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Cellular Organization of Neuroimmune Interactions in the Gastrointestinal Tract

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Abstract

The gastrointestinal (GI) tract is the largest immune organ; in vertebrates, it is the only organ whose function is controlled by its own intrinsic enteric nervous system (ENS), but it is additionally regulated by extrinsic (sympathetic and parasympathetic) innervation. The GI nervous and immune systems are highly integrated in their common goal, which is to unite digestive functions with protection from ingested environmental threats. This review discusses the physiological relevance of enteric neuroimmune integration by summarizing the current knowledge of evolutionary and developmental pathways, cellular organization, and molecular mechanisms of neuroimmune interactions in health and disease.

Neural Regulation of GI Physiology

Food intake and digestion are complex physiological events that rely on the coordinated efforts of many cell types, including smooth muscle, epithelia, and the vasculature. GI functions are regulated by a complex integration of signals arising from intrinsic neurons within the GI wall as well as extrinsic neurons whose soma lie outside the GI tract. Neurally mediated responses are triggered by the activation of sensory afferents in response to changes in luminal content or pressure, muscle distention, or inflammation and are transmitted to target cells through reflexes including: local enteric reflexes within the gut wall; extraspinal reflexes originating in the gut wall that pass through prevertebral sympathetic ganglia without involving the **central nervous system (CNS)** (see Glossary); and reflexes that pass to and from the gut via the CNS [1–5] Figure 1.

The discovery of large neuronal ganglia in the bowel wall organized in submucosal and intramuscular myenteric neural plexuses [6,7] led to the first description of the **ENS**. In terms of neuronal numbers, the ENS is by far the largest of the three divisions that Langley included in his original definition of the **autonomic nervous system (ANS)** [8–10]. In

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contrast to the upper GI tract, which operates under a greater degree of extrinsic (CNS) control, the ENS of the bowel is able to function independently of extrinsic inputs. The functional independence of the ENS was first demonstrated by Bayliss and Starling, who showed that increases in intraluminal pressure caused stereotypic propulsive responses, characterized by oral contraction and anal relaxation, in the dog intestine [11]. This reflex, termed ‘the law of the intestine’, is evoked even after all extrinsic inputs to the intestine are removed and was attributed, therefore, to the ‘local nervous mechanism’ of the gut; Trendelenburg confirmed these observations when he showed that this ‘peristaltic reflex’ could also be elicited in the guinea pig *in vitro* [12]. Since its initial discovery, much research has been devoted to understanding the neuronal mechanisms that underlie the peristaltic reflex. Intrinsic primary afferent neurons (IPANs) are sensory neurons with cell bodies in the submucosal and the myenteric plexuses and peripheral projections that reach the mucosa; IPANs then synapse with interneurons of the ENS that mediate the ascending and descending limbs of the peristaltic reflex [13]. Peristaltic reflexes are triggered by these IPANs, which are mechanosensitive; IPANs can also be activated by mucosal pressure, which causes neurohormone release from epithelial **enteroendocrine cells** [9,14,15], as well as by direct distortion of the gut wall [9,14,16,17]. Enteric motor neurons innervate the intestinal musculature as well as the epithelium, epithelial secretory glands, and blood–lymphatic vasculature. Compared with peristaltic reflexes, however, the neurocircuitry responsible for secretion is relatively simple, involving a two-neuron reflex that includes an afferent neuron, which can be activated by secretory products of enteroendocrine cells, and a secretomotor neuron, which projects into the lamina propria [13].

Although the ENS can control intestinal **motility** and other GI functions in the absence of extrinsic input, vagal, sacral, and sympathetic inputs all influence these functions. While these extrinsic nerves are not responsible for detailed motility patterns, they modulate activities that are hardwired within the ENS [13]. The gut, moreover, transmits a great deal of information to the brain; the significant majority of vagal fibers are afferents, carrying signals from the viscera to the CNS [18,19]. Some afferent signals from the gut cause discomfort, such as nausea [20] and pain [21], but most do not reach consciousness and are therefore ‘homeostatic’ [22].

The GI tract is a portal through which nutrients, but also toxins and pathogens, gain entrance to the body, requiring it to act, therefore, as a ‘bodyguard’ to screen ingested material such that beneficial nutrients are absorbed while toxic substances are excluded. Because no barrier is impermeable, the intestine is equipped with a system that can remove or neutralize potentially dangerous elements, involving multiple effector cell types including stromal (epithelial, vascular, smooth muscle) and enteroendocrine cells, all of which are under neural control [2,3]. Reflex activation of these sensory–effector systems induces physiological responses to ‘danger’ such as vomiting or diarrhea. A more sophisticated response to danger such as microbial invasion cannot be achieved without the immune system, however, whose diverse cells types can provide both sensory signals and effector responses to dangerous ‘non-self’ (Figure 2). Analysis of the evolutionary, developmental, and functional interactions between the enteric nervous and immune systems suggests that, in adult organisms, both systems are integral to gut protection.

