Neuro-immune interactions in the intestine

Antonia Wallrapp[1] and Isaac M. Chiu[1]

[1]Department of Immunology, Harvard Medical School, Blavatnik Institute, Boston, Massachusetts 02115, USA; email: antonia_wallrapp@hms.harvard.edu, isaac_chiu@hms.harvard.edu

Antonia Wallrapp's ORCID ID: 0000-0002-3176-1553

Isaac M. Chiu's ORCID ID: 0000-0002-0723-4841

Corresponding author's contact information:

Isaac M. Chiu

4 Blackfan Circle, Room 806

Boston, MA 02115

USA

isaac_chiu@hms.harvard.edu

Keywords:

Neuro-immune interactions, mucosal immunology, intestine, gut-brain axis, visceral pain, neuropeptides

Abstract:

Recent advances have contributed to a mechanistic understanding of neuro-immune interactions in the intestine and revealed an essential role of this crosstalk for gut homeostasis and modulation of inflammatory and infectious intestinal diseases. In this review, we describe the innervation of the intestine by intrinsic and extrinsic neurons, and then focus on the bi-directional communication between neurons and immune cells. First, we highlight the contribution of neuronal subtypes to the development of colitis and discuss the different immune and epithelial cell types that are regulated by neurons via the release of neuropeptides and neurotransmitters. Next, we review the role of intestinal inflammation in the development of visceral hypersensitivity and summarize how inflammatory mediators induce peripheral and central sensitization of gut-innervating sensory neurons. Finally, we outline the importance of immune cells and gut microbiota for the survival and function of different neuronal populations at homeostasis and during bacterial and helminth infection.

1 INTRODUCTION

The nervous system and immune system share many similarities as both systems monitor the environment and internal stimuli, integrating these signals to regulate homeostasis and to mediate defense responses to potential threats (1). Both systems also express receptors that recognize inflammatory mediators and pathogens (1). In response to challenges, both nervous and immune systems undergo adaptations and are able to recall memory responses to subsequent stimuli (1). Based on these similarities, it is not surprising that neuronal and immune cells harbor molecular signals and receptors that allow them to communicate with each other in a reciprocal manner (**Figure 1**) (2). Upon activation, the nervous system responds within seconds and is relatively fixed in location, whereas the immune system responds relatively slower and is more motile (3). Therefore, a coordinate neuroimmune crosstalk maximizes the ability of the organism to be able to optimally regulate homeostasis and to drive a successful host defense and inflammatory response.

An involvement of neurons in inflammatory processes has already been described two thousand years ago by Cornelius Celsus with his observation that *dolor* (pain) is one of the four cardinal signs of inflammation (4, 5). In the early twentieth century, several scientists showed using denervation and electrical stimulation that peripheral neurons can directly drive vasodilation and inflammatory processes, a process that was termed neurogenic inflammation (6). This process starts with the activation of sensory neurons, which generate action potentials that not only travel towards the central nervous system

in an afferent direction, but also propagate at branch points antidromically back to nerve terminals (7, 8). There, action potentials initiate calcium-dependent release of neuropeptides from dense core vesicles in nerve terminals into the tissue environment (7, 9). These neuropeptides include substance P and calcitonin gene-related peptide (CGRP), which were found to promote vascular permeability and vasodilation, respectively (10). Further research showed that these neuropeptides can regulate the function of immune cells, which either ameliorates or worsens inflammatory and infectious diseases (2, 8). Conversely, immune cells also potently regulate the function of neurons, who express receptors for immune-derived factors such as cytokines and lipid mediators (11). For example, immune mediators lower the threshold of nociceptor sensory neurons, leading to peripheral sensitization to noxious stimuli and pain hypersensitivity (12).

The nervous and immune system are highly diverse with intricate cellular networks in tissues. Recently we have started to gain a much better understanding of the exact cellular and molecular mechanisms of neuro-immune interactions due to technical and genetic advances in single-cell RNA-sequencing, intravital multiphoton microscopy, tissue-clearing techniques, genetic calcium indicators, optogenetic and chemogenetic technology (1, 13). In particular the gastrointestinal tract is a highly specialized and complex organ that is in close contact to the environment, needing to balance nutrient uptake, digestion and motility with the ability to mediate host defense (14). The gastrointestinal tract is densely innervated by the nervous system, housing millions of neurons and receiving input from the brain and spinal cord (15). It is also populated by

numerous innate and adaptive immune cells, which are essential for mediating tolerance to food antigens and mounting defense responses to invading pathogens (14, 16). The intestine not only receives input from the brain but also communicates with the brain, a bidirectional pathway that is termed gut-brain axis (17). The effector function of immune and non-immune cells in the brain is regulated by bacterial metabolites in the intestine and immune cells have been shown to migrate from the intestine to the brain in a disease context (17). Therefore, the focus of this review is the bidirectional neuro-immune crosstalk in the intestine since interactions between neurons and gut-resident immune cells have important implications for homeostasis and intestinal inflammation.

2 INTESTINAL ANATOMY AND INNERVATION

The intestine consists of the small and large intestine, which are composed of different layers, including the epithelium, lamina propria, submucosa and muscle layer (**Figure 2a**) (16). The intestinal layers are innervated by different types of neurons, which can be classified into gut-intrinsic and extrinsic neurons (18). While the cell bodies of intrinsic neurons are located within the intestinal wall, the cell bodies of extrinsic neurons are located outside of the gut in sensory or autonomic ganglia and brainstem, sending projections to the gastrointestinal tract (18). Intrinsic and extrinsic intestinal neurons in the mouse have been characterized in the past based on morphology, electrophysiological recordings and immunohistochemical methods (19-24) and more recently with the advent of RNA-sequencing based on transcriptional expression profiles.

These neurons constitute both sensory and autonomic branches of the peripheral nervous system (2).

2.1 Intrinsic neurons

Intrinsic neurons, whose cell bodies are fully contained within the gastrointestinal tract, form the enteric nervous system (ENS), which functions to mediate sensory, peristaltic and secretory function independently of the central nervous system (CNS) (15, 18). The ENS consists of two plexuses, which are comprised of enteric ganglia containing neuronal cell bodies and networks of neuronal processes (**Figure 2a**) (15, 25, 26). The myenteric plexus, also termed Auerbach's plexus, is dispersed between the circular and longitudinal smooth muscles of the muscle layer (18, 27) and predominantly involved in the regulation of intestinal motility (28). The submucosal plexus, also termed Meissner's plexus, is positioned within the submucosa (18, 27) and regulates nutrient uptake and secretion (28).

Enteric neurons consist of different neuronal types, including: 1) intrinsic primary afferent neurons (IPANs), which are sensory neurons that can be activated directly by mechanical stretch or indirectly via serotonin (5-HT) produced by enterochromaffin cells, 2) ascending and descending interneurons, and 3) excitatory and inhibitory motor neurons that innervate smooth muscles (15, 27). These three types of neurons form the peristaltic reflex circuit of the ENS, which mediates sensing of distension of the intestinal wall and promotes forward propulsion of the food (15, 27). Another subset of enteric neurons that

are less well studied are intestinofugal neurons, which have axonal projections outside of the intestine (15, 29) and respond directly to mechanical stimuli or are activated by other enteric neurons (28, 29).

While the function of these neuronal subtypes is broadly defined, the molecular identities and specific subtypes that mediate these functions are still being elucidated. Single-cell RNA-sequencing (ScRNA-seq) of nuclei from the mouse enteric nervous system have revealed 12 neuron subsets in the ileum and 21 neuron subsets in the colon of adult mice (30). The neurons in the colon expressed either *Nos1* (nitrergic), *Chat* (cholinergic) or both and were further categorized as putative sensory neurons (*Calcb*$^{+}$ *Calca*$^{+/-}$), interneurons (*Penk*$^{+}$), excitatory motor neurons (*Chat*$^{+}$ *Tac1*$^{+}$), inhibitory motor neurons (*Nos1*$^{+}$) and secretomotor/vasodilator neurons (*Glp2r*$^{+}$) (30). While this study analyzed enteric neurons from all intestinal layers (30), other studies specifically analyzed enteric neurons from the mouse myenteric plexus by population-based RNA-seq (31) and scRNA-seq (13, 32-34). Transcriptional analysis revealed that transcriptional profiles of enteric neurons are specific to the location within the gastrointestinal tract and that their gene expression is also modulated by the microbiota (31, 35). In addition to these studies of the mouse enteric nervous system, the human enteric nervous system was also analyzed transcriptionally recently by molecular profiling (30, 33, 34). Despite these recent advances in the transcriptional characterization of enteric neurons, it remains to be determined how each of these distinct neuronal cell types regulates different intestinal processes and crosstalks with the immune system.

2.2 Extrinsic neurons

Extrinsic neurons connect the gut to the brain and spinal cord, and can be further divided into extrinsic sensory afferent neurons, that transmit sensory information to the CNS, and sympathetic and parasympathetic neurons, that innervate and modulate cell types in the gut as well as the ENS (18, 36).

2.2.1 Extrinsic sensory afferent neurons

The intestine is innervated by extrinsic sensory afferent neurons, which transmit sensory information from the intestine to the spinal cord and brain (**Figure 2b**) (29, 37). Extrinsic sensory afferent neurons are pseudo-unipolar neurons, whose peripheral axon is innervating the intestine and whose central axon is projecting to the brainstem or spinal cord (18, 38-40). Depending on the location of the cell bodies within the different ganglia, extrinsic sensory afferent neurons can be further divided: Vagal afferent neurons are located in nodose and jugular ganglia, with central projections to the brainstem and peripheral axons projecting via the vagus nerve to the small intestine and proximal colon (29, 41). The small intestine and proximal colon are also innervated by thoracolumbar spinal afferent neurons, whose cell bodies are located in the thoracolumbar dorsal root ganglia (DRGs) and innervate the small intestine and colon via the splanchnic nerves (29, 42). Thoracolumbar spinal afferent neurons also innervate the lower colon and rectum, which receives additional innervation via pelvic and rectal nerves from lumbosacral spinal afferent neurons (29, 42-44). Based on their terminal morphology and location of

innervation, sensory afferents can be classified into intraganglionic laminar afferents, mucosal afferents, muscular-mucosal afferents, intramuscular afferents, and vascular afferents (29, 43). These sensory subtypes can respond to different stimuli including mechanical stretch, hormones, and chemical irritants (29, 43). Silent afferents are also thought to exist that at homeostasis are unresponsive to mechanical stimuli but can become responsive during inflammation (29, 43).

Vagal and DRG sensory neurons are highly diverse at a molecular level. Almost a decade ago, bulk transcriptional analysis started revealing subpopulations of sensory neurons (45-48). Several groups then performed single-cell RNA-sequencing of vagal ganglia (49) and DRGs from mice (13, 50-52) as well as DRGs from non-human primates (53), identifying up to 24 neuronal clusters in vagal ganglia and 17 different neuronal clusters in DRGs. While these studies provide an unbiased characterization of neuron subtypes present in DRGs, it was unclear which specific molecular subtypes innervate the intestine. This drawback was addressed by Hockley and colleagues, who specifically targeted sensory neurons innervating the mouse colon by labeling them retrogradely with a fluorescent dye and subsequently isolating them from thoracolumbar and lumbosacral DRGs (54). ScRNA-seq identified 7 neuronal clusters, including peptidergic, non-peptidergic and neurofilament containing subtypes (54). Of these gut-innervating neuronal clusters, 5 were found in both thoracolumbar and lumbosacral DRGs, whereas 2 clusters were specific for lumbosacral DRGs (54) suggesting functional differences. Peptidergic subtypes express the neuropeptide CGRP, encoded by *Calca*, and substance

P, encoded by *Tac1* (54). These subtypes also express transient receptor potential cation channel subfamily V member 1 (TRPV1), which is activated by heat, capsaicin, and protons (54-56). The ion channel TRPA1 is expressed by peptidergic subsets observed in both thoracolumbar and lumbosacral DRGs and senses inflammatory products as well as specific chemicals (54-56). The voltage-gated sodium channel $Na_v1.8$, encoded by *Scn10a*, is expressed by different neuronal subtypes and involved in the generation of action potentials (54, 55). Besides these ion channels, DRG sensory neurons also express different Toll-like receptors (TLRs) including TLR4 and TLR5 and can directly respond to microbes and their products including lipopolysaccharide (LPS), an outer wall glycolipid from Gram-negative bacteria (57-60).

2.2.2 Extrinsic sympathetic efferent neurons

The sympathetic pathway consists of a two neuron relay, with a cholinergic preganglionic neuron, which is located in the thoracolumbar spinal cord, which projects to paravertebral and prevertebral ganglia, where it synapses with noradrenergic postganglionic neurons, which then project to peripheral tissues including the gut (**Figure 2b**) (26, 61, 62). Postganglionic neurons of the paravertebral ganglia innervate blood vessels in the intestine and induce vasoconstriction (63). Postganglionic neurons of prevertebral ganglia have a similar function but also innervate other targets, such as neurons in the myenteric and submucosal ganglia to inhibit motility and secretion, as well as Peyer's patches and mucosa (63, 64).

2.2.3 Extrinsic parasympathetic efferent neurons

The parasympathetic pathway consists of a cholinergic preganglionic neuron that synapses with cholinergic postganglionic neurons (**Figure 2b**) (65). Parasympathetic preganglionic neurons located in the dorsal motor nucleus of the vagus and the nucleus ambiguous project via the vagus nerve to the myenteric plexus in the gut, where they synapse with enteric neurons located in the proximal small intestine and proximal colon (18, 26, 62). In contrast, parasympathetic preganglionic neurons located in the sacral spinal cord directly innervate the distal colon or project to pelvic ganglia, where they synapse on postganglionic neurons that innervate the distal colon via rectal nerves (18, 62).

3 REGULATION OF IMMUNE CELLS BY NEURONS

Although it has been known since the last century that nociceptor sensory neurons can induce neurogenic inflammation (6), the extent to which neuro-immune interactions regulate tissue inflammation and host defenses has long been underappreciated. Recent studies revealed that besides sensory neurons also other neuronal populations communicate with immune cells in the intestine and modulate intestinal inflammation. While several studies focused initially on the role of neurons in colitis, more recently the field moved towards a characterization of the signaling pathways that are used by neurons to regulate the function of particular immune cells. Therefore, in this chapter we will first summarize the contribution of extrinsic and intrinsic neurons to the development

of colitis and then review the molecular mechanisms by which neurons signal to different intestinal immune cells.

3.1 Role of different neuronal populations in colitis

Inflammatory bowel diseases (IBD) are defined by chronic relapsing intestinal inflammation and encompass ulcerative colitis and Crohn's disease (66). While the mucosa and submucosa of the colon is inflamed in ulcerative colitis, all layers of the intestinal wall of the distal ileum and colon are inflamed in Crohn's disease (66). IBD is also characterized by pain and stress, which are mediated by the nervous system (43, 67-69). Increasing evidence shows that the nervous system also plays a role in regulating outcome of colitis.

Neuroimmune regulation and a signaling pathway between the brain and periphery that regulates inflammatory responses was discovered over two decades ago and termed the "cholinergic anti-inflammatory pathway" (11). Electrical stimulation of the vagus nerve reduced serum tumor necrosis factor (TNF) levels and endotoxic shock in response to a lethal dose of LPS (70). Further experiments revealed that the vagus nerve signals to the splenic nerve, which induces acetylcholine production by T cells in the spleen. Acetylcholine then negatively regulates TNF production by splenic macrophages in a nicotinic acetylcholine receptor α7 subunit-dependent manner (71-74). This anti-inflammatory pathway not only has a protective effect in LPS-induced endotoxic shock but also in colitis. Vagus nerve stimulation decreased disease severity in models of

trinitrobenzene sulfonic acid (TNBS)- or dextran sulfate sodium (DSS)-induced colitis (75-77) and increased the survival rate in a model of oxazolone-induced colitis (78). Similarly, stimulation of cholinergic signaling pathways decreased disease severity of DSS-induced colitis (79). In contrast, vagotomy increased severity of DSS-induced colitis as well as colonic IL-6 and TNFα levels (80, 81).

Similar to the anti-inflammatory pathway, sympathetic neuron activation could ameliorate colitis. Daily optogenetic activation of sympathetic neurons during DSS-induced colitis reduced weight loss and intestinal inflammation in a noradrenaline-dependent manner (82). Interestingly, systemic administration of noradrenaline resulted in increased body weight loss and inflammation, suggesting that local versus systemic release of noradrenaline may determine the effect (82). This detrimental effects of systemic noradrenaline on DSS-induced colitis has also been reported by another group that blocked the α_{2A}-adrenoceptor during colitis by systemic administration of an antagonist (83). Further experiments revealed that optogenetic activation of sympathetic neurons decreased expression of the cell adhesion molecule MAdCAM-1 on endothelial cells (82). MAdCAM-1 is important for lymphocyte extravasation during inflammation suggesting that sympathetic neurons negatively regulate immune cell infiltration, which ameliorates intestinal inflammation (82).

