

# The Enteric Network: Interactions between the Immune and Nervous Systems of the Gut

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Interactions between the nervous and immune systems enable the gut to respond to the variety of dietary products that it absorbs, the broad spectrum of pathogens that it encounters, and the diverse microbiome that it harbors. The enteric nervous system (ENS) senses and reacts to the dynamic ecosystem of the gastrointestinal (GI) tract by translating chemical cues from the environment into neuronal impulses that propagate throughout the gut and into other organs in the body, including the central nervous system (CNS). This review will describe the current understanding of the anatomy and physiology of the GI tract by focusing on the ENS and the mucosal immune system. We highlight emerging literature that the ENS is essential for important aspects of microbe-induced immune responses in the gut. Although most basic and applied research in neuroscience has focused on the brain, the proximity of the ENS to the immune system and its interface with the external environment suggest that novel paradigms for nervous system function await discovery.

## Introduction

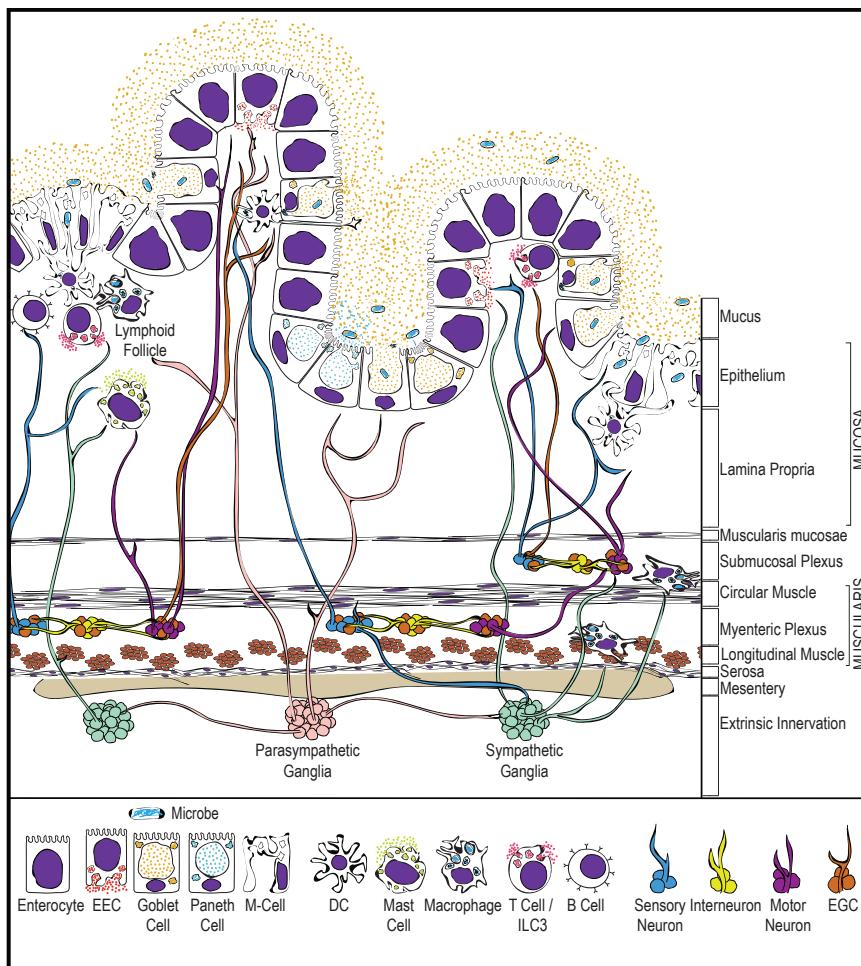
The gastrointestinal (GI) tract spans 5 m in length and has an epithelial surface area of ~32 m<sup>2</sup> (Helander and Fändriks, 2014). It is the home to 70%–80% of the body's immune cells (Kagnoff, 1987), over 100 million neurons (Furness et al., 2014), and up to 100,000 extrinsic nerve endings (Grundy and Brookes, 2011). The microbiome consists of as many as 40 trillion cells and at least hundreds of different species (Lozupone et al., 2012; Sender et al., 2016). An important function of the GI tract is to sense and respond to external cues. Diverse cellular interactions are responsible for interpreting them, and these interactions must be amenable to the change and flux in the molecular environment of the GI tract. Thus, countless components necessitate GI function, and equally complex changes can affect it. Accordingly, 70 million people in the United States (>20% of the population) are affected by digestive diseases every year (Peery et al., 2012), and GI dysfunction is often comorbid with numerous non-intestinal conditions. As such, interactions between interdependent, cellular pathways in the gut and the periphery could underlie processes involved in health and disease.

The GI ecosystem can be largely characterized by the exchange of molecules between and within luminal constituents and the host. Environmental and dietary molecules are necessary for host survival, but they are also important factors that affect gut microbes and the factors they produce. How these luminal components, as a whole, interact with the cells and molecules of the intestine elucidates complex and coordinated events that occur in the GI tract. Homeostatic communication across the intestinal epithelium involves contributions by diet and the microbiota, which interact with the mucosal immune system and the enteric nervous system (ENS). In the GI tract, multiple distinct cell types can produce a given modulatory molecule, and conversely, a given molecule is able to affect various cell types. This molecular synchrony, and deviations from it, affects expansive GI and non-GI physiologies. Yet, the cellular and molecular interconnectivity at this critical interface between the body and the environment remains largely unexplored. This re-

view will focus on the diverse cellular anatomy of the GI system and discuss the molecules and receptors that various cell types use to communicate and function. The GI tract represents a direct portal to the molecular universe, and the unique juxtaposition of its nervous and immune systems suggests a vital role for neuro-immune interactions in the gut.

## Structural Anatomy of the GI Tract

Digestion, absorption, and secretion in the GI tract primarily occur in the stomach and small and large intestines. Anatomically, the intestinal tissue can generally be compartmentalized by the mesentery, serosa, muscularis, submucosa, lamina propria, epithelium, and lumen (Figure 1). Major extrinsic arteries, veins, lymphatics, and nerve fibers enter and exit the tissue through the mesentery. It also encloses the mesenteric lymph nodes (MLNs), which are the draining lymph nodes of the intestines. Immunological development and lymphocyte transport occur within the intestinal tissue, but movement to and from peripheral lymphoid tissues requires passage through the mesentery. The mesentery is contiguous with the serosa, which is the outermost layer of the mesothelium and encapsulates and lubricates the GI tract so that peristaltic contractions are uninhibited. The outermost layers of intestinal tissue proper are collectively called the muscularis. This region is made up of an outer longitudinal and an inner circular muscle layer. These layers are orthogonal to each other, providing stretch and shear flexibility, and also resident to many immune cells (Kalff et al., 1998). The myenteric plexus lies between the two layers of smooth muscle, and the submucosal plexus is luminal to the muscularis. Both consist of vast networks of neurons and glia that extend throughout the GI tract, and they send impulses and sense inputs to and from the various intestinal compartments, including the mucosa. Vasculature extends throughout the submucosal layer, bringing within it circulating immune cells that infiltrate and exit the tissue. Immune structures such as Peyer's patches and lymphoid follicles emanate from the submucosa and extend into the mucosa, which consists of the lamina propria and innermost epithelial



**Figure 1. Anatomy of the GI Tract**

The GI tract is composed of distinct cross-sectional compartments. Extrinsic, sympathetic, and parasympathetic nerve fibers enter the GI tract through the mesentery and can extend throughout all layers of intestinal tissue. The myenteric and submucosal plexuses form the dense nerve network that innervates the entire length and depth of the GI tract. Various immune cells reside in the muscularis but are also highly abundant in the lamina propria, especially in Peyer's patches and lymphoid follicles. These immune cells are also in close proximity to neurons and glia. The epithelium shown here is made up of five different cell types, including absorptive enterocytes, EECs, goblet cells, Paneth cells, and M cells.

layer. The lamina propria contains many innate and adaptive immune cells but is also made of the connective tissue that is important for GI structural identity. Furthermore, vascular, neuronal, and glial processes extend throughout the lamina propria and mucosa, and the diverse cells and molecules associated with these structures are important for enteric function. As will be described below, distinct subtypes of intestinal epithelial cells (IECs) are responsible for absorption, communication, and protection, and these roles are mediated by diverse effector molecules that function apically and basolaterally. Finally, the lumen contains the molecules excreted and consumed by the host and the microbiome that has adapted to survive the GI tract. This structural and cellular compartmentalization, described in detail below, will provide anatomical reference for the vast neuro-immune interactions that can occur in the GI tract.

### The Intestinal Epithelium

The intestinal epithelium is composed of distinct IEC types that mediate communication between the host and the luminal environment. The four most abundant IECs are enterocytes, goblet cells, Paneth cells, and enteroendocrine cells (EECs) (Figure 3). Enterocytes are the primary absorptive cells in the epithelium, and they increase their surface area with apical microvilli structures. Goblet cells are responsible for producing and secreting

mucin proteins into the lumen. Mucins are heavily glycosylated proteins that not only provide a protective barrier from the lumen but also provide a medium for facilitating molecular exchange between the epithelium and the environment (Johansson and Hansson, 2016). Paneth cells reside mainly in ileal intestinal crypts and secrete potent antimicrobial products via release of granule contents (Bevins and Salzman, 2011). As such, antimicrobial peptides (AMPs) are secreted when the epithelium senses microbe-associated molecular patterns and are downregulated in germ-free (GF) mice (mice free of all microorganisms) (Ayabe et al., 2000; Satoh et al., 1986; Vaishnava et al., 2011). Finally, EECs produce a variety of modulatory

neuroendocrine molecules. These cells have been commonly referred to as the “taste” cells of the gut (Sternini et al., 2008) because they are popularly known for their chemosensation and production of molecules that control aspects of feeding, such as appetite.

Other IECs are important for directing innate and adaptive immunological responses to luminal antigens. In particular, microfold cells (M cells) aid in the immunological sampling of antigens across the epithelium (Lelouard et al., 2012). M cells are also capable of transporting intact bacteria across the epithelium and to gut-associated lymphoid tissue (GALT) (Clark et al., 1998). Thus, M cells are often found in close association with dendritic cells (DCs) on the luminal side of Peyer's patches and lymphoid follicles. This gives immune cells a prime position to induce an adaptive immune response. Finally, cup and tuft cells are not well characterized, but the latter have recently been shown to be important for inducing type 2 immune responses against parasitic helminths (Gerbe et al., 2016; Howitt et al., 2016).

Transport of molecules to and from the lumen occurs both transepithelial (through epithelial cells) and paracellularly (between epithelial cells) (Pácha, 2000). Paracellular transport is mediated by selective cellular junctions known as tight junctions. Transepithelial transport can occur through primary or secondary active transport mechanisms—the former requires ATP and

carrier proteins, and the latter employs concentration gradients (Bröer, 2008). Furthermore, receptor-mediated transcytosis also occurs across the epithelium, as is the case with immunoglobulin A (IgA) (Hansen et al., 1999). These transport mechanisms are vital for giving immune cells and neuronal processes in the mucosa access to diverse luminal cues. Although a variety of apical stimuli are important in mediating IEC function, as will be discussed, basolateral signals can also incite effector functions in IECs. Thus, the epithelium mediates critical barrier functions in a dynamic fashion by interacting with both the luminal (e.g., gut bacteria) and basolateral (e.g., immune and neuronal cells) compartments of the gut to initiate and transmit bilateral signals at the interface of the host and its environment.

### The Enteric Nervous System

The mammalian nervous system is divided into two arms, the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS encompasses the brain and the spinal cord, and the PNS includes the ganglia, which are aggregates of neural cell bodies from which nerve bundles arise in the head, neck, and viscera. The autonomic (involuntary) nervous system, a division of the PNS, is characterized as sympathetic or parasympathetic, and the main neurotransmitters produced are catecholamines (CCBs: norepinephrine, epinephrine, and dopamine) or acetylcholine (ACh), respectively. Extrinsic connectivity from the CNS to the ENS is composed of both sympathetic and parasympathetic nerve fibers. Upon leaving the hindbrain, the parasympathetic and sympathetic nerves can synapse directly onto the GI tract. For example, the parasympathetic vagus nerve, upon leaving the hindbrain, travels along the esophagus through the diaphragm and ultimately synapses onto the GI tract. Sympathetic nerves originate in the spinal column and synapse onto sympathetic visceral ganglia, such as the celiac, superior, and inferior mesenteric ganglia. Both parasympathetic and sympathetic nerves can synapse directly onto the myenteric ganglia, smooth muscle, and mucosa (Hansen et al., 1999). Additionally, pelvic nerves, which originate in the spinal cord and leave via the sacral spinal nerve, innervate the distal colon and rectum. Pelvic nerves have traditionally been characterized as parasympathetic, but recent, controversial data by Espinosa-Medina et al. suggest that these could be sympathetic in their developmental origin (Espinosa-Medina et al., 2016). Adding a layer of complexity to the neural connectivity of the GI tract is the innervation of sympathetic ganglia by parasympathetic nerves. Finally, the intrinsic ENS is the expansive network of neurons and glia along the GI tract; these can function autonomously but are also tunable by the ENS's connectivity to extrinsic sympathetic and parasympathetic nerves (Furness and Costa, 1980). Thus, communication between the CNS and ENS is bidirectional.

Receptors on enteric neurons mediate important GI functions. Mechanoreceptors are responsive to mucosal abrasion, and tension receptors are responsive to stretch. Chemoreceptors respond to various chemical stimuli in the lumen, such as pH, osmolarity, and nutrients. Furthermore, various receptors are responsible for regulating fluid exchange within the gut (Derrien et al., 2004). Subsets of neurons can generally be categorized by their connectivity. Intrinsic primary afferent neurons (IPANs) are large, multi-axonal sensory neurons that are responsible

for detecting molecular and mechanical aberrations of the GI tract. These neurons relay sensory impulses to other IPANs, interneurons, and ultimately intrinsic motor neurons that induce effector functions. IPANs make up 10%–30% of the neurons in both the myenteric and submucosal plexuses. Intrinsic intestinofugal afferent neurons (IFANs) send neuronal impulses from the GI tract to extrinsic, visceral ganglia, where sympathetic impulses are sent back to the ENS to complete the reflex arc (Bevins and Salzman, 2011). Interneurons are responsible for connecting sensory and motor neurons and thus propagate neuronal impulses. Muscle motor neurons are found along the entire GI tract in the longitudinal and circular muscle layers, and they respond to signals initiated by mechano- and tension receptors. Finally, vasodilator and secretomotor neurons manage fluid and molecular exchange between the GI vasculature, tissue, and lumen (Ayabe et al., 2000; Satoh et al., 1986) (Figures 1 and 2).