Evolution of Neuroimmune Integration

The survival advantage inherent in distinguishing nutrients from potential invaders and ingested toxins is so fundamental that it is likely to have arisen early in evolution. Virtually every living organism faces environmental threats; as animals became more highly evolved, their ability to recognize and neutralize these threats became increasingly sophisticated. Innate immune mechanisms, involving phagocytic cells, are among the earliest of these defenses; later in evolution this innate immunity was supplemented with adaptive immunity, endocrine messaging, and neuronal signaling. Macrophages, and their antecedent phagocytic predecessors, are thus central to this process. In various forms, macrophages or related cells communicate with the lymphocytes that mediate adaptive immune responses, and both are subject to hormonal regulation and neuroimmune interactions [23–25]. Primitive multicellular organisms such as sponges, which evolved over 500 000 000 years ago, contain a heterogeneous population of pluripotent mesenchymal ameboid cells (archeocytes) that are phagocytic and function as stem cells. In addition to nutrient uptake, distribution, and storage, they also provide a means of organismal defense [26]. Even phagocytic cells must be mobilized, however, which requires intercellular communication, and the use of secreted, diffusible signals is phylogenetically older than neurotransmission. Organisms lacking a nervous system (e.g., sponges, Placozoa) produce a wide array of hormones that are homologous, sometimes identical, to the corresponding signaling molecules found in higher organisms. In primitive multicellular organisms, specialized cells in the epidermis and the intestinal lining, analogous to the enteroendocrine cells found in modern vertebrates, appear to have evolved the ability to secrete signaling metabolites in response to chemical or physical environmental stimuli [27]. These signaling molecules, which diffuse throughout the body, stimulate other cells to mount a defensive reaction. Some of these primitive endocrine cells evolved additional specializations, ultimately separating from the epithelium to become neurons or endocrine cells [28]. Along with cellular interactions, a pool of signaling molecules also evolved that enabled intercellular communication. These signaling molecules appear to have been evolutionarily conserved and, with the addition of new cells and systems, have been reused to coordinate specific and rapid defensive reactions. Primitive phagocytes can produce a wide array of signaling molecules including cytokines, nitric oxide (NO), the proopiomelanocortin-derived peptides, adrenocorticotrophic hormone (ACTH), alpha melanocyte-stimulating hormone (α -MSH), and β -endorphin, which are utilized as endocrine and/or neurocrine messengers in higher animals. A similar common pool of shared immune and neuroendocrine signaling molecules has been identified in many other invertebrates and vertebrates, suggesting that the integration between the immune, endocrine, and neurocrine systems has been conserved during evolution [23–25].

Common Developmental Pathways of Enteric Nervous and Immune Systems

As predicted by the theory of recapitulation, common signaling pathways control the development of enteric nervous and immune systems. The tyrosine kinase receptor rearranged during transfection (Ret) and its ligands drive ENS development; Ret promotes the proliferation, survival, and migration of the **neural crest-derived** precursors of enteric

neurons and glia in the fetal gut and its deficiency causes intestinal aganglionosis, such as **Hirschsprung's disease** [29,30]. Ret also directs the development of Peyer's patches by controlling the clustering of CD11c⁺ cKit⁺ hematopoietic progenitors called lymphoid tissue initiator (LTi) cells in the intestinal mucosa [31,32]. Ret-activating ligands are glial cell line-derived neurotrophic factor (GDNF) growth factors, which include GDNF, neurturin, artemin, and persephin. These ligands bind to one of four members of the GDNF family of coreceptors (GFR α 1–4) and the resulting complex binds to Ret, which dimerizes and becomes active. All four GDNF and GFR α ligands are produced in the enteric mesenchyme during fetal development. Although the crest-derived precursors of enteric neurons require stimulation by GDNF and recruitment of endogenous GFR α 1, the actions of Ret on LTis can be induced by multiple GDNF family ligands and GFR α s derived from other cells. This implies consecutive and interdependent activation of enteric neuronal and immune progenitors that initiate the gut-associated lymphoid tissue (GALT) [32].

Vitamin A, a well-known modulator of immune responses and a substrate for retinoic acid [34], is also required for normal ENS development. During embryogenesis, retinoic acid signaling promotes efficient GDNF-induced migration of ENS precursors through the developing bowel, and vitamin A deficiency leads to Hirschsprung's disease [35,36]. Prenatal vitamin A deficiency also causes underdevelopment of Peyer's patches, likely to be triggered by the combined lack of retinoic acid receptor signaling in immune cells and neurons [33,37]. In fetal mesenteric lymph nodes, neuronal retinaldehyde dehydrogenase (RALDH) induces the retinoic acid-dependent production of CXCL13 by stromal organizer cells followed by the clustering of lymphoid tissue inducer (LTi) cells [33]; similar mechanisms may also be involved in formation of Peyer's patches. Observations in patients suggest an association of Hirschsprung's disease with mucosal immunodeficiency and mice with neural crest-specific depletion of *EdnrB*, which closely models human Hirschsprung's disease, have small lymphopenic Peyer's patches and gut-specific deficiency in secretory IgA [38].

