Title

Neural Immune Communication in the Control of Host-Bacterial Pathogen Interactions in the Gastrointestinal Tract.

Journal

Infection and Immunity, 88(9)

ISSN

0019-9567

Authors

Ramirez, Valerie
Swain, Samantha
Murray, Kaitlin
et al.

Publication Date

2020-08-19

DOI

10.1128/iai.00928-19

Peer reviewed

AMERICAN SOCIETY FOR MICROBIOLOGY | Infection and Immunity®

MINIREVIEW

Neural Immune Communication in the Control of Host-Bacterial Pathogen Interactions in the Gastrointestinal Tract

Valerie Ramirez,[a] Samantha Swain,[a] Kaitlin Murray,[a] Colin Reardon[a]

[a]Department. of Anatomy, Physiology, and Cell Biology, UC Davis School of Veterinary Medicine, Davis, California, USA

ABSTRACT The orchestration of host immune responses to enteric bacterial pathogens is a complex process involving the integration of numerous signals, including from the nervous system. Despite the recent progress in understanding the contribution of neuroimmune interactions in the regulation of inflammation, the mechanisms and effects of this communication during enteric bacterial infection are only beginning to be characterized. As part of this neuroimmune communication, neurons specialized to detect painful or otherwise noxious stimuli can respond to bacterial pathogens. Highlighting the complexity of these systems, the immunological consequences of sensory neuron activation can be either host adaptive or maladaptive, depending on the pathogen and organ system. These are but one of many types of neuroimmune circuits, with the vagus nerve and sympathetic innervation of numerous organs now known to modulate immune cell function and therefore dictate immunological outcomes during health and disease. Here, we review the evidence for neuroimmune communication in response to bacterial pathogens, and then discuss the consequences to host morbidity and mortality during infection of the gastrointestinal tract.

KEYWORDS gastrointestinal inflammation, host defense, neuroimmunology, sensory neurons, vagus nerve, cholinergic anti-inflammatory pathway, enteric nervous system, enteric bacterial pathogens, *Citrobacter*, enteric pathogens, gastrointestinal infection

It is becoming increasingly clear that the nervous and immune systems are intertwined, with complex bidirectional communication shaping the function of both systems. The peripheral nervous system exerts a fundamental role in monitoring and controlling the physiological processes of the organ systems that they innervate. This includes the detection of inflammation and pathogens, and the regulation of immune cell activation. In the digestive system, this neuronal control is accomplished by extrinsic innervation, neurons that originate from outside the intestine, and by the intrinsic innervation (i.e., the enteric nervous system) that resides within the wall of the gastrointestinal tract (1). Like the rest of the body, noxious substances in the intestine can be detected by nociceptors that are specialized sensory neurons (2). These extrinsic neurons originate from the dorsal root ganglion (DRG) of the spinal cord projecting to organs throughout the body including the gastrointestinal (GI) tract, and can be activated by noxious stimuli such as excessive heat or bacterial pathogens and toxins. Activation can induce both highly localized release of neuropeptides, or initiation of a reflex arc through the DRG and back to the organ system. While recent studies have identified the ability of specific bacterial pathogens to activate nociceptors and induce maladaptive host responses that reduce immune cell activation (3–5), the role of these sensory neurons during enteric bacterial infection is not well established. In addition to these nociceptive neurons, the vagus nerve also provides sensory innervation from the duodenum through to the proximal colon, with the cell bodies of these afferent

Citation Ramirez V, Swain S, Murray K, Reardon C. 2020. Neural immune communication in the control of host-bacterial pathogen interactions in the gastrointestinal tract. Infect Immun 88:e00928-19. https://doi.org/10.1128/IAI.00928-19.

Editor Anthony R. Richardson, University of Pittsburgh

Address correspondence to Colin Reardon, creardon@ucdavis.edu.