Besides the parasympathetic and sympathetic pathways, nociceptors have also been shown to play a protective role against DSS-induced colitis in mice (84, 85). Chemical

ablation of TRPV1$^+$ neurons or chemogenetic silencing of neurons using designer receptors exclusively activated by designer drugs (DREADDs) resulted in increased body weight loss and disease severity as well as worse intestinal inflammation during DSS-induced colitis compared to control mice (84, 85). In contrast, chemogenetic activation of TRPV1$^+$ neurons was protective during DSS-induced colitis (84, 85). In the absence of sensory neurons, the neuropeptides substance P and CGRP were reduced and microbial dysbiosis was observed suggesting that nociceptors exert their protective effect via these neuropeptides and the microbiota (84, 85). Substance P-deficient mice showed increased susceptibility to DSS-induced colitis, which could be transferred into germ-free mice by microbial transfer suggesting that substance P ameliorates intestinal inflammation by influencing the microbial composition (84). However, in a different model of colitis induced by the chemical oxazolone, substance P plays a pro-inflammatory role as substance P-deficient mice developed less severe oxazolone-induced colitis compared to wildtype mice (86). In contrast, CGRP shows an anti-inflammatory effect in this model of colitis, as histological disease score was higher in CGRP-deficient mice compared to wildtype mice (86). Besides substance P and CGRP, the neuropeptide vasoactive intestinal peptide (VIP), which is expressed in both vagal sensory neuron and enteric neuron subsets (30, 49), seems to play a role in colitis, although the effect varies between studies. While two studies showed that VIP ameliorates disease severity during TNBS-induced colitis (87, 88), another study did not observe any protective effect of VIP (89). Moreover, germline deletion of VIP resulted in slightly reduced weight loss and decreased levels of IL-6 and TNFα in TNBS-induced colitis indicating a pro-inflammatory role of endogenous VIP (90).

A recent study suggests that the nervous system may induce memory of inflammatory challenge in the gut and recalls information related to these previous immune responses with subsequent challenge in the context of DSS-induced colitis (91). The authors injected a neuron-specific viral vector encoding a Cre-dependent fluorophore into the brain of $Fos^{2A\text{-}iCreERT2}$ mice to label activated neurons at a specific anatomical location upon tamoxifen treatment (91). This viral vector also expressed a stimulatory DREADD, which allows reactivation of neurons with the chemical ligand clozapine *N*-oxide (CNO) (91). Using these genetic and viral approaches, the authors showed that reactivation of neurons in the insular cortex that had been active during previous DSS-induced colitis induces intestinal inflammation partly similar to the inflammatory response observed during colitis (91). In contrast, unspecific inhibition of neurons in the insular cortex during colitis using a viral vector expressing an inhibitory DREADD, ameliorated some signs of inflammation (91).

3.2 Effect of neurons on immune cells

Neurons not only regulate colitis but also other intestinal diseases and enteric infections by modulating the function of immune cells (**Figure 3**). The intestine contains many different immune cells that are located within different areas, such as intraepithelial lymphocytes in the epithelium, and innate lymphoid cells, eosinophils, mast cells, macrophages, dendritic cells, T cells and IgA-producing plasma cells in the lamina propria (16). Immune cells express cognate receptors for neurotransmitters and neuropeptides,

allowing them to respond to mediators produced by different neuronal subtypes (92). We discuss recent findings showing how neurons regulate the function of different immune cells.

3.2.1 Neuron-ILC interactions

Innate lymphoid cells (ILCs) are resident innate immune cells present in different tissues, including the intestine (93). Based on their effector function as well as developmental pathway ILCs can be subdivided into natural killer cells, ILC1s, ILC2s, ILC3s, and lymphoid tissue-inducer cells (93, 94). Despite their functional similarities to T cells, ILCs do not express rearranged antigen receptors but instead respond to cytokines and inflammatory mediators (95). ILC2s promote defense against helminths but also contribute to the development of allergies, whereas ILC3s are important for intestinal homeostasis and defense against extracellular bacteria and fungi (93, 94).

The neuropeptide neuromedin U (NMU) is expressed by cholinergic neurons in the intestine (96). The type 2 alarmin cytokine IL-33 or helminth *Nippostrongylus brasiliensis* products promote NMU expression in neuronal organoid cultures in a MYD88-dependent manner (97) suggesting that cholinergic neurons can sense the presence of helminths via Toll-like receptors. ILC2s are in close proximity to cholinergic neurons (96) and the receptor for NMU, neuromedin U receptor 1 (Nmur1) is found exclusively on ILC2s (96-98) indicating a potential interaction. Indeed, upon stimulation with NMU, intestinal ILC2s proliferate and express increased levels of type 2 cytokines in a Nmur1-dependent

manner (96, 97). In a model of *N. brasiliensis* infection, recombinant NMU promotes eosinophilia and helminth expulsion (96, 97). Adoptive transfer of wildtype versus Nmur1-deficient ILC2s into *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ mice confirmed the important role of Nmur1-expressing ILC2s in mounting a type 2 immune response against helminths (96, 97). This neuro-immune interaction seems to be conserved between tissues, since NMU, in particular in combination with IL-25, has a similar pro-inflammatory effect on lung ILC2s resulting in exacerbated airway inflammation (96, 98).

Another neuropeptide that activates ILC2s is VIP, which is expressed in the intestine by enteric neurons that do not express substance P or tyrosine hydroxylase (99, 100). The receptors for VIP, *Vipr1* and *Vipr2*, are expressed by intestinal ILC2s and signaling via Vipr2 promotes IL-5 production by ILC2s in vitro (101). Since VIP is upregulated after food intake (100, 102) and IL-5 induces eosinophilia, this neuro-immune pathway may be important in promoting eosinophil recruitment after nutrient intake (101). Another study showed that VIP synergizes with IL-33 to promote IL-5 production by ILC2s (102). In a model of helminth infection with *Trichuris muris*, VIP signaling in ILC2s is important for potentiating ILC2 responses and helminth expulsion (102). This signaling pathway could also potentially promote an allergic immune response in the intestine since in the lung VIP has been shown to contribute to the development of allergic airway inflammation by promoting type 2 cytokine production by ILC2s and Th2 cells (103).

In contrast to NMU and VIP, other neuropeptides and neurotransmitters can have an inhibitory effect on ILC2s. CGRP is expressed in the small intestine by choline acetyltransferase $(ChAT)^+$ enteric neurons (104). ILC2s are in close contact to these neurons and express the receptor for CGRP, which consists of a coreceptor complex formed by Ramp1 and Calcrl (104). Short-term cultures revealed a differential effect of CGRP on activated intestinal ILC2s: While CGRP increased IL-5 expression, it inhibited IL-13 expression as well as proliferation (104). These results suggest a detrimental role of CGRP in helminth infection as it inhibits IL-13 expression important for mucus production and helminth expulsion (93). Indeed, adoptive transfer of wildtype or Ramp1-deficient bone marrow into *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ mice revealed a decreased worm burden in the absence of Ramp1 signaling (105). In a model of OVA-induced food allergy, recombinant CGRP also inhibits ILC2 expansion (104). This neuro-immune pathway is also observed in the lung where it reduces the severity of IL-33-induced airway inflammation in the absence of adaptive immune cells (105, 106).

Another negative regulator of ILC2 responses is norepinephrine expressed by adrenergic neurons in the small intestine (107). ILC2s are neighboring adrenergic neurons and among other immune cells express the norepinephrine receptor, β_2-adrenergic receptor (107). In a model of *N. brasiliensis* infection, signaling via the β_2-adrenergic receptor inhibits ILC2 expansion resulting in increased worm burden (107). A similar inhibitory effect is observed in the lung during airway inflammation suggesting a conserved neuro-immune pathway (107).

Besides ILC2s, ILC3s are also regulated by neuropeptides. Similar to ILC2s, ILC3s are located next to VIP$^+$ neurons in the intestine and express *Vipr2*, one of the receptors for VIP (99, 100, 102). Stimulation of intestinal ILC3s with VIP induces IL-22 production and this effect is potentiated when given in combination with IL-23 (100, 102). Since IL-22 is important for epithelial barrier function (108), the VIP-ILC3-IL-22 axis might play an important role in intestinal inflammation and infection. Indeed, in a DSS-induced colitis model, *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ mice with adoptively transferred Vipr2-deficient ILC3s develop stronger intestinal inflammation compared to control mice with wildtype ILC3s (100). Similarly, in a model of bacterial infection with *Citrobacter rodentium*, the bacterial burden is increased in mice with a conditional deletion of Vipr2 in ILC3s (Rorγt-Cre$^+$ Vipr2$^{fl/fl}$) compared to controls (102). While the aforementioned studies observe an increase in IL-22 production in ILC3s upon VIP stimulation, another study reports the contrary effect (99). The authors show that chemogenetic activation of VIP$^+$ neurons increases susceptibility to *C. rodentium* infection and inhibition of neurons decreases bacterial load (99). Their data supports that signaling via Vipr2 negatively regulates IL-22 production by CCR6$^+$ ILC3s, which results in a decreased expression of antimicrobial peptides (99). Using mice with a Rorγt$^+$ cell-specific deletion of Vipr2 they found decreased length of segmented filamentous bacteria (SFB) and diminished uptake of lipids (99). The difference in outcome between these studies could be due to different experimental approaches and timing of experiments, as well as the microbiota. In addition to the coordination of intestinal responses to food intake, VIP is also associated with circadian

rhythms (109). Cytokine expression of intestinal ILC3s follows circadian oscillations, which are regulated by the light-dark cycle as well as brain-derived signals (110-112). Expression of Arntl, a key regulator of the circadian rhythm, is important for ILC3 homeostasis (111, 112) and defense against bacterial infection (112).

Notably, recent research has revealed that neuropeptides and neurotransmitters are not only expressed by neurons but also by ILC2s and ILC3s themselves. CGRP is expressed by intestinal and lung ILC2s, which upregulate its expression during intestinal inflammation and helminth infection, suggesting a negative feedback circuit to suppress ILC2 responses during tissue inflammation (104-106). ChAT, an enzyme involved in the synthesis of acetylcholine, is expressed by lung and intestinal ILC2s during helminth infection and conditional deletion in IL-7R^{+} cells or Rorα^{+} cells results in reduced ILC2 numbers and decreased worm expulsion suggesting that ILC2-derived acetylcholine contributes to anti-helminth responses (113, 114). Similarly, tryptophan hydroxylase 1 (Tph1), the enzyme involved in serotonin synthesis, is also expressed in intestinal ILC2s and lymphocyte-specific deletion of Tph1 results in diminished inflammatory ILC2 responses and impaired helminth expulsion (115). These studies show that immune cells can develop neuronal-like characteristics, and this could lead to functional impacts on their phenotype.

3.2.2 Neuron-mast cell interactions

Mast cells play important roles during helminth infection by promoting a type 2 immune response (116) and in allergies by releasing preformed mediators, such as histamine, which induce vasodilation, vascular permeability and bronchoconstriction (117). Two subsets of mast cells exist in mice, the connective-tissue mast cells (CTMCs) located in the muscularis propria and the mucosal mast cells (MMCs) located in the mucosa (118).

MMCs are observed close to $CGRP^+$ cholinergic nerves in the colon of mice with food allergy (119) and in close proximity to peptidergic neurons in the intestines of helminth-infected rats (120). Similarly, mast cells were also neighboring nerves in colons from patients with irritable bowel syndrome (121). Substance P and CGRP are able to induce degranulation of rodent MMCs (122, 123), whereas substance P and VIP promote degranulation of primary cultured human mast cells (124). While these results suggest that mast cell degranulation is regulated by neuropeptides, another study reports that unpurified mast cells isolated from human intestines do not degranulate in response to substance P, VIP or CGRP (125). However, upregulation of NK-1, the receptor for substance P, was observed upon IgE receptor crosslinking in this study (125).

The neurotransmitter acetylcholine also presumably negatively regulates mast cells. Mucosal-type murine bone marrow-derived mast cells express the $\alpha 7$ nicotinic acetylcholine receptor (nAChR) subunit and a specific agonist for this subunit reduced the degranulation induced by IgE-mediated activation (126). In a model of OVA-induced

food allergy, administration of a nAChR agonist reduced colonic MMC numbers and development of allergic diarrhea (119). Another study reports that vagus nerve stimulation during OVA-induced food allergy reduced intestinal mast cell numbers and mMCP-1 levels, however, this effect was independent of α7nAChR signaling (127). Myenteric neurons can also activate mucosal-type bone marrow-derived mast cells based on co-culture experiments, which was among others dependent on adenosine A3 receptor signaling in mast cells (128).

3.2.3 Neuron-macrophage interactions

Intestinal macrophages can be classified into lamina propria macrophages and muscularis macrophages depending on their molecular profiles as well as location within the tissue (129). Lamina propria macrophages are located close to the intestinal epithelium and are important for the clearance of dead cells and pathogens, transfer of luminal antigens as well as regulation of intestinal ILC and T cell responses (129, 130). In contrast, muscularis macrophages are located in the muscle layers of the intestine and play an important role in intestinal homeostasis and colonic motility (129).

Enteric neurons provide crucial support for muscularis macrophages in the intestine. Muscularis macrophages are located in proximity to enteric neurons and express *Csf1r*, the receptor for colony stimulatory factor 1 (CSF1) (131). Enteric neurons produce the ligand CSF1, which is an essential growth factor for the development of muscularis macrophages (131). Sympathetic neurons protect the tissue and enteric neurons by

regulating muscularis macrophages during bacterial infection. In response to bacterial infection with *Salmonella Typhimurium* mutant *Spib* extrinsic sympathetic neurons produce norepinephrine, which stimulates β_2 adrenergic receptors on muscularis macrophages to promote a tissue-protective cell state (132). Moreover, β_2 adrenergic signaling in muscularis macrophages promotes arginase 1-mediated production of polyamines, which reduces death of enteric neurons after enteric bacterial infection (133).

Besides sympathetic pathways, parasympathetic pathways are also involved in intestinal inflammation. Macrophages are located next to cholinergic nerves in the myenteric plexus (134) and express α7 nAChR (135). Anterograde tracing of vagal efferents innervating the intestine revealed that macrophages are not in close proximity to cholinergic vagal efferents but to cholinergic myenteric neurons suggesting that muscularis macrophages are regulated by acetylcholine released by enteric neurons (135). Indeed, stimulation of LPS-activated peritoneal macrophages with nicotine decreased the secretion of TNF and IL-6 in vitro (134). In a model of surgery-induced intestinal inflammation, vagus nerve stimulation negatively regulated peritoneal IL-6 levels and intestinal inflammation (134). This inhibitory effect was independent of splenic innervation and T cells suggesting that the vagus nerve innervates cholinergic enteric neurons, which upon release of acetylcholine inhibit IL-6 release by muscularis macrophages (135). In a model of OVA-induced food allergy vagus nerve stimulation promoted the uptake of OVA by CX3CR1^{hi} macrophages suggesting that the vagus nerve also regulates antigen uptake in different immune cell populations (127).

Enteric serotonergic neurons also regulate macrophage function in the intestine. In response to valeric acid, a metabolite produced by intestinal microbiota, enteric serotonergic neurons express tryptophan hydroxylase-2, an enzyme involved in serotonin synthesis (136). Serotonin subsequently promotes PGE2 release by macrophages, which positively regulates intestinal stem cell renewal (136).

Research on neuron-macrophage interactions in the skin suggests that macrophages could also be potentially controlled by sensory neurons during intestinal inflammation. In the skin, GINIP^{+} sensory neurons support tissue healing by releasing the neuropeptide TAFA4, which acts on dermal macrophages to enhance IL-10 production after UV-induced damage (137).

3.2.4 Neuron-dendritic cell interactions

Dendritic cells are essential for mounting an adaptive immune response as they present antigen via MHC molecules to naïve T cells (138, 139). In the intestine, four different subsets of classical dendritic cells (cDCs) are located within the lamina propria and Peyer's patches as well as solitary intestinal lymphoid tissues (138). Upon antigen uptake cDCs migrate via the lymph to draining mesenteric lymph nodes, where they present antigen to naïve T cells (138). Another subset of DCs present in the lamina propria, the plasmacytoid DCs (pDCs), are non-migratory but promote oral tolerance and migration of cDCs to draining lymph nodes (138).

In other tissues, DCs are regulated by VIP, CGRP and substance P. Since these neuropeptides are also expressed by neurons in the intestine (30), they might also regulate the function of intestinal DCs. Using bone marrow-derived DCs it was shown that VIP promotes IL-10 expression and limits the expression of costimulatory molecules in response to LPS suggesting that VIP induces tolerogenic DCs (140). In a co-culture system of DRG neurons and bone marrow-derived DCs, neuron-derived CGRP induces transcriptional changes in DCs resulting in an increase in sentinel functions (141). In addition, DRG neurons attract DCs via release of the chemokine CCL2 and their electrical activity induces calcium influx in DCs, leading to an increased cytokine response to immune stimuli (141). In the skin, sensory neurons release substance P in response to protease allergens, which induces migration of DCs to draining lymph nodes, where they promote the differentiation of T helper cells to Th2 cells (142).

Antigen-presenting cells (APCs), including $CD103^+$ dendritic cells and $CX3CR1^+$ mononuclear phagocytes, express the muscarinic acetylcholine receptor (mAChR) and upon stimulation with acetylcholine express *Aldh1a1* and *Aldh1a2*, which encode enzymes important for the synthesis of retinoic acid (143). Retinoic acid has been shown to be important for peripheral regulatory T cell (Treg) generation (144). Indeed, during DSS-induced colitis surgical division of the hepatic vagal branch led to a decline in the number of colonic $Foxp3^+$ Tregs and increased intestinal inflammation. Further experiments revealed a neuronal circuit between the liver, brain and intestine that limits

tissue inflammation by inducing retinoic acid production by APCs, which supports intestinal Tregs (143).