Postnatal Organization of Neuroimmune Interactions

Neuroimmune interactions begin before birth and modulate gut function throughout life. To respond to dynamic changes in intestinal homeostasis, the interplay between the nervous and immune systems is maintained through bidirectional signaling pathways. Many peripheral neurons respond to cytokines secreted by immune cells, which themselves express receptors for, and respond to, neurotransmitters secreted by neurons [39–41]. The close proximity of nerve fibers to intestinal immune cells may facilitate neuroimmune signaling, but neither the anatomical nature of functional neuroimmune interaction sites (synapses) nor the locations of receptors in relation to proximate sources of neurotransmitters in the GI tract has been well characterized. The most comprehensive anatomical evidence of interconnection between the immune and nervous systems has come from studies of the innervation that the ENS and peripheral nerves provide to Peyer's patches and in studies of muscularis macrophages and mast cells (Figure 3).

Payer's patches are organized aggregates of immune cells comprising a few B cell follicles separated by T cell-enriched regions, covered by a subepithelial dome rich in dendritic cells

(DCs). Analysis of intestinal mucosa in different mammalian species consistently demonstrates much denser innervation of Peyer's patches compared with the rest of the intestinal mucosa. Peyer's patches are surrounded by mesh-like ganglionated neuronal plexuses situated at the base of, or between, the follicles and with radial projections of nerve bundles towards the center of the dome area [42]. Innervation of Peyer's patches by the ENS includes projections from the submucosal plexus that include both intrinsic sensory and various neurochemically distinct motor neurons [43,44]. Extrinsic sympathetic (noradrenergic) fibers project to Peyer's patches [45] whereas parasympathetic fibers terminate at neurons of the myenteric plexus [46,47]. T cell areas, and the dome region of Peyer's patches, are highly innervated and some neural fibers enter the follicles [45]. Most noradrenergic fibers are associated with the vasculature, but vasculature-independent fibers are also present [48]. Close associations between neural fibers and CD11c⁺ or MHC class II⁺ cells, which can represent both DCs and macrophages [49,50], have been found within the follicles and the dome region [42,45]. Close contacts of neural fibers with CD3⁺ T cells, B220⁺ B cells [45], and IgA-producing plasma cells [42] have also been observed, consistent with the expression of the type 2 muscarinic acetylcholine (ACh) receptor (m2AChR) by some Peyer's patch CD11c⁺, B220⁺, CD3⁺, and CD4⁺ cells [45].

Muscularis macrophages [51] are a phenotypically and transcriptionally distinct population of intestinal macrophages that reside in the outer smooth muscle layer of the intestine (muscularis externa). Within the muscle, macrophages are positioned along nerve fibers and adjacent to enteric neuronal cell bodies, being most abundant in the myenteric and deep muscular plexuses. In the intact tissue, muscularis macrophages are characterized by bipolar or stellate morphology, with two or more pseudopods stretched along, or wrapped around, nerve fibers [52–54]. The analogous macrophage population is likely to be associated with the neural network in the submucosa (our unpublished observation) and mucosa [45], although this requires confirmation. At steady state, muscularis macrophages modulate peristalsis via secretion of the growth factor bone morphogenetic protein 2 (BMP2), which acts on enteric neurons. Enteric neurons, in turn, regulate macrophage numbers through secretion of colony stimulatory factor 1 (CSF1), also known as macrophage colony stimulatory factor (M-CSF), a cytokine that controls macrophage development. This crosstalk is dynamic and appears responsive to microbial changes in the lumen, since both BMP2 and CSF1 expression are enhanced by commensal bacteria [52]. Beside the ENS, interactions between muscularis macrophages and sympathetic nerve terminals have been described [53,54].

Mast cells congregate in close vicinity to intestinal nerves, mainly in the mucosa and submucosa [55], and this close mast cell–nerve fiber relationship is supported by the findings that mast cells express Kit, a receptor tyrosine kinase that is essential for their generation and maintenance [56]. Nerve fibers in the gut produce and secrete the growth factor Kit ligand [57], the neural expression of which is often plasma membrane associated [58], suggesting a direct contact between mast cells and nerve fibers. In contrast to muscularis macrophages, mast cells attached to nerve fibers are mainly round [59]. Because of their sparse presence in the normal gut, the mast cell role in neuroimmune interaction is more likely to be relevant to inflammatory conditions characterized by a dramatic increase

of mast cells, such as food allergies, parasite infection, and some forms of **irritable bowel syndrome (IBS)** [60].