Accepted manuscript posted online 27 April 2020
Published 19 August 2020

neurons residing within the cervical nodose ganglion (6). Although it is beyond the scope of this review to detail the complete innervation to each organ, it is important to note that vagal afferents innervate and receive sensory information from numerous abdominal organs (7). Perhaps it is for this reason that in most species vagal afferent (sensory) fibers comprise approximately 80% of the vagus, with the remainder being efferent (motor) fibers. Despite the contentious nature of peripheral neuroimmune circuits, vagal efferent fibers have been characterized as part of an anti-inflammatory reflex termed the cholinergic anti-inflammatory pathway that reduces aberrant macrophage activation during septic shock, and part of a discrete neuroimmune circuit that directly suppresses intestinal macrophage activation (8). Unlike other organ systems, the intrinsic innervation or enteric nervous system (ENS) of the gastrointestinal tract can control intestinal physiology independently of the central nervous system (CNS). Despite this unique ability, the ENS and CNS are tightly integrated in the control of physiology modulating motility and the absorption of nutrients and water (1). In this review, we will describe the role of neuroimmune circuits that exert or have the potential to exert control over the interactions between the host and bacterial pathogens in the intestinal tract, while comparing and contrasting these to other organ systems. As a mucosal surface, it should not be surprising that in the intestine various types of pathogens other than bacteria, such as parasites, viruses, and fungi, attempt to interface with or enter the host. While there is a rich literature in neuroimmune regulation during parasitic infection (9–13), there is a near complete absence of studies characterizing neuroimmune circuits elicited during viral or fungal infection. While there are undoubtedly unique neuroimmune responses to these types of pathogens, we propose that there will be commonalities in the neuronal detection of the host immune responses.

SENSORY NOCICEPTIVE NEURONS

Detection of noxious stimuli ranging from extremes in heat or cold to the presence of discrete chemical substances (2) has been long appreciated to occur through highly specialized "sensory nociceptive" neurons. This ability is conferred through the expression of a family of transient receptor potential (TRP) cation channels that open upon stimulation, such as ligand binding, resulting in neuronal activation (2). While there are a multitude of TRP channel family members, we will limit our scope to those expressed by sensory nociceptive neurons that are responsive to bacterial pathogens, their components, or the host response to infection. Interested readers are directed to the comprehensive reviews on TRP channels and their expression on various cell types (14).

Perhaps the most well-known of these TRP channels is the transient receptor potential cation channel subfamily V member 1 (TRPV1) that is activated by temperatures in excess of 43°C, low pH, and compounds such as ATP and capsaicin (14–17). Activation of TRPV1 on nociceptive neurons consequently induces signaling in the DRG of the spinal cord, initiating efferent signaling in a classical reflex arc (18). This signaling culminates in the release of neuropeptides such as substance P (SP), calcitonin-gene related protein (CGRP), and neurokinin A (NKA) into the organ. Activation can also cause localized release of these neuropeptides at the initial site or organ system through antidromic activation (2, 19). Although sensory nociceptors can be found throughout the body, the density, distribution, and sensitivity of these neurons can vary dramatically depending on the organ system. As a specific example, the GI tract is densely innervated by sensory nociceptors; however, unlike other organs such as the skin, the majority of these nociceptive neurons in healthy individuals are quiescent (20). It is important to note that these TRPV1-expressing sensory afferents in visceral organs can also function to support normal physiology, with these neurons in the small intestine and colon functioning in mechanosensation of organ distension (21). From a neuroanatomical perspective, sensory afferent neurons in the colon are derived from lumbar splanchnic and pelvic nerve of the thoracolumbar and lumbosacral regions of the spinal cord, respectively (22). Although historically these sensory neurons have been identified based on morphometry or electrical properties, single-cell sequencing

of colonic projecting neurons from the thoracolumbar and lumbosacral DRG identified TRPV1 expression in three of seven discrete types of colonic sensory afferents (22). These data highlight that TRPV1$^+$ sensory neurons are heterogenous in composition and respond to unique stimuli, and could result in different functional outcomes.

ROLES OF SENSORY AFFERENTS IN BACTERIAL INFECTION

Despite these differences between types of sensory innervation within different organs, the release of nociceptive neuropeptides has a profound effect on the molecular underpinnings of inflammation and host responses to pathogens. For example, SP acts directly as a chemotactic molecule (23), while increasing chemokine expression, endothelial permeability (24, 25), and lymphatic drainage (26). Complicating this generalized proinflammatory role of nociceptive neuropeptides (26), CGRP can increase vasodilation (27) and act synergistically with cytokines to increase neutrophil recruitment (28) *in vivo*, yet reduces *in vitro* T-cell proliferation (29). Thus, the overall effect of sensory nociceptive activation on host immune responses is likely to be determined by several variables, including the nature of the stimuli, the organ site, and the immune cells involved.