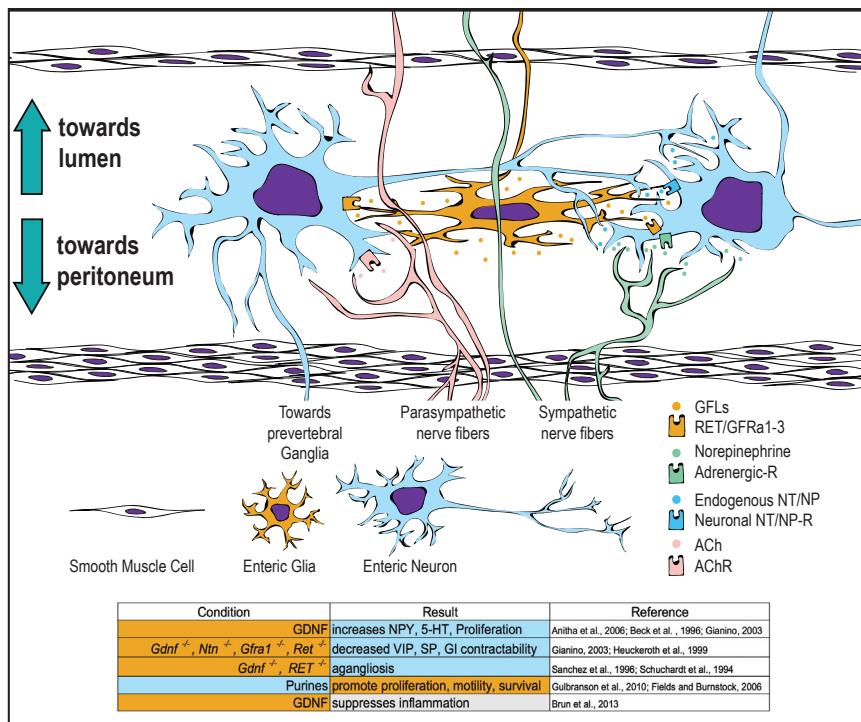
Enteric glial cells (EGCs) are also significant components of the ENS and have been shown to be important benefactors toward mucosal health (Vaishnava et al., 2011). EGCs in the myenteric and submucosal plexuses have been shown to envelop enteric neurons but also associate with blood vessels and lymphatics (Sternini et al., 2008). As will be described in later sections, EGC-derived signaling molecules implicate the functional development of cell types in GI immunity.

### Molecular Constituents of Gastrointestinal Neuroimmunity

The extensive GI lymphatic system mediates the flux of immune cells that occupy the GI tract. Cells and molecules of the innate and adaptive immune system require antigens and stimulating molecules that, as will be discussed, can come from a variety of cellular sources. The GI tract represents one of the most expansive immune organs, with all the necessary components for innate and adaptive immunity, but is unique because of the diverse factors that influence immunological development. In the subsequent sections, we will describe how classic neuro-, immuno-, and microbe-associated molecules have effects on noncanonical cellular partners and how these interactions could underlie neuro-immune responses to gut bacteria.

#### Catecholamines

CCBs are a class of neuroactive molecules secreted at sympathetic nerve endings. The bone marrow, thymus, spleen, and peripheral lymph nodes are innervated by sympathetic nerves (Rescigno et al., 2001), and accordingly, functional adrenergic receptors are expressed on virtually all leukocytes (Serafini et al., 2015). Thus, immunologists have long been intrigued by the role of sympathetic, catecholaminergic signaling in the immune system, and their effects have been extensively studied (Elenkov et al., 2000). Recently, Gabanyi et al. posited the role of sympathetic innervation of the GI tract and discovered potential mechanisms by which the ENS functions to polarize intestinal macrophages both spatially and immunologically (Gabanyi et al., 2016). In this study, intestinal macrophages were found to be phenotypically compartmentalized in the muscularis and mucosa, putting them in close proximity to extrinsic and mucosal nerve fibers, respectively. Muscularis macrophages were found to be phenotypically similar to M2 (regulatory) macrophages, whereas those isolated in the lamina propria were similar to M1



**Figure 2. Connectivity of Enteric Neurons and Glia**

Enteric neurons are located in either the submucosal or myenteric plexus. Both plexuses are located between two muscle layers (see Figure 1). Parasympathetic nerve fibers release ACh, and sympathetic nerves release norepinephrine. These extrinsic nerve fibers can innervate enteric neurons but also associate with the cells in the smooth muscle, lamina propria, and epithelium. Enteric neurons can innervate one another or extend toward the lamina propria, and specific IFANs can synapse onto sympathetic ganglia. Enteric glial cells make and release neurotrophic factors, associate with enteric neurons, and extend throughout the mucosa. The left and middle columns are color coded to represent cells and molecules that generate specific conditions and the results that are produced from those conditions, respectively.

(pro-inflammatory) macrophages. When naive peritoneal macrophages were cultured with enteric neurospheres, they became more similar to muscularis macrophages, and this determination was dependent upon engagement of the  $\beta_2$  adrenergic receptor ( $\beta$ 2AR) (Figure 4). Furthermore, sympathetic ganglia were activated during *Salmonella* infection, and this induced a  $\beta$ 2AR-activation-dependent expression of genes associated with muscularis macrophages. In another study, ex vivo catecholaminergic treatment of Peyer's patches exhibited lower rates of *Salmonella* translocation (Brown and Price, 2008), potentially mitigating infection. These studies suggest that adrenergic signaling occurs in the GI tract. However, tyrosine hydroxylase (an enzyme critical for CCh biosynthesis from tyrosine) immunoreactivity persists in the GI tract after extrinsic denervation (Li et al., 2004), and this suggests that intrinsic, catecholaminergic synthesis and signaling could also be involved in modulating GI immune responses.

In addition to the direct effects that CChs can have on the regulation of immune responses, similar effects can occur via modulation of their availability in the GI tract. UDP-glucuronyltransferase is an enzyme that has been studied in the liver, kidneys, brain, and GI tract (King et al., 2000) for its ability to transfer glucuronic acid onto endogenous and xenobiotic molecules, making them more hydrophilic and subject to excretion in the urine and feces. Hormones are also glucuronidated, giving them their rapid systemic effect. 90% of the dopamine found in the lumen of the GF mouse is glucuronidated, and 90% of the dopamine found in the GI tract of specific-pathogen-free (SPF) mice is not. Colonizing GF mice with an SPF microbiota or a consortium of *Clostridia* species can reverse skewing toward glucuronidated products (Asano et al., 2012). Interestingly, microbes isolated from rodent GI tracts have been shown to

have endogenous beta-glucuronidase (Gus) activity (Gadelle et al., 1985), and thus colonizing GF mice with *E. coli* that produce functional Gus enzymes is sufficient for decreasing levels of glucuronidated CChs. Conversely, mutant *E. coli* deficient in Gus production cannot (Asano et al., 2012). Thus, gut microbes,

and specifically microbial Gus activity, can shape the molecular activity and availability of CChs in the GI tract, and this could ultimately affect how leukocytes behave.

Remarkably, bacteria also express functional adrenergic receptors, QseC and QseE, through which epinephrine and norepinephrine regulate expression of virulence genes in enteric pathogens (Hadjifrangiskou et al., 2011; Moreira and Sperandio, 2012; Njoroge and Sperandio, 2012). Pathogenicity is diminished in mutant *Citrobacter rodentium* (an enteric pathogen) strains that are deficient in QseC and QseE. Also, wild-type *C. rodentium* is ineffective at colonizing mice that do not express dopamine beta-hydroxylase (Moreira et al., 2016), the enzyme required for norepinephrine and epinephrine synthesis. However, whether glucuronidated CChs are ineffective at modulating virulence or whether bacteria that produce Gus can enhance virulence in a mouse is unknown. Regardless, this intriguingly links host CCh biosynthetic pathways to bacterial infections and could provide novel, antibiotic-independent strategies for mitigating intestinal bacterial infections. All in all, CChs in the GI tract are impactful signaling molecules that affect cells of multiple phyla. However, the way in which the cellular source of CChs exerts its effect on the immune system remains poorly understood.

#### Acetylcholine

ACh is the primary parasympathetic neurotransmitter that is released by preganglionic nerve fibers (these fibers arise from ganglia along the spinal cord) and the vagus nerve. ACh has been studied for its powerful anti-inflammatory effects in the periphery. It was first found that vagal stimulation was sufficient in suppressing systemic inflammation in response to endotoxin (Borovikova et al., 2000). It was later discovered that endotoxemic mice deficient in  $\alpha$ 7-nicotinic acetylcholine receptor

(*Chrna7*<sup>-/-</sup>) have increased systemic levels of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and IL-6, and these mice could not suppress TNF- $\alpha$  levels upon vagal stimulation. Specifically, CHRNA7 expression in macrophages was necessary for the observed ACh-mediated TNF- $\alpha$  suppression (Wang et al., 2003). In another study that followed, splenic nerve stimulation produced similar inhibition of the inflammatory TNF- $\alpha$  response to liposaccharide (LPS) (Rosas-Ballina et al., 2008). However, vagal innervation results in ACh release on the celiac ganglion, which in turn sends noradrenergic signals to the spleen via the splenic nerve (Berthoud and Powley, 1993). Thus, the question remained as to how vagal and splenic nerve stimulation are able to produce similar effects when the vagus nerve does not directly innervate the spleen and the splenic nerve does not produce acetylcholine; meanwhile, the immunoregulatory effects appeared to function through ACh receptors. In a paradigm-shifting study in neuro-immunology, ACh-producing T cells were discovered to mediate these effects. These T cells secreted ACh in response to the activation of their  $\beta$  adrenergic receptors, and the resulting ACh activated CHRNA7 on macrophages to suppress TNF- $\alpha$  production (Rosas-Ballina et al., 2011). The functional discovery of ACh-producing T cells has demonstrated the remarkable molecular reflexes at the interface of the peripheral nervous and immune systems, and such discoveries have immensely expanded the breadth and appreciation of previously known neuro-immune interactions. However, research regarding cholinergic pathways in the GI tract is only just beginning to appear in the literature. For example, it has recently been shown that specific depletion of ACh-producing T cells results in reduced intestinal AMP levels and relative changes to the microbiota (Dhawan et al., 2016). Mice deficient in type 3 muscarinic ACh receptors (*Chrm3*<sup>-/-</sup>) have a leaky intestinal barrier, higher basal levels of interferon- $\gamma$  (IFN- $\gamma$ ), IL-17A, and TNF- $\alpha$ , and exhibit delayed clearance of *C. rodentium* (McLean et al., 2015). Interestingly, these mice also express lower levels of IL-4 and IL-13, resulting in the delayed clearance of intestinal parasites (McLean et al., 2016).

Although the vagus and pelvic nerves have connections along the length of the GI tract, where some nerve endings directly innervate the mucosa, they are not the only source of intestinal ACh. Intrinsic neurons are also immunoreactive against choline acetyltransferase (CHAT), the rate-limiting enzyme in ACh synthesis (Furness, 2012). Furthermore, these extrinsic nerve endings often synapse onto neurons in the myenteric plexus. Thus, ACh from parasympathetic nerves can induce the activation of noncholinergic neurons in the ENS and the subsequent release of other neuromodulatory compounds. However, because the ENS can initiate its own neuronal reflexes independently of extrinsic inputs, it is possible that intrinsic circuitry alone can influence immune function, and similar neuro-immune interactions existing in the spleen and the periphery could also exist in the GI tract.

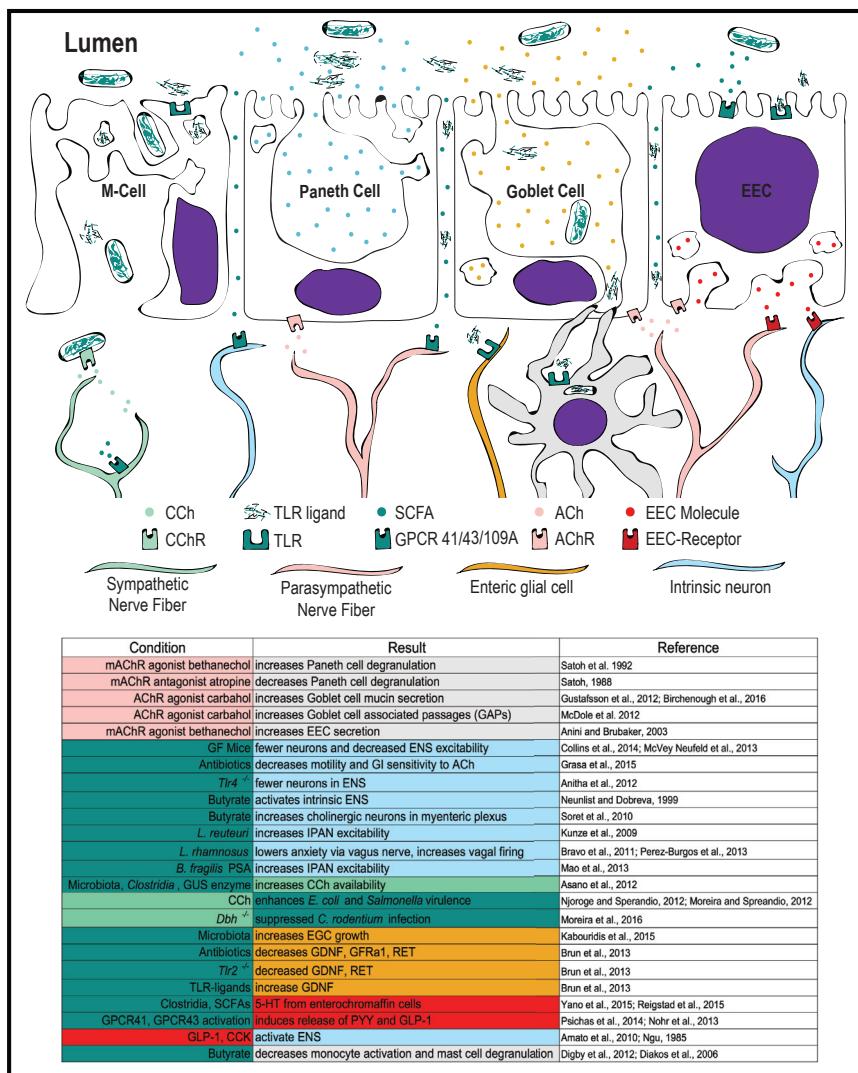
IECs, like immune cells, are in close proximity to neuronal projections in the mucosa. However, unlike immune cells, every IEC is in direct association with the lumen. This apical and basolateral dichotomy makes IECs an interesting and potential mediator of neuro-immune communications that occur between the mucosa and the microbiota. Specifically, ACh has been well studied for its effects on Paneth and goblet cells (Figure 3). Acetylcholine

receptor (AChR) activation is important for both Paneth and goblet cell degranulation. As such, bethanechol, a muscarinic AChR (mAChR) agonist, stimulates degranulation (Satoh et al., 1992), whereas the mAChR antagonist atropine suppresses this function (Satoh, 1988). Similarly, AChR activation on goblet cells induces mucin secretion (Gustafsson et al., 2012; Birchenough et al., 2016). In mice, AChR activation also results in increased goblet-cell-associated antigen passages (GAPs), which allow goblet cells to take up luminal antigens and deliver them to DCs in the lamina propria (McDole et al., 2012). This is particularly interesting because it directly implicates ACh in the immunological sampling of luminal contents and microbes. Finally, like for goblet and Paneth cells, cholinergic stimulation of EECs induces secretion of neuroendocrine molecules (Anini and Brubaker, 2003). Ultimately, ACh is a powerful mediator of intestinal function, but again, it is unclear what the endogenous source of ACh is and whether this distinction affects its functional output on IECs, the ENS, and GI immune cells.