Immune Regulation of ENS Homeostasis

Intestinal Microbiota

The interaction between the enteric microbiota and the ENS begins at birth. Germ-free mice exhibit significant ENS abnormalities, including a decline in the myenteric, particularly nitrergic, neurons in the jejunum, ileum, and colon [61,62]. Similar but less pronounced changes have been found in the ENS of Toll-like receptor 4 (TLR4)^{-/-} and MyD88^{-/-} mice, as well as mice that lack MyD88 in enteric neurons [62]. Lack of a microbiota, or the blockade of microbiota-dependent signaling pathways, diminishes GI motility [52,61,62]. The lack of TLR2 has been also associated with structural abnormalities of the submucosal plexus and reduced chloride secretion [63], observations that highlight the importance of innate immune pathways in ENS homeostasis, possibly by enhancing neurogenesis and/or the survival of enteric neurons. GDNF, for example, is not only essential for the formation of the entire ENS [64], but in later life modulates microbiota-dependent ENS development. The ENS defects in *Thr2*^{-/-} mice, which lack GDNF, can be partially corrected by administration of exogenous GDNF or a TLR2 agonist [63]. Remarkably, intestinal smooth muscles, not glia, have been shown to be the main source of GDNF in mature mice [65].

Enteric glia are an important structural and functional constituent of the ENS and postnatal enteric gliogenesis is also regulated by the microbiota (Figure 4). Lamina propria colonization by glial cells begins during early postnatal life and reaches steady-state levels after weaning. In adult mice myenteric plexus glial cells migrate centripetally to continuously renew mucosal glial cells; this process is aberrant in germ-free mice but resumes if the animals are ‘commensalized’ [66]. The innate immune mechanisms of postnatal control of gliogenesis are unknown.

Macrophages

Macrophages are essential regulators of organ development and postnatal homeostasis and remodeling [67]. The potential importance of macrophages in ENS development is supported by studies of macrophage-deficient mice. The nearly complete absence of macrophages in CSF1-deficient *Csf1*^{op/op} mice, which are characterized by abnormal bone growth and toothlessness [68,69], for example, results in structural ENS defects including increased myenteric neuronal numbers and a less organized architecture of the neuronal network [52]. Similar ENS changes are found in NSE-noggin mice, in which BMP receptor (BMPR) neuronal signaling is inhibited by overexpression of the endogenous BMP inhibitor noggin under the control of the neuron-specific enolase (NSE) promoter [70,71]. BMPs, a group of secreted proteins in the transforming growth factor beta (TGF-β) superfamily that are thought to mainly control organ development [72], strongly influence development of the ENS. BMP2 is highly expressed in the fetal gut and its expression is downregulated quickly after birth [70,71,73]. While the cellular source of BMP2 and other BMP family members in the developing gut has not been identified, muscularis macrophages are the major source of

BMP2 in the adult gut [52], suggesting that fetal macrophages or their predecessors in the enteric mesenchyme might produce BMPs.

BMPR signaling is also involved in postnatal homeostasis of enteric neurons. In *Hipk2*^{-/-} mice, in which BMPR2 signaling is excessive due to the lack of the negative regulator *Hipk2*, the number of myenteric neurons decreases progressively after birth [74]. The role of macrophages in postnatal enteric neuronal homeostasis is, however, unknown, as is the nature of neuronal functions regulated by macrophages and macrophage-derived BMP2 in adult mice. Studies of BMPR signaling in motor neurons of developing *Drosophila* larvae suggest actions to regulate axon microtubule stability, fast axonal transport, and synaptic growth and stability [75–79]. BMPs have also been demonstrated to inhibit constitutive electrical activity in differentiating neurons of the developing *Xenopus* spinal cord [80] and to guide the axons of CNS neurons [81]. Macrophage-derived BMPs may exert similar effects on adult enteric neurons, although such functions remain to be elucidated. Furthermore, microglia, the resident macrophages of the CNS, promote learning-dependent synapse formation in adult mice by providing brain-derived neurotrophic factor (BDNF) to neurons [82]; whether macrophages support synapse formation in the ENS is unknown.

Inflammation

Inflammatory insults to the GI mucosa often cause profound and irreversible changes of GI homeostasis that result in the development of, for example, IBS [83]. Mucosal inflammation induces rapid death of enteric neurons, which can be observed even before immune cell infiltration of the muscularis [5,84,85]. By contrast, chronically inflamed intestinal regions from patients with **inflammatory bowel disease (IBD)** are often hyperinnervated [86,87], most likely through neurogenesis [88] from activated stem cells that are retained in the mature intestine [89]. Inflammation-induced pathophysiological alterations of enteric neurons, including altered neurochemical phenotypes and hyperexcitability, were observed in both patient samples and animal models; such inflammation-induced enteric alterations persist long after resolution of the initial inflammation [4,5] and similar dysfunctions have been observed in extrinsic afferent and efferent neurons [5].

The cellular and molecular mechanisms of neuronal death caused by inflammation are poorly understood. The activation of neuronal P2X7 receptors, Panx1, and caspases by extraganglionic ATP released by inflamed tissue is thought to be one mechanism responsible for neuronal loss during colitis [85]. Studies *in vitro* suggest that the inflammatory cytokines IL-1 β and tumor necrosis factor alpha (TNF α) exert a trophic effect on enteric neurons via upregulation of smooth muscle cell GDNF, which in turn induces neurite overgrowth [90]. It is unclear, however, whether intestinal hyperinnervation results from neuronal death and regeneration or from ENS restructuring. Similarly, the role of immune cells, particularly muscularis macrophages, in the regulation of neuronal homeostasis during colitis is also unknown. Additional studies will require the unraveling of the nature of post-inflammatory changes in GI innervation.