It is becoming increasingly clear that pathogens, or the resulting infection-induced inflammation, can activate sensory nociceptors. While these neurons express a range of Toll-like receptors (TLRs) to detect components of pathogens, typically TLR activation by pathogen-associated molecular patterns (PAMPS), such as lipopolysaccharide (LPS), reduces the threshold of neuronal activation (30). It is an important caveat that exquisite care and appropriate culture techniques must be used to exclude nonneuronal cells and the potential contribution of nonneural cells, such as satellite glial cells, in the assay of these transcripts. Activation of TLRs not only can reduce the threshold of activation but can further induce expression of proinflammatory cytokines, and stimulate prostaglandin synthesis. This further complicates our interpretation of bacterial activation of sensory nociceptors, since these neurons are activated by TNF-α, IL-1β, IL-6, IL-17A, IL-31, histamine, and prostaglandins (31–33). Additionally, bacterial infection can increase TRPV1 signaling through TLR4-dependent increases in TRPV1 expression, altering sensitivity to exogenous or endogenous ligands (18). As a polymodal nociceptive channel, TRPV1 can also function as a direct detector of bacterial components, becoming activated by LPS binding at a discrete site from the capsaicin-binding site (34). Other host-produced compounds besides cytokines, such as the lipid-derived compounds *N*-arachidonyldopamine (35), lysophosphatidic acid (36), and hydroxyeicosatetraenoic acid (20-HETE) (37), have been identified as TRPV1 ligands. However, at this time it is not certain what role if any these substances have during host immune responses to enteric bacterial pathogens. Further complicating analysis, activation of TRPV1 has also been shown to "gate" or reduce the activity of other ion channels, including the mechanically activated Piezo cation channels (38). This would suggest that activation of TRPV1 can induce changes in other signaling pathways, thus further complicating analysis of "TRPV1-dependent" responses. With this in mind, it would seem that infection and the resulting immune response would be expected to activate or facilitate a reduced threshold for activation of sensory nociceptive neurons through a variety of discrete mechanisms. While the focus has been on neuronal expression of this channel, care is required in the interpretation of results from constitutive knockout (KO) mice or neuronal ablation studies due to expression on nonneuronal cells such as epithelial cells and T cells (39, 40). In practice, these concerns can be mitigated by use of bone marrow chimeras, conditional knockout mice, or in ablation studies allowing sufficient time for replenishment of nonneuronal cells.

Adding to this complexity of the role of the nervous system during enteric infection is the expression of a diverse array of other TRP channels, which are specialized sensors of unique stimuli. The TRPA1 cation channel is activated by noxious cold ($<$17°C) and chemical irritants such as mustard oils (41), endocannabinoids, lipid peroxidation products generated during oxidative stress and inflammation (42, 43), and components of bacteria such as LPS (44). This ability suggests that sensory nociceptors expressing

TRPA1 could become activated not only by LPS, but also by products of the host immune response during infection. Although the role of TRPA1 during enteric bacterial infection is unknown, in the bladder, LPS instillation induced nociceptive hypersensitivity, together with bladder voiding in wild-type (WT) but not TRPA1 KO mice, with no change in overt inflammation. These findings indicate that TRPA1$^+$ nociceptors are responsive to LPS instillation; however, it is uncertain if this is a direct effect of LPS on this channel or if these changes are a consequence of the local induced inflammation (45). Similar to TRPV1, TRPA1 can also be activated by cytokines such as IL-4 and IL-13, suggesting that expression of this TRP channel confers neurons with the ability to monitor immune responses (32). This role of TRPA1 in monitoring of inflammation appears to be widespread, with TRPA1 activation in the lung increasing immune cell recruitment and airway hyperreactivity during ovalbumin (OVA)-induced airway hypersensitivity. Reduced inflammation and changes in lung function were correlated with reduced SP, CGRP, and NKA release in sensitized TRPA1 KO mice challenged with OVA compared to WT mice. Although these data demonstrate that endogenously produced immune reactive products activate TRPA1 to enhance lung inflammation, the location of TRPA1 was not evaluated (46). As a whole, these findings suggest that TRPA1-dependent nociceptor activation can amplify local innate immune responses to promote host protection.

