biphasic stimulus pulses of 200 μ A and 2 ms (1 ms per phase) applied to the superior mesenteric nerve at 10 Hz for 5 min, twice per day for 6 days. This caused a substantial reduction in the disease activity index, when compared to unstimulated DSS-treated controls. It is feasible that the sympathetic anti-inflammatory pathway to the gut is activated by vagal afferents, which connect to centers in the brain stem that in turn connect with the efferent sympathetic anti-inflammatory pathway. VNS activates sympathetic pathways to suppress systemic inflammation.⁶⁰

The vagal efferent pathways connect with enteric neurons, but vagal efferent fibers do not directly innervate effector tissues in the gut. Thus, it is very likely that the effects of vagal efferents are mediated through enteric nerve circuits. Just where in the enteric circuits the vagal neurons make their contacts is unknown. However, it is likely to involve modulation of activities of IPANs, which are neurons that have been identified to have receptors for inflammatory mediators, and to produce neurotransmitters (NMU, CGRP, tachykinins and ACh) that modulate the functions of immune cells. This remains speculation until direct experimental evidence is obtained.

Sympathetic, noradrenergic, neurons could affect inflammation both by direct effects of noradrenaline on immune cells and indirectly by their innervation of enteric neurons. The majority of sympathetic nerve endings are around enteric neurons, where they have inhibitory effects, although inhibition via innervation of enteric neurons has been shown for modulation of contractile and secretomotor activity, not immune or inflammatory control.⁸

6 | TRANSLATIONAL PERSPECTIVE

Available data points to the feasibility of developing neuromodulation therapies for IBD and suggests a range of

stimulus sites that could be exploited, but there are substantial knowledge gaps that restrict progress. The only human study has been of cervical VNS for Crohn's disease, which was shown to reduce disease in some patients.^{6,7} Only one stimulus regime was tested and in animal studies there are far too few data to conclude what stimulus parameters may be most effective. Thus there is an urgent need to explore the stimulus parameter space (pulse frequency, pulse duration and form, train length, time of day, etc.¹⁰⁵) for regulation of the gut immune system and gut inflammation in disease states. The effectiveness of sympathetic nerve stimulation in an animal model⁴⁷ strongly suggests that this may be an approach that could be developed if potential off-target effects, for example vasoconstriction, can be avoided. Another promising direction could be to target the hepatic branch of the vagus, which clearly carries anti-inflammatory signals to reflex centers in the CNS.³⁵ It is additionally possible that better outcomes may be achieved if conventional medical treatments are combined with neuro-modulation therapies, or if receptors for neurotransmitters recently discovered to be involved in control of gut inflammation, such as NMU, VIP and CGRP are also targeted. It is necessary to use animal models to investigate the utility of the possible range of stimulus parameters, stimulus sites and combination therapies. Investigations have been limited by the use of acute chemically-induced inflammation for almost all animal work, which does not properly model the long lasting, remitting, and relapsing nature of the human condition.¹⁹

In conclusion, there is compelling evidence that inflammation and immune cell activities within the gastrointestinal tract are under regulatory control through vagal, sympathetic and enteric nerve pathways. This regulation is an essential component of immune homeostasis in the gut. It will be important to determine how an integrated neural control of defense of the gut against microbiota and other challenges is achieved. This understanding applied in appropriate animal models is predicted to lead to new therapies, including neuromodulatory therapies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the review of literature, to writing the article and gave final approval for submission.

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