### Neuropeptides

Neuropeptides are small protein molecules that are mainly produced by neuroendocrine cells, such as EECs (Nogueira and Barbosa, 1994). They can be used to communicate between neurons, but they also have endocrine function. In the GI tract, many subtypes of EECs produce a variety of neuropeptides in response to luminal and GI cues (Gribble and Reimann, 2016; Gunawardene et al., 2011). Intriguingly, enteric neurons can have similar neurochemical profiles to EECs and are also responsive to the neuropeptides produced by EECs (Mongardi Fantaguzzi et al., 2009; Merchant, 2007). As such, the molecules glucagon-like peptide 1 (GLP-1) (Amato et al., 2010) and cholecystokinin (CCK) (Ngu, 1985) are known effector molecules of EECs, but they also modulate ENS activity. Furthermore, enteric neurons have been shown to “synapse” onto EECs (Bohórquez et al., 2015), and EECs have been shown to secrete their intracellular stores of effector molecules in response to membrane depolarization (Matsumura et al., 2005; Reimann et al., 2012) and heightened calcium levels (Hira et al., 2008). However, the functionality of these physical neuro-epithelial circuits is not well understood. Specifically, the secretory output of an EEC in response to an action potential from a synapsed enteric neuron is unknown.

Other GI constituents can regulate aspects of EEC neuropeptide production. For example, mice with mutant T cell receptor have lower numbers of CCK-producing EECs in the colon (Rubin et al., 2000), potentially linking adaptive immune function to EEC peptide production. GF mice not only have fewer EECs (Duca et al., 2012) but also have altered neuropeptide levels in the CNS (Covasa et al., 2016; Schéle et al., 2013) and GI tract (Duca et al., 2012). Furthermore, EECs express functional Toll-like receptors (TLRs) (Bogunovic et al., 2007), and treatment of mice with TLR agonists induces CCK release (Palazzo et al., 2007), but these effects might not be limited to CCK. As previously described, EECs are oriented such that apical stimuli are translated into basolateral signals (Figure 3). Thus, EECs could represent a direct means by which microbes can actively remodel the neuromodulatory environment across the epithelium to potentially change the activity of sensory neurons in the mucosa. Given the similarity of the neuropeptide molecules that EECs and the ENS utilize to communicate, and their



**Figure 3. Interactions at the Intestinal Epithelium**

The intestinal epithelium is where luminal constituents are actively or passively transported into the tissue. Extrinsic nerves and neurons are found near the epithelium, and thus the molecules that cross the epithelium and those that are secreted basolaterally can potentially affect their activity. Microbes or microbial parts can cross the epithelium and affect other cell types through M cells, immunoglobulin-mediated transcytosis, GAPs, and general leakiness of the epithelium. DCs and macrophages can phagocytose microbial antigens and secrete cytokines that can have an effect on neurons as well. Parasympathetic fibers release ACh and induce secretion of intracellular stores of molecules. In goblet cells, ACh also increases rates of DC luminal sampling via GAPs. EECs can also release neuroendocrine molecules in response to TLR stimulation and SCFAs. Basolaterally released molecules can potentially regulate the activity of neurons. Enteric glial cells can also project toward the epithelium, potentially allowing microbes to affect their function. Enteric neurons can be activated by commensals and pathogenic bacteria, as well as SCFAs that diffuse across the epithelium. The left and middle columns are color coded to represent cells and molecules that generate specific conditions and the results that are produced from those conditions, respectively.

potential effects on the immune system, perhaps EECs are capable of translating luminal cues to the GI neuro-immune system.

Additionally, neuropeptide effects on immunological function have been well documented. Peyer's patches are innervated (Figure 1) by intrinsic, peptidergic neurons (Vulchanova et al., 2007), and immunoglobulin synthesis and lymphocyte proliferation in peripheral lymph nodes and GALT are affected by neuropeptide exposure (Stanisz et al., 1986). Substance P (SP) was the first example of an immunomodulatory neuropeptide, and it was found to enhance the production of IL-1, TNF- $\alpha$ , and IL-6 in human monocytes (Lotz et al., 1988). SP also regulates intestinal ion and fluid transport across the epithelium, and these effects are abolished by the blocking of neural transmission with tetrodotoxin. Often, intestinal inflammation is concomitant with excessive ion and fluid secretion, leading to symptoms such as diarrhea. As such, SP is more abundant in the GI tract of patients with ulcerative colitis and Crohn's disease (Mantyh et al., 1988). Subsequent research has shown that many other neuropeptides and their associated receptors are important for immu-

nity. For example, in macrophages, neuropeptide Y (NPY) and peptide YY (PYY) enhance phagocytosis (De la Fuente et al., 1993), and mice deficient in NPY receptor 1 display impaired macrophage response to endotoxin. Furthermore, these mice have fewer naive CD4 $^{+}$  T cells in peripheral lymph nodes and reduced IFN- $\gamma$  production after dextran sulfate sodium (DSS)-induced colitis (Wheway et al., 2005). This suggests that immune cells harbor receptors

for neuropeptides, and these receptors have an important role in shaping the maturation of the immune system. In another study, Th1- and Th2-lineage-polarized T cell lines were created to have T cell receptors specific to an exogenous antigen. Thus, antigen exposure should have led to the production of cytokines representative of each respective lineage. Surprisingly, T cell exposure to neuropeptides was able to induce both canonical and non-canonical production of T helper cytokines without exposure of the antigen (Levite, 1998). These results point to the intriguing possibility of neuropeptide-mediated immunological plasticity and innate-like function from adaptive lymphocytes. These initial studies broadened the known impact of neuropeptides but also elucidated the complexity of their immunological effects.

In contrast to SP and NPY, vasoactive intestinal peptide (VIP) has been the best studied anti-inflammatory peptide. VIP induces regulatory (Chorny et al., 2005) and tolerogenic DCs (Delgado et al., 2005b; Ganea et al., 2006), both of which are able to induce differentiation of regulatory T (Treg) cells (Delgado et al., 2005a). Furthermore, VIP inhibits TGF- $\beta$ 1 (Sun et al., 2000) and

enhances IL-10 (Delgado et al., 1999) production in murine macrophages. Also in macrophages, TLR2 and TLR4 stimulation up-regulates expression of VIP receptor type 2 (Herrera et al., 2009), and conversely, VIP inhibits LPS-mediated upregulation of TLRs and suppresses macrophage differentiation (Foster et al., 2007). In the GI tract, VIP mitigates trinitrobenzene sulfonic acid (TNBS)-induced intestinal inflammation, another mouse model of colitis. In this model, VIP also inhibits TLR expression in macrophages, DCs, lymphocytes, and colon protein extracts (Abad et al., 2003; Gomariz et al., 2005). Similarly, VIP ameliorates intestinal barrier dysfunction in *C. rodentium*-induced colitis in mice (Conlin et al., 2009). Furthermore, toxin B from the colitogenic bacterium *Clostridium difficile* can activate VIP-producing neurons in the submucosa (Neunlist et al., 2003). However, over time, *C. difficile* infection decreases overall levels of VIP (Nassif et al., 1995), and perhaps by depleting VIP, *C. difficile* can ultimately exacerbate enteric infection. Finally, VIP-deficient mice have multiple GI abnormalities, including deficits in goblet cell secretion (Lelievre et al., 2007), which potentially compromises the protective mucus barrier in the lumen. All in all, neuropeptide balance is imperative for proper immune function and GI homeostasis, and skewing in either direction leads to physiological irregularity, potential disease pathology, and increased infection susceptibility.

#### Serotonin and Histamine

Serotonin (5-HT) is produced by various cell types in the GI tract and has multiple physiological roles. 5-HT was first identified for its roles in vasoconstriction and was found to be stored in platelets (Rapport et al., 1948). It has also long been established that in the GI tract, mast cells and enterochromaffin cells, a specific EEC, are the major sources of 5-HT (Erspamer and Asero, 1952). Currently, enteric neurons (Gutknecht et al., 2009) and various leukocyte types, including macrophages, DCs, B and T cells, have been found to express either tryptophan hydroxylase (TPH-1/2, the rate-limiting enzyme in 5-HT biosynthesis) or 5-HT transporters, which are necessary mediators of extracellular 5-HT uptake (Meredith et al., 2005; Rudd et al., 2005; O'Connell et al., 2006). Furthermore, a variety of 5-HT receptors have been identified in lymphoid tissues (Stefulj et al., 2000), and as a result, their expression and function in a variety of immune cells have been extensively studied (Shajib and Khan, 2015). In general, serotonergic signaling in leukocytes appears to promote immune function by either enhancing DC-mediated T cell activation (León-Ponte et al., 2007; O'Connell et al., 2006) or affecting macrophage polarization (de las Casas-Engel et al., 2013) and phagocytosis (Csaba et al., 1975).

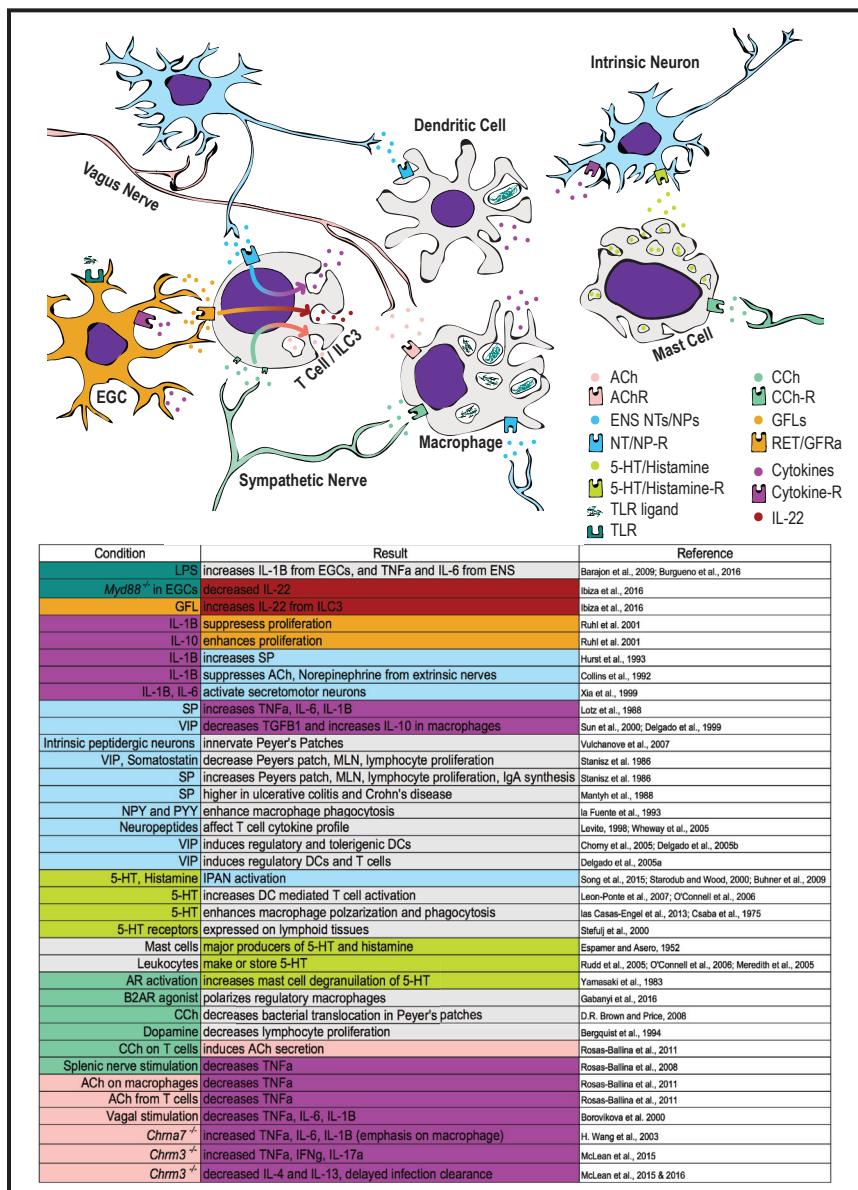
5-HT is also one of the earliest known molecules involved in GI peristalsis (Bülbbring and Lin, 1958), and it does so by directly modulating ENS activity (Costa and Furness, 1979; Hillsley and Grundy, 1998). In addition to producing 5-HT, mast cells produce histamine, which can activate IPANs (Song et al., 2015; Starodub and Wood, 2000), and expectedly, heightened levels of histamine and 5-HT correlate with increased activation of submucosal neurons. This increase in activation is dampened by 5-HT and histamine receptor antagonists (Buhner et al., 2009), and although mast-cell-mediated ENS activation is compelling, its significance and physiological implications are poorly understood. Interestingly, vagal stimulation initiates release of 5-HT in enterochromaffin cells, and this appears to be mediated by

adrenergic receptor activation (Ahlman et al., 1976). Mast cells also express functional adrenergic receptors, and receptor binding enhances IgE-mediated degranulation of 5-HT and histamine (Yamasaki et al., 1983). Furthermore, amitriptyline, a 5-HT- and norepinephrine-uptake inhibitor, inhibits mast cell secretion of histamine but not 5-HT (Theoharides et al., 1982). This suggests that histamine secretion from mast cells can be modulated by CChs, whereas intrinsic production of 5-HT, as opposed to the reuptake of 5-HT, is needed for adrenergic-receptor-mediated 5-HT secretion. These data show that similar neural paradigms observed in the cholinergic anti-inflammatory pathway could also affect the levels of 5-HT and histamine in the GI tract, but similar shortcomings in mechanistic understanding persist, such as the contribution of adrenergic signals from extrinsic sympathetic nerves versus those that are produced intrinsically. Nonetheless, these results show how molecules that are traditionally associated with leukocytes are capable of modulating the ENS and vice versa, providing more evidence of complex neuro-immune interactions in the GI tract.

In a recent study, Yano et al. found indigenous, spore-forming bacteria, which are primarily *Clostridia*, to be sufficient in rescuing the 5-HT deficiency observed in GF mice (Yano et al., 2015), further associating the microbiota with potential neuro-immune interactions in the GI tract. Consistent with this finding, short-chain fatty acids (SCFAs), which are the fermentation products of dietary fibers metabolized by the intestinal microbiota, were also sufficient to upregulate 5-HT (Reigstad et al., 2015; Yano et al., 2015). *Clostridia*, and specifically butyrate-producing strains, are important for the induction of Treg cells (Atarashi et al., 2013; 2011; Furusawa et al., 2013), and interestingly, patients with depression have lower levels of serum 5-HT and Treg cells that express lower levels of the 5-HT<sub>1a</sub> receptor (Li et al., 2010). Furthermore, anti-CD25 depletion of Treg cells lowers 5-HT levels and leads to depression-like behaviors in mice. Additionally, *C. rodentium* infection decreases levels of 5-HT-producing EECs (O'Hara et al., 2006), whereas Treg cells confer protection from it (Wang et al., 2014). It is possible that specific gut bacteria are capable of modulating CNS-associated phenotypes by inducing immunological changes that are mediated by neuromodulatory compounds. These types of studies would provide a powerful, mechanistic understanding of how gut bacteria incite changes beyond the GI tract and implicate more global physiologies. At the moment, it is appreciated that mucosal 5-HT is an important paracrine signaling molecule in the GI tract; however, it is less understood how diverse cell types contribute to such a unified physiological response that is dependent on 5-HT.