Although TRPA1 and TRPV1 are established to influence a variety of immune responses, it is important to note that not all TRP channels expressed by sensory nociceptors appear to exert an influence over immune outcomes. While the cold- and menthol-sensing channel TRPM8 is expressed by sensory nociceptive neurons, immunological effects have been attributed to TRPM8 expression by macrophages (47, 48). These data suggest that careful dissection of the roles of sensory afferent neuron activation and TRP channels in immune responses is required.

Despite the well-established ability of SP and CGRP to drive neurogenic inflammation, activation of sensory nociceptors has paradoxically been found to drive maladaptive host immune responses by select pathogens. Although the majority of studies on nociceptive neurons during infection have been conducted in the skin or lung, there appear to be some commonalities between these sites and responses evoked in the GI tract. Infection of wounded skin with *Staphylococcus aureus* activates local sensory nociceptors in the immediate vicinity of the site that is not proportional to the wound size, indicating that the infection triggered activation of sensory nociceptive neurons. Using cultured DRG neurons, Ca^{+2} signaling indicative of activation occurred *in vitro* following addition of live and heat-killed *S. aureus* and *S. pneumoniae*. This nociceptor activation, however, is detrimental to the host, as *in vivo* this nociceptor activation leads to a significantly inhibited host response. Confirming the role of sensory neurons, prior neural ablation with the excitotoxic TRPV1 agonist resiniferatoxin (RTX) (49) increased host protective immune responses. Using this approach, maladaptive immune responses were identified to be due to the release of CGRP and inhibition of neutrophil recruitment to the wound site. This ability to subvert host immune responses through sensory nociceptors was not restricted to a single bacterial pathogen, as *Streptococcus pyogenes* induced similar maladaptive responses due to TRPV1$^+$-dependent neuronal activation and release of CGRP. Highlighting that these effects of neuronally induced immune subversion are not limited to the skin, CGRP released by sensory neurons in the lung reduced host survival during methicillin-resistant *Staphylococcus aureus* pneumonia (5). It is interesting to note that at this mucosal site, prior ablation of TRPV1$^+$ neurons increased neutrophil recruitment, and $\gamma\delta$ T-cell recruitment due to increased expression of the neutrophil chemotactic protein CXCL1 and TNF-α (5). While these studies suggest that bacterial activation of sensory nociceptors is a general mechanism of immune subversion, it is critical to note that different heat-killed bacterial pathogens elicited various degrees of nociceptor activation *in vitro* (3). These findings suggest that the response of sensory afferent neurons to bacterial pathogens is likely to be highly complex. In support of this, silencing of sensory afferents using the voltage-gated sodium channel blocker QX-314 reduced infection-induced neuronal excitation and

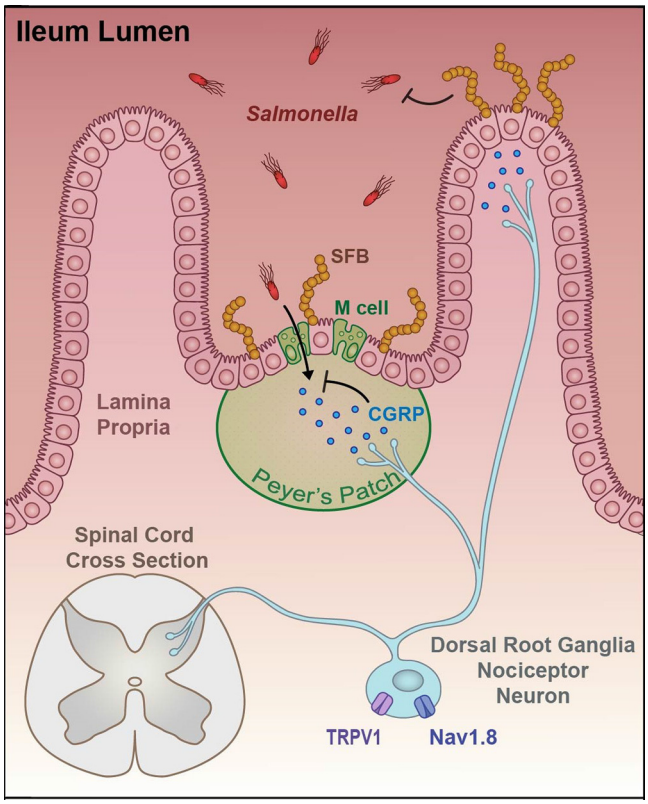

FIG 1 Extrinsic sensory innervation of the colon drives maladaptive responses to *Salmonella* Typhimurium infection. Sensory nociceptive neurons that originate in the dorsal root ganglion (DRG) release CGRP in the small intestinal mucosa, thereby supporting luminal segmented filamentous bacteria (SFB) that consequently reduce susceptibility to *S.* Typhimurium infection. Release of CGRP also inhibits the differentiation of microfold M cells in Peyer's patches, reducing bacterial burden and disease.