3.2.5 Neuron-T cell interactions

Regulatory T cells (Tregs) express the transcription factor Foxp3 and are crucial for tissue homeostasis and tolerance (145). Depending on their developmental origin, Tregs are categorized as thymus-derived Tregs (tTregs) or peripherally derived Tregs (pTregs) (144). While tTregs develop in the thymus in response to binding of self-antigen and can be identified in the intestine as Gata3$^+$ Helios$^+$ Tregs, pTregs are induced in the periphery by intestinal microbiota and are RORγt$^+$ Helios$^-$ cells (144, 145). In the small intestine, another subset of RORγt$^-$ Helios$^-$ Tegs has been identified that develops in response to food antigens (145).

Regulatory T cells are observed in close proximity to CGRP$^+$ sensory neurons or nitric oxide synthase (NOS1)$^+$ motor neurons in the lamina propria (146). Experiments revealed that the bacterium *Clostridium ramosum* negatively regulates expression of the neuropeptide substance P (*Tac1*) in the gut (60), while also inducing Rorγ^+ Tregs in the colon (147). Further experiments suggested that substance P either directly or indirectly suppresses colonic Treg differentiation since Treg frequencies were increased in substance P-deficient mice, and capsaicin-induced activation of intestinal TRPV1$^+$ sensory neurons reduced the frequency of Tregs in the colon (60). A subsequent study revealed that enteric neurons can regulate the differentiation of Tregs via release of IL-6

(146). Supernatant from enteric neurons suppressed the differentiation of conventional T cells to Foxp3$^+$ regulatory T cells under regulatory T cell differentiating conditions in an IL-6-dependent manner and conditional deletion of IL-6 in neurons resulted in higher Treg frequencies in the colon (146).

3.2.6 Neuron-immune cell interactions in other tissues

Although not described in the intestine, interactions between neurons, neutrophils and γδ T cells have been observed in other tissues suggesting that they might also play a role in the intestine. In the skin, nociceptors secrete CGRP in response to *Streptococcus pyogenes* infection, which negatively regulates the recruitment of neutrophils and neutrophil-mediated killing of the bacterium (148). Another study showed that optogenetic activation of TRPV1$^+$ neurons in the skin induces a type 17 immune response, which reduces the bacterial and fungal load during *Staphylococcus aureus* and *Candida albicans* infection, respectively (149). In the lung, *S. aureus* induces CGRP release by TRPV1$^+$ neurons, which inhibits neutrophil recruitment and γδ T cell responses (150). In addition to these interactions, many other neuro-immune interactions were described in other tissues at homeostasis and in the context of infections and diseases (reviewed in (2, 17, 65, 151, 152)).

3.2.7 Enteric glial cell-immune cell interactions

In addition to neurons, also enteric glial cells communicate with intestinal immune cells, such as type 3 innate lymphoid cells (153), monocytes (154), muscularis macrophages

(155), and contribute to tissue repair after helminth infection (156). We will not cover this particular crosstalk in this review but refer the reader to other excellent reviews on glial-immune interactions (157, 158).

3.3 Effect of neurons on epithelial cells

Recent work revealed that neuronal populations also regulate intestinal diseases by communicating with epithelial cells. The intestinal epithelium consists of different epithelial cell types with separate functions, such as microfold (M) cells, which are important for antigen uptake, and mucus-producing goblet cells (159).

Upon infection with *Salmonella enterica*, mice with depleted nociceptors had significantly increased bacterial load in the ileum, Peyer's patches and mesenteric lymph nodes as well as elevated bacterial dissemination to other organs suggesting that nociceptors play a protective role in enteric bacterial infections (160). Further experiments revealed that TRPV1$^+$ nociceptors sense *Salmonella enterica* and in response release CGRP, which negatively regulates the density of M cells in ileum Peyer's patch follicle-associated epithelia, thereby minimizing the entry points for *Salmonella* (160). In addition, CGRP also promotes levels of segmented filamentous bacteria (SFB), a microbial strain that offers protection against *Salmonella* infection (160).

Besides M cells, nociceptors also regulate intestinal goblet cells and their production of mucus in the colon (85). ScRNA-seq of ileal and colonic epithelial cells revealed that

goblet cells express Ramp1, which is part of the co-receptor complex for CGRP, suggesting that goblet cells are regulated by nociceptors via CGRP (85). Nociceptors are close to goblet cells in the colon and release CGRP in response to microbiota and dietary cues (85). Moreover, a thinner mucus layer is observed both in Calca-deficient mice as well as in mice with an epithelial cell-specific Ramp1 deletion indicating that goblet cell emptying is positively regulated by CGRP signaling (85). During DSS-induced colitis mice with ablated nociceptors develop more severe intestinal inflammation, which is rescued by administration of CGRP, indicating an important role of this signaling pathway for epithelial barrier protection (85).

Another study showed that goblet cell function is also regulated by enteric neurons. Mice infected with *S. typhimurium* that lack IL-18 in enteric neurons show increased bacterial dissemination and succumb faster to infection (161). Further experiments revealed that enteric neuron-derived IL-18 mediates its protective effect by inducing the production of antimicrobial proteins by goblet cells in the colon (161).

Goblet cells can also contribute to an immune response by creating goblet cell-associated antigen passages (GAPs), which transport antigens from the lumen to APCs in the lamina propria (162, 163). Intestinal goblet cells express the muscarinic acetylcholine receptor 4 (mAChR4) and administration of a cholinergic agonist enhances the formation of GAPs in the small intestine (164). In contrast, blockade with a selective antagonist prevents the

formation of GAPs indicating that acetylcholine signals via mAChR4 (164). These results suggest that acetylcholine positively regulates GAP formation (164).

Several studies suggest that VIP regulates the epithelial barrier in the intestine. Co-culture of the human submucosa and colonic epithelial cells revealed that activation of submucosal neurons reduces the permeability of the epithelium, presumably in a VIP-dependent manner (165). Mice lacking VIP have a dysregulated epithelial barrier (166) and administration of VIP limits epithelial permeability and body weight loss induced by *Citrobacter rodentium* infection (167). While these studies suggest a protective role for VIP, another study reports that blockade of VIP signaling negatively regulates bacterial passage over human colonic epithelium ex vivo indicating that VIP may promote epithelial permeability (168).

4 REGULATION OF NEURONS BY IMMUNE CELLS

4.1 Sensitization of gut-innervating sensory neurons by immune cells

4.1.1 Intestinal inflammation and visceral pain hypersensitivity

Abdominal pain is frequently experienced by patients with inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) (43). IBS is a chronic intestinal disorder, which results in visceral discomfort and pain and an altered stool pattern (43, 169). Risk factors are gastrointestinal infections, atopic diseases, psychological stress as well as consumption of certain foods (169). Visceral pain develops when painful stimuli are

transmitted from internal organs via afferent sensory neurons to the CNS (169). The majority of IBS patients experience visceral hypersensitivity, which describes a state when either an usually innocuous stimulus is perceived as painful or a painful stimulus elicits higher sensitivity than normal in the viscera (169). In these individuals, visceral pain is long-term, and does not resolve after resolution of inflammation (**Figure 4a**) (43). High-threshold, distension-sensitive sensory afferents and vasculature-innervating sensory neurons become sensitized, in addition to activation of usually mechanically-insensitive afferents (43). Besides sensitization at nerve terminals, changes are also observed in the spinal cord, where colonic afferents innervate additional areas leading to amplified activation of dorsal horn neurons (43).

Mouse models have revealed that visceral hypersensitivity develops and accompanies intestinal inflammation. Increased visceromotor responses were measured in response to colorectal distension in mice with TNBS-induced colitis compared to control mice (170) indicating that these mice developed visceral hypersensitivity. Moreover, a higher frequency of colon-innervating DRG neurons responded to colorectal distension during TNBS-induced colitis compared to controls (171). Mice also experienced visceral pain not only during acute DSS-induced colitis but also after disease resolution (172). Notably, intestinal inflammation-induced hypersensitivity also seems to affect other distal tissue sites. During colitis, an increased withdrawal response to mechanical stimulation of the hind paws was observed in mice (170, 171). Neurons located in DRGs that innervate both the colon and low-back skin, responded stronger to skin brushes during colitis compared

to controls suggesting that visceral sensitivity can also lead to somatic hypersensitivity (171). Recently, it was reported that mice sensitized to ovalbumin (OVA) during bacterial infection develop visceral hypersensitivity when challenged orally with OVA after infection (173), suggesting that food-induced immune responses can also be a major contributor to abdominal pain. Indeed, mucosal changes in IBS patients with likely food intolerance were immediately visible when food allergens were applied onto the mucosa of the duodenum during endoscopy (174). If the food antigen that the patient responded to was subsequently excluded from the diet, abdominal symptoms improved in the majority of patients (174).

Recent work suggests that TRPV1$^+$ neurons promote abdominal pain. Despite similar disease severity, visceral pain was significantly reduced in mice lacking TRPV1 after resolution of DSS-induced colitis (172). Similarly, chemogenetic inhibition of TRPV1$^+$ neurons during DSS-induced colitis did not influence disease severity but reduced visceral hypersensitivity (175). In contrast, chemogenetic activation of TRPV1$^+$ neurons resulted in visceral hypersensitivity, in addition to the induction of intestinal inflammation (175).

4.1.2 Peripheral sensitization

Research over the last two decades revealed the involvement of immune cells in the peripheral sensitization of neurons in the intestine. Patients with irritable bowel syndrome (IBS) had elevated numbers of degranulating mast cells, and mast cells located close to

nerve fibers in the mucosa of the colon correlated with abdominal pain (121). Supernatants of mucosal biopsies from IBS patients contained higher levels of the mast cell mediators histamine and tryptase compared to those from healthy individuals (176-178). In vivo, increased visceral sensitivity in response to colorectal distension was observed in mice that had received colon supernatants from IBS patient biopsies compared to supernatants from control biopsies (178). These results suggest that mast cells promote visceral pain by sensitizing afferent sensory neurons in the intestine. Indeed, mediators released from murine mast cells and supernatant from IBS patients activated DRG neurons in vitro (123, 176, 178). Supernatant from IBS patients, especially diarrhea-predominant IBS patients, was shown to increase the excitability of DRG neurons, which was diminished in PAR_2-deficient DRGs suggesting that sensitization of sensory neurons occurs in a protease-dependent manner (**Figure 4a**) (178, 179). Moreover, histamine contributes to DRG neuron activation by sensitizing the TRPV1, TRPA1 and TRPV4 ion channels via histamine receptor H1 (169, 180). In contrast, resolvins, which are pro-resolving lipid mediators, inhibited TRPV1 sensitization in DRG neurons by histamine or substance P in vitro, and negatively regulated the development of visceral hypersensitivity in vivo (181-183).

Although not in the context of colitis, a recent study showed that during IgE-mediated anaphylaxis, the mast cell-derived protease chymase activates $TRPV1^+$ sensory neurons by binding to its receptor protease-activated receptor-1 (PAR1) (184). As a result, warm-sensing neural circuits in the brain are activated leading to decreased brown adipose

tissue thermogenesis and hypothermia (184). In a model of food-induced visceral hypersensitivity, colonic supernatants from mice with visceral hypersensitivity promoted sensitization of $TRPV1^+$ sensory neurons in a histamine-1 receptor-dependent manner (173) suggesting that histamine-mediated sensitization of sensory neurons promotes abdominal pain in response to food allergens.

Besides mast cells, enterochromaffin cells have also been shown to play a key role in sensitizing sensory neurons in the gut to drive visceral pain. Enterochromaffin cells are an epithelial cell type that is activated by microbial metabolites, chemical irritants as well as epinephrine, norepinephrine and dopamine (185). In response, enterochromaffin cells release 5-HT (serotonin), which acts on afferent neurons via the $5HT_3$ receptor (185). Chemogenetic activation of enterochromaffin cells promotes visceral hypersensitivity and anxiety-like behavior (186). Further experiments revealed that this phenotype can be caused by enterochromaffin cells interacting with mucosal afferents that are distension-insensitive but respond to local mucosal deformation (186).

In addition to peripheral sensitization of nerve terminals, also increased neuron sprouting is associated with inflammation. A higher neuron density was observed in colons from patients with IBS along with elevated expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) compared to healthy individuals (187, 188) suggesting that both factors promote nerve sprouting.

In the skin, IL-17A from microbiota-induced Th17 cells contributed to neuronal regeneration after skin injury (189) and IL-31 released from conventional type 2 dendritic cells induced itch during wound healing by increasing the sensitivity of itch sensory neurons (190). These observations from other tissues suggest that these cytokines could also be involved in the sensitization of neurons in the intestine.

4.1.3 Central sensitization

Central sensitization describes the process that nociceptive signals increase the sensitivity of nociceptive circuits by promoting synaptic plasticity and excitability of neurons, as well as diminishing inhibition in the central nervous system (12). One study showed that granulocyte-colony-stimulating factor (G-CSF) promotes the development of visceral hypersensitivity in mice recovering from DSS-induced colitis by activating spinal microglia (**Figure 4a**) (191). Experiments suggest that G-CSF binds to the G-CSF receptor on microglia and induces the release of Cathepsin S, which cleaves fractalkine (191). Subsequently, soluble fractalkine binds to CX3CR1 on microglia, which promotes nitric oxide production resulting in the hyperexcitability of DRG neurons (191). Another study revealed that activated TRPV1$^+$ neurons release adenosine triphosphate (ATP), which via the microglial receptor P2RY12 promotes microglia activation and neuronal sensitization (175).

4.2 Regulation of neuronal survival and function

4.2.1 Effect of immune cells on enteric neurons

Recent work revealed an important role of different immune cell types for function and survival of enteric neurons at homeostasis and during intestinal infection and inflammation (**Figure 4b**).

Intestinal macrophages have been shown to support the survival and function of enteric neurons. Self-maintaining gut-resident macrophages are observed in proximity to myenteric neuronal ganglia and submucosal enteric neurons and their depletion results in neuronal apoptosis in both submucosal and myenteric plexus leading to decreased secretion and gut motility (192). During both bacterial and helminth infection muscularis macrophages obtain a tissue-protective phenotype and support neuronal survival (193). During infection with *Salmonella Typhimurium spiB* muscularis macrophages decrease infection-induced death of enteric neurons in the intestine (133). This protective effect is dependent on β_2-adrenergic receptor signaling in muscularis macrophages and arginase 1 expression and polyamine synthesis (133). Muscularis macrophages also play an important role in preventing further neuronal loss upon re-infection. Infection with the bacterium *Yersinia pseudotuberculosis* induces loss of myenteric neurons but protects from further neuronal loss during subsequent infection with *S. Typhimurium spiB* in a muscularis macrophage-dependent manner (193). In contrast, infection with the helminth *Strongyloides venezulenesis* does not induce neuronal loss and still prevents *spiB*-induced loss of myenteric neurons (193). While this protective effect is independent of β_2-

adrenergic receptor signaling (193), it instead requires the induction of Th2 cells and eosinophils, which via IL-4 and IL-13 release promote Arginase 1 expression by muscularis macrophages (193). In addition to their protective effect muscularis macrophages also modulate colonic peristalsis by producing bone morphogenetic protein (BMP) 2, which binds to BMP receptor on enteric neurons (131).

In contrast to muscularis macrophages, CD8 T cells have been shown to be detrimental for enteric neuron survival. Inoculation of mice with the flavivirus West Nile virus results in infection of submucosal and myenteric neurons, which leads to neuronal death and decreased intestinal motility due to the infiltration of antigen-specific CD8 T cells (194). Similarly, adoptive transfer of OVA-specific CD8 T cells into transgenic mice expressing OVA in submucosal and myenteric neurons results in enteric ganglionitis and neuronal loss (195).

B cells presumably regulate intestinal neurons via the secretion of IgE. A recent study showed that the majority of cholinergic neurons in the murine myenteric plexus express FcεRI (128). In vitro calcium imaging revealed that cross-linking with DNP-BSA induces activation of some myenteric neurons that were pre-incubated with anti-DNP IgE (128).

4.2.2 Effect of microbiota on intrinsic and extrinsic neurons

Besides immune cells, the gut microbiome is important for normal intestinal motility. Mice treated with antibiotics had reduced intestinal motility (131, 196, 197), which could be

rescued by administration of LPS (131). Primary enteric neurons can directly sense LPS as stimulation of them in vitro with LPS promoted the expression of *Csf1*, a growth factor for muscularis macrophages (131). Since muscularis macrophages are important for intestinal peristalsis, the presence of microbiota promotes functional intestinal motility (131). In addition, administration of short-chain fatty acids (SCFAs) restored reduced propulsive colonic motility in germ-free mice compared to colonized mice (198). Another study revealed that SCFAs produced by certain bacterial consortia inhibit activation of extrinsic sympathetic neurons (199). Using anterograde and retrograde tracing as well as translational profiling and chemogenetic approaches, the authors identified a neuronal circuit between the intestine and brain involving vagal afferents and brainstem neurons that regulates efferent sympathetic neuron signaling and intestinal transit in response to luminal SCFA (199). Another study showed that intrinsic primary afferent neurons in the myenteric plexus are affected by the microbiota (200). Sensory neuron excitability was lower in germ-free mice compared to specific pathogen-free (SPF) mice and was improved after colonization with microbiota (200). Further work has revealed that myenteric neurons in the colon express *Ahr*, which encodes the transcription factor aryl hydrocarbon receptor (AHR), in a microbiota-dependent manner (31). Conditional deletion of *Ahr* in enteric neurons resulted in reduced expression of a K^+ channel important for neuronal excitability, decreased colonic peristalsis and prolonged intestinal transit time, suggesting that in response to microbial signals neuronal AHR promotes intestinal motility (31).