#### Neurotrophic Factors

Neurotrophic factors (NTFs) are small protein molecules that have classically been studied for their roles in sustaining neuronal growth and maturation. In the GI tract, the most abundant NTFs are the glial-cell-derived neurotrophic factor (GDNF) family of ligands (GFLs, which include GDNF, neurturin, and artemin), and they are produced by EGCs and smooth muscle cells (Bär et al., 1997; Brun et al., 2015; Han et al., 2015). However, unlike their name suggests, NTFs can modulate neuronal activity and produce effects on non-neuronal cell types. First, EGCs potentially affect neurotransmission by modulating production of neuropeptides. For example, GDNF promotes enteric neuron



release of NPY (Anitha et al., 2006; Figure 2), and *Gdnf*-, *Ntn*-, *Gfra1* (receptor for GDNF)-, and *Ret* (whose co-receptors are GFL receptors  $\alpha 1-\alpha 3$ )-deficient mice have defects in stimulus-evoked release of VIP and SP. These mice also display reduced GI contractility (Gianino et al., 2003; Heuckeroth et al., 1999), suggesting that EGC-mediated changes in neuropeptide levels are sufficient to cause dysregulated ENS activity. As previously described, GFL-mediated modulation of neuropeptides could have downstream effects on immune cell function. This illustrates the potential indirect effects of GFLs on neuro-immune interactions in the GI tract; however, EGC-derived NTFs have recently been shown to directly affect the development of specific GI immunological compartments. It was previously known that GFL-mediated RET signaling is necessary for the proper development of Peyer's patches (Patel et al., 2012; Veiga-Fernandes et al., 2007), but in a more recent study, Ibiza et al. found

**Figure 4. Interactions between GI Immune Cells and the ENS**

Extrinsic nerves, intrinsic neurons, and enteric glial cells are in close proximity to each other and to the immune cells in the GI tract. Thus, the molecules that are produced by one cell can have an effect on another cell, given that the latter expresses a receptor to recognize the molecule. By these parameters, interactions between neurons, glia, and immune cells are, in theory, abundant, and some of these putative interactions are presented here. Immune cells can be influenced by neurotransmitters and neuropeptides produced by intrinsic neurons of the ENS (as well as those produced by extrinsic nerve fibers), and cytokines produced by immune cells can have a reciprocal effect on neurons. The left and middle columns are color coded to represent cells and molecules that generate specific conditions and the results that are produced from those conditions, respectively.

that GFL-mediated RET signaling is necessary for the functional development of IL-22-producing type 3 innate lymphoid cells (ILC3s) (Ibiza et al., 2016). ILC3s are the first innate immune cells to expand in the GI tract after bacterial infection (Sonnenberg et al., 2011), and via IL-22, ILC3s have broadly protective effects on the intestinal epithelium (Liang et al., 2006; Lindemans et al., 2015; Sugimoto et al., 2008; Zheng et al., 2008). Ibiza et al. found that GFLs directly activate RET expressed on ILC3s, and depletion of RET diminishes intestinal IL-22 levels and exacerbates enteric inflammation (Figure 4). ILC3s were also found in close proximity to EGCs, and as previous studies also presented (Brun et al., 2013), TLR activation induced expression of GFLs. Importantly, glial-specific depletion of Myd88 reduced levels of GFLs and IL-22 and also recapitulated inflammatory pathologies observed in mice with ILC3-specific deletion of *Ret* (Ibiza et al., 2016).

These findings are the first to show that EGCs directly sense the microbial environment to induce GFL production, and this sensing is necessary for the development of GI immunity. This seminal study elucidates mechanisms by which components of the ENS can directly shape the immunological environment in the GI tract.

EGCs and NTFs are also implicated in intestinal neuropathies and inflammatory pathologies. Ablation of EGCs results in epithelial and neuronal damage as well as severe intestinal inflammation (Bush et al., 1998). Aganglionosis (a lack of enteric ganglia) occurs in mice that are *Gdnf*<sup>-/-</sup> (Sánchez et al., 1996) and *Ret*<sup>-/-</sup> (Schuchardt et al., 1994) and also in humans with Hirschsprung's disease. Furthermore, pathology of Hirschsprung's disease is often comorbid with enterocolitis (Austin, 2012; Fujimoto et al., 1988; Imamura et al., 1992; Murphy and Puri, 2005), and although

the precise etiology of this colitic pathology is unknown, patients with Hirschsprung's disease often display germline mutations in *RET* (Brooks et al., 2005) and *GDNF* (Angrist et al., 1996; Bär et al., 1997). In addition to producing GI pathologies similar to those found in Hirschsprung's disease, *Ret*<sup>-/-</sup> mice also display a marked reduction in *Vip* transcription (Heaney and Pachnis, 2006; Lelievre et al., 2007), and as previously mentioned, VIP has anti-inflammatory effects that are protective effects against chemically and microbe-induced colitis. Thus, glial constituents could also be important in maintaining homeostatic levels of immunoregulatory neuromodulatory compounds.

Finally, EGC proliferation is linked to intestinal inflammation (Bradley et al., 1997; Rühl et al., 2001). Accordingly, GDNF is increased during enteric parasitic infections (Starke-Buzetti and Oaks, 2008), found in intestinal biopsies from humans with colitogenic *C. difficile* infection, and upregulated in humans who suffer from ulcerative colitis and Crohn's disease (von Boyen et al., 2011). However, in culture, IL-1 $\beta$  appears to dampen EGC proliferation, whereas IL-10 enhances it (Rühl et al., 2001). Although this might appear contradictory, it is possible that intestinal inflammation promotes immunoregulatory functions in EGCs through proliferation and upregulation of GFLs but that persistent, hyperinflammatory responses result in inhibition of these processes altogether, thus leading to enteric pathology and disease. All in all, it is apparent that GFLs and EGCs have a significant role in maintaining intestinal immune homeostasis. Although it is possible that enteric neurons might interface EGCs and GFLs to modulate GI immunity, it is currently unknown whether these compartments are in fact interdependent.

### Cytokines

Cytokines and chemokines are the main effector molecules of immune cells. However, enteric neurons and glia are also capable of producing cytokines. As previously alluded to, EGCs express functional TLRs (Barajon et al., 2009; Ibiza et al., 2016), and upon LPS stimulation of EGCS, heightened levels of IL-1 $\beta$  are observed. Furthermore, ENS cultures produce TNF- $\alpha$  and IL-6 in response to LPS, and this production is abrogated when NF- $\kappa$ B signaling is inhibited (Burgueño et al., 2016). Curiously, IL-6 and its receptor promote the growth and survival of enteric neurons in culture (Schäfer et al., 1999), but just as IL-6 has both pro- and anti-inflammatory effects in the peripheral immune system (Scheller et al., 2011), it could also have dichotomous effects on the ENS.

As detailed previously, neurotransmitters and neuropeptides alter immune function. However, reciprocally, cytokines are also capable of modulating neuronal activity. The abundance and close proximity of neuronal varicosities to immune cells should make this unsurprising, but it is important in the study of immune responses to consider the effector functions of cytokine molecules on non-immune cell types. For example, IL-1 $\beta$  is heightened in the intestines of patients with inflammatory bowel disease (IBD) (Ligumsky et al., 1990), and in accordance with prior discussions, IL-1 $\beta$  also induces SP production in the ENS (Hurst et al., 1993). Furthermore, IL-1 $\beta$  engagement with its cognate receptor in ex vivo ENS preparations suppresses electric-field stimulation (EFS)-evoked release of ACh and norepinephrine (Collins et al., 1992). IL-1 $\beta$  and IL6 can excite enteric secretomotor neurons (Figure 4), and this appears to occur via

the suppression of extrinsic sympathetic and parasympathetic nerves (Xia et al., 1999). Thus, it is possible that enteric inflammatory symptoms are not only a product of pro-inflammatory cytokines but also a result of IL-1 $\beta$ -mediated upregulation of SP and subsequent increases in secretomotor activity and fluid secretion and decreased anti-inflammatory signaling from cholinergic and adrenergic neurons. This would explain many of the aforementioned phenotypes in IBD patients, including the heightened levels of SP, IL-1 $\beta$ , and TNF- $\alpha$  (Xavier and Podolsky, 2007).

### Short-Chain Fatty Acids

Again, SCFAs are microbial metabolic products of dietary fibers, and the most studied SCFAs are butyrate, propionate, and acetate (Campbell et al., 1997; Wolin, 1981). These metabolites are sensed by the intestinal epithelium but can also diffuse across the epithelium (Charney et al., 1998), where they can be accessed by the enteric nervous and immune systems (Figure 3). G-protein-coupled receptors 41 and 43 (GPR41 and GPR43, respectively) are activated by acetate and propionate (Brown et al., 2003; Tazoe et al., 2008), whereas GPR109A is specific to butyrate (Thangaraju et al., 2009).

IECs from mice deficient in *Gpr41* or *Gpr43* display reduced inflammatory chemokine and cytokine profiles in response to TNBS-induced colitis and *C. rodentium* infection. These mice also exhibit delayed clearance of *C. rodentium* itself (Kim et al., 2013). Furthermore, GPR43 is necessary for propionate-mediated secretion of PYY and GLP-1 (Psichas et al., 2015), and selective agonists for GPR41 and GPR43 induce GLP-1 secretion from colonic crypt cultures. Additionally, purified *Gpr41*<sup>+</sup> cells exhibit higher expression levels for neuropeptide and neuroendocrine hormone precursors (Nøhr et al., 2013), suggesting that an association between SCFAs and the production of neuropeptides exists. Also, PYY inhibits gastrointestinal transit, and *Gpr41* deficiency is associated with decreased PYY production (Samuel et al., 2008). Accordingly, SCFA-mediated induction of PYY inhibits colonic motility (Cherbut et al., 1998), which suggests that SCFA-mediated modulation of enteric neuronal activity exists. Intriguingly, it was found that enteric neurons in both the myenteric and submucosal plexuses express *Gpr41*. In contrast, immune cells in the lamina propria preferentially express *Gpr43* (Karaki et al., 2006; Nøhr et al., 2013). Treg cells isolated from the colon and small intestines express high levels of *Gpr43*, through which propionate enhances their immunosuppressive capabilities (Smith et al., 2013). Accordingly, propionate administration alleviates symptoms during experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis, and the mitigating effects of propionate were found to be concomitant with small intestinal Treg cell induction (Haghikia et al., 2015). Mucosal mast cells (Karaki et al., 2006) and neutrophils (Le Poul et al., 2003) also express GPR43. In neutrophils, bacterial-derived SCFAs enhance chemotaxis (Eftimadi et al., 1987), and propionate induces degranulation (Carretta et al., 2013). Activation of GPR109A reduces intestinal inflammation and colon cancer susceptibility in mice (Singh et al., 2014). In human monocytes, activation of GPR109A by nicotinic acid has anti-inflammatory effects (Digby et al., 2012), and this is further illustrated by the ability of butyrate to inhibit mast cell activation and degranulation (Diakos et al., 2006). Butyrate- and butyrate-producing bacteria, as previously described, are also sufficient to induce differentiation of Treg cells in mice (Atarashi et al.,

2013; Furusawa et al., 2013). Finally, butyrate is also capable of inducing action potentials in both submucosal and myenteric neurons of the ENS (Kunze et al., 2009; Neunlist et al., 1999), and this is correlated with higher numbers of intrinsic, cholinergic neurons of the myenteric plexus (Soret et al., 2010). This is in agreement with prior evidence showing the potent anti-inflammatory effects of ACh, and thus it is possible that ENS sensing of butyrate might be an important aspect of coordinating intrinsic, ACh-mediated neuro-immunoregulatory responses.

### Microbes and the Enteric Immune and Nervous Systems

The intestinal microbiota is the collection of microorganisms in the GI tract, and its constituents are known to be representative of an animal's diet, habitat, and even phylogenetic order (Ley et al., 2008). It has long been appreciated that the indigenous microbiota of the GI tract is important for its development and susceptibility to enteric infections (Dubos and Schaedler, 1960), but it was not until much later that researchers demonstrated how commensal bacteria have an active role in maintaining GI immune homeostasis. In 1996, it was found that anaerobic bacteria in human feces were specifically enriched in coating by IgA (van der Waaij et al., 1996). A few years later, Macpherson et al. discovered T-cell-independent mechanisms of IgA binding to commensal gut microbes, suggesting an evolutionarily innate, immunological adaptation to the intestinal microbiota (Macpherson et al., 2000). In 2001, Hooper et al. showed that *Bacteroides thetaiotaomicron* modulates host genes important for IEC homeostasis (Hooper et al., 2001), and in 2005, Mazmanian et al. discovered that polysaccharide A (PSA) from *Bacteroides fragilis* is sufficient for the development of adaptive immune responses (Mazmanian et al., 2005). These influential studies expanded the knowledge and incited curiosity toward the microbiome, but more importantly, they empirically defined previously unappreciated homeostatic roles of the intestinal microbiota.

In 1932, Dr. James Reyniers created the first GF animal in the Laboratories of Bacteriology at the University of Notre Dame (Reyniers, 1932). Later, GF rats were examined and characterized in the 1950s (Orland et al., 1954) for the study of dental hygiene and were later used for the study of microbial colonization resistance (van der Waaij et al., 1971). More recently, research groups have implicated the microbiota in stress, anxiety, behavioral developmental disorders, and neurological deficits (Diaz Heijtz et al., 2011; Hoban et al., 2016; Hsiao et al., 2013; Sampson et al., 2016). This has popularized hypotheses on the “gut-brain axis.” However, the role of the ENS and GI-resident immune functions are currently understudied as conduits for the gut-brain effect, and the abundance of immune cells in the GI tract and the vast connectivity of the ENS (and its direct connectivity to the CNS) make it unlikely that the axis is independent of these interactions.

The microbiota has been shown to be altered during intestinal inflammation and immunological disease. Severity of inflammation is mediated and ameliorated by pathogenic and commensal bacteria, respectively (Aas et al., 2003; Mazmanian et al., 2008). As previously mentioned, microbes or microbial products can actively change TLR expression in most cellular compartments of the GI tract, and this alters the host's ability to sense and respond to the microbiota. Antibiotic depletion of the microbiota alters TLR expression in mice and also results in concomitant

changes to GI motility and sensitivity to ACh (Grasa et al., 2015). Mice with mutant TLR4 have fewer neurons in the ENS, and this is also seen in MyD88-knockout and antibiotic-treated mice (Anitha et al., 2012). Furthermore, GF animals display defects in ENS morphology (Collins et al., 2014) and excitability (McVey Neufeld et al., 2013), and these deficits are reversed by colonization with a complex microbiota. EGC growth, maturation, and signaling are also affected by the microbiota. For example, GF mice and antibiotic-mediated depletion of the microbiota result in non-migratory glial cells that fail to extend through the mucosa and into villi structures (Kabouridis et al., 2015). Antibiotic treatments also reduce levels of GDNF, GFR $\alpha$ 1, and RET, and these deficiencies can be reversed by TLR2 stimulation (Brun et al., 2013). Furthermore, TLR2-deficient mice also display lower levels of GDNF, impaired RET signaling, and heightened inflammation in response to DSS-induced colitis. Accordingly, these phenotypes can be mitigated by exogenous GDNF administration (Brun et al., 2013). Although the exact mechanisms by which the microbiota modulates neural activity in the GI tract are unknown, these findings suggest that there is an active, post-developmental role of the microbiota in ENS form and function, potentially explaining disparities in their molecular output.