Afferent Neural Sensing via Immune Recognition

Sensing Microbial Signals

The enteric microbiome impacts the functional properties of myenteric IPANs, which are less excitable in germ-free mice, although such IPAN abnormalities are corrected on commensalization of the bowel [93]. Little is known about how neurons sense signals from the enteric microbiota; afferents may recognize mucosal microbial products directly through numerous mechanisms including chemosensation of bacterial metabolites, activation of innate immune pathways by microbe- and pathogen-associated molecular patterns (MAMPs and PAMPs), and microbiota-produced neurotransmitters. The ability of bacterial products to activate neurons directly has been demonstrated in other species and organ systems that may also be applicable to the human bowel. Thus, exposure of the soil-dwelling nematode *Caenorhabditis elegans* to a pathogenic bacterium, *Pseudomonas aeruginosa*, induces chemotactic behavior to avoid the pathogen. This avoidance behavior is driven by direct activation of G protein signaling pathways in chemosensory neurons in response to the metabolites phenazine-1-carboxamide and pyochelin produced by *P. aeruginosa* [94]. In murine skin infected with *Staphylococcus aureus*, sensory nociceptors are activated by bacterial *N*-formyl peptides and the pore-forming toxin α -hemolysin, inducing pain [95]. Similarly, pain and acute vascular reactions caused by lipopolysaccharide (LPS) from Gram-negative bacteria can stimulate mouse nodose (i.e., vagal) sensory neurons innervating the viscera via activation of the transient receptor potential cation channel TRPA1 independently of TLR4 [96]. The TLR/MyD88 signaling pathway may still be involved in regulating IPAN (as well as extrinsic sensory neuron) functions. Application of LPS to cultured enteric glial cells produces IL-1 β [97], while application to cultured enteric neurons produces TNF α [98] and enhances the constitutive expression of CSF1 [52]. Furthermore, in transgenic mice the constitutive depletion of MyD88 in enteric neurons causes structural and functional ENS changes [62]. Finally, bacteria are able to produce neurotransmitters such as 5-hydroxytryptamine (5-HT), dopamine, norepinephrine, γ -aminobutyric acid (GABA), ACh, tyramine, tryptamine, and histamine [99,100], although the amounts of these compounds produced *in vivo* are unknown.

Small molecules with bioactive properties – for example, short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate – are generated by the gut microbiota through the digestion of complex carbohydrates, principally from dietary fiber [101]. The SCFA receptor GPR41 (also known as FFAR3) is expressed by a subset of enteric neurons; GPR41 and GPR43 (FFAR2) are expressed by most intestinal enteroendocrine cells, whereas GPR43 is expressed only by lamina propria leukocytes [102], mainly myeloid cells such as neutrophils, eosinophils, and activated macrophages [103]. Besides being an important energy source, SCFAs serve as signal molecules that regulate electrolyte secretion, smooth muscle contraction, and enteroendocrine cell differentiation [104–106] and as immunoregulatory molecules with anti-inflammatory potential [103].

Microbial signals may activate enteric neurons indirectly; enteroendocrine cells are able to transmit luminal chemosensory signals to neurons through the release of hormones as well as through synaptic transmission to sensory neurons [107]. Recent studies have

demonstrated that commensal spore-forming bacteria expand numbers of 5-HT⁺ enteroendocrine cells and upregulate their *Tph1* expression (Figure 4) [108]. Microbiota-dependent increase of mucosal 5-HT impacts host physiology, including GI motility, and is driven by selected microbial metabolites [108] such as SCFAs [108,109]. Additionally, human 5-HT⁺ enteroendocrine cells and mouse enteroendocrine cell lines have been shown to express multiple bacterial ligand-specific TLRs including TLR1, 2, 4, 5, 6, and 9 [110,111]. LPS activates mouse enteroendocrine cell lines to release cholecystokinin (CCK) and upregulate inflammatory cytokine and chemokine secretion [110]. *In vivo*, oral administration of the TLR ligands flagellin, CpG, or LPS increases systemic levels of CCK, a response that is more pronounced in mice receiving antibiotics. By contrast, there is no CCK increase in *Thr4*^{-/-} mice in response to the TLR4 ligand LPS or *Thr9*^{-/-} mice treated with the TLR9 ligand CpG [111]. Interactions of enteroendocrine cells with bacterial toxins have been also described; cholera toxin, a secretory enterotoxin produced by *Vibrio cholerae*, evokes intestinal fluid secretion via binding to its receptor GM1 ganglioside on enteroendocrine cells, followed by 5-HT release and activation of the ENS [112].