Besides neuronal function, the structure of the enteric nervous system has been shown to be dependent on the microbiome and is abnormal in early postnatal germ-free mice (201). The density of intrinsic enteric-associated neurons (iEANs) in the myenteric plexus was decreased in the duodenum and ileum but not in the colon of germ-free mice compared to SPF mice (35). Fecal transfer rescued the loss of iEANs in the ileum of germ-free mice (35). One potential mechanism how the microbiota supports ENS function could be via serotonin (5-HT), which promoted innervation of the mucosa in germ-free mice (202).

The microbiota also promotes neuronal survival and recovery at homeostasis and during infection. Viscerofugal neurons that express cocaine- and amphetamine-regulated transcript (CART) were reduced in the ileum and colon upon microbial depletion (35) indicating that the microbiota supports this neuronal subset. This neuronal subset has an important physiological role as it regulates blood glucose levels and food intake (35). A functional microbiome is required for the recovery of enteric neurons after bacterial infection-induced death (133). Experiments revealed that bacterial enteric infection induces dysbiosis and neuronal loss, both of which are rescued after recolonization with SPF microbiota (133).

5 OUTLOOK

Research over the last years has revealed an essential role for neuro-immune crosstalk in the intestine both in maintaining tissue homeostasis and in regulating the outcome of intestinal inflammation. This is an exciting time for this research field as the recent technological and genetic advances in biology will allow an in-depth characterization of neuro-immune interactions that will lead to the discovery of novel interactions and a better mechanistic understanding. Despite the increased interest in this field there are still many outstanding questions and avenues that could be pursued, including determining how neuro-immune networks are established in the gut, both at the spatial and molecular levels, how neuro-immune interactions affect inter-organ communication, and applying our knowledge of these interactions to therapeutic applications. We summarize and discuss some of these points below. We believe that over the next decade studies will reveal exciting new conceptual and mechanistic insights into neuro-immune interactions with important implications for the development of novel therapies for the treatment of intestinal diseases.

5.1 SUMMARY POINTS

1. Neuro-immune interactions are a rapidly developing research field with therapeutic implications for inflammatory diseases, such as inflammatory bowel diseases, irritable bowel syndrome, and enteric infections.
2. The intestine is innervated by intrinsic enteric neurons and extrinsic sensory, sympathetic, and parasympathetic neurons. The intrinsic enteric neurons form the

enteric nervous system and regulate intestinal motility as well as nutrient uptake and secretion, whereas extrinsic neurons transmit visceral sensory information to the central nervous system via afferent sensory neurons and in response modulate the enteric nervous system via efferent neurons.

3. Mouse models of intestinal inflammation and infection revealed the important role of different neuronal subtypes in the development of colitis and showed that neurons release neuropeptides, neurotransmitters and cytokines, which regulate the effector function of different immune and epithelial cell types in the intestine.
4. Intestinal inflammation promotes visceral hypersensitivity by inducing peripheral and central sensitization of gut-innervating sensory afferent neurons via inflammatory mediators.
5. Survival and function of different neuronal populations at homeostasis and during bacterial and helminth infection depends on the presence of immune cells and functional microbiota.

5.2 FUTURE ISSUES

1. Enteric neurons and colon-innervating sensory neurons have been characterized on a transcriptional level by scRNA-seq at homeostasis. However, how are these neuronal populations changing over the lifetime and in response to inflammatory or infectious stimuli?

2. Research on neuro-immune interactions in other tissues revealed additional interactions between neurons and immune cells. Are these interactions conserved between tissues and also play a role in the intestine?
3. Neuropeptides and neurotransmitters have distinct effects on different immune cell types. How is the release of these distinct mediators regulated? Are they released in response to different stimuli or released at different time points or different anatomical locations?
4. How is the gut-brain axis involved in different intestinal diseases? Are there neuronal circuits connecting the intestine to other organs besides the CNS that are active during intestinal inflammation?
5. How can neuro-immune interactions be targeted for therapies? Examples of a successful translation of scientific discoveries to potential therapies already exist such as a beneficial effect of vagus nerve stimulation in Crohn's disease patients (203, 204) and an improvement of visceral hypersensitivity and abdominal pain in IBS patients after treatment with a mast cell stabilizer or an antagonist of histamine receptor H1 (180, 205).

DISCLOSURE STATEMENT

I.M.C. consults for GSK Pharmaceuticals, Kallyope, Inc, and LIMM therapeutics. The Chiu lab receives sponsored research support from Moderna, Inc.

ACKNOWLEDGMENTS

The figures were created with BioRender.com. The Chiu lab acknowledges funding support from NIH (R01DK127257, R01AI168005), Food Allergy Science Initiative, Burroughs Wellcome Fund, Chan-Zuckerberg Initiative, Kenneth Rainin Foundation, Jackson-Wijaya Fund, and Drako Fund.

LITERATURE CITED

1. Veiga-Fernandes H, Mucida D. 2016. Neuro-Immune Interactions at Barrier Surfaces. *Cell* 165: 801-11

2. Godinho-Silva C, Cardoso F, Veiga-Fernandes H. 2019. Neuro-Immune Cell Units: A New Paradigm in Physiology. *Annu Rev Immunol* 37: 19-46

3. McMahon SB, La Russa F, Bennett DL. 2015. Crosstalk between the nociceptive and immune systems in host defence and disease. *Nat Rev Neurosci* 16: 389-402

4. Foster SL, Seehus CR, Woolf CJ, Talbot S. 2017. Sense and Immunity: Context-Dependent Neuro-Immune Interplay. *Front Immunol* 8: 1463

5. Medzhitov R. 2010. Inflammation 2010: new adventures of an old flame. *Cell* 140: 771-6

6. Sousa-Valente J, Brain SD. 2018. A historical perspective on the role of sensory nerves in neurogenic inflammation. *Semin Immunopathol* 40: 229-36

7. Dubin AE, Patapoutian A. 2010. Nociceptors: the sensors of the pain pathway. *J Clin Invest* 120: 3760-72

8. Pavlov VA, Tracey KJ. 2017. Neural regulation of immunity: molecular mechanisms and clinical translation. *Nat Neurosci* 20: 156-66

9. Ludwig M, Leng G. 2006. Dendritic peptide release and peptide-dependent behaviours. *Nat Rev Neurosci* 7: 126-36

10. Basbaum AI, Bautista DM, Scherrer G, Julius D. 2009. Cellular and molecular mechanisms of pain. *Cell* 139: 267-84

11. Chavan SS, Pavlov VA, Tracey KJ. 2017. Mechanisms and Therapeutic Relevance of Neuro-immune Communication. *Immunity* 46: 927-42

12. Latremoliere A, Woolf CJ. 2009. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *J Pain* 10: 895-926

13. Zeisel A, Hochgerner H, Lonnerberg P, Johnsson A, Memic F, van der Zwan J, Haring M, Braun E, Borm LE, La Manno G, Codeluppi S, Furlan A, Lee K, Skene N, Harris KD, Hjerling-Leffler J, Arenas E, Ernfors P, Marklund U, Linnarsson S. 2018. Molecular Architecture of the Mouse Nervous System. *Cell* 174: 999-1014 e22

14. Margolis KG, Gershon MD. 2016. Enteric Neuronal Regulation of Intestinal Inflammation. *Trends Neurosci* 39: 614-24

15. Furness JB. 2012. The enteric nervous system and neurogastroenterology. *Nat Rev Gastroenterol Hepatol* 9: 286-94

16. Mowat AM, Agace WW. 2014. Regional specialization within the intestinal immune system. *Nat Rev Immunol* 14: 667-85

17. Huh JR, Veiga-Fernandes H. 2020. Neuroimmune circuits in inter-organ communication. *Nat Rev Immunol* 20: 217-28

18. Brierley SM, Linden DR. 2014. Neuroplasticity and dysfunction after gastrointestinal inflammation. *Nat Rev Gastroenterol Hepatol* 11: 611-27

19. Sang Q, Young HM. 1996. Chemical coding of neurons in the myenteric plexus and external muscle of the small and large intestine of the mouse. *Cell Tissue Res* 284: 39-53

20. Sang Q, Williamson S, Young HM. 1997. Projections of chemically identified myenteric neurons of the small and large intestine of the mouse. *J Anat* 190 (Pt 2): 209-22

21. Sang Q, Young HM. 1998. The identification and chemical coding of cholinergic neurons in the small and large intestine of the mouse. *The Anatomical Record* 251: 185-99

22. De Jonge F, Van Nassauw L, Adriaensen D, Van Meir F, Miller HR, Van Marck E, Timmermans JP. 2003. Effect of intestinal inflammation on capsaicin-sensitive afferents in the ileum of Schistosoma mansoni-infected mice. *Histochem Cell Biol* 119: 477-84

23. Mao Y, Wang B, Kunze W. 2006. Characterization of myenteric sensory neurons in the mouse small intestine. *J Neurophysiol* 96: 998-1010

24. Qu ZD, Thacker M, Castelucci P, Bagyanszki M, Epstein ML, Furness JB. 2008. Immunohistochemical analysis of neuron types in the mouse small intestine. *Cell Tissue Res* 334: 147-61

25. Payne SC, Furness JB, Stebbing MJ. 2019. Bioelectric neuromodulation for gastrointestinal disorders: effectiveness and mechanisms. *Nat Rev Gastroenterol Hepatol* 16: 89-105

26. Jänig W. 2006. The Integrative Action of the Autonomic Nervous System. *Cambridge University Press, New York*

27. Rao M, Gershon MD. 2016. The bowel and beyond: the enteric nervous system in neurological disorders. *Nat Rev Gastroenterol Hepatol* 13: 517-28

28. Spencer NJ, Hu H. 2020. Enteric nervous system: sensory transduction, neural circuits and gastrointestinal motility. *Nat Rev Gastroenterol Hepatol* 17: 338-51

29. Brookes SJ, Spencer NJ, Costa M, Zagorodnyuk VP. 2013. Extrinsic primary afferent signalling in the gut. *Nat Rev Gastroenterol Hepatol* 10: 286-96

30. Drokhlyansky E, Smillie CS, Van Wittenberghe N, Ericsson M, Griffin GK, Eraslan G, Dionne D, Cuoco MS, Goder-Reiser MN, Sharova T, Kuksenko O, Aguirre AJ, Boland GM, Graham D, Rozenblatt-Rosen O, Xavier RJ, Regev A. 2020. The Human and Mouse Enteric Nervous System at Single-Cell Resolution. *Cell* 182: 1606-22 e23

31. Obata Y, Castano A, Boeing S, Bon-Frauches AC, Fung C, Fallesen T, de Aguero MG, Yilmaz B, Lopes R, Huseynova A, Horswell S, Maradana MR, Boesmans W, Vanden Berghe P, Murray AJ, Stockinger B, Macpherson AJ, Pachnis V. 2020. Neuronal programming by microbiota regulates intestinal physiology. *Nature* 578: 284-9

32. Morarach K, Mikhailova A, Knoflach V, Memic F, Kumar R, Li W, Ernfors P, Marklund U. 2021. Diversification of molecularly defined myenteric neuron classes revealed by single-cell RNA sequencing. *Nat Neurosci* 24: 34-46

33. May-Zhang AA, Tycksen E, Southard-Smith AN, Deal KK, Benthal JT, Buehler DP, Adam M, Simmons AJ, Monaghan JR, Matlock BK, Flaherty DK, Potter SS, Lau KS, Southard-Smith EM. 2021. Combinatorial Transcriptional Profiling of Mouse and Human Enteric Neurons Identifies Shared and Disparate Subtypes In Situ. *Gastroenterology* 160: 755-70 e26

34. Wright CM, Schneider S, Smith-Edwards KM, Mafra F, Leembruggen AJL, Gonzalez MV, Kothakapa DR, Anderson JB, Maguire BA, Gao T, Missall TA, Howard MJ, Bornstein JC, Davis BM, Heuckeroth RO. 2021. scRNA-Seq Reveals

New Enteric Nervous System Roles for GDNF, NRTN, and TBX3. *Cell Mol Gastroenterol Hepatol* 11: 1548-92 e1

35. Muller PA, Matheis F, Schneeberger M, Kerner Z, Jove V, Mucida D. 2020. Microbiota-modulated CART(+) enteric neurons autonomously regulate blood glucose. *Science* 370: 314-21

36. Gershon MD, Margolis KG. 2021. The gut, its microbiome, and the brain: connections and communications. *J Clin Invest* 131

37. Mayer EA. 2011. Gut feelings: the emerging biology of gut-brain communication. *Nat Rev Neurosci* 12: 453-66

38. Furness JB, Rivera LR, Cho HJ, Bravo DM, Callaghan B. 2013. The gut as a sensory organ. *Nat Rev Gastroenterol Hepatol* 10: 729-40

39. Jacobson A, Yang D, Vella M, Chiu IM. 2021. The intestinal neuro-immune axis: crosstalk between neurons, immune cells, and microbes. *Mucosal Immunol* 14: 555-65

40. Zhong F, Christianson JA, Davis BM, Bielefeldt K. 2008. Dichotomizing axons in spinal and vagal afferents of the mouse stomach. *Dig Dis Sci* 53: 194-203

41. Prescott SL, Liberles SD. 2022. Internal senses of the vagus nerve. *Neuron* 110: 579-99

42. Kyloh M, Nicholas S, Zagorodnyuk VP, Brookes SJ, Spencer NJ. 2011. Identification of the visceral pain pathway activated by noxious colorectal distension in mice. *Front Neurosci* 5: 16

43. Grundy L, Erickson A, Brierley SM. 2019. Visceral Pain. *Annu Rev Physiol* 81: 261-84

44. Christianson JA, Liang R, Ustinova EE, Davis BM, Fraser MO, Pezzone MA. 2007. Convergence of bladder and colon sensory innervation occurs at the primary afferent level. *Pain* 128: 235-43

45. Thakur M, Crow M, Richards N, Davey GI, Levine E, Kelleher JH, Agley CC, Denk F, Harridge SD, McMahon SB. 2014. Defining the nociceptor transcriptome. *Front Mol Neurosci* 7: 87

46. Goswami SC, Mishra SK, Maric D, Kaszas K, Gonnella GL, Clokie SJ, Kominsky HD, Gross JR, Keller JM, Mannes AJ, Hoon MA, Iadarola MJ. 2014. Molecular Signatures of Mouse TRPV1-Lineage Neurons Revealed by RNA-Seq Transcriptome Analysis. *The Journal of Pain* 15: 1338-59

47. Zheng Y, Liu P, Bai L, Trimmer JS, Bean BP, Ginty DD. 2019. Deep Sequencing of Somatosensory Neurons Reveals Molecular Determinants of Intrinsic Physiological Properties. *Neuron* 103: 598-616 e7

48. Chiu IM, Barrett LB, Williams EK, Strochlic DE, Lee S, Weyer AD, Lou S, Bryman GS, Roberson DP, Ghasemlou N, Piccoli C, Ahat E, Wang V, Cobos EJ, Stucky CL, Ma Q, Liberles SD, Woolf CJ. 2014. Transcriptional profiling at whole population and single cell levels reveals somatosensory neuron molecular diversity. *Elife* 3

49. Kupari J, Haring M, Agirre E, Castelo-Branco G, Ernfors P. 2019. An Atlas of Vagal Sensory Neurons and Their Molecular Specialization. *Cell Rep* 27: 2508-23 e4

50. Usoskin D, Furlan A, Islam S, Abdo H, Lonnerberg P, Lou D, Hjerling-Leffler J, Haeggstrom J, Kharchenko O, Kharchenko PV, Linnarsson S, Ernfors P. 2015. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci* 18: 145-53

51. Li CL, Li KC, Wu D, Chen Y, Luo H, Zhao JR, Wang SS, Sun MM, Lu YJ, Zhong YQ, Hu XY, Hou R, Zhou BB, Bao L, Xiao HS, Zhang X. 2016. Somatosensory neuron types identified by high-coverage single-cell RNA-sequencing and functional heterogeneity. *Cell Res* 26: 83-102

52. Sharma N, Flaherty K, Lezgiyeva K, Wagner DE, Klein AM, Ginty DD. 2020. The emergence of transcriptional identity in somatosensory neurons. *Nature* 577: 392-8

53. Kupari J, Usoskin D, Parisien M, Lou D, Hu Y, Fatt M, Lonnerberg P, Spangberg M, Eriksson B, Barkas N, Kharchenko PV, Lore K, Khoury S, Diatchenko L, Ernfors P. 2021. Single cell transcriptomics of primate sensory neurons identifies cell types associated with chronic pain. *Nat Commun* 12: 1510

54. Hockley JRF, Taylor TS, Callejo G, Wilbrey AL, Gutteridge A, Bach K, Winchester WJ, Bulmer DC, McMurray G, Smith ESJ. 2019. Single-cell RNAseq reveals seven classes of colonic sensory neuron. *Gut* 68: 633-44