### Lactobacilli and Bacteroides

Many *Lactobacillus* species have been studied for their immunoregulatory role. Species such as *L. rhamnosus*, *L. salivarius*, *L. reuteri*, *L. planatrum*, *L. fermentum*, and *L. casei* have been shown to induce Treg cells and decrease levels of inflammatory cytokines, such as IFN- $\gamma$  and TNF- $\alpha$  (Foligne et al., 2007; Mad-sen et al., 1999; Valeur et al., 2004). *Lactobacilli* have also been studied for their neuromodulatory capabilities. Rats colonized with *L. reuteri* display lower thresholds for IPAN activation, higher frequencies of action potentials (Kunze et al., 2009; Mao et al., 2013), and shorter hyperpolarizing potentials, all of which are proxies of enhanced excitability. Interestingly, in an influential study describing the gut-brain axis, Bravo et al. discovered that commensal microbes modulate physiologies beyond the GI tract and influence behaviors commonly associated with maladies to the CNS. Specifically, *L. rhamnosus* reduces stress-induced corticosterone levels and decreases expression of  $\gamma$ -aminobutyric acid (GABA) receptors in the CNS, leading to concomitant reductions in anxiety and depression-like symptoms in mice. Interestingly, these behavioral and neurochemical phenotypes were abolished after chemically induced vagotomy (Bravo et al., 2011). In accordance, it was later shown that *L. rhamnosus* increases the rate of spontaneous firing in vagal afferent nerves (Perez-Burgos et al., 2013). Furthermore, certain strains of *L. rhamnosus* are known to produce GABA under certain conditions (Lin, 2013), and *L. reuteri* can produce GABA from its precursor, glutamate (Barrett et al., 2012; Siragusa et al., 2007). Thus, direct production of neuroactive molecules by gut microbes also has the potential to modulate ENS activity, but this has not been specifically addressed.

The *Bacteroides* genus also has immunoregulatory effects in the GI tract, and studies have predominantly been focused on *B. thetaiotaomicron* (Kelly et al., 2004) and *B. fragilis* (Mazmanian et al., 2008). As described previously, *B. fragilis* has been studied for its role in inducing IL-10-producing Foxp3 $^{+}$  Treg cells (Chu

et al., 2016; Round and Mazmanian, 2010). Interestingly, *B. fragilis*-mono-associated mice also display enhanced IPAN excitability, and PSA is both sufficient and necessary for this phenotype (Mao et al., 2013). These data illustrate the active role that bacteria and bacteria-associated molecules have on the excitability and activity of intrinsic sensory neurons in the ENS, but whether microbe-induced ENS activation is necessary for modulating GI immunity is unknown.

#### **Trichinella spiralis**

*Trichinella spiralis* is a parasitic nematode that infects the intestines (Wright, 1979) and induces IL-1 production (Stadnyk and Kearsey, 1996). In mice, *T. spiralis* infection causes hypophagia, a decrease in food intake (McDermott et al., 2006). This infection causes upregulation of CCK and 5-HT expression in EECs, both of which are implicated in hypophagia (Burton-Freeman et al., 1999; Hayes et al., 2006). Furthermore, CCK and IL-4 induce contractions in the longitudinal muscle (Lv et al., 2010; Zhao et al., 2003), and increased contractility of the GI tract is associated with *T. spiralis* expulsion (Vallance et al., 1997). Interestingly, prior *T. spiralis* infection increases sensitivity of mouse enteric neurons to *T. spiralis* antigens, and this heightened sensitivity is abrogated by histamine receptor antagonism (Frieling et al., 1994). IgE-mediated mast cell activation also induces histamine secretion (Ishizaka et al., 1980) and enhances *T. spiralis* expulsion (Ahmad et al., 1991). Together, these studies put forth a potential histamine-mediated, adaptive, neuro-immune response to pathogenic microorganisms that could be important for their clearance.

#### **Perspective**

The GI tract is positioned to sense and respond to diverse fluxes of cues, which can be intrinsic, extrinsic, and environmental. In the viscera, nerves from the CNS and PNS synapse onto the GI tract. At the mucosa, the host readily and selectively absorbs molecules within and across the epithelium. And in the lumen, the microbiota is under constant selective pressure from factors such as diet, environmental exposure, and immune and exocrine function. This exchange of molecular information in the GI tract illustrates intestinal “reflex loops” that direct how our body utilizes the ENS to physiologically sense and respond to changes in the environment. However, mechanisms by which functional enteric circuits integrate with microbe-mediated GI immune responses are poorly understood. For example, it is largely unknown whether the ENS senses the microbial environment and initiates an immune response or whether the ENS senses immune responses to subsequently modify, amplify, or propagate those signals. It is also unknown whether or how the ENS reciprocally affects the luminal environment to shape microbial communities in the gut and whether this effect is mediated by the immune system. Further characterization of the connectivity between the ENS and itself and other cell types will help uncover the GI tract molecules that dictate the body’s ability to immunologically mature and adapt to the microbiota. However, truly dissecting mechanisms by which the ENS mediates, potentiates, or responds to microbe-induced GI immunological changes will require spatial and temporal manipulation of the ENS. Accordingly, modern advances in neuroscience, such as optogenetics and chemogenetics, have recently been implemented in the context of the ENS (Chang et al., 2015; Grubišić and Gulbransen, 2017; Rakhilin et al., 2016). Although discoveries of neuro-im-

mune interactions in the GI tract indicate ENS participation in mucosal immune development (Gabanyi et al., 2016; Ibiza et al., 2016), effects of the microbiota on these immunological changes remain largely unknown. A recent study by Yissachar et al. utilized an intestinal organ-culture system to show that gut bacteria can modulate the neuro-immune environment of the intestines (Yissachar et al., 2017); however, it remains unknown whether neural components are sufficient or necessary for this regulation. Thus, additional studies uncoupling the effects of the ENS on GI immune function will provide the foundation for studying how the microbiota affects interactions between the neurological and immunological systems of the body. Furthermore, it remains an intriguing possibility that enteric neuro-immune interactions might be the preferred route by which the microbiota has more global effects on CNS-related behavior.

Communication between the microbiome and the mucosa is essential for the maintenance of intestinal homeostasis. Given the diversity and reciprocity of extracellular signaling molecules in the GI tract, the ENS has evolved over time to receive and interpret these diverse cues and transmit them throughout the GI tract and, ultimately, the entire body. As such, the ENS is best adapted to coordinate physiologically related responses between different cell types and propagate them over vast regions of the GI tract. The cellular biology of the GI tract is inherently complex, necessitating the development and testing of interdisciplinary hypotheses, a challenge with immense implications for human physiology.

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#### **REFERENCES**

- Aas, J., Gessert, C.E., and Bakken, J.S. (2003). Recurrent *Clostridium difficile* colitis: case series involving 18 patients treated with donor stool administered via a nasogastric tube. *Clin. Infect. Dis.* 36, 580–585.
- Abad, C., Martinez, C., Juarranz, M.G., Arranz, A., Leceta, J., Delgado, M., and Gomariz, R.P. (2003). Therapeutic effects of vasoactive intestinal peptide in the trinitrobenzene sulfonic acid mice model of Crohn’s disease. *Gastroenterology* 124, 961–971.
- Ahlman, H., Lundberg, J., Dahlström, A., and Kewenter, J. (1976). A possible vagal adrenergic release of serotonin from enterochromaffin cells in the cat. *Acta Physiol. Scand.* 98, 366–375.
- Ahmad, A., Wang, C.H., and Bell, R.G. (1991). A role for IgE in intestinal immunity. Expression of rapid expulsion of *Trichinella spiralis* in rats transfused with IgE and thoracic duct lymphocytes. *J. Immunol.* 146, 3563–3570.
- Amato, A., Cinci, L., Rotondo, A., Serio, R., Faussone-Pellegrini, M.S., Vannucchi, M.G., and Mulè, F. (2010). Peripheral motor action of glucagon-like peptide-1 through enteric neuronal receptors. *Neurogastroenterol. Motil.* 22, 664–e203.
- Angrist, M., Bolk, S., Halushka, M., Lapchak, P.A., and Chakravarti, A. (1996). Germline mutations in glial cell line-derived neurotrophic factor (GDNF) and RET in a Hirschsprung disease patient. *Nat. Genet.* 14, 341–344.
- Anini, Y., and Brubaker, P.L. (2003). Muscarinic receptors control glucagon-like peptide 1 secretion by human endocrine L cells. *Endocrinology* 144, 3244–3250.

- Anitha, M., Chandrasekharan, B., Salgado, J.R., Grouzmann, E., Mwangi, S., Sitaraman, S.V., and Srinivasan, S. (2006). Glial-derived neurotrophic factor modulates enteric neuronal survival and proliferation through neuropeptide Y. *Gastroenterology* 131, 1164–1178.
- Anitha, M., Vijay-Kumar, M., Sitaraman, S.V., Gewirtz, A.T., and Srinivasan, S. (2012). Gut microbial products regulate murine gastrointestinal motility via Toll-like receptor 4 signaling. *Gastroenterology* 143, 1006–16.e4.
- Asano, Y., Hiramoto, T., Nishino, R., Aiba, Y., Kimura, T., Yoshihara, K., Koga, Y., and Sudo, N. (2012). Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 303, G1288–G1295.
- Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., et al. (2011). Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* 331, 337–341.
- Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K., et al. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 500, 232–236.
- Austin, K.M. (2012). The pathogenesis of Hirschsprung's disease-associated enterocolitis. *Semin. Pediatr. Surg.* 21, 319–327.
- Ayabe, T., Satchell, D.P., Wilson, C.L., Parks, W.C., Selsted, M.E., and Ouellette, A.J. (2000). Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. *Nat. Immunol.* 1, 113–118.
- Bár, K.J., Facer, P., Williams, N.S., Tam, P.K., and Anand, P. (1997). Glial-derived neurotrophic factor in human adult and fetal intestine and in Hirschsprung's disease. *Gastroenterology* 112, 1381–1385.
- Barajon, I., Serrao, G., Arnaboldi, F., Opizzi, E., Ripamonti, G., Balsari, A., and 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, 1013–1023.
- Barrett, E., Ross, R.P., O'Toole, P.W., Fitzgerald, G.F., and Stanton, C. (2012). γ-Aminobutyric acid production by culturable bacteria from the human intestine. *J. Appl. Microbiol.* 113, 411–417.
- Berthoud, H.-R., and Powley, T.L. (1993). Characterization of vagal innervation to the rat celiac, suprarenal and mesenteric ganglia. *J. Auton. Nerv. Syst.* 42, 153–169.
- Bevins, C.L., and Salzman, N.H. (2011). Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nat. Rev. Microbiol.* 9, 356–368.
- Birchenough, G.M.H., Nyström, E.E.L., Johansson, M.E.V., and Hansson, G.C. (2016). A sentinel goblet cell guards the colonic crypt by triggering Nlrp6-dependent Muc2 secretion. *Science* 352, 1535–1542.
- Bogunovic, M., Davé, S.H., Tilstra, J.S., Chang, D.T.W., Harpaz, N., Xiong, H., Mayer, L.F., and Plevy, S.E. (2007). Enteroendocrine cells express functional Toll-like receptors. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292, G1770–G1783.
- Bohorquez, D.V., Shahid, R.A., Erdmann, A., Kreger, A.M., Wang, Y., Calakos, N., Wang, F., and Liddle, R.A. (2015). Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *J. Clin. Invest.* 125, 782–786.
- Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Wang, H., Abumrad, N., Eaton, J.W., and Tracey, K.J. (2000). Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405, 458–462.
- Bradley, J.S., Jr., Parr, E.J., and Sharkey, K.A. (1997). Effects of inflammation on cell proliferation in the myenteric plexus of the guinea-pig ileum. *Cell Tissue Res.* 289, 455–461.
- Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., and Cryan, J.F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. USA* 108, 16050–16055.
- Bröer, S. (2008). Amino acid transport across mammalian intestinal and renal epithelia. *Physiol. Rev.* 88, 249–286.
- Brooks, A.S., Oostra, B.A., and Hofstra, R.M.W. (2005). Studying the genetics of Hirschsprung's disease: unraveling an oligogenic disorder. *Clin. Genet.* 67, 6–14.
- Brown, D.R., and Price, L.D. (2008). Catecholamines and sympathomimetic drugs decrease early *Salmonella Typhimurium* uptake into porcine Peyer's patches. *FEMS Immunol. Med. Microbiol.* 52, 29–35.
- Brown, A.J., Goldsworthy, S.M., Barnes, A.A., Eilert, M.M., Tcheang, L., Daniels, D., Muir, A.I., Wigglesworth, M.J., Kinghorn, I., Fraser, N.J., et al. (2003). The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.* 278, 11312–11319.
- Brun, P., Giron, M.C., Qesari, M., Porzionato, A., Caputi, V., Zoppellaro, C., Banzato, S., Grillo, A.R., Spagnol, L., De Caro, R., et al. (2013). Toll-like receptor 2 regulates intestinal inflammation by controlling integrity of the enteric nervous system. *Gastroenterology* 145, 1323–1333.
- Brun, P., Gobbo, S., Caputi, V., Spagnol, L., Schirato, G., Pasqualin, M., Levorato, E., Palù, G., Giron, M.C., and Castagliuolo, I. (2015). Toll like receptor-2 regulates production of glial-derived neurotrophic factors in murine intestinal smooth muscle cells. *Mol. Cell. Neurosci.* 68, 24–35.
- Buhner, S., Li, Q., Vignali, S., Barbara, G., De Giorgio, R., Stanghellini, V., Cremon, C., Zeller, F., Langer, R., Daniel, H., et al. (2009). Activation of human enteric neurons by supernatants of colonic biopsy specimens from patients with irritable bowel syndrome. *Gastroenterology* 137, 1425–1434.
- Büllbring, E., and Lin, R.C.Y. (1958). The effect of intraluminal application of 5-hydroxytryptamine and 5-hydroxytryptophan on peristalsis; the local production of 5-HT and its release in relation to intraluminal pressure and propulsive activity. *J. Physiol.* 140, 381–407.
- Burgueño, J.F., Barba, A., Eyre, E., Romero, C., Neunlist, M., and Fernández, E. (2016). TLR2 and TLR9 modulate enteric nervous system inflammatory responses to lipopolysaccharide. *J. Neuroinflammation* 13, 187.
- Burton-Freeman, B., Gietzen, D.W., and Schneeman, B.O. (1999). Cholecystokinin and serotonin receptors in the regulation of fat-induced satiety in rats. *Am. J. Physiol.* 276, R429–R434.
- Bush, T.G., Savidge, T.C., Freeman, T.C., Cox, H.J., Campbell, E.A., Mucke, L., Johnson, M.H., and Sofroniew, M.V. (1998). Fulminant jejunio-ileitis following ablation of enteric glia in adult transgenic mice. *Cell* 93, 189–201.
- Campbell, J.M., Fahey, G.C., Jr., and Wolf, B.W. (1997). Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. *J. Nutr.* 127, 130–136.
- Carretta, M.D., Conejeros, I., Hidalgo, M.A., and Burgos, R.A. (2013). Propionate induces the release of granules from bovine neutrophils. *J. Dairy Sci.* 96, 2507–2520.
- Chang, R.B., Strochlic, D.E., Williams, E.K., Umans, B.D., and Liberles, S.D. (2015). Vagal Sensory Neuron Subtypes that Differentially Control Breathing. *Cell* 161, 622–633.
- Charney, A.N., Micic, L., and Egnor, R.W. (1998). Nonionic diffusion of short-chain fatty acids across rat colon. *Am. J. Physiol.* 274, G518–G524.
- Cherbut, C., Ferrier, L., Rozé, C., Anini, Y., Blottière, H., Lecanu, G., and Galimberti, J.-P. (1998). Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat. *Am. J. Physiol.* 275, G1415–G1422.
- Chorniy, A., Gonzalez-Rey, E., Fernandez-Martin, A., Pozo, D., Ganea, D., and Delgado, M. (2005). Vasoactive intestinal peptide induces regulatory dendritic cells with therapeutic effects on autoimmune disorders. *Proc. Natl. Acad. Sci. USA* 102, 13562–13567.
- Chu, H., Khosravi, A., Kusumawardhani, I.P., Kwon, A.H.K., Vasconcelos, A.C., Cunha, L.D., Mayer, A.E., Shen, Y., Wu, W.-L., Kambal, A., et al. (2016). Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. *Science* 352, 1116–1120.
- Clark, M.A., Hirst, B.H., and Jepson, M.A. (1998). M-cell surface β1 integrin expression and invasin-mediated targeting of *Yersinia pseudotuberculosis* to mouse Peyer's patch M cells. *Infect. Immun.* 66, 1237–1243.
- Collins, S.M., Hurst, S.M., Main, C., Stanley, E., Khan, I., Blennerhassett, P., and Swain, M. (1992). Effect of inflammation of enteric nerves. Cytokine-induced changes in neurotransmitter content and release. *Ann. N.Y Acad. Sci.* 664, 415–424.