hyperresponsiveness to noxious stimuli, with no reduction in *S. aureus* burden (50). These data could be indicative of different types of sensory nerves in the dorsal skin compared to the hind paw of the mouse, or that neural ablation versus silencing could target different types of sensory nerves. Either of these possibilities suggests that nociceptors in the GI tract or other organ systems could respond uniquely to bacterial pathogens.

This ability of sensory nociceptors to dictate host immune responses is not limited to the lung and skin. In the GI tract, neuropeptides released by sensory nociceptors can exert host-protective and proinflammatory effects. At this mucosal site, SP has long been regarded as proinflammatory, with increased release occurring in animal models of nonbacterial-induced colitis, and with selective SP receptor antagonists reducing the severity of inflammation (51, 52). During enteric bacterial infection, treatment of WT mice with SP receptor antagonists, or knockout mice for the gene encoding the SP receptor, *Tacr1*, infected with the invasive enteric pathogen *Salmonella enterica* serovar Dublin, have significantly reduced IL-12 and IFN-γ production, and consequently reduced host survival (53). The source of host-protective SP during enteric infection was not established, and is complicated by the ability of monocytes (54), macrophages (54, 55), and lymphocytes (56) to serve as nonneuronal sources. Similar to the skin, sensory nociceptors express not only SP but also CGRP and have been shown to exert a protective role during *Salmonella enterica* serovar Typhimurium infection. Sensory nociceptive neuron-derived CGRP was shown to support maintenance of segmented filamentous bacterial in the ileum, and reduce the differentiation of microfold "M" cells (57), a site of *S.* Typhimurium invasion (Fig. 1) (58). Although the extrinsic sensory innervation of the GI tract originates from either the DRG or nodose ganglion, and can