55. Baral P, Udit S, Chiu IM. 2019. Pain and immunity: implications for host defence. *Nat Rev Immunol* 19: 433-47

56. Julius D. 2013. TRP channels and pain. *Annu Rev Cell Dev Biol* 29: 355-84

57. Ochoa-Cortes F, Ramos-Lomas T, Miranda-Morales M, Spreadbury I, Ibeakanma C, Barajas-Lopez C, Vanner S. 2010. Bacterial cell products signal to mouse colonic nociceptive dorsal root ganglia neurons. *Am J Physiol Gastrointest Liver Physiol* 299: G723-32

58. Meseguer V, Alpizar YA, Luis E, Tajada S, Denlinger B, Fajardo O, Manenschijn JA, Fernandez-Pena C, Talavera A, Kichko T, Navia B, Sanchez A, Senaris R, Reeh P, Perez-Garcia MT, Lopez-Lopez JR, Voets T, Belmonte C, Talavera K, Viana F. 2014. TRPA1 channels mediate acute neurogenic inflammation and pain produced by bacterial endotoxins. *Nat Commun* 5: 3125

59. Barajon I, Serrao G, Arnaboldi F, Opizzi E, Ripamonti G, Balsari A, Rumio C. 2009. Toll-like receptors 3, 4, and 7 are expressed in the enteric nervous system and dorsal root ganglia. *J Histochem Cytochem* 57: 1013-23

60. Yissachar N, Zhou Y, Ung L, Lai NY, Mohan JF, Ehrlicher A, Weitz DA, Kasper DL, Chiu IM, Mathis D, Benoist C. 2017. An Intestinal Organ Culture System Uncovers a Role for the Nervous System in Microbe-Immune Crosstalk. *Cell* 168: 1135-48 e12

61. Cervi AL, Lukewich MK, Lomax AE. 2014. Neural regulation of gastrointestinal inflammation: role of the sympathetic nervous system. *Auton Neurosci* 182: 83-8

62. Costes LM, Boeckxstaens GE, de Jonge WJ, Cailotto C. 2013. Neural networks in intestinal immunoregulation. *Organogenesis* 9: 216-23

63. Lomax AE, Sharkey KA, Furness JB. 2010. The participation of the sympathetic innervation of the gastrointestinal tract in disease states. *Neurogastroenterol Motil* 22: 7-18

64. Smith-Edwards KM, Edwards BS, Wright CM, Schneider S, Meerschaert KA, Ejoh LL, Najjar SA, Howard MJ, Albers KM, Heuckeroth RO, Davis BM. 2021. Sympathetic Input to Multiple Cell Types in Mouse and Human Colon Produces Region-Specific Responses. *Gastroenterology* 160: 1208-23 e4

65. Klose CSN, Veiga-Fernandes H. 2021. Neuroimmune interactions in peripheral tissues. *Eur J Immunol* 51: 1602-14

66. Neurath MF. 2014. Cytokines in inflammatory bowel disease. *Nat Rev Immunol* 14: 329-42

67. Bakshi N, Hart AL, Lee MC, Williams ACC, Lackner JM, Norton C, Croft P. 2021. Chronic pain in patients with inflammatory bowel disease. *Pain* 162: 2466-71

68. Black J, Sweeney L, Yuan Y, Singh H, Norton C, Czuber-Dochan W. 2022. Systematic review: the role of psychological stress in inflammatory bowel disease. *Aliment Pharmacol Ther* 56: 1235-49

69. Mawdsley JE, Rampton DS. 2005. Psychological stress in IBD: new insights into pathogenic and therapeutic implications. *Gut* 54: 1481-91

70. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ. 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405: 458-62

71. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang H, Yang H, Ulloa L, Al-Abed Y, Czura CJ, Tracey KJ. 2003. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 421: 384-8

72. Huston JM, Ochani M, Rosas-Ballina M, Liao H, Ochani K, Pavlov VA, Gallowitsch-Puerta M, Ashok M, Czura CJ, Foxwell B, Tracey KJ, Ulloa L. 2006. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. *J Exp Med* 203: 1623-8

73. Rosas-Ballina M, Ochani M, Parrish WR, Ochani K, Harris YT, Huston JM, Chavan S, Tracey KJ. 2008. Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. *Proc Natl Acad Sci U S A* 105: 11008-13

74. Rosas-Ballina M, Olofsson PS, Ochani M, Valdes-Ferrer SI, Levine YA, Reardon C, Tusche MW, Pavlov VA, Andersson U, Chavan S, Mak TW, Tracey KJ. 2011. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science* 334: 98-101

75. Meregnani J, Clarencon D, Vivier M, Peinnequin A, Mouret C, Sinniger V, Picq C, Job A, Canini F, Jacquier-Sarlin M, Bonaz B. 2011. Anti-inflammatory effect of vagus nerve stimulation in a rat model of inflammatory bowel disease. *Auton Neurosci* 160: 82-9

76. Sun P, Zhou K, Wang S, Li P, Chen S, Lin G, Zhao Y, Wang T. 2013. Involvement of MAPK/NF-kappaB signaling in the activation of the cholinergic anti-inflammatory pathway in experimental colitis by chronic vagus nerve stimulation. *PLoS One* 8: e69424

77. Meroni E, Stakenborg N, Gomez-Pinilla PJ, Stakenborg M, Aguilera-Lizarraga J, Florens M, Delfini M, de Simone V, De Hertogh G, Goverse G, Matteoli G, Boeckxstaens GE. 2021. Vagus Nerve Stimulation Promotes Epithelial Proliferation and Controls Colon Monocyte Infiltration During DSS-Induced Colitis. *Front Med (Lausanne)* 8: 694268

78. Meroni E, Stakenborg N, Gomez-Pinilla PJ, De Hertogh G, Goverse G, Matteoli G, Verheijden S, Boeckxstaens GE. 2018. Functional characterization of oxazolone-induced colitis and survival improvement by vagus nerve stimulation. *PLoS One* 13: e0197487

79. Ji H, Rabbi MF, Labis B, Pavlov VA, Tracey KJ, Ghia JE. 2014. Central cholinergic activation of a vagus nerve-to-spleen circuit alleviates experimental colitis. *Mucosal Immunol* 7: 335-47

80. Ghia JE, Blennerhassett P, Kumar-Ondiveeran H, Verdu EF, Collins SM. 2006. The vagus nerve: a tonic inhibitory influence associated with inflammatory bowel disease in a murine model. *Gastroenterology* 131: 1122-30

81. Di Giovangiulio M, Bosmans G, Meroni E, Stakenborg N, Florens M, Farro G, Gomez-Pinilla PJ, Matteoli G, Boeckxstaens GE. 2016. Vagotomy affects the development of oral tolerance and increases susceptibility to develop colitis independently of the alpha-7 nicotinic receptor. *Mol Med* 22: 464-76

82. Schiller M, Azulay-Debby H, Boshnak N, Elyahu Y, Korin B, Ben-Shaanan TL, Koren T, Krot M, Hakim F, Rolls A. 2021. Optogenetic activation of local colonic sympathetic innervations attenuates colitis by limiting immune cell extravasation. *Immunity* 54: 1022-36 e8

83. Zadori ZS, Toth VE, Feher A, Al-Khrasani M, Puskar Z, Kozsurek M, Timar J, Tabi T, Helyes Z, Hein L, Holzer P, Gyires K. 2016. Inhibition of alpha2A-Adrenoceptors Ameliorates Dextran Sulfate Sodium-Induced Acute Intestinal Inflammation in Mice. *J Pharmacol Exp Ther* 358: 483-91

84. Zhang W, Lyu M, Bessman NJ, Xie Z, Arifuzzaman M, Yano H, Parkhurst CN, Chu C, Zhou L, Putzel GG, Li TT, Jin WB, Zhou J, Bank JRILC, Hu H, Tsou AM, Guo CJ, Artis D. 2022. Gut-innervating nociceptors regulate the intestinal microbiota to promote tissue protection. *Cell* 185: 4170-89 e20

85. Yang D, Jacobson A, Meerschaert KA, Sifakis JJ, Wu M, Chen X, Yang T, Zhou Y, Anekal PV, Rucker RA, Sharma D, Sontheimer-Phelps A, Wu GS, Deng L, Anderson MD, Choi S, Neel D, Lee N, Kasper DL, Jabri B, Huh JR, Johansson M, Thiagarajah JR, Riesenfeld SJ, Chiu IM. 2022. Nociceptor neurons direct goblet cells via a CGRP-RAMP1 axis to drive mucus production and gut barrier protection. *Cell* 185: 4190-205 e25

86. Engel MA, Khalil M, Siklosi N, Mueller-Tribbensee SM, Neuhuber WL, Neurath MF, Becker C, Reeh PW. 2012. Opposite effects of substance P and calcitonin gene-related peptide in oxazolone colitis. *Dig Liver Dis* 44: 24-9

87. Abad C, Martinez C, Juarranz MG, Arranz A, Leceta J, Delgado M, Gomariz RP. 2003. Therapeutic effects of vasoactive intestinal peptide in the trinitrobenzene sulfonic acid mice model of Crohn's disease. *Gastroenterology* 124: 961-71

88. Abad C, Juarranz Y, Martinez C, Arranz A, Rosignoli F, Garcia-Gomez M, Leceta J, Gomariz RP. 2005. cDNA array analysis of cytokines, chemokines, and receptors involved in the development of TNBS-induced colitis: homeostatic role of VIP. *Inflamm Bowel Dis* 11: 674-84

89. Newman R, Cuan N, Hampartzoumian T, Connor SJ, Lloyd AR, Grimm MC. 2005. Vasoactive intestinal peptide impairs leucocyte migration but fails to modify experimental murine colitis. *Clin Exp Immunol* 139: 411-20

90. Abad C, Cheung-Lau G, Coute-Monvoisin AC, Waschek JA. 2015. Vasoactive intestinal peptide-deficient mice exhibit reduced pathology in trinitrobenzene sulfonic acid-induced colitis. *Neuroimmunomodulation* 22: 203-12

91. Koren T, Yifa R, Amer M, Krot M, Boshnak N, Ben-Shaanan TL, Azulay-Debby H, Zalayat I, Avishai E, Hajjo H, Schiller M, Haykin H, Korin B, Farfara D, Hakim F, Kobiler O, Rosenblum K, Rolls A. 2021. Insular cortex neurons encode and retrieve specific immune responses. *Cell* 184: 5902-15 e17

92. Klein Wolterink RGJ, Wu GS, Chiu IM, Veiga-Fernandes H. 2022. Neuroimmune Interactions in Peripheral Organs. *Annu Rev Neurosci* 45: 339-60

93. Wallrapp A, Riesenfeld SJ, Burkett PR, Kuchroo VK. 2018. Type 2 innate lymphoid cells in the induction and resolution of tissue inflammation. *Immunol Rev* 286: 53-73

94. Vivier E, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, McKenzie ANJ, Mebius RE, Powrie F, Spits H. 2018. Innate Lymphoid Cells: 10 Years On. *Cell* 174: 1054-66

95. Klose CSN, Artis D. 2020. Innate lymphoid cells control signaling circuits to regulate tissue-specific immunity. *Cell Res* 30: 475-91

96. Klose CSN, Mahlakoiv T, Moeller JB, Rankin LC, Flamar AL, Kabata H, Monticelli LA, Moriyama S, Putzel GG, Rakhilin N, Shen X, Kostenis E, Konig GM, Senda T, Carpenter D, Farber DL, Artis D. 2017. The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. *Nature* 549: 282-6

97. Cardoso V, Chesne J, Ribeiro H, Garcia-Cassani B, Carvalho T, Bouchery T, Shah K, Barbosa-Morais NL, Harris N, Veiga-Fernandes H. 2017. Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature* 549: 277-81

98. Wallrapp A, Riesenfeld SJ, Burkett PR, Abdulnour RE, Nyman J, Dionne D, Hofree M, Cuoco MS, Rodman C, Farouq D, Haas BJ, Tickle TL, Trombetta JJ, Baral P, Klose CSN, Mahlakoiv T, Artis D, Rozenblatt-Rosen O, Chiu IM, Levy BD, Kowalczyk MS, Regev A, Kuchroo VK. 2017. The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation. *Nature* 549: 351-6

99. Talbot J, Hahn P, Kroehling L, Nguyen H, Li D, Littman DR. 2020. Feeding-dependent VIP neuron-ILC3 circuit regulates the intestinal barrier. *Nature* 579: 575-80

100. Seillet C, Luong K, Tellier J, Jacquelot N, Shen RD, Hickey P, Wimmer VC, Whitehead L, Rogers K, Smyth GK, Garnham AL, Ritchie ME, Belz GT. 2020. The neuropeptide VIP confers anticipatory mucosal immunity by regulating ILC3 activity. *Nat Immunol* 21: 168-77

101. Nussbaum JC, Van Dyken SJ, von Moltke J, Cheng LE, Mohapatra A, Molofsky AB, Thornton EE, Krummel MF, Chawla A, Liang HE, Locksley RM. 2013. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* 502: 245-8

102. Pascal M, Kazakov A, Chevalier G, Dubrule L, Deyrat J, Dupin A, Saha S, Jagot F, Sailor K, Dulauroy S, Moigneu C, Belkaid Y, Lepousez G, Lledo PM, Wilhelm C, Eberl G. 2022. The neuropeptide VIP potentiates intestinal innate type 2 and type 3 immunity in response to feeding. *Mucosal Immunol* 15: 629-41

103. Talbot S, Abdulnour RE, Burkett PR, Lee S, Cronin SJ, Pascal MA, Laedermann C, Foster SL, Tran JV, Lai N, Chiu IM, Ghasemlou N, DiBiase M, Roberson D, Von Hehn C, Agac B, Haworth O, Seki H, Penninger JM, Kuchroo VK, Bean BP, Levy BD, Woolf CJ. 2015. Silencing Nociceptor Neurons Reduces Allergic Airway Inflammation. *Neuron* 87: 341-54

104. Xu H, Ding J, Porter CBM, Wallrapp A, Tabaka M, Ma S, Fu S, Guo X, Riesenfeld SJ, Su C, Dionne D, Nguyen LT, Lefkovith A, Ashenberg O, Burkett PR, Shi HN, Rozenblatt-Rosen O, Graham DB, Kuchroo VK, Regev A, Xavier RJ. 2019. Transcriptional Atlas of Intestinal Immune Cells Reveals that Neuropeptide alpha-CGRP Modulates Group 2 Innate Lymphoid Cell Responses. *Immunity* 51: 696-708 e9

105. Nagashima H, Mahlakoiv T, Shih HY, Davis FP, Meylan F, Huang Y, Harrison OJ, Yao C, Mikami Y, Urban JF, Jr., Caron KM, Belkaid Y, Kanno Y, Artis D, O'Shea JJ. 2019. Neuropeptide CGRP Limits Group 2 Innate Lymphoid Cell Responses and Constrains Type 2 Inflammation. *Immunity* 51: 682-95 e6

106. Wallrapp A, Burkett PR, Riesenfeld SJ, Kim SJ, Christian E, Abdulnour RE, Thakore PI, Schnell A, Lambden C, Herbst RH, Khan P, Tsujikawa K, Xavier RJ, Chiu IM, Levy BD, Regev A, Kuchroo VK. 2019. Calcitonin Gene-Related Peptide Negatively Regulates Alarmin-Driven Type 2 Innate Lymphoid Cell Responses. *Immunity* 51: 709-23 e6

107. Moriyama S, Brestoff JR, Flamar AL, Moeller JB, Klose CSN, Rankin LC, Yudanin NA, Monticelli LA, Putzel GG, Rodewald HR, Artis D. 2018. beta2-adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses. *Science* 359: 1056-61

108. Keir M, Yi Y, Lu T, Ghilardi N. 2020. The role of IL-22 in intestinal health and disease. *J Exp Med* 217: e20192195

109. Harmar AJ, Marston HM, Shen S, Spratt C, West KM, Sheward WJ, Morrison CF, Dorin JR, Piggins HD, Reubi JC, Kelly JS, Maywood ES, Hastings MH. 2002. The VPAC(2) receptor is essential for circadian function in the mouse suprachiasmatic nuclei. *Cell* 109: 497-508

110. Wang Q, Robinette ML, Billon C, Collins PL, Bando JK, Fachi JL, Secca C, Porter SI, Saini A, Gilfillan S, Solt LA, Musiek ES, Oltz EM, Burris TP, Colonna M. 2019. Circadian rhythm-dependent and circadian rhythm-independent impacts of the molecular clock on type 3 innate lymphoid cells. *Sci Immunol* 4

111. Teng F, Goc J, Zhou L, Chu C, Shah MA, Eberl G, Sonnenberg GF. 2019. A circadian clock is essential for homeostasis of group 3 innate lymphoid cells in the gut. *Sci Immunol* 4

112. Godinho-Silva C, Domingues RG, Rendas M, Raposo B, Ribeiro H, da Silva JA, Vieira A, Costa RM, Barbosa-Morais NL, Carvalho T, Veiga-Fernandes H. 2019. Light-entrained and brain-tuned circadian circuits regulate ILC3s and gut homeostasis. *Nature* 574: 254-8

113. Chu C, Parkhurst CN, Zhang W, Zhou L, Yano H, Arifuzzaman M, Artis D. 2021. The ChAT-acetylcholine pathway promotes group 2 innate lymphoid cell responses and anti-helminth immunity. *Sci Immunol* 6