- Collins, J., Borojevic, R., Verdu, E.F., Huizinga, J.D., and Ratcliffe, E.M. (2014). Intestinal microbiota influence the early postnatal development of the enteric nervous system. *Neurogastroenterol. Motil.* 26, 98–107.
- Conlin, V.S., Wu, X., Nguyen, C., Dai, C., Vallance, B.A., Buchan, A.M.J., Boyer, L., and Jacobson, K. (2009). Vasoactive intestinal peptide ameliorates intestinal barrier disruption associated with *Citrobacter rodentium*-induced colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 297, G735–G750.
- Costa, M., and Furness, J.B. (1979). The sites of action of 5-hydroxytryptamine in nerve-muscle preparations from the guinea-pig small intestine and colon. *Br. J. Pharmacol.* 65, 237–248.
- Covasa, M., Duca, F.A., and Swartz, T.D. (2016). Gut Microbiota Restores Central Neuropeptide Deficits Present in Germ Free Animals. *FASEB J.* 30, 1166.9.
- Csaba, G., Kapa, E., and Cserhalmi, M. (1975). Hormone receptor studies on frog macrophage cells by means of histamine, serotonin and indoleacetic acid. *Endocrinologie* 65, 219–223.
- De la Fuente, M., Bernaez, I., Del Rio, M., and Hernanz, A. (1993). Stimulation of murine peritoneal macrophage functions by neuropeptide Y and peptide YY. Involvement of protein kinase C. *Immunology* 80, 259–265.
- de las Casas-Engel, M., Domínguez-Soto, A., Sierra-Filardi, E., Bragado, R., Nieto, C., Puig-Kroger, A., Samaniego, R., Loza, M., Corcueria, M.T., Gómez-Aguado, F., et al. (2013). Serotonin skews human macrophage polarization through HTR2B and HTR7. *J. Immunol.* 190, 2301–2310.
- Delgado, M., Munoz-Elias, E.J., Gomariz, R.P., and Ganea, D. (1999). Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide enhance IL-10 production by murine macrophages: *in vitro* and *in vivo* studies. *J. Immunol.* 162, 1707–1716.
- Delgado, M., Chorny, A., Gonzalez-Rey, E., and Ganea, D. (2005a). Vasoactive intestinal peptide generates CD4+CD25+ regulatory T cells *in vivo*. *J. Leukoc. Biol.* 78, 1327–1338.
- Delgado, M., Gonzalez-Rey, E., and Ganea, D. (2005b). The neuropeptide vasoactive intestinal peptide generates tolerogenic dendritic cells. *J. Immunol.* 175, 7311–7324.
- Derrien, M., Vaughan, E.E., Plugge, C.M., and de Vos, W.M. (2004). Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int. J. Syst. Evol. Microbiol.* 54, 1469–1476.
- Dhawan, S., De Palma, G., Willemze, R.A., Hilbers, F.W., Verseijden, C., Luyer, M.D., Nuding, S., Wehkamp, J., Souwer, Y., de Jong, E.C., et al. (2016). Acetylcholine-producing T cells in the intestine regulate antimicrobial peptide expression and microbial diversity. *Am. J. Physiol. Gastrointest. Liver Physiol.* 311, G920–G933.
- Diakos, C., Prieschl, E.E., Säemann, M.D., Böhmig, G.A., Csonga, R., Sobanov, Y., Baumruker, T., and Zlabinger, G.J. (2006). n-Butyrate inhibits Jun NH(2)-terminal kinase activation and cytokine transcription in mast cells. *Biochem. Biophys. Res. Commun.* 349, 863–868.
- Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, M.L., Forssberg, H., and Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci. USA* 108, 3047–3052.
- Digby, J.E., Martinez, F., Jefferson, A., Ruparelia, N., Chai, J., Wamil, M., Greaves, D.R., and Choudhury, R.P. (2012). Anti-inflammatory effects of nicotinic acid in human monocytes are mediated by GPR109A dependent mechanisms. *Arterioscler. Thromb. Vasc. Biol.* 32, 669–676.
- Dubos, R.J., and Schaedler, R.W. (1960). The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. *J. Exp. Med.* 111, 407–417.
- Duca, F.A., Swartz, T.D., Sakar, Y., and Covasa, M. (2012). Increased oral detection, but decreased intestinal signaling for fats in mice lacking gut microbiota. *PLoS ONE* 7, e39748.
- Eftimia, C., Buzzi, E., Tonetti, M., Buffa, P., Buffa, D., van Steenberghe, M.T., de Graaff, J., and Botta, G.A. (1987). Short-chain fatty acids produced by anaerobic bacteria alter the physiological responses of human neutrophils to chemotactic peptide. *J. Infect.* 14, 43–53.
- Elenkov, I.J., Wilder, R.L., Chrousos, G.P., and Vizi, E.S. (2000). The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. *Pharmacol. Rev.* 52, 595–638.
- Erspamer, V., and Asero, B. (1952). Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature* 169, 800–801.
- Espinosa-Medina, I., Saha, O., Boismoreau, F., Chettouh, Z., Rossi, F., Richardson, W.D., and Brunet, J.F. (2016). The sacral autonomic outflow is sympathetic. *Science* 354, 893–897.
- Foligne, B., Nutten, S., Grangette, C., Dennin, V., Goudercourt, D., Poiret, S., Dewulf, J., Brassart, D., Mercenier, A., and Pot, B. (2007). Correlation between *in vitro* and *in vivo* immunomodulatory properties of lactic acid bacteria. *World J. Gastroenterol.* 13, 236–243.
- Foster, N., Lea, S.R., Preshaw, P.M., and Taylor, J.J. (2007). Pivotal advance: vasoactive intestinal peptide inhibits up-regulation of human monocyte TLR2 and TLR4 by LPS and differentiation of monocytes to macrophages. *J. Leukoc. Biol.* 87, 893–903.
- Frieling, T., Palmer, J.M., Cooke, H.J., and Wood, J.D. (1994). Neuroimmune communication in the submucous plexus of guinea pig colon after infection with *Trichinella spiralis*. *Gastroenterology* 107, 1602–1609.
- Fujimoto, T., Reen, D.J., and Puri, P. (1988). Inflammatory response in entero-colitis in the piebald lethal mouse model of Hirschsprung's disease. *Pediatr. Res.* 24, 152–155.
- Furness, J.B. (2012). The enteric nervous system and neurogastroenterology. *Nat. Rev. Gastroenterol. Hepatol.* 9, 286–294.
- Furness, J.B., and Costa, M. (1980). Types of nerves in the enteric nervous system. *Neuroscience* 5, 1–20.
- Furness, J.B., Callaghan, B.P., Rivera, L.R., and Cho, H.-J. (2014). The enteric nervous system and gastrointestinal innervation: integrated local and central control. *Adv. Exp. Med. Biol.* 817, 39–71.
- Furusawa, Y., Obata, Y., Fukuda, S., Endo, T.A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T., et al. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504, 446–450.
- Gabanyi, I., Muller, P.A., Feighery, L., Oliveira, T.Y., Costa-Pinto, F.A., and Mucida, D. (2016). Neuro-immune Interactions Drive Tissue Programming in Intestinal Macrophages. *Cell* 164, 378–391.
- Gadelle, D., Raibaud, P., and Sacquet, E. (1985). beta-Glucuronidase activities of intestinal bacteria determined both *in vitro* and *in vivo* in gnotobiotic rats. *Appl. Environ. Microbiol.* 49, 682–685.
- Ganea, D., Gonzalez-Rey, E., and Delgado, M. (2006). A novel mechanism for immunosuppression: from neuropeptides to regulatory T cells. *J. Neuroimmune Pharmacol.* 1, 400–409.
- Gerbe, F., Sidot, E., Smyth, D.J., Ohmoto, M., Matsumoto, I., Dardalhon, V., Cesses, P., Garnier, L., Pouzolles, M., Brulin, B., et al. (2016). Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature* 529, 226–230.
- Gianino, S., Grider, J.R., Cresswell, J., Enomoto, H., and Heuckeroth, R.O. (2003). GDNF availability determines enteric neuron number by controlling precursor proliferation. *Development* 130, 2187–2198.
- Gomariz, R.P., Arranz, A., Abad, C., Torroba, M., Martinez, C., Rosignoli, F., Garcia-Gómez, M., Leceta, J., and Juarranz, Y. (2005). Time-course expression of Toll-like receptors 2 and 4 in inflammatory bowel disease and homeostatic effect of VIP. *J. Leukoc. Biol.* 78, 491–502.
- Grasa, L., Abecia, L., Forcén, R., Castro, M., de Jalón, J.A.G., Latorre, E., Alcalde, A.I., and Murillo, M.D. (2015). Antibiotic-Induced Depletion of Murine Microbiota Induces Mild Inflammation and Changes in Toll-Like Receptor Patterns and Intestinal Motility. *Microb. Ecol.* 70, 835–848.
- Gribble, F.M., and Reimann, F. (2016). Enteroendocrine Cells: Chemosensors in the Intestinal Epithelium. *Annu. Rev. Physiol.* 78, 277–299.
- Grubišić, V., and Gulbransen, B.D. (2017). Enteric glial activity regulates secretomotor function in the mouse colon but does not acutely affect gut permeability. *J. Physiol.* <http://dx.doi.org/10.1113/JP273492>.