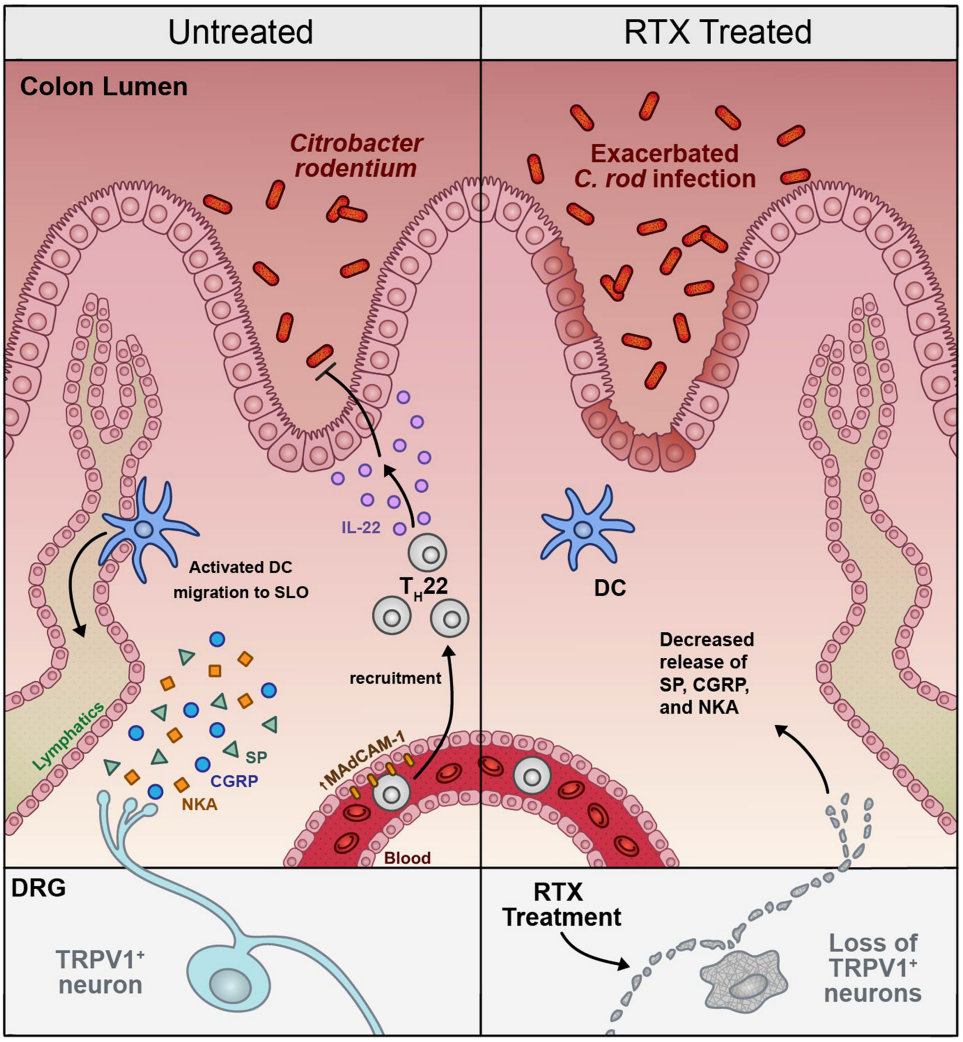

FIG 2 Extrinsic sensory neurons in the colon coordinate protection during enteric infection. In mice with intact sensory innervation, infection with *C. rodentium* drives increased recruitment of IL-22-producing T cells into the colonic lamina propria. Ablation of TRPV1$^+$ sensory nociceptors with RTX reduces expression of MadCAM-1 and consequently T cell recruitment during *C. rodentium* infection.

express TRP receptors, including TRPV1, and SP (59), there are few CGRP$^+$ vagal afferent neurons that innervate the intestinal tract (60). In addition, while these data suggest that CGRP is released by sensory neurons from the DRG during infection, there are two isoforms of CGRP, with CGRPα predominantly expressed by extrinsic neurons and CGRPβ by intrinsic innervation (61). The source of this host-protective CGRP has not been formally proven; however, given that CGRPβ-expressing intrinsic neurons do not express TRPV1 and are insensitive to capsaicin (61), these cells would be unaffected by sensory nociceptor ablation methods that are dependent on TRPV1 expression. Together, this would suggest that host protection is afforded by the sensory nociceptors arising in the DRG.

Coordination of protective host immune responses to enteric bacterial pathogens by sensory nociceptors appears to be a generalized phenomenon, regardless of the nature of the bacterial pathogen. Using the model noninvasive enteric pathogen *Citrobacter rodentium*, we found that ablation of nociceptive neurons with RTX significantly increased the severity of disease and delayed bacterial clearance (62). As TRPV1-deficient mice also exhibited increased bacterial burden and delayed clearance, this host-protective function of sensory nociceptive neurons appears to be TRPV1 dependent (Fig. 2) (62). Increased fecal and colonic adherent pathogenic bacteria were

found to be due to reduced IL-22 production, a cytokine vital to the host response against *C. rodentium*. This reduction in host-protective immune function 10 days postinfection was due to delayed recruitment of $CD3^+$ T cells into the infected colonic lamina propria. Ablation of sensory neurons appeared to reduce T-cell recruitment due to reduced expression of the mucosal addressin molecule MAdCAM-1 (62). In determining the potential role for CGRP in mediating the host-protective effects of the sensory neurons, mice were administered a highly selective and potent small molecule CGRP receptor antagonist (63, 64). Blockade of CGRP receptors did not increase the fecal or colonic adherent *C. rodentium*, or significantly reduce T-cell recruitment (62). In keeping with prior studies where *C. rodentium* infection did not increase colonic CGRP expression (65), our data demonstrate that nociceptor-mediated protection is independent of CGRP. These data highlight that neuroimmune interactions during enteric bacterial infection are complex and dependent on the region and the pathogen involved.