114. Roberts LB, Schnoeller C, Berkachy R, Darby M, Pillaye J, Oudhoff MJ, Parmar N, Mackowiak C, Sedda D, Quesniaux V, Ryffel B, Vaux R, Gounaris K, Berrard S, Withers DR, Horsnell WGC, Selkirk ME. 2021. Acetylcholine production by group 2 innate lymphoid cells promotes mucosal immunity to helminths. *Sci Immunol* 6

115. Flamar AL, Klose CSN, Moeller JB, Mahlakoiv T, Bessman NJ, Zhang W, Moriyama S, Stokic-Trtica V, Rankin LC, Putzel GG, Rodewald HR, He Z, Chen L, Lira SA, Karsenty G, Artis D. 2020. Interleukin-33 Induces the Enzyme Tryptophan Hydroxylase 1 to Promote Inflammatory Group 2 Innate Lymphoid Cell-Mediated Immunity. *Immunity* 52: 606-19 e6

116. Shimokawa C, Kanaya T, Hachisuka M, Ishiwata K, Hisaeda H, Kurashima Y, Kiyono H, Yoshimoto T, Kaisho T, Ohno H. 2017. Mast Cells Are Crucial for Induction of Group 2 Innate Lymphoid Cells and Clearance of Helminth Infections. *Immunity* 46: 863-74 e4

117. Galli SJ, Tsai M. 2012. IgE and mast cells in allergic disease. *Nat Med* 18: 693-704

118. Galli SJ, Gaudenzio N, Tsai M. 2020. Mast Cells in Inflammation and Disease: Recent Progress and Ongoing Concerns. *Annu Rev Immunol* 38: 49-77

119. Yamamoto T, Kodama T, Lee J, Utsunomiya N, Hayashi S, Sakamoto H, Kuramoto H, Kadowaki M. 2014. Anti-allergic role of cholinergic neuronal pathway via alpha7 nicotinic ACh receptors on mucosal mast cells in a murine food allergy model. *PLoS One* 9: e85888

120. Stead RH, Tomioka M, Quinonez G, Simon GT, Felten SY, Bienenstock J. 1987. Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc Natl Acad Sci U S A* 84: 2975-9

121. Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, Corinaldesi R. 2004. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 126: 693-702

122. Shanahan F, Denburg JA, Fox J, Bienenstock J, Befus D. 1985. Mast cell heterogeneity: effects of neuroenteric peptides on histamine release. *The Journal of Immunology* 135: 1331-7

123. De Jonge F, De Laet A, Van Nassauw L, Brown JK, Miller HR, van Bogaert PP, Timmermans JP, Kroese AB. 2004. In vitro activation of murine DRG neurons by CGRP-mediated mucosal mast cell degranulation. *Am J Physiol Gastrointest Liver Physiol* 287: G178-91

124. Kulka M, Sheen CH, Tancowny BP, Grammer LC, Schleimer RP. 2008. Neuropeptides activate human mast cell degranulation and chemokine production. *Immunology* 123: 398-410

125. Bischoff SC, Schwengberg S, Lorentz A, Manns MP, Bektas H, Sann H, Levi-Schaffer F, Shanahan F, Schemann M. 2004. Substance P and other neuropeptides do not induce mediator release in isolated human intestinal mast cells. *Neurogastroenterol Motil* 16: 185-93

126. Kageyama-Yahara N, Suehiro Y, Yamamoto T, Kadowaki M. 2008. IgE-induced degranulation of mucosal mast cells is negatively regulated via nicotinic acetylcholine receptors. *Biochem Biophys Res Commun* 377: 321-5

127. Bosmans G, Appeltans I, Stakenborg N, Gomez-Pinilla PJ, Florens MV, Aguilera-Lizarraga J, Matteoli G, Boeckxstaens GE. 2019. Vagus nerve stimulation dampens intestinal inflammation in a murine model of experimental food allergy. *Allergy* 74: 1748-59

128. Yashiro T, Ogata H, Zaidi SF, Lee J, Hayashi S, Yamamoto T, Kadowaki M. 2021. Pathophysiological Roles of Neuro-Immune Interactions between Enteric Neurons and Mucosal Mast Cells in the Gut of Food Allergy Mice. *Cells* 10

129. Muller PA, Matheis F, Mucida D. 2020. Gut macrophages: key players in intestinal immunity and tissue physiology. *Curr Opin Immunol* 62: 54-61

130. Na YR, Stakenborg M, Seok SH, Matteoli G. 2019. Macrophages in intestinal inflammation and resolution: a potential therapeutic target in IBD. *Nat Rev Gastroenterol Hepatol* 16: 531-43

131. Muller PA, Koscso B, Rajani GM, Stevanovic K, Berres ML, Hashimoto D, Mortha A, Leboeuf M, Li XM, Mucida D, Stanley ER, Dahan S, Margolis KG, Gershon MD, Merad M, Bogunovic M. 2014. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell* 158: 300-13

132. Gabanyi I, Muller PA, Feighery L, Oliveira TY, Costa-Pinto FA, Mucida D. 2016. Neuro-immune Interactions Drive Tissue Programming in Intestinal Macrophages. *Cell* 164: 378-91

133. Matheis F, Muller PA, Graves CL, Gabanyi I, Kerner ZJ, Costa-Borges D, Ahrends T, Rosenstiel P, Mucida D. 2020. Adrenergic Signaling in Muscularis Macrophages Limits Infection-Induced Neuronal Loss. *Cell* 180: 64-78 e16

134. de Jonge WJ, van der Zanden EP, The FO, Bijlsma MF, van Westerloo DJ, Bennink RJ, Berthoud HR, Uematsu S, Akira S, van den Wijngaard RM, Boeckxstaens GE. 2005. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. *Nat Immunol* 6: 844-51

135. Matteoli G, Gomez-Pinilla PJ, Nemethova A, Di Giovangiulio M, Cailotto C, van Bree SH, Michel K, Tracey KJ, Schemann M, Boesmans W, Vanden Berghe P, Boeckxstaens GE. 2014. A distinct vagal anti-inflammatory pathway modulates intestinal muscularis resident macrophages independent of the spleen. *Gut* 63: 938-48

136. Zhu P, Lu T, Wu J, Fan D, Liu B, Zhu X, Guo H, Du Y, Liu F, Tian Y, Fan Z. 2022. Gut microbiota drives macrophage-dependent self-renewal of intestinal stem cells via niche enteric serotonergic neurons. *Cell Res* 32: 555-69

137. Hoeffel G, Debroas G, Roger A, Rossignol R, Gouilly J, Laprie C, Chasson L, Barbon P-V, Balsamo A, Reynders A, Moqrich A, Ugolini S. 2021. Sensory neuron-derived TAFA4 promotes macrophage tissue repair functions. *Nature* 594: 94-9

138. Worbs T, Hammerschmidt SI, Forster R. 2017. Dendritic cell migration in health and disease. *Nat Rev Immunol* 17: 30-48

139. Eisenbarth SC. 2019. Dendritic cell subsets in T cell programming: location dictates function. *Nat Rev Immunol* 19: 89-103

140. Chorny A, Gonzalez-Rey E, Fernandez-Martin A, Pozo D, Ganea D, Delgado M. 2005. Vasoactive intestinal peptide induces regulatory dendritic cells with therapeutic effects on autoimmune disorders. *Proc Natl Acad Sci U S A* 102: 13562-7

141. Hanc P, Gonzalez RJ, Mazo IB, Wang Y, Lambert T, Ortiz G, Miller EW, von Andrian UH. 2023. Multimodal control of dendritic cell functions by nociceptors. *Science* 379: eabm5658

142. Perner C, Flayer CH, Zhu X, Aderhold PA, Dewan ZNA, Voisin T, Camire RB, Chow OA, Chiu IM, Sokol CL. 2020. Substance P Release by Sensory Neurons Triggers Dendritic Cell Migration and Initiates the Type-2 Immune Response to Allergens. *Immunity* 53: 1063-77 e7

143. Teratani T, Mikami Y, Nakamoto N, Suzuki T, Harada Y, Okabayashi K, Hagihara Y, Taniki N, Kohno K, Shibata S, Miyamoto K, Ishigame H, Chu PS, Sujino T, Suda W, Hattori M, Matsui M, Okada T, Okano H, Inoue M, Yada T, Kitagawa Y, Yoshimura A, Tanida M, Tsuda M, Iwasaki Y, Kanai T. 2020. The liver-brain-gut neural arc maintains the T(reg) cell niche in the gut. *Nature* 585: 591-6

144. Whibley N, Tucci A, Powrie F. 2019. Regulatory T cell adaptation in the intestine and skin. *Nat Immunol* 20: 386-96

145. Sakaguchi S, Mikami N, Wing JB, Tanaka A, Ichiyama K, Ohkura N. 2020. Regulatory T Cells and Human Disease. *Annu Rev Immunol* 38: 541-66

146. Yan Y, Ramanan D, Rozenberg M, McGovern K, Rastelli D, Vijaykumar B, Yaghi O, Voisin T, Mosaheb M, Chiu I, Itzkovitz S, Rao M, Mathis D, Benoist C. 2021. Interleukin-6 produced by enteric neurons regulates the number and phenotype of microbe-responsive regulatory T cells in the gut. *Immunity* 54: 499-513 e5

147. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, McGuire AM, Burzyn D, Ortiz-Lopez A, Lobera M, Yang J, Ghosh S, Earl A, Snapper SB, Jupp R, Kasper D, Mathis D, Benoist C. 2015. MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. *Science* 349: 993-7

148. Pinho-Ribeiro FA, Baddal B, Haarsma R, O'Seaghdha M, Yang NJ, Blake KJ, Portley M, Verri WA, Dale JB, Wessels MR, Chiu IM. 2018. Blocking Neuronal Signaling to Immune Cells Treats Streptococcal Invasive Infection. *Cell* 173: 1083-97.e22

149. Cohen JA, Edwards TN, Liu AW, Hirai T, Jones MR, Wu J, Li Y, Zhang S, Ho J, Davis BM, Albers KM, Kaplan DH. 2019. Cutaneous TRPV1(+) Neurons Trigger Protective Innate Type 17 Anticipatory Immunity. *Cell* 178: 919-32 e14

150. Baral P, Umans BD, Li L, Wallrapp A, Bist M, Kirschbaum T, Wei Y, Zhou Y, Kuchroo VK, Burkett PR, Yipp BG, Liberles SD, Chiu IM. 2018. Nociceptor sensory neurons suppress neutrophil and gammadelta T cell responses in bacterial lung infections and lethal pneumonia. *Nat Med* 24: 417-26

151. Chu C, Artis D, Chiu IM. 2020. Neuro-immune Interactions in the Tissues. *Immunity* 52: 464-74

152. Pavlov VA, Chavan SS, Tracey KJ. 2018. Molecular and Functional Neuroscience in Immunity. *Annu Rev Immunol* 36: 783-812

153. Ibiza S, Garcia-Cassani B, Ribeiro H, Carvalho T, Almeida L, Marques R, Misic AM, Bartow-McKenney C, Larson DM, Pavan WJ, Eberl G, Grice EA, Veiga-Fernandes H. 2016. Glial-cell-derived neuroregulators control type 3 innate lymphoid cells and gut defence. *Nature* 535: 440-3

154. Stakenborg M, Abdurahiman S, De Simone V, Goverse G, Stakenborg N, van Baarle L, Wu Q, Pirottin D, Kim JS, Chappell-Maor L, Pintelon I, Thys S, Pollenus E, Boon L, Van den Steen P, Hao M, Van Ginderachter JA, Boeckxstaens GE, Timmermans JP, Jung S, Marichal T, Ibiza S, Matteoli G. 2022. Enteric glial cells favor accumulation of anti-inflammatory macrophages during the resolution of muscularis inflammation. *Mucosal Immunol* 15: 1296-308

155. Grubisic V, McClain JL, Fried DE, Grants I, Rajasekhar P, Csizmadia E, Ajijola OA, Watson RE, Poole DP, Robson SC, Christofi FL, Gulbransen BD. 2020. Enteric Glia Modulate Macrophage Phenotype and Visceral Sensitivity following Inflammation. *Cell Rep* 32: 108100

156. Progatzky F, Shapiro M, Chng SH, Garcia-Cassani B, Classon CH, Sevgi S, Laddach A, Bon-Frauches AC, Lasrado R, Rahim M, Amaniti EM, Boeing S, Shah K, Entwistle LJ, Suarez-Bonnet A, Wilson MS, Stockinger B, Pachnis V. 2021. Regulation of intestinal immunity and tissue repair by enteric glia. *Nature* 599: 125-30

157. Rosenberg HJ, Rao M. 2021. Enteric glia in homeostasis and disease: From fundamental biology to human pathology. *iScience* 24: 102863

158. Progatzky F, Pachnis V. 2022. The role of enteric glia in intestinal immunity. *Curr Opin Immunol* 77: 102183

159. Allaire JM, Crowley SM, Law HT, Chang SY, Ko HJ, Vallance BA. 2018. The Intestinal Epithelium: Central Coordinator of Mucosal Immunity. *Trends Immunol* 39: 677-96

160. Lai NY, Musser MA, Pinho-Ribeiro FA, Baral P, Jacobson A, Ma P, Potts DE, Chen Z, Paik D, Soualhi S, Yan Y, Misra A, Goldstein K, Lagomarsino VN, Nordstrom A,

Sivanathan KN, Wallrapp A, Kuchroo VK, Nowarski R, Starnbach MN, Shi H, Surana NK, An D, Wu C, Huh JR, Rao M, Chiu IM. 2020. Gut-Innervating Nociceptor Neurons Regulate Peyer's Patch Microfold Cells and SFB Levels to Mediate Salmonella Host Defense. *Cell* 180: 33-49 e22

161. Jarret A, Jackson R, Duizer C, Healy ME, Zhao J, Rone JM, Bielecki P, Sefik E, Roulis M, Rice T, Sivanathan KN, Zhou T, Solis AG, Honcharova-Biletska H, Velez K, Hartner S, Low JS, Qu R, de Zoete MR, Palm NW, Ring AM, Weber A, Moor AE, Kluger Y, Nowarski R, Flavell RA. 2020. Enteric Nervous System-Derived IL-18 Orchestrates Mucosal Barrier Immunity. *Cell* 180: 50-63 e12

162. Knoop KA, Newberry RD. 2018. Goblet cells: multifaceted players in immunity at mucosal surfaces. *Mucosal Immunol* 11: 1551-7

163. McDole JR, Wheeler LW, McDonald KG, Wang B, Konjufca V, Knoop KA, Newberry RD, Miller MJ. 2012. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature* 483: 345-9

164. Knoop KA, McDonald KG, McCrate S, McDole JR, Newberry RD. 2015. Microbial sensing by goblet cells controls immune surveillance of luminal antigens in the colon. *Mucosal Immunol* 8: 198-210

165. Neunlist M, Toumi F, Oreschkova T, Denis M, Leborgne J, Laboisse CL, Galmiche JP, Jarry A. 2003. Human ENS regulates the intestinal epithelial barrier permeability and a tight junction-associated protein ZO-1 via VIPergic pathways. *Am J Physiol Gastrointest Liver Physiol* 285: G1028-36

166. Lelievre V, Favrais G, Abad C, Adle-Biassette H, Lu Y, Germano PM, Cheung-Lau G, Pisegna JR, Gressens P, Lawson G, Waschek JA. 2007. Gastrointestinal dysfunction in mice with a targeted mutation in the gene encoding vasoactive intestinal polypeptide: a model for the study of intestinal ileus and Hirschsprung's disease. *Peptides* 28: 1688-99

167. Conlin VS, Wu X, Nguyen C, Dai C, Vallance BA, Buchan AM, Boyer L, Jacobson K. 2009. Vasoactive intestinal peptide ameliorates intestinal barrier disruption associated with Citrobacter rodentium-induced colitis. *Am J Physiol Gastrointest Liver Physiol* 297: G735-50

168. Bednarska O, Walter SA, Casado-Bedmar M, Strom M, Salvo-Romero E, Vicario M, Mayer EA, Keita AV. 2017. Vasoactive Intestinal Polypeptide and Mast Cells Regulate Increased Passage of Colonic Bacteria in Patients With Irritable Bowel Syndrome. *Gastroenterology* 153: 948-60 e3

169. Aguilera-Lizarraga J, Hussein H, Boeckxstaens GE. 2022. Immune activation in irritable bowel syndrome: what is the evidence? *Nat Rev Immunol* 22: 674-86

170. Lamb K, Zhong F, Gebhart GF, Bielefeldt K. 2006. Experimental colitis in mice and sensitization of converging visceral and somatic afferent pathways. *Am J Physiol Gastrointest Liver Physiol* 290: G451-7

171. Gao X, Han S, Huang Q, He SQ, Ford NC, Zheng Q, Chen Z, Yu S, Dong X, Guan Y. 2021. Calcium imaging in population of dorsal root ganglion neurons unravels novel mechanisms of visceral pain sensitization and referred somatic hypersensitivity. *Pain* 162: 1068-81

172. Lapointe TK, Basso L, Iftinca MC, Flynn R, Chapman K, Dietrich G, Vergnolle N, Altier C. 2015. TRPV1 sensitization mediates postinflammatory visceral pain following acute colitis. *Am J Physiol Gastrointest Liver Physiol* 309: G87-99