Mucosal immune cells, particularly myeloid cells, are equipped to recognize a wide range of microbial components and are the major producers of inflammatory cytokines in response to microbial challenge [113]. The contributions of specific immune cell types and cytokine receptor pathways to ENS activation, however, are not well established. Development of proctitis, an inflammatory infiltrate of the submucosal and myenteric plexuses in patients with IBD [5], suggests that ENS-associated macrophages, including muscularis macrophages, might contribute to the transmural spread of inflammation by providing local inflammatory signals within the ENS. These macrophages may act as sensors that translate environmental cues for neurons; both the luminal microbiota and mucosal infection regulate the gene expression profile of muscularis macrophages (Figure 4) [52,53]. Their spatial separation from the lumen raises questions regarding the mechanisms by which muscularis macrophages recognize luminal signals. While the rapid response to infection can be neurally mediated [53], the sustained long-term response to the microbiota and infection may be driven by direct sensing of systemically circulating MAMPs and PAMPs [62].

Sensing Inflammation

Mucosal inflammation induces various functional changes both in the ENS and in the extrinsic innervation, including hypersensitivity and hyperexcitability [4,5]. The direct effect of inflammatory cytokines on neuronal function has not been studied extensively; TNF α , IL-1 β , IL-6, and cysteinyl leukotrienes alter the activity of acutely dissociated dorsal root ganglion (DRG) neurons [114] as well as enteric neurons in tissue explants [115–120]. This effect may be direct, as both myenteric and DRG neurons express TNF receptors [114,121]; however, the contribution of intermediate cell types, particularly ENS-associated macrophages, cannot be excluded. In fact, muscularis macrophages have been implicated in the pathogenesis of **postoperative ileus (POI)**, a transient inflammatory condition of the GI tract that results in intestinal paralysis, likely by providing inflammatory stimuli to the ENS [91,92].

Like neurons, innate and adaptive immune cells have been shown to produce neurotransmitters and neuromodulators, such as 5-HT, ACh, vasoactive intestinal peptide (VIP), and endorphins [122–127], whose production in the intestinal mucosa might regulate the nociceptive threshold of sensory nerves [128]. Mast cells, in particular, secrete a complex mixture of mediators including proteases, prostaglandins, histamine, 5-HT, cytokines, and chemokines, which may modulate the activity of intestinal nerves [60].

Efferent Neural Regulation of Immune Cell Function

Accumulating evidence indicates that the nervous system has established evolutionarily conserved inflammatory reflexes to mount and to restrict immune responses [129,130] that, in higher vertebrates, utilize both the sympathetic nervous system (SNS) [131] and the parasympathetic nervous system (PNS) [132,133]. In rodents chemical sympathectomy reduced acute dextran sodium sulfate (DSS)-induced colitis but increased the inflammatory response in both a chronic model of DSS-induced colitis and a model of spontaneous colitis in IL-10^{-/-} mice [134]. Furthermore, both surgical sympathectomy and pharmacological blockade of adrenoceptors improved the clinical symptoms of POI [135]. By contrast, vagotomy exacerbated the inflammatory response in a chronic model of DSS-induced colitis [136], while vagus nerve stimulation alleviated colonic inflammation in a model of trinitrobenzene sulfonic acid (TNBS)-induced colitis [137] and both vagus nerve stimulation and pharmacological activation of cholinergic pathways prevented POI [138]. Additionally, NSE-noggin mice, with a hyperplastic ENS due to an increase in enteric neuronal number [71], develop more severe DSS- and TNBS-induced colitis; by contrast, *Hand2*^{+/-} mice, with a hypoplastic ENS [139], have reduced severity of colitis in the same experimental models [140], suggesting that abnormalities of the ENS also influence intestinal inflammation.

The mechanisms by which neural signals regulate intestinal immunity remain unclear but are likely to involve the direct interaction of neurons with immune cells, particularly macrophages. Vagotomy, for example, had no effect on the severity of colitis in macrophage-deficient *Csf1*^{op/op} mice [136], while vagus nerve stimulation attenuated the tissue inflammatory response driven by muscularis macrophages in mice after intestinal manipulation that modeled POI. Moreover, stimulation of muscularis macrophages via alpha-7 nicotinic ACh receptors (α7AChRs) modulates their ATP-induced Ca²⁺ response [141,142]. Anterograde labeling of efferent vagal fibers, however, failed to reveal direct contact between vagal fiber terminals and muscularis macrophages. Instead, vagal fibers contact cholinergic, VIP⁺, and neuronal NO synthase (nNOS)⁺ enteric neurons, which are likely to transduce vagal signals to macrophages [143]. More recently, muscularis (but not mucosal) macrophages were shown to be targeted by sympathetic fibers through β2-adrenoceptor (β2AR) activation. Mucosal application of noninvasive *Salmonella* led to rapid (within 2 h) activation of muscularis macrophages, which was prevented by pharmacological blockade or absence of β2AR, suggesting a role for the SNS [53], although the physiological relevance remains unclear. Genes uniquely upregulated by muscularis (but not mucosal) macrophages during *Salmonella* infection include *Arg1* (encodes Arginase 1) and *Chi3l3* (encodes Chitinase-like 3) [53]; both are expressed by alternatively activated murine microglia and are upregulated in the frontal lobe cortical extracts of patients with Alzheimer's disease [144], suggesting a possible link to intestinal neuroplasticity.