In these studies of the role of nociceptive neurons during infection of skin, lung, and intestine, the site of infection has received focus without considering the effects on developing immune responses in secondary lymphoid tissues, such as the spleen and lymph nodes. In support of nociceptive neuropeptides modulating responses in these immune tissues, SP increases lymphatic flow by causing the contraction of lymphatic muscle cells (66–69). Control over afferent lymphatics by nociceptive neurotransmitters appears to be a feature of the intertwining of SP-expressing neurons with lymphatic vessels (70). In contrast to SP-induced increased flow rates of afferent lymph, CGRP induces smooth muscle relaxation, consequently reducing lymph flow (71). Anatomically, this control is provided by the peri- and paravasulcar innervation of mesenteric, axillary, inguinal lymph nodes and spleen that coexpress SP and CGRP (72, 73). Nonvascular associated fibers have been found throughout the lymph node (74), although recent studies using a genetic fate map approach with tdTomato expressed in $Nav1.8^+$ sensory neurons revealed only perivascular sensory nociceptor innervation of the lymph nodes (75). This disparity suggests discrete patterns of innervation in different strains of mice, or that SP and CGRP are expressed by other $Nav1.8^-$ neuronal cell types. Beyond lymphatic endothelial cells, these neuropeptides activate other stromal cells, or immune cells in the lymphatic tissues (76). At this point, it has not been experimentally determined whether nociceptor-induced host-protective responses require signaling in these immune tissues, or what cell types are involved. These studies would require use of conditional knockout mice for the receptors of these neuropeptides, and for techniques to perform organ-selective neuronal ablation to be developed.

VAGAL AFFERENTS AND THEIR ROLE IN BACTERIAL INFECTION

Maintenance of a wide range of homeostatic functions in the body is provided through the sensory information detected by vagal afferents. As the longest cranial nerve, the vagus innervates numerous visceral organs, including the heart, lungs, spleen, gastrointestinal tract, liver, and pancreas, placing vagal sensory afferents at important interfaces between the host and the environment.

The cell bodies of afferent vagal sensory neurons reside in the nodose and jugular ganglia, with projections to the brainstem where most synapse to neurons in the nucleus tractus solitarius (NTS). Information is coordinated in the NTS complex with resulting outflow, i.e., efferent vagal nerve signaling, occurring through the activation of neuronal cell bodies of the dorsal motor nucleus (DMN). Given the positioning of vagal afferent sensory neurons at the interface between the body and the external environment, it is not surprising that the vagus is implicated as a critical sensor of pathogens and inflammation. As evidence of this role, infection of mice with the enteric bacterial pathogen *Campylobacter jejuni* activates vagal afferent neurons, evidenced by expression of the immediate early gene c-*fos* in neurons of the nodose and NTS (77). These data, in concert with the absence of detectable serum cytokines, suggested that pathogens could be directly detected by vagal afferent neurons (77). It is, however, worth noting that vagal afferent activation could occur indirectly due to pathogen-

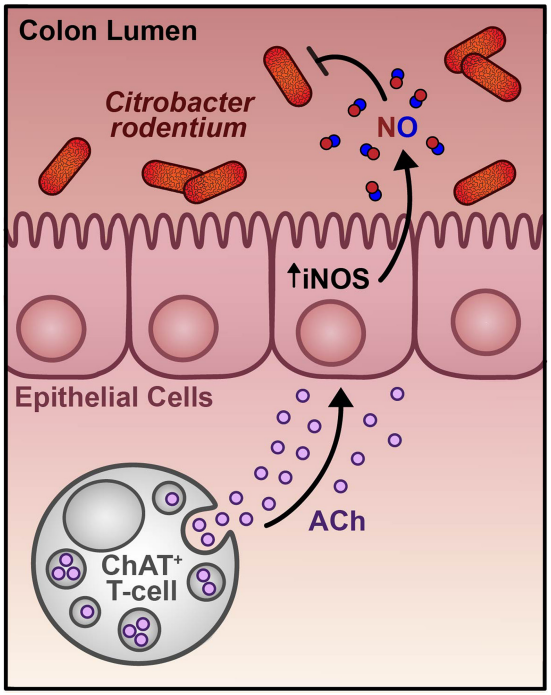

FIG 3 ChAT$^+$ T cells induce host-protective responses during *C. rodentium* infection. T cells expressing ChAT and producing acetylcholine home to the colonic lamina propria during infection with *C. rodentium*. Production of ACh enhances expression of inducible nitric oxide synthase (iNOS), a known host-protective molecule.

induced changes to host physiology or activation of intestinal enteroendocrine cells (78).