- Grundy, D., and Brookes, S. (2011). Neural Control of Gastrointestinal Function, Colloquium Series on Integrated Systems Physiology: From Molecule to Function (Morgan & Claypool Publishers).
- Gunawardene, A.R., Corfe, B.M., and Staton, C.A. (2011). Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. *Int. J. Exp. Pathol.* 92, 219–231.
- Gustafsson, J.K., Ermund, A., Johansson, M.E.V., Schütte, A., Hansson, G.C., and Sjövall, H. (2012). An ex vivo method for studying mucus formation, properties, and thickness in human colonic biopsies and mouse small and large intestinal explants. *Am. J. Physiol. Gastrointest. Liver Physiol.* 302, G430–G438.
- Gutknecht, L., Kriegebaum, C., Waider, J., Schmitt, A., and Lesch, K.-P. (2009). Spatio-temporal expression of tryptophan hydroxylase isoforms in murine and human brain: convergent data from Tph2 knockout mice. *Eur. Neuropsychopharmacol.* 19, 266–282.
- Hadjifrangiskou, M., Kostakioti, M., Chen, S.L., Henderson, J.P., Greene, S.E., and Hultgren, S.J. (2011). A central metabolic circuit controlled by QseC in pathogenic Escherichia coli. *Mol. Microbiol.* 80, 1516–1529.
- Haghikia, A., Jörg, S., Duscha, A., Berg, J., Manzel, A., Waschbisch, A., Hammer, A., Lee, D.-H., May, C., Wilck, N., et al. (2015). Dietary Fatty Acids Directly Impact Central Nervous System Autoimmunity via the Small Intestine. *Immunity* 43, 817–829.
- Han, T.Y., Lourenssen, S., Miller, K.G., and Blennerhassett, M.G. (2015). Intestinal smooth muscle phenotype determines enteric neuronal survival via GDNF expression. *Neuroscience* 290, 357–368.
- Hansen, G.H., Niels-Christiansen, L.L., Immerdal, L., Hunziker, W., Kenny, A.J., and Danielsen, E.M. (1999). Transcytosis of immunoglobulin A in the mouse enterocyte occurs through glycolipid raft- and rab17-containing compartments. *Gastroenterology* 116, 610–622.
- Hayes, M.R., Chory, F.M., Gallagher, C.A., and Covasa, M. (2006). Serotonin type-3 receptors mediate cholecystokinin-induced satiation through gastric distension. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 291, R115–R123.
- Heanue, T.A., and Pachnis, V. (2006). Expression profiling the developing mammalian enteric nervous system identifies marker and candidate Hirschsprung disease genes. *Proc. Natl. Acad. Sci. USA* 103, 6919–6924.
- Helander, H.F., and Fändriks, L. (2014). Surface area of the digestive tract - revisited. *Scand. J. Gastroenterol.* 49, 681–689.
- Herrera, J.L., Gonzalez-Rey, E., Fernandez-Montesinos, R., Quintana, F.J., Najmanovich, R., and Pozo, D. (2009). Toll-like receptor stimulation differentially regulates vasoactive intestinal peptide type 2 receptor in macrophages. *J. Cell. Mol. Med.* 13 (9B), 3209–3217.
- Heuckeroth, R.O., Enomoto, H., Grider, J.R., Golden, J.P., Hanke, J.A., Jackman, A., Molliver, D.C., Bardgett, M.E., Snider, W.D., Johnson, E.M., Jr., and Milbrandt, J. (1999). Gene targeting reveals a critical role for neurturin in the development and maintenance of enteric, sensory, and parasympathetic neurons. *Neuron* 22, 253–263.
- Hillsley, K., and Grundy, D. (1998). Sensitivity to 5-hydroxytryptamine in different afferent subpopulations within mesenteric nerves supplying the rat jejunum. *J. Physiol.* 509, 717–727.
- Hira, T., Nakajima, S., Eto, Y., and Hara, H. (2008). Calcium-sensing receptor mediates phenylalanine-induced cholecystokinin secretion in enteroendocrine STC-1 cells. *FEBS J.* 275, 4620–4626.
- Hoban, A.E., Stilling, R.M., Ryan, F.J., Shanahan, F., Dinan, T.G., Claesson, M.J., Clarke, G., and Cryan, J.F. (2016). Regulation of prefrontal cortex myelination by the microbiota. *Transl. Psychiatry* 6, e774.
- Hooper, L.V., Wong, M.H., Thelin, A., Hansson, L., Falk, P.G., and Gordon, J.I. (2001). Molecular analysis of commensal host-microbial relationships in the intestine. *Science* 291, 881–884.
- Howitt, M.R., Lavoie, S., Michaud, M., Blum, A.M., Tran, S.V., Weinstock, J.V., Gallini, C.A., Redding, K., Margolskee, R.F., Osborne, L.C., et al. (2016). Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* 351, 1329–1333.
- Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., Codelli, J.A., Chow, J., Reisman, S.E., Petrosino, J.F., et al. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* 155, 1451–1463.
- Hurst, S.M., Stanisz, A.M., Sharkey, K.A., and Collins, S.M. (1993). Interleukin-1-Beta Induced Increase in Substance-P in Rat Myenteric Plexus. *Gastroenterology* 105, 1754–1760.
- Ibiza, S., García-Cassani, B., Ribeiro, H., Carvalho, T., Almeida, L., Marques, R., Misic, A.M., Bartow-McKenney, C., Larson, D.M., Pavan, W.J., et al. (2016). Glial-cell-derived neuroregulators control type 3 innate lymphoid cells and gut defence. *Nature* 535, 440–443.
- Imamura, A., Puri, P., O'Briain, D.S., and Reen, D.J. (1992). Mucosal immune defence mechanisms in enterocolitis complicating Hirschsprung's disease. *Gut* 33, 801–806.
- Ishizaka, T., Hirata, F., Ishizaka, K., and Axelrod, J. (1980). Stimulation of phospholipid methylation, Ca<sup>2+</sup> influx, and histamine release by bridging of IgE receptors on rat mast cells. *Proc. Natl. Acad. Sci. USA* 77, 1903–1906.
- Johansson, M.E.V., and Hansson, G.C. (2016). Immunological aspects of intestinal mucus and mucins. *Nat. Rev. Immunol.* 16, 639–649.
- Kabouridis, P.S., Lasrado, R., McCallum, S., Chng, S.H., Snippert, H.J., Clevers, H., Pettersson, S., and Pachnis, V. (2015). Microbiota controls the homeostasis of glial cells in the gut lamina propria. *Neuron* 85, 289–295.
- Kagnoff, M.F., 1987. Immunology of the digestive system. *Physiology of the gastrointestinal tract*.
- Kalff, J.C., Schwarz, N.T., Walgenbach, K.J., Schraut, W.H., and Bauer, A.J. (1998). Leukocytes of the intestinal muscularis: their phenotype and isolation. *J. Leukoc. Biol.* 63, 683–691.
- Karaki, S., Mitsui, R., Hayashi, H., Kato, I., Sugiya, H., Iwanaga, T., Furness, J.B., and Kuwahara, A. (2006). Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. *Cell Tissue Res.* 324, 353–360.
- Kelly, D., Campbell, J.I., King, T.P., Grant, G., Jansson, E.A., Coutts, A.G.P., Pettersson, S., and Conway, S. (2004). Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat. Immunol.* 5, 104–112.
- Kim, M.H., Kang, S.G., Park, J.H., Yanagisawa, M., and Kim, C.H. (2013). Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology* 145, 396–406.e1, 10.
- King, C.D., Rios, G.R., Green, M.D., and Tephly, T.R. (2000). UDP-glucuronosyltransferases. *Curr. Drug Metab.* 1, 143–161.
- Kunze, W.A., Mao, Y.K., Wang, B., Huizinga, J.D., Ma, X., Forsythe, P., and Bienenstock, J. (2009). Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *J. Cell. Mol. Med.* 13 (8B), 2261–2270.
- Le Poul, E., Loison, C., Struyf, S., Springael, J.-Y., Lannoy, V., Decobecq, M.-E., Brezillon, S., Dupriez, V., Vassart, G., Van Damme, J., et al. (2003). Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J. Biol. Chem.* 278, 25481–25489.
- Lelievre, V., Favrais, G., Abad, C., Adle-Biassette, H., Lu, Y., Germano, P.M., Cheung-Lau, G., Pisegna, J.R., Gressens, P., Lawson, G., and Waschek, J.A. (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, 1688–1699.
- Lelouard, H., Fallet, M., de Bovis, B., Méresse, S., and Gorvel, J.P. (2012). Peyer's patch dendritic cells sample antigens by extending dendrites through M cell-specific transcellular pores. *Gastroenterology* 142, 592–601.e3.
- León-Ponte, M., Ahern, G.P., and O'Connell, P.J. (2007). Serotonin provides an accessory signal to enhance T-cell activation by signaling through the 5-HT7 receptor. *Blood* 109, 3139–3146.
- Levite, M. (1998). Neuropeptides, by direct interaction with T cells, induce cytokine secretion and break the commitment to a distinct T helper phenotype. *Proc. Natl. Acad. Sci. USA* 95, 12544–12549.
- Ley, R.E., Hamady, M., Lozupone, C., Turnbaugh, P.J., Ramey, R.R., Bircher, J.S., Schlegel, M.L., Tucker, T.A., Schrenzel, M.D., Knight, R., and Gordon, J.I. (2008). Evolution of mammals and their gut microbes. *Science* 320, 1647–1651.

- Li, Z.S., Pham, T.D., Tamir, H., Chen, J.J., and Gershon, M.D. (2004). Enteric dopaminergic neurons: definition, developmental lineage, and effects of extrinsic denervation. *J. Neurosci.* 24, 1330–1339.
- Li, Y., Xiao, B., Qiu, W., Yang, L., Hu, B., Tian, X., and Yang, H. (2010). Altered expression of CD4(+)CD25(+) regulatory T cells and its 5-HT(1a) receptor in patients with major depression disorder. *J. Affect. Disord.* 124, 68–75.
- Liang, S.C., Tan, X.-Y., Luxenberg, D.P., Karim, R., Durussi-Joannopoulos, K., Collins, M., and Fouser, L.A. (2006). Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J. Exp. Med.* 203, 2271–2279.
- Ligumsky, M., Simon, P.L., Karmeli, F., and Rachmilewitz, D. (1990). Role of interleukin 1 in inflammatory bowel disease-enhanced production during active disease. *Gut* 31, 686–689.
- Lin, Q. (2013). Submerged fermentation of *Lactobacillus rhamnosus* YS9 for γ-aminobutyric acid (GABA) production. *Braz. J. Microbiol.* 44, 183–187.
- Lindemans, C.A., Calafiore, M., Mertelsmann, A.M., O'Connor, M.H., Dudakov, J.A., Jenq, R.R., Velardi, E., Young, L.F., Smith, O.M., Lawrence, G., et al. (2015). Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature* 528, 560–564.
- Lotz, M., Vaughan, J.H., and Carson, D.A. (1988). Effect of neuropeptides on production of inflammatory cytokines by human monocytes. *Science* 241, 1218–1221.
- Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K., and Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature* 489, 220–230.
- Lv, Y., Feng, M., Che, T., Sun, H., Luo, Y., Liu, K., and Liu, C. (2010). CCK mediated the inhibitory effect of oxytocin on the contraction of longitudinal muscle strips of duodenum in male rats. *Pflugers Arch.* 460, 1063–1071.
- Macpherson, A.J., Gatto, D., Sainsbury, E., Harriman, G.R., Hengartner, H., and Zinkernagel, R.M. (2000). A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science* 288, 2222–2226.
- Madsen, K.L., Doyle, J.S., Jewell, L.D., Tavernini, M.M., and Fedorak, R.N. (1999). *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 116, 1107–1114.
- Mantyh, C.R., Gates, T.S., Zimmerman, R.P., Welton, M.L., Passaro, E.P., Jr., Vigna, S.R., Maggio, J.E., Kruger, L., and Mantyh, P.W. (1988). Receptor binding sites for substance P, but not substance K or neuromedin K, are expressed in high concentrations by arterioles, venules, and lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn disease. *Proc. Natl. Acad. Sci. USA* 85, 3235–3239.
- Mao, Y.K., Kasper, D.L., Wang, B., Forsythe, P., Bienenstock, J., and Kunze, W.A. (2013). *Bacteroides fragilis* polysaccharide A is necessary and sufficient for acute activation of intestinal sensory neurons. *Nat. Commun.* 4, 1465.
- Matsumura, K., Miki, T., Jhomori, T., Gonoi, T., and Seino, S. (2005). Possible role of PEPT1 in gastrointestinal hormone secretion. *Biochem. Biophys. Res. Commun.* 336, 1028–1032.
- Mazmanian, S.K., Liu, C.H., Tzianabos, A.O., and Kasper, D.L. (2005). An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 122, 107–118.
- Mazmanian, S.K., Round, J.L., and Kasper, D.L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 453, 620–625.
- McDermott, J.R., Leslie, F.C., D'Amato, M., Thompson, D.G., Grincis, R.K., and McLaughlin, J.T. (2006). Immune control of food intake: enteroendocrine cells are regulated by CD4+ T lymphocytes during small intestinal inflammation. *Gut* 55, 492–497.
- McDole, J.R., Wheeler, L.W., McDonald, K.G., Wang, B., Konjufca, V., Knoop, K.A., Newberry, R.D., and Miller, M.J. (2012). Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. *Nature* 483, 345–349.
- McLean, L.P., Smith, A., Cheung, L., Sun, R., Grinchuk, V., Vanuytsel, T., Desai, N., Urban, J.F., Jr., Zhao, A., Raufman, J.-P., and Shea-Donohue, T. (2015). Type 3 Muscarinic Receptors Contribute to Clearance of *Citrobacter rodentium*. *Inflamm. Bowel Dis.* 21, 1860–1871.
- McLean, L.P., Smith, A., Cheung, L., Urban, J.F., Sun, R., Grinchuk, V., Desai, N., Zhao, A., Raufman, J.-P., and Shea-Donohue, T. (2016). Type 3 Muscarinic Receptors Contribute to Intestinal Mucosal Homeostasis and Clearance of *Nippostrongylus brasiliensis* through Induction of Th2 Cytokines. *Am. J. Physiol. Gastrointest. Liver Physiol.* 311, G130–G141.
- McVey Neufeld, K.A., Mao, Y.K., Bienenstock, J., Foster, J.A., and Kunze, W.A. (2013). The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterol. Motil.* 25, 183–e88.
- Merchant, J.L. (2007). Tales from the crypts: regulatory peptides and cytokines in gastrointestinal homeostasis and disease. *J. Clin. Invest.* 117, 6–12.
- Meredith, E.J., Holder, M.J., Chamba, A., Challa, A., Drake-Lee, A., Bunce, C.M., Drayson, M.T., Pilkington, G., Blakely, R.D., Dyer, M.J., et al. (2005). The serotonin transporter (SLC6A4) is present in B-cell clones of diverse malignant origin: probing a potential anti-tumor target for psychotropics. *FASEB J.* 19, 1187–1189.
- Mongardi Fantaguzzi, C., Thacker, M., Chiocchetti, R., and Furness, J.B. (2009). Identification of neuron types in the submucosal ganglia of the mouse ileum. *Cell Tissue Res.* 336, 179–189.
- Moreira, C.G., and Sperandio, V. (2012). Interplay between the QseC and QseE bacterial adrenergic sensor kinases in *Salmonella enterica* serovar Typhimurium pathogenesis. *Infect. Immun.* 80, 4344–4353.
- Moreira, C.G., Russell, R., Mishra, A.A., Narayanan, S., Ritchie, J.M., Waldor, M.K., Curtis, M.M., Winter, S.E., Weisshenker, D., and Sperandio, V. (2016). Bacterial Adrenergic Sensors Regulate Virulence of Enteric Pathogens in the Gut. *MBio* 7, e00826-16.
- Murphy, F., and Puri, P. (2005). New insights into the pathogenesis of Hirschsprung's associated enterocolitis. *Pediatr. Surg. Int.* 21, 773–779.
- Nassif, A., Longo, W.E., Sexe, R., Stratton, M., Standeven, J., Vernava, A.M., and Kaminski, D.L. (1995). *Clostridium difficile* suppresses colonic vasoactive intestinal peptide associated with altered motility. *Mediators Inflamm.* 4, 452–453.
- Neunlist, M., Dobreva, G., and Schemann, M. (1999). Characteristics of mucosally projecting myenteric neurones in the guinea-pig proximal colon. *J. Physiol.* 517, 533–546.
- Neunlist, M., Barouk, J., Michel, K., Just, I., Oreshkova, T., Schemann, M., and Galmiche, J.P. (2003). Toxin B of *Clostridium difficile* activates human VIP submucosal neurons, in part via an IL-1beta-dependent pathway. *Am. J. Physiol. Gastrointest. Liver Physiol.* 285, G1049–G1055.
- Ngu, M.C. (1985). Activation of enteric nerve pathways in the guinea-pig duodenum by cholecystokinin octapeptide and pentagastrin. *J. Physiol.* 364, 31–43.
- Njoroge, J., and Sperandio, V. (2012). Enterohemorrhagic *Escherichia coli* virulence regulation by two bacterial adrenergic kinases, QseC and QseE. *Infect. Immun.* 80, 688–703.
- Nogueira, A.M., and Barbosa, A.J. (1994). Immunocytochemical study of intestinal endocrine cells in germ-free mice. *Eur. J. Histochem.* 38, 213–218.
- Nöhr, M.K., Pedersen, M.H., Gille, A., Egerod, K.L., Engelstoft, M.S., Husted, A.S., Sichlau, R.M., Grunddal, K.V., Poulsen, S.S., Han, S., et al. (2013). GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology* 154, 3552–3564.
- O'Connell, P.J., Wang, X., Leon-Ponte, M., Griffiths, C., Pingle, S.C., and Ahern, G.P. (2006). A novel form of immune signaling revealed by transmission of the inflammatory mediator serotonin between dendritic cells and T cells. *Blood* 107, 1010–1017.
- O'Hara, J.R., Skinn, A.C., MacNaughton, W.K., Sherman, P.M., and Sharkey, K.A. (2006). Consequences of *Citrobacter rodentium* infection on enteroendocrine cells and the enteric nervous system in the mouse colon. *Cell. Microbiol.* 8, 646–660.
- Orland, F.J., Blayney, J.R., Harrison, R.W., Reyniers, J.A., Trexler, P.C., Wagner, M., Gordon, H.A., and Luckey, T.D. (1954). Use of the germfree animal technic in the study of experimental dental caries. I. Basic observations on rats reared free of all microorganisms. *J. Dent. Res.* 33, 147–174.