173. Aguilera-Lizarraga J, Florens MV, Viola MF, Jain P, Decraecker L, Appeltans I, Cuende-Estevez M, Fabre N, Van Beek K, Perna E, Balemans D, Stakenborg N, Theofanous S, Bosmans G, Mondelaers SU, Matteoli G, Ibiza Martinez S, Lopez-Lopez C, Jaramillo-Polanco J, Talavera K, Alpizar YA, Feyerabend TB, Rodewald HR, Farre R, Redegeld FA, Si J, Raes J, Breynaert C, Schrijvers R, Bosteels C, Lambrecht BN, Boyd SD, Hoh RA, Cabooter D, Nelis M, Augustijns P, Hendrix S, Strid J, Bisschops R, Reed DE, Vanner SJ, Denadai-Souza A, Wouters MM, Boeckxstaens GE. 2021. Local immune response to food antigens drives meal-induced abdominal pain. *Nature* 590: 151-6

174. Fritscher-Ravens A, Schuppan D, Ellrichmann M, Schoch S, Rocken C, Brasch J, Bethge J, Bottner M, Klose J, Milla PJ. 2014. Confocal endomicroscopy shows food-associated changes in the intestinal mucosa of patients with irritable bowel syndrome. *Gastroenterology* 147: 1012-20 e4

175. Defaye M, Abdullah NS, Iftinca M, Hassan A, Agosti F, Zhang Z, Cumenal M, Zamponi GW, Altier C. 2022. Gut-innervating TRPV1+ Neurons Drive Chronic Visceral Pain via Microglial P2Y12 Receptor. *Cell Mol Gastroenterol Hepatol* 13: 977-99

176. Barbara G, Wang B, Stanghellini V, de Giorgio R, Cremon C, Di Nardo G, Trevisani M, Campi B, Geppetti P, Tonini M, Bunnett NW, Grundy D, Corinaldesi R. 2007. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology* 132: 26-37

177. Buhner S, Li Q, Vignali S, Barbara G, De Giorgio R, Stanghellini V, Cremon C, Zeller F, Langer R, Daniel H, Michel K, Schemann M. 2009. Activation of human enteric neurons by supernatants of colonic biopsy specimens from patients with irritable bowel syndrome. *Gastroenterology* 137: 1425-34

178. Cenac N, Andrews CN, Holzhausen M, Chapman K, Cottrell G, Andrade-Gordon P, Steinhoff M, Barbara G, Beck P, Bunnett NW, Sharkey KA, Ferraz JG, Shaffer

E, Vergnolle N. 2007. Role for protease activity in visceral pain in irritable bowel syndrome. *J Clin Invest* 117: 636-47

179. Valdez-Morales EE, Overington J, Guerrero-Alba R, Ochoa-Cortes F, Ibeakanma CO, Spreadbury I, Bunnett NW, Beyak M, Vanner SJ. 2013. Sensitization of peripheral sensory nerves by mediators from colonic biopsies of diarrhea-predominant irritable bowel syndrome patients: a role for PAR2. *Am J Gastroenterol* 108: 1634-43

180. Wouters MM, Balemans D, Van Wanrooy S, Dooley J, Cibert-Goton V, Alpizar YA, Valdez-Morales EE, Nasser Y, Van Veldhoven PP, Vanbrabant W, Van der Merwe S, Mols R, Ghesquiere B, Cirillo C, Kortekaas I, Carmeliet P, Peetermans WE, Vermeire S, Rutgeerts P, Augustijns P, Hellings PW, Belmans A, Vanner S, Bulmer DC, Talavera K, Vanden Berghe P, Liston A, Boeckxstaens GE. 2016. Histamine Receptor H1-Mediated Sensitization of TRPV1 Mediates Visceral Hypersensitivity and Symptoms in Patients With Irritable Bowel Syndrome. *Gastroenterology* 150: 875-87 e9

181. Perna E, Aguilera-Lizarraga J, Florens MV, Jain P, Theofanous SA, Hanning N, De Man JG, Berg M, De Winter B, Alpizar YA, Talavera K, Vanden Berghe P, Wouters M, Boeckxstaens G. 2021. Effect of resolvins on sensitisation of TRPV1 and visceral hypersensitivity in IBS. *Gut* 70: 1275-86

182. Jo YY, Lee JY, Park CK. 2016. Resolvin E1 Inhibits Substance P-Induced Potentiation of TRPV1 in Primary Sensory Neurons. *Mediators Inflamm* 2016: 5259321

183. Serhan CN, Levy BD. 2018. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. *J Clin Invest* 128: 2657-69

184. Bao C, Chen O, Sheng H, Zhang J, Luo Y, Hayes BW, Liang H, Liedtke W, Ji RR, Abraham SN. 2023. A mast cell-thermoregulatory neuron circuit axis regulates hypothermia in anaphylaxis. *Sci Immunol* 8: eadc9417

185. Bellono NW, Bayrer JR, Leitch DB, Castro J, Zhang C, O'Donnell TA, Brierley SM, Ingraham HA, Julius D. 2017. Enterochromaffin Cells Are Gut Chemosensors that Couple to Sensory Neural Pathways. *Cell* 170: 185-98 e16

186. Bayrer JR, Castro J, Venkataraman A, Touhara KK, Rossen ND, Morrie RD, Maddern J, Hendry A, Braverman KN, Garcia-Caraballo S, Schober G, Brizuela M, Castro Navarro FM, Bueno-Silva C, Ingraham HA, Brierley SM, Julius D. 2023. Gut enterochromaffin cells drive visceral pain and anxiety. *Nature* 616: 137-42

187. Yu YB, Zuo XL, Zhao QJ, Chen FX, Yang J, Dong YY, Wang P, Li YQ. 2012. Brain-derived neurotrophic factor contributes to abdominal pain in irritable bowel syndrome. *Gut* 61: 685-94

188. Dothel G, Barbaro MR, Boudin H, Vasina V, Cremon C, Gargano L, Bellacosa L, De Giorgio R, Le Berre-Scoul C, Aubert P, Neunlist M, De Ponti F, Stanghellini V, Barbara G. 2015. Nerve fiber outgrowth is increased in the intestinal mucosa of patients with irritable bowel syndrome. *Gastroenterology* 148: 1002-11 e4

189. Enamorado M, Kulalert W, Han SJ, Rao I, Delaleu J, Link VM, Yong D, Smelkinson M, Gil L, Nakajima S, Linehan JL, Bouladoux N, Wlaschin J, Kabat J, Kamenyeva O, Deng L, Gribonika I, Chesler AT, Chiu IM, Le Pichon CE, Belkaid Y. 2023. Immunity to the microbiota promotes sensory neuron regeneration. *Cell* 186: 607-20 e17

190. Xu J, Zanvit P, Hu L, Tseng PY, Liu N, Wang F, Liu O, Zhang D, Jin W, Guo N, Han Y, Yin J, Cain A, Hoon MA, Wang S, Chen W. 2020. The Cytokine TGF-beta Induces Interleukin-31 Expression from Dermal Dendritic Cells to Activate Sensory Neurons and Stimulate Wound Itching. *Immunity* 53: 371-83 e5

191. Basso L, Lapointe TK, Iftinca M, Marsters C, Hollenberg MD, Kurrasch DM, Altier C. 2017. Granulocyte-colony-stimulating factor (G-CSF) signaling in spinal microglia drives visceral sensitization following colitis. *Proc Natl Acad Sci U S A* 114: 11235-40

192. De Schepper S, Verheijden S, Aguilera-Lizarraga J, Viola MF, Boesmans W, Stakenborg N, Voytyuk I, Schmidt I, Boeckx B, Dierckx de Casterlé I, Baekelandt V, Gonzalez Dominguez E, Mack M, Depoortere I, De Strooper B, Sprangers B, Himmelreich U, Soenen S, Guilliams M, Vanden Berghe P, Jones E, Lambrechts D, Boeckxstaens G. 2018. Self-Maintaining Gut Macrophages Are Essential for Intestinal Homeostasis. *Cell* 175: 400-15.e13

193. Ahrends T, Aydin B, Matheis F, Classon CH, Marchildon F, Furtado GC, Lira SA, Mucida D. 2021. Enteric pathogens induce tissue tolerance and prevent neuronal loss from subsequent infections. *Cell* 184: 5715-27 e12

194. White JP, Xiong S, Malvin NP, Khoury-Hanold W, Heuckeroth RO, Stappenbeck TS, Diamond MS. 2018. Intestinal Dysmotility Syndromes following Systemic Infection by Flaviviruses. *Cell* 175: 1198-212 e12

195. Sanchez-Ruiz M, Brunn A, Montesinos-Rongen M, Rudroff C, Hartmann M, Schluter D, Pfitzer G, Deckert M. 2019. Enteric Murine Ganglionitis Induced by Autoimmune CD8 T Cells Mimics Human Gastrointestinal Dysmotility. *Am J Pathol* 189: 540-51

196. Anitha M, Vijay-Kumar M, Sitaraman SV, Gewirtz AT, Srinivasan S. 2012. Gut microbial products regulate murine gastrointestinal motility via Toll-like receptor 4 signaling. *Gastroenterology* 143: 1006-16 e4

197. Ge X, Ding C, Zhao W, Xu L, Tian H, Gong J, Zhu M, Li J, Li N. 2017. Antibiotics-induced depletion of mice microbiota induces changes in host serotonin biosynthesis and intestinal motility. *J Transl Med* 15: 13

198. Vincent AD, Wang XY, Parsons SP, Khan WI, Huizinga JD. 2018. Abnormal absorptive colonic motor activity in germ-free mice is rectified by butyrate, an effect possibly mediated by mucosal serotonin. *Am J Physiol Gastrointest Liver Physiol* 315: G896-G907

199. Muller PA, Schneeberger M, Matheis F, Wang P, Kerner Z, Ilanges A, Pellegrino K, Del Marmol J, Castro TBR, Furuichi M, Perkins M, Han W, Rao A, Pickard AJ, Cross JR, Honda K, de Araujo I, Mucida D. 2020. Microbiota modulate sympathetic neurons via a gut-brain circuit. *Nature* 583: 441-6

200. McVey Neufeld KA, Mao YK, Bienenstock J, Foster JA, Kunze WA. 2013. The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterol Motil* 25: 183-e88

201. Collins J, Borojevic R, Verdu EF, Huizinga JD, Ratcliffe EM. 2014. Intestinal microbiota influence the early postnatal development of the enteric nervous system. *Neurogastroenterol Motil* 26: 98-107

202. De Vadder F, Grasset E, Manneras Holm L, Karsenty G, Macpherson AJ, Olofsson LE, Backhed F. 2018. Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. *Proc Natl Acad Sci U S A* 115: 6458-63

203. Bonaz B, Sinniger V, Hoffmann D, Clarencon D, Mathieu N, Dantzer C, Vercueil L, Picq C, Trocme C, Faure P, Cracowski JL, Pellissier S. 2016. Chronic vagus nerve stimulation in Crohn's disease: a 6-month follow-up pilot study. *Neurogastroenterol Motil* 28: 948-53

204. Sinniger V, Pellissier S, Fauvelle F, Trocme C, Hoffmann D, Vercueil L, Cracowski JL, David O, Bonaz B. 2020. A 12-month pilot study outcomes of vagus nerve stimulation in Crohn's disease. *Neurogastroenterol Motil* 32: e13911

205. Klooker TK, Braak B, Koopman KE, Welting O, Wouters MM, van der Heide S, Schemann M, Bischoff SC, van den Wijngaard RM, Boeckxstaens GE. 2010. The mast cell stabiliser ketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patients with irritable bowel syndrome. *Gut* 59: 1213-21

TERMS AND DEFINITIONS LIST

Neuropeptide: a small peptide that is produced and released by neurons

Neurotransmitter: a small molecule released by neurons at the synapse to transmit information

Ganglia: aggregation of neuronal cell bodies

Paravertebral ganglia: sympathetic ganglia that form a chain and are located next to the vertebral column

Prevertebral ganglia: sympathetic ganglia that are localized in front of the vertebral column

Cholinergic: neurons that express the neurotransmitter acetylcholine

Noradrenergic: neurons that express the neurotransmitter noradrenaline

Nociceptor: Sensory neurons that are activated by noxious stimuli and induce the sensation of pain

Preganglionic neuron: The cell bodies of preganglionic neurons are located in the spinal cord and their axons synapse on postganglionic neurons.

Postganglionic neuron: The cell bodies of postganglionic neurons are located in ganglia and innervate the target organ.

FIGURE LEGENDS

Figure 1: Neuro-immune interactions in the intestine.

Immune cells are located in proximity to different neuronal populations in the intestine. Neurons are able to communicate with immune cells either via direct mechanisms or through neural reflex circuits. Upon detection of mechanical, chemical or microbial stimuli in the gut, sensory neurons are activated, leading to generation of action potentials that propagate to the central nervous system (CNS) to mediate visceral sensations such as pain. Sensory neurons signal to immune cells via the axon reflex, whereby action potentials back-propagate along neighboring nerve branches towards the nerve terminals, which causes neuropeptide release that can signal to immune cells. Immune cells release inflammatory mediators, such as cytokines, lipid mediators and proteases, during inflammation or infection, which can also directly activate sensory neurons or sensitize them, thereby lowering their activation threshold. Signals from the gut also induce neural reflex circuits via the brainstem and spinal cord to parasympathetic or sympathetic neurons of the autonomic nervous system which signal to the gut via neurotransmitters that act on immune cells. Locally within the gut, enteric neurons release neuropeptides and neurotransmitters which bind to receptors expressed on immune cells and regulate their effector function.

Figure 2: Intrinsic and extrinsic innervation of the intestine

a) Anatomy of the enteric nervous system. The layers of the intestine consist of the epithelium, followed by the lamina propria, muscularis mucosae, and submucosa.

Adjacent lies the muscularis, consisting of an inner circular and outer longitudinal smooth muscle layer, and serosa. Enteric neurons reside in the submucosal plexus, located within the submucosa, and the myenteric plexus, between the circular and longitudinal smooth muscle layer. The myenteric plexus houses intrinsic primary afferent neurons (IPANs), interneurons, excitatory and inhibitory muscle motor neurons that innervate the muscle layers to coordinate intestinal motility. The submucosal plexus houses IPANs and secretomotor/vasodilator neurons that innervate the mucosa, where they regulate secretion and vasodilation. Another subset of neurons, the intestinofugal neurons, project to sympathetic prevertebral ganglia.

b) Extrinsic innervation of the intestine. The gut is also innervated by multiple types of extrinsic sensory and motor neurons, whose cell bodies reside outside the gut. Vagal sensory afferents (purple) have cell bodies located in nodose and jugular ganglia, and project via the vagus nerve to the small intestine and proximal colon. Spinal sensory afferents (purple) have cell bodies in the dorsal root ganglia (DRG), and project either via splanchnic nerves to the small intestine and colon, or via the pelvic and rectal nerve to the distal colon and rectum. Sympathetic efferents (red) arise in the thoracolumbar spinal cord and project to paravertebral and prevertebral ganglia, where they synapse onto neurons that innervate the small intestine and colon. Parasympathetic efferents (green) arise in the dorsal motor nucleus of the vagus (DMV) and nucleus ambiguous (NA) or the sacral spinal cord. DMV and NA neurons innervate the small intestine and proximal colon

via the vagus nerve and sacral spinal cord neurons innervate the colon via the rectal nerve or pelvic ganglia.

Figure 3: Neuronal regulation of immune cell effector function

a) Neuropeptides and neurotransmitters are produced by different gut-innervating neuronal populations, which are able to signal to both neuronal and immune cells. Peptidergic subtypes of DRG sensory neurons express calcitonin gene-related peptide (CGRP) and substance P (SP) (13, 54). Postganglionic sympathetic neurons express norepinephrine (NE) and postganglionic parasympathetic neurons express acetylcholine (ACh). Within the enteric nervous system, a subset of intrinsic primary afferent neurons (IPANs) expresses ChAT, which mediates synthesis of acetylcholine, neuromedin U (NMU), and CGRP (30, 32). Subsets of enteric interneurons express ChAT, SP (30) or serotonin (32). Excitatory motor neurons express ChAT and SP, whereas inhibitory motor neurons express vasoactive intestinal peptide (VIP) and IL-6 (30, 32, 146). A subset of secretomotor/vasodilator (S/V) neurons expresses VIP (30). IL-6 and IL-18 are expressed by different subtypes of enteric neurons (13).

b) The effector function of different intestinal immune cell populations is regulated by neuronal mediators including neuropeptides, neurotransmitters, and cytokines. NMU and VIP both promote type 2 innate lymphoid cell (ILC2) cytokine production, which facilitates helminth expulsion. In contrast, CGRP negatively regulates IL-13 expression and NE inhibits proliferation, leading to a diminished anti-helminth response. VIP promotes IL-22

production by type 3 innate lymphoid cells (ILC3s), which reduces bacterial infection and intestinal inflammation. NE induces tissue-protective macrophages, whereas ACh negatively regulates macrophage production of IL-6, which inhibits intestinal inflammation. Serotonin induces the release of PGE2 from macrophages, which promotes stem cell renewal. ACh also promotes retinoic acid production by dendritic cells, which supports regulatory T cell (Treg) generation. The differentiation of Tregs is inhibited by neuronal production of IL-6. Mast cells are regulated by substance P, which can promote degranulation, and acetylcholine (ACh), which was shown to inhibit degranulation. Neuropeptides and neurotransmitters also regulate epithelial cell function. CGRP reduces M cell density and supports segmented filamentous bacteria (SFB), which protects against bacterial infection. CGRP also promotes goblet cell mucus release, which limits intestinal inflammation during colitis. IL-18 produced by enteric neurons promotes the production of antimicrobial proteins by goblet cells, thereby limiting bacterial infection, and ACh induces formation of goblet cell-associated antigen passages (GAPs).