The impact of neural signaling on the function of mucosal immune cells is less clear. Chemical sympathectomy increases the numbers of mucosal IgM- and IgA-producing plasma cells without affecting *de novo* generation of antigen-specific plasma cells in response to immunization [145]. Together with the finding that vagal efferent stimulation upregulates CXCL13 production in the mouse ileum [33], this suggests that extrinsic innervation to the gut regulates trafficking of immune cells in the mucosa, which might be required to rapidly mobilize dispersed immune cells during infection. Alternatively, β 2AR-driven retention of activated lymphocytes in the mesenteric lymph nodes might control mucosal inflammation by restricting gut homing [146]. Finally, nociceptive sensory neurons have been shown to drive inflammatory cytokine (IL-23) production by resident phagocytes in the skin; the existence of similar inflammatory reflexes in the intestinal mucosa has not been tested [147,148].

Concluding Remarks

Interest in neuroimmune interactions in intestinal homeostasis and GI pathology is growing rapidly. These interactions are complex, involving active participation of the immune, nervous, and epithelial neuroendocrine systems together with environmental factors including diet, the microbiota, and infection. Despite recent progress, much remains to be discovered (see Outstanding Questions) and critical questions remain regarding the means by and extent to which neuroimmunity affects human physiology and disease. It will also be important to determine whether the neuroimmune alterations that initially induce dysfunction are the same as those that drive chronic pathology. Unraveling the developmental pathways of enteric nervous and immune systems, identifying triggers and cellular targets of inflammatory and anti-inflammatory reflexes, and understanding the immune mechanisms of neuroplasticity may improve therapeutic interventions for Hirschsprung's disease, POI, IBS, and IBD.

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Glossary

Autonomic nervous system (ANS)

a functional division of the nervous system that regulates involuntary functions of peripheral organs. The ANS comprises two complementary branches, the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), along with the ENS.

Central nervous system (CNS)

an anatomical division of the nervous system that includes the brain and the spinal cord.

Enteric glia

astrocyte-like cells that create a net surrounding neural ganglia and bundles of neural fibers. Enteric glia provide structural and functional support to enteric neurons and are a required component of the ENS.

Enteric nervous system (ENS)

the gastrointestinal intrinsic nervous system, comprising neurons housed in the GI wall. Enteric neurons are organized in two mesh-like plexuses surrounded by glial cells: the submucosal plexus, which mainly regulates the function of the epithelium; and the myenteric plexus, which controls the propulsive contractility of intestinal smooth muscles.

Enteroendocrine cells

sensory epithelial cell type with neuroendocrine function. At least 15 subtypes exist, each secreting different bioactive hormones. The serotonin (5-HT)-producing subtype, the most common type of enteroendocrine cell in the colon and rectum, is responsible for most of the serotonin produced in the body and in the gut. Enteroendocrine cell-derived hormones regulate GI motility and secretion by exerting local and systemic effects on the ENS.

Gastrointestinal (GI) motility

coordinated propulsive contractions of the smooth muscles that pass the luminal contents along the GI tract.

Hirschsprung's disease

polygenic congenital abnormality characterized by functional bowel obstruction caused by aganglionosis of distal bowel due to failed migration of enteric neural crest-derived cells.

Inflammatory bowel disease (IBD)

chronic intestinal inflammatory conditions, including Crohn's disease and ulcerative colitis, thought to be primarily caused by immune dysregulation in the gut mucosa. Patients with IBD commonly show signs of GI dysfunction including dysmotility even when in remission.

Irritable bowel syndrome (IBS)

functional GI disorder characterized by altered GI motility (dysmotility) accompanied by chronic abdominal pain or discomfort. The clinical presentation of IBS widely varies and can be divided into a few major groups: constipation predominant, diarrhea predominant, and mixed defecation pattern as well as a history of post-infectious versus not post-infectious symptom development. The etiology of IBS is unknown and the pathophysiology is poorly understood.

Neural crest-derived cells

migratory cells that originate during embryogenesis by delaminating from the dorsal side of the neural tube following neurulation and migrate towards predetermined regions of the embryo to give rise to diverse tissues. Enteric neural crest-derived cells colonize the fetal bowel to give rise to neurons and the glia of the ENS.

Postoperative (paralytic) ileus (POI)

inflammatory condition mainly affecting the muscularis externa in which motility of the entire GI tract is impaired. It occurs after abdominal surgery and causes nausea, vomiting, food intolerance, and constipation.

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Trends

Comparison of the evolutionary and embryonic development of the enteric nervous system (ENS) and immune system suggests functional integration between the two systems. Macrophages, the evolutionarily most ancient immunocytes, play a special role in the development, postnatal homeostasis, and functional modulation of the ENS.

Intestinal immune cells are an integrated element of the neural sensory system; neural circuits triggered through immune mechanisms link the immune response to infection to defensive physiological adaptations.