- Pácha, J. (2000). Development of intestinal transport function in mammals. *Physiol. Rev.* 80, 1633–1667.
- Palazzo, M., Balsari, A., Rossini, A., Selleri, S., Calcaterra, C., Gariboldi, S., Zanobbio, L., Arnaboldi, F., Shirai, Y.F., Serrao, G., and Rumio, C. (2007). Activation of enteroendocrine cells via TLRs induces hormone, chemokine, and defensin secretion. *J. Immunol.* 178, 4296–4303.
- Patel, A., Harker, N., Moreira-Santos, L., Ferreira, M., Alden, K., Timmis, J., Foster, K., Garefalaki, A., Pachnis, P., Andrews, P., et al. (2012). Differential RET signaling pathways drive development of the enteric lymphoid and nervous systems. *Sci. Signal.* 5, ra55.
- Peery, A.F., Dellow, E.S., Lund, J., Crockett, S.D., McGowan, C.E., Bulsiewicz, W.J., Gangarosa, L.M., Thiny, M.T., Stizenberg, K., Morgan, D.R., et al. (2012). Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 143, 1179–87.e1, 3.
- Perez-Burgos, A., Wang, B., Mao, Y.K., Mistry, B., McVey Neufeld, K.A., Bienenstock, J., and Kunze, W. (2013). Psychoactive bacteria *Lactobacillus rhamnosus* (JB-1) elicits rapid frequency facilitation in vagal afferents. *Am. J. Physiol. Gastrointest. Liver Physiol.* 304, G211–G220.
- Psichas, A., Sleeth, M.L., Murphy, K.G., Brooks, L., Bewick, G.A., Hanyaloglu, A.C., Ghatei, M.A., Bloom, S.R., and Frost, G. (2015). The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int. J. Obes.* 39, 424–429.
- Rakhilin, N., Barth, B., Choi, J., Muñoz, N.L., Kulkarni, S., Jones, J.S., Small, D.M., Cheng, Y.T., Cao, Y., LaVinka, C., et al. (2016). Simultaneous optical and electrical in vivo analysis of the enteric nervous system. *Nat. Commun.* 7, 11800.
- Rapport, M.M., Green, A.A., and Page, I.H. (1948). Serum vasoconstrictor, serotonin; isolation and characterization. *J. Biol. Chem.* 176, 1243–1251.
- Reigstad, C.S., Salmonson, C.E., Rainey, J.F., 3rd, Szurszewski, J.H., Linden, D.R., Sonnenburg, J.L., Farrugia, G., and Kashyap, P.C. (2015). Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J.* 29, 1395–1403.
- Reimann, F., Tolhurst, G., and Gribble, F.M. (2012). G-protein-coupled receptors in intestinal chemosensation. *Cell Metab.* 15, 421–431.
- Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J.-P., and Ricciardi-Castagnoli, P. (2001). Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat. Immunol.* 2, 361–367.
- Reyniers, J.A. (1932). The use of germ-free guinea pigs in bacteriology. *Proc. Ind. Acad. Sci.* 42, 35–37.
- Rosas-Ballina, M., Ochani, M., Parrish, W.R., Ochani, K., Harris, Y.T., Huston, J.M., Chavan, S., and Tracey, K.J. (2008). Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. *Proc. Natl. Acad. Sci. USA* 105, 11008–11013.
- Rosas-Ballina, M., Olofsson, P.S., Ochani, M., Valdés-Ferrer, S.I., Levine, Y.A., Reardon, C., Tusche, M.W., Pavlov, V.A., Andersson, U., Chavan, S., et al. (2011). Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science* 334, 98–101.
- Round, J.L., and Mazmanian, S.K. (2010). Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc. Natl. Acad. Sci. USA* 107, 12204–12209.
- Rubin, D.C., Zhang, H., Qian, P., Lorenz, R.G., Hutton, K., and Peters, M.G. (2000). Altered enteroendocrine cell expression in T cell receptor alpha chain knock-out mice. *Microsc. Res. Tech.* 51, 112–120.
- Rudd, M.L., Nicolas, A.N., Brown, B.L., Fischer-Stenger, K., and Stewart, J.K. (2005). Peritoneal macrophages express the serotonin transporter. *J. Neuroimmunol.* 159, 113–118.
- Rühl, A., Franzke, S., and Stremmel, W. (2001). IL-1 $\beta$  and IL-10 have dual effects on enteric glial cell proliferation. *Neurogastroenterol. Motil.* 13, 89–94.
- Sampson, T.R., Debelius, J.W., Thron, T., Janssen, S., Shastri, G.G., Ilhan, Z.E., Challis, C., Schretter, C.E., Rocha, S., Gradiaru, V., et al. (2016). Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* 167, 1469–1480.e12.
- Samuel, B.S., Shaito, A., Motoike, T., Rey, F.E., Bäckhed, F., Manchester, J.K., Hammer, R.E., Williams, S.C., Crowley, J., Yanagisawa, M., and Gordon, J.I. (2008). Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc. Natl. Acad. Sci. USA* 105, 16767–16772.
- Sánchez, M.P., Silos-Santiago, I., Frisén, J., He, B., Lira, S.A., and Barbacid, M. (1996). Renal agenesis and the absence of enteric neurons in mice lacking GDNF. *Nature* 382, 70–73.
- Satoh, Y. (1988). Atropine inhibits the degranulation of Paneth cells in ex-germ-free mice. *Cell Tissue Res.* 253, 397–402.
- Satoh, Y., Ishikawa, K., Ono, K., and Vollrath, L. (1986). Quantitative light microscopic observations on Paneth cells of germ-free and ex-germ-free Wisistar rats. *Digestion* 34, 115–121.
- Satoh, Y., Ishikawa, K., Oomori, Y., Takeda, S., and Ono, K. (1992). Bethanechol and a G-protein activator, NaF/AlCl<sub>3</sub>, induce secretory response in Paneth cells of mouse intestine. *Cell Tissue Res.* 269, 213–220.
- Schäfer, K.-H., Mestres, P., März, P., and Rose-John, S. (1999). The IL-6/sIL-6R fusion protein hyper-IL-6 promotes neurite outgrowth and neuron survival in cultured enteric neurons. *J. Interferon Cytokine Res.* 19, 527–532.
- Schéle, E., Grahnemo, L., Anesten, F., Hallén, A., Bäckhed, F., and Jansson, J.-O. (2013). The gut microbiota reduces leptin sensitivity and the expression of the obesity-suppressing neuropeptides proglucagon (Gcg) and brain-derived neurotrophic factor (Bdnf) in the central nervous system. *Endocrinology* 154, 3643–3651.
- Scheller, J., Chalaris, A., Schmidt-Arras, D., and Rose-John, S. (2011). The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim. Biophys. Acta* 1813, 878–888.
- Schuchardt, A., D'Agati, V., Larsson-Blomberg, L., Costantini, F., and Pachnis, V. (1994). Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. *Nature* 367, 380–383.
- Sender, R., Fuchs, S., and Milo, R. (2016). Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. *Cell* 164, 337–340.
- Serafini, N., Vosshenrich, C.A.J., and Di Santo, J.P. (2015). Transcriptional regulation of innate lymphoid cell fate. *Nat. Rev. Immunol.* 15, 415–428.
- Shajib, M.S., and Khan, W.I. (2015). The role of serotonin and its receptors in activation of immune responses and inflammation. *Acta Physiol. (Oxf.)* 213, 561–574.
- Singh, N., Gurav, A., Sivaprakasam, S., Brady, E., Padia, R., Shi, H., Thangaraju, M., Prasad, P.D., Manicassamy, S., Munn, D.H., et al. (2014). Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* 40, 128–139.
- Siragusa, S., De Angelis, M., Di Cagno, R., Rizzello, C.G., Coda, R., and Gobbetti, M. (2007). Synthesis of gamma-aminobutyric acid by lactic acid bacteria isolated from a variety of Italian cheeses. *Appl. Environ. Microbiol.* 73, 7283–7290.
- Smith, P.M., Howitt, M.R., Panikov, N., Michaud, M., Gallini, C.A., Bohlooly-Y, M., Glickman, J.N., and Garrett, W.S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341, 569–573.
- Song, J., Zhang, L., Bai, T., Qian, W., Li, R., and Hou, X. (2015). Mast Cell-dependent Mesenteric Afferent Activation by Mucosal Supernatant From Different Bowel Segments of Guinea Pigs With Post-infectious Irritable Bowel Syndrome. *J. Neurogastroenterol. Motil.* 21, 236–246.
- Sonnenberg, G.F., Monticelli, L.A., Elloso, M.M., Fousser, L.A., and Artis, D. (2011). CD4(+) lymphoid tissue-inducer cells promote innate immunity in the gut. *Immunity* 34, 122–134.
- Soret, R., Chevalier, J., De Coppet, P., Poupeau, G., Derkinderen, P., Segain, J.P., and Neunlist, M. (2010). Short-chain fatty acids regulate the enteric neurons and control gastrointestinal motility in rats. *Gastroenterology* 138, 1772–1782.
- Stadnyk, A.W., and Kearsey, J.A. (1996). Pattern of proinflammatory cytokine mRNA expression during *Trichinella spiralis* infection of the rat. *Infect. Immun.* 64, 5138–5143.

- Stanisz, A.M., Befus, D., and Bienenstock, J. (1986). Differential effects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferations by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. *J. Immunol.* **136**, 152–156.
- Starke-Buzetti, W.A., and Oaks, J.A. (2008). Increased glial-derived neurotrophic factor in the small intestine of rats infected with the tapeworm, *Hymenolepis diminuta*. *Int. J. Exp. Pathol.* **89**, 458–465.
- Starodub, A.M., and Wood, J.D. (2000). Histamine suppresses A-type potassium current in myenteric neurons from guinea pig small intestine. *J. Pharmacol. Exp. Ther.* **294**, 555–561.
- Stefulj, J., Jernej, B., Cicin-Sain, L., Rinner, I., and Schauenstein, K. (2000). mRNA expression of serotonin receptors in cells of the immune tissues of the rat. *Brain Behav. Immun.* **14**, 219–224.
- Sternini, C., Anselmi, L., and Rozengurt, E. (2008). Enteroendocrine cells: a site of ‘taste’ in gastrointestinal chemosensing. *Curr. Opin. Endocrinol. Diabetes Obes.* **15**, 73–78.
- Sugimoto, K., Ogawa, A., Mizoguchi, E., Shimomura, Y., Andoh, A., Bhan, A.K., Blumberg, R.S., Xavier, R.J., and Mizoguchi, A. (2008). IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *J. Clin. Invest.* **118**, 534–544.
- Sun, W., Tadmori, I., Yang, L., Delgado, M., and Ganea, D. (2000). Vasoactive intestinal peptide (VIP) inhibits TGF- $\beta$ 1 production in murine macrophages. *J. Neuroimmunol.* **107**, 88–99.
- Tazoe, H., Otomo, Y., Kaji, I., Tanaka, R., Karaki, S.I., and Kuwahara, A. (2008). Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic functions. *J. Physiol. Pharmacol.* **59** (Suppl 2), 251–262.
- Thangaraju, M., Cresci, G.A., Liu, K., Ananth, S., Gnanaprakasam, J.P., Browning, D.D., Mellinger, J.D., Smith, S.B., Digby, G.J., Lambert, N.A., et al. (2009). GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. *Cancer Res.* **69**, 2826–2832.
- Theoharides, T.C., Bondy, P.K., Tsakalos, N.D., and Askenase, P.W. (1982). Differential release of serotonin and histamine from mast cells. *Nature* **297**, 229–231.
- Vaishnavi, S., Yamamoto, M., Severson, K.M., Ruhn, K.A., Yu, X., Koren, O., Ley, R., Wakeland, E.K., and Hooper, L.V. (2011). The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. *Science* **334**, 255–258.
- Valeur, N., Engel, P., Carbalal, N., Connolly, E., and Ladefoged, K. (2004). Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract. *Appl. Environ. Microbiol.* **70**, 1176–1181.
- Vallance, B.A., Blennerhassett, P.A., and Collins, S.M. (1997). Increased intestinal muscle contractility and worm expulsion in nematode-infected mice. *Am. J. Physiol.* **272**, G321–G327.
- van der Waaij, D., Berghuis-de Vries, J.M., and Lekkerkerk Lekkerkerk-v. (1971). Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. *J. Hyg. (Lond.)* **69**, 405–411.
- van der Waaij, L.A., Limburg, P.C., Mesander, G., and van der Waaij, D. (1996). In vivo IgA coating of anaerobic bacteria in human faeces. *Gut* **38**, 348–354.
- Veiga-Fernandes, H., Coles, M.C., Foster, K.E., Patel, A., Williams, A., Natarajan, D., Barlow, A., Pachnis, V., and Kioussis, D. (2007). Tyrosine kinase receptor RET is a key regulator of Peyer's patch organogenesis. *Nature* **446**, 547–551.
- von Boyen, G.B., Schulte, N., Pflüger, C., Spaniol, U., Hartmann, C., and Stein-kamp, M. (2011). Distribution of enteric glia and GDNF during gut inflammation. *BMC Gastroenterol.* **11**, 3.
- Vulchanova, L., Casey, M.A., Crabb, G.W., Kennedy, W.R., and Brown, D.R. (2007). Anatomical evidence for enteric neuroimmune interactions in Peyer's patches. *J. Neuroimmunol.* **185**, 64–74.
- Wang, H., Yu, M., Ochani, M., Amella, C.A., Tanovic, M., Susarla, S., Li, J.H., Wang, H., Yang, H., Ulloa, L., et al. (2003). Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* **421**, 384–388.
- Wang, Z., Friedrich, C., Hagemann, S.C., Korte, W.H., Goharani, N., Cording, S., Eberl, G., Sparwasser, T., and Lochner, M. (2014). Regulatory T cells promote a protective Th17-associated immune response to intestinal bacterial infection with *C. rodentium*. *Mucosal Immunol.* **7**, 1290–1301.
- Wheway, J., Mackay, C.R., Newton, R.A., Sainsbury, A., Boey, D., Herzog, H., and Mackay, F. (2005). A fundamental bimodal role for neuropeptide Y1 receptor in the immune system. *J. Exp. Med.* **202**, 1527–1538.
- Wolin, M.J. (1981). Fermentation in the rumen and human large intestine. *Science* **213**, 1463–1468.
- Wright, K.A. (1979). *Trichinella spiralis*: an intracellular parasite in the intestinal phase. *J. Parasitol.* **65**, 441–445.
- Xavier, R.J., and Podolsky, D.K. (2007). Unravelling the pathogenesis of inflammatory bowel disease. *Nature* **448**, 427–434.
- Xia, Y., Hu, H.-Z., Liu, S., Ren, J., Zafirov, D.H., and Wood, J.D. (1999). IL-1 $\beta$  and IL-6 excite neurons and suppress nicotinic and noradrenergic neurotransmission in guinea pig enteric nervous system. *J. Clin. Invest.* **103**, 1309–1316.
- Yamasaki, Y., Shimamura, O., Kizu, A., Nakagawa, M., and Ijichi, H. (1983). Modulation by alpha 2-adrenergic stimulation of IgE-mediated 14C-serotonin release from rat mast cells. *Agents Actions* **13**, 310–317.
- Yano, J.M., Yu, K., Donaldson, G.P., Shastri, G.G., Ann, P., Ma, L., Nagler, C.R., Ismagilov, R.F., Mazmanian, S.K., and Hsiao, E.Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* **161**, 264–276.
- Yissachar, N., Zhou, Y., Ung, L., Lai, N.Y., Mohan, J.F., Ehrlicher, A., Weitz, D.A., Kasper, D.L., Chiu, I.M., Mathis, D., and Benoist, C. (2017). An Intestinal Organ Culture System Uncovers a Role for the Nervous System in Microbe-Immune Crosstalk. *Cell* **168**, 1135–1148.e12.
- Zhao, A., McDermott, J., Urban, J.F., Jr., Gause, W., Madden, K.B., Yeung, K.A., Morris, S.C., Finkelman, F.D., and Shea-Donohue, T. (2003). Dependence of IL-4, IL-13, and nematode-induced alterations in murine small intestinal smooth muscle contractility on Stat6 and enteric nerves. *J. Immunol.* **171**, 948–954.
- Zheng, Y., Valdez, P.A., Danilenko, D.M., Hu, Y., Sa, S.M., Gong, Q., Abbas, A.R., Modrusan, Z., Ghilardi, N., de Sauvage, F.J., and Ouyang, W. (2008). Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat. Med.* **14**, 282–289.