Figure 4: Immune cell-mediated regulation of neurons

a) Visceral pain hypersensitivity develops in response to intestinal inflammation by different mechanisms, including peripheral sensitization, nerve sprouting and central sensitization. Peripheral sensitization of sensory neurons is induced by 5-HT released by enterochromaffin cells and chymase and histamine released by mast cells. Resolvins inhibit histamine-induced sensitization of transient receptor potential cation channel subfamily V member 1 (TRPV1). Another mechanism is nerve sprouting, which is

associated with increased levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Central sensitization is induced by microglia, which sensitize $Trpv1^+$ neurons upon binding of adenosine triphosphate (ATP) to P2RY12 on microglia. Microglia sensitize sensory neurons via nitric oxide (NO), which is induced by binding of granulocyte-colony-stimulating factor (G-CSF) to G-CSFR on microglia and subsequent release of Cathepsin S, which cleaves fractalkine and thereby promotes CX3CR1 signaling.

b) Immune cells regulate the survival and function of neurons. Self-maintaining macrophages support the survival of enteric neurons, and muscularis macrophages promote colonic peristalsis via the secretion of bone morphogenetic protein 2 (BMP2). During bacterial enteric infection, neuron-derived norepinephrine binds to β_2-adrenergic receptor (β_2-AR) on muscularis macrophages and promotes Arginase 1 expression and polyamine production to protect enteric neurons. During helminth infection, T helper type 2 (Th2)-derived IL-5 recruits eosinophils, which via IL-4 and IL-13 promote arginase 1 expression by muscularis macrophages. During food allergy, antigen-specific IgE antibodies have been shown to activate myenteric neurons. During viral infection, antigen-specific CD8 T cells are involved in the death of enteric neurons.

FIGURES

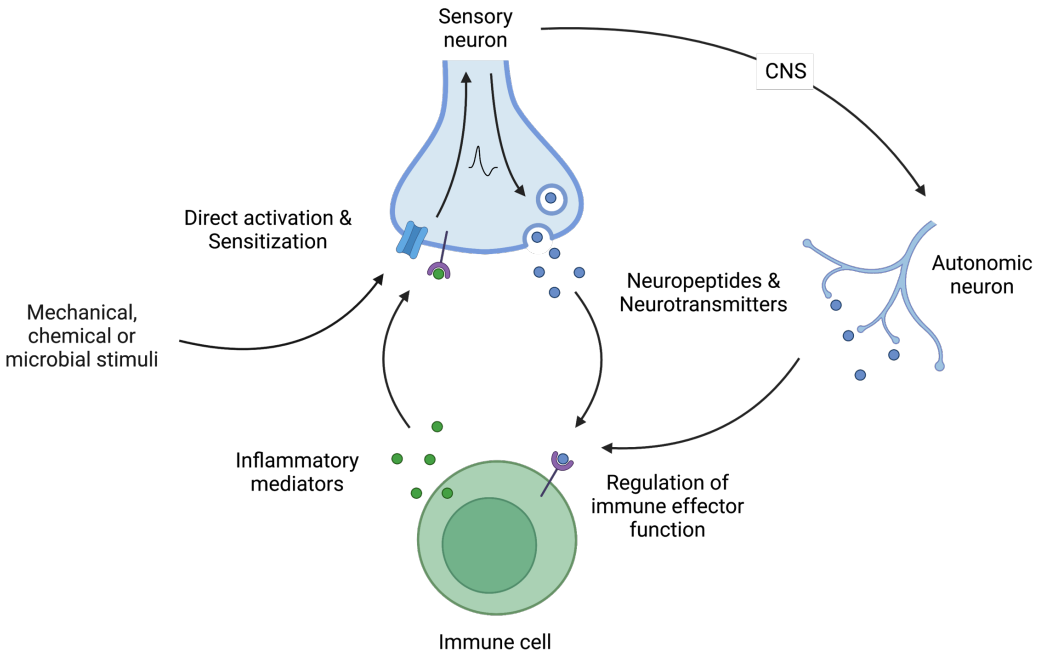

Figure 1

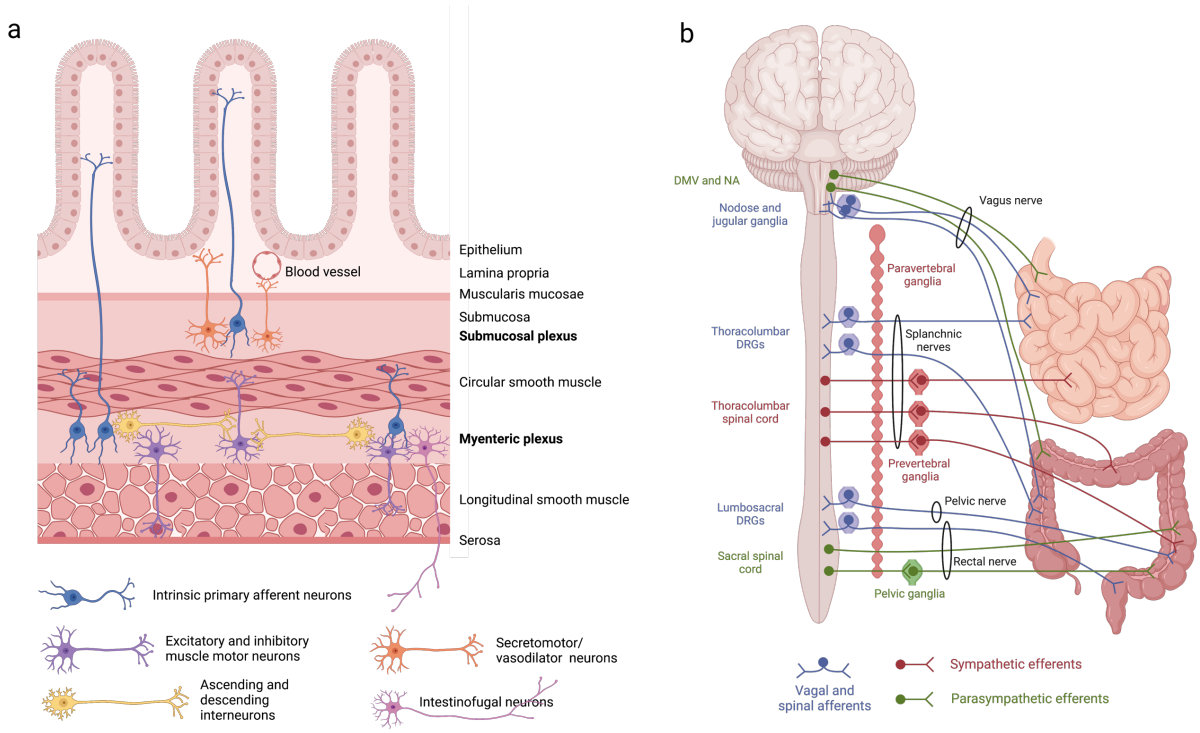
a
Blood vessel
Epithelium
Lamina propria
Muscularis mucosae
Submucosa
Submucosal plexus
Circular smooth muscle
Myenteric plexus
Longitudinal smooth muscle
Serosa
Intrinsic primary afferent neurons
Excitatory and inhibitory muscle motor neurons
Ascending and descending interneurons
Secretomotor/ vasodilator neurons
Intestinofugal neurons
b
DMV and NA
Nodose and jugular ganglia
Vagus nerve
Paravertebral ganglia
Thoracolumbar DRGs
Splanchnic nerves
Thoracolumbar spinal cord
Prevertebral ganglia
Lumbosacral DRGs
Pelvic nerve
Sacral spinal cord
Rectal nerve
Pelvic ganglia
Vagal and spinal afferents
Sympathetic efferents
Parasympathetic efferents

Figure 2

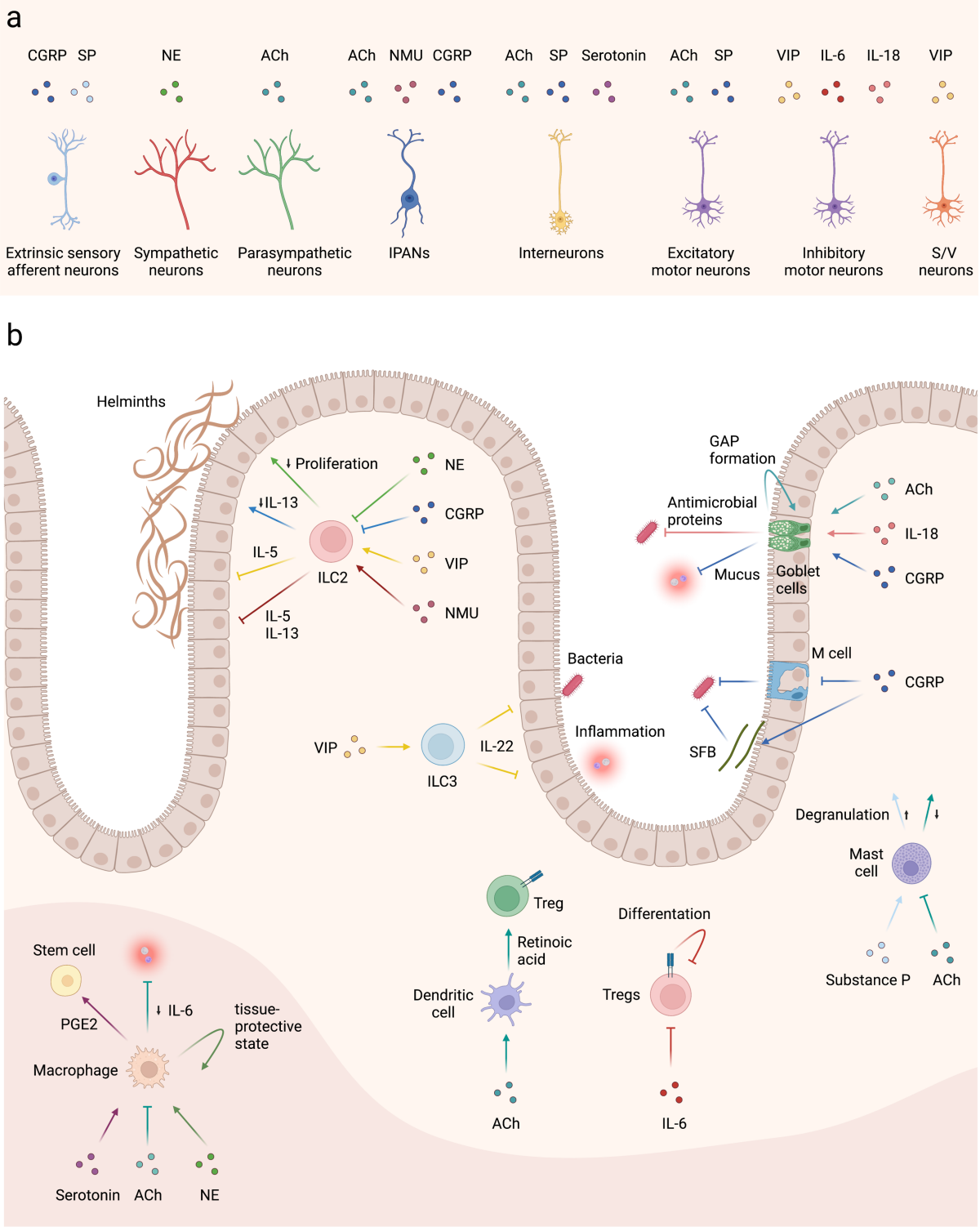
a
CGRP SP
NE
ACh
ACh NMU CGRP
ACh SP Serotonin
ACh SP
VIP IL-6 IL-18
VIP
Extrinsic sensory afferent neurons
Sympathetic neurons
Parasympathetic neurons
IPANs
Interneurons
Excitatory motor neurons
Inhibitory motor neurons
S/V neurons
b
Helminths
Proliferation
IL-13
IL-5
IL-5
IL-13
ILC2
NE
CGRP
VIP
NMU
GAP formation
Antimicrobial proteins
Mucus
Goblet cells
ACh
IL-18
CGRP
Bacteria
M cell
CGRP
Inflammation
SFB
VIP
ILC3
IL-22
Degranulation
Mast cell
Substance P
ACh
Treg
Retinoic acid
Dendritic cell
ACh
Differentation
Tregs
IL-6
Stem cell
PGE2
IL-6
Macrophage
tissue-protective state
Serotonin
ACh
NE

Figure 3

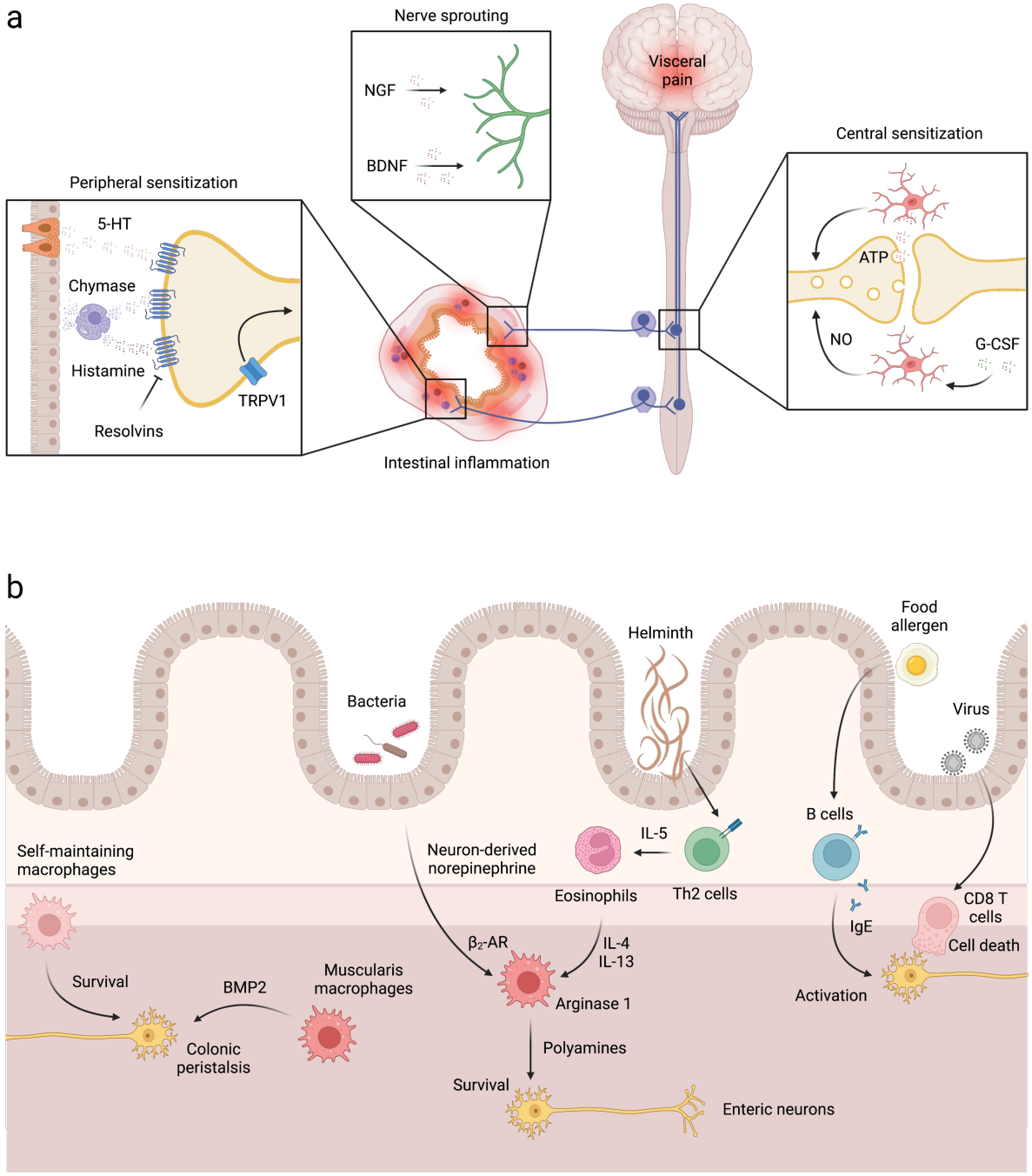
a
Nerve sprouting
NGF
BDNF
Visceral pain
Central sensitization
Peripheral sensitization
5-HT
Chymase
Histamine
TRPV1
Resolvins
ATP
NO
G-CSF
Intestinal inflammation
b
Food allergen
Helminth
Bacteria
Virus
B cells
IL-5
Self-maintaining macrophages
Neuron-derived norepinephrine
Eosinophils
Th2 cells
IgE
CD8 T cells
Cell death
β2-AR
IL-4
IL-13
Survival
BMP2
Muscularis macrophages
Arginase 1
Activation
Polyamines
Colonic peristalsis
Survival
Enteric neurons

Figure 4