The nervous system connects and mobilizes dispersed intestinal immune cells through efferent neuroimmune inflammatory reflex pathways. It also provides feedback to limit excessive inflammatory response through anti-inflammatory reflexes.

Neuroimmune interactions in the gut are plastic and are interdependently influenced by intrinsic factors (diet, microbiota, tissue homeostasis) as well as by extrinsic neural regulations (Figure 1).

Outstanding Questions

Anatomy of neuroimmune interactions

What cell types are involved in neuro-immune interactions in the gut mucosa?

What is the morphology of functional neuroimmune interaction sites?

What the ligand/receptor pairs are involved in neuro-immune interactions?

Neuronal Sensing of Commensal Micro-organisms, Infection, and Inflammation

How do neurons sense commensals, infection, and inflammation?

How do enteroendocrine cells, enteric glia, and innate immune cells, particularly macrophages and mast cells, “inform” neurons about changes in the lumen and tissue?

Inflammatory and Anti-inflammatory Reflexes

What neural circuits regulate immune cell function in GI homeostasis, infection, and inflammatory disorders?

Which immune cell functions are regulated by neural circuits?

Macrophages and the ENS

How do muscularis macrophages and macrophage-derived BMP2, regulate the development, postnatal homeostasis, and function of enteric neurons?

Is there a specialized macrophage population associated with the ENS in the mucosa?

Inflammation-Driven Neuroplasticity

How does inflammation regulate neuronal death and regeneration?

What are the roles of muscularis macrophages and macrophage-derived BMP2, Arginase 1, and Chitinase-like 3 in neuroplasticity?

Trends in Immunology

Figure 1. A Multidimensional Interplay between the Luminal Environment of the Gastrointestinal (GI) Tract, Homeostasis of Intestinal Tissue, and Extrinsic Neural Regulation of GI Functions

The GI tract is a portal through which food and fluids enter the body. It is also a home for trillions of commensal bacteria and is an entry site for microbes and parasites invading the host. Diet, which is a source of nutrients, water, electrolytes, and essential vitamins, provides the substrate for commensal and infectious organisms and is often a source of poisonous substances and infection. The commensal microbiota aids in digestion. By completing the breakdown of partially digested nutrients, the microbiota generates dietary metabolites, releases additional vitamins, and synthesizes new vitamins. Colonization of the lumen with commensal bacteria induces physiological inflammation through innate immune pathways and reduces the invasiveness of some pathogens. Infection of the lumen induces inflammation and commonly suppresses food consumption. To respond to dynamic changes in intestinal homeostasis, the interplay between the intestinal nervous and immune systems is maintained through bidirectional communication between neurons and immune cells. GI pathology is capable of dysregulating neuroimmune interactions; likewise, disease may arise from abnormal neuroimmune interactions. Neuroimmune exchanges are not restricted to the wall of the gut. Because the bowel and the central nervous system (CNS) also communicate, neuroimmune interactions within the gut can affect the CNS and, in turn, input from the CNS can regulate enteric neuroimmunity. GI disorders that lead to discomfort or pain, therefore, are stressful, while stress and/or a depressed mood may worsen GI disorders.

Figure 2. Neuroimmune Pathways in the Gastrointestinal (GI) Tract

Immune cells are able to activate sensory neurons through production of cytokines and bioactive molecules with neurotransmitter properties (left). Immune cell function can be influenced by signals from motor neurons (middle). Immune cells can be positioned at both the afferent and efferent arms of a neural circuit (right). Abbreviation: EECs, enteroendocrine cells.

Figure 3. Model of Cellular Organization of Neuroimmune Interactions in the Gut

Epithelial enteroendocrine cells (EECs) provide the first line of defense against luminal microbes and respond to microbial stimuli by the production and release of neuropeptides such as 5-hydroxytryptamine (5-HT), chemokines, and cytokines. Activated immune cells including mucosal macrophages and mast cells stimulate intrinsic and extrinsic (not shown) sensory neurons through secretion of cytokines and bioactive molecules. Enteric glia and a specialized subset of enteric nervous system (ENS)-associated macrophages provide homeostatic and functional support to enteric neurons. Intrinsic and extrinsic motor neurons regulate the function of stromal and immune cells by paracrine release of neurotransmitters. The ENS and sympathetic nervous system (SNS) innervate Peyer's patches in the mucosa and muscularis macrophages. The para-sympathetic nervous system (PNS) connects with the ENS.

Figure 4. Known Mechanisms of Microbiota-Driven Regulation of Intestinal Neuroimmune Interactions

At the mucosal–luminal interphase, microbiota-produced dietary metabolites increase 5-hydroxytryptamine (5-HT) production by enteroendocrine cells (EECs). In the mucosa, the microbiota promotes postnatal gliogenesis through unknown mechanisms. In the myenteric plexus, the microbiota promotes crosstalk between muscularis macrophages and enteric neurons by supporting macrophage development and survival through increased colony stimulatory factor 1 (CSF1) production by enteric neurons and by controlling the homeostasis and function of enteric neurons through increased bone morphogenetic protein 2 (BMP2) production by macrophages.