VAGAL EFFERENT NEURONS AND THEIR ROLE IN IMMUNE REGULATION AND BACTERIAL INFECTION

Stimulated or induced efferent vagal nerve fiber activity can impact host immune processes by directly communicating with immune cells in organs such as lung and intestine, or through intermediaries in secondary lymphoid tissues (79). For example, it is well appreciated that inflammation due to activation of tissue resident macrophages in the small intestine following surgery can be reduced by stimulation of vagal nerve efferents. Release of acetylcholine from these nerve terminals in the intestinal muscularis binds to nicotinic acetylcholine receptors on these macrophages to prevent NF-κB signaling and consequently reduce proinflammatory gene expression (80). Vagal innervation of secondary lymphoid organs, such as the spleen, is indirect, with efferent vagal nerve fibers synapsing onto sympathetic neuronal cell bodies in the superior mesenteric ganglion/celiac ganglion complex (81). These sympathetic fibers project into the spleen and mesenteric lymph nodes and, when activated, release norepinephrine (NE) into these tissues. This indirect neuroimmune circuit requires specialized T cells to release acetylcholine (ACh) that can inhibit NF-κB-dependent signaling transduction in macrophages (82). Although the consequences of vagal efferent signaling directly or indirectly to macrophages during enteric bacterial infection are unknown, we have shown that Ach-producing T cells exert a host-protective effect during infection with an enteric bacterial pathogen. While ChAT T cells are rare in the GI tract of naive mice, an inability of T cells to produce ACh caused significant changes in the commensal microbiota (83). Based on these observations during health, we assessed the contributions to host-pathogen interactions. Increased numbers of ChAT$^+$ T cells are found in *C. rodentium*-infected colon, but not during chemically induced colitis, and conditional T-cell knockout mice have significantly increased bacterial burdens and delayed pathogen clearance (Fig. 3) (84). Despite the known role for sympathetic innervation to

induce ACh release from these cells in the spleen and MLN (81, 82), the role of sympathetic nerves in communicating to ChAT$^+$ T cells in the colon during infection is unknown.

Vagal innervation and stimulation can also enhance innate immunity and host protection in the peritoneal cavity by controlling production of enzymatically produced factors during the resolution of inflammation, termed specialized pro-resolving mediators (SPM) (85). This term applies to a broad class of mediators, including resolvins, lipoxins, and protectins, and protein conjugates of these lipids that display unique biological activity (86). Vagotomy in mice significantly altered the production of a wide variety of lipid mediators, including the peptide-lipid conjugate termed protection conjugates in tissue regeneration 1 (PCTR1) (87). While it is unknown if PCTR1 is produced in the GI tract, this protectin conjugate reduces inflammation following peritoneal infection with *Escherichia coli* by enhancing chemotaxis of monocytes and macrophages, and increasing bacterial phagocytosis (88). During peritoneal *E. coli* infection, vagotomy significantly delayed bacterial clearance, reduced macrophage phagocytotic capacity, and decreased the number of type 3 innate lymphoid cells (ILC3s) (87). Vagotomy-induced changes were due to reduced levels of ACh in the peritoneal cavity, as ACh induced PCTR1 production by ILC3s, and administration of either ILC3s or PTCR1 to vagotomized mice improved the resolution of inflammation (87). As ILC3s are a critical component of the host defense against *C. rodentium* (89), the effect of a vagal-protectin conjugate axis on ILC3 in the GI tract could be an important mechanism. Although Ach-producing cells were found in close proximity to ILC in the omentum, these cells were not T cells, supporting our observation of a large ChAT$^+$ B cell population in the peritoneum that could regulate neutrophil migration (90). As a whole, these studies suggest that a multitude of vagus nerve-mediated neuroimmune circuits could exist to aid host protection or resolution following infection of the GI tract.

SYMPATHETIC INNERVATION

Neuroimmune circuitry that utilizes the sympathetic nervous system consists of the cholinergic anti-inflammatory pathway (CAIP) and pathways descending the spinal cord to prevertebral ganglia that provide sympathetic innervation to secondary lymph nodes, and the GI tract, including the liver (91). In the spleen, a sympathetic neuroimmune circuit has been characterized that is discrete from the CAIP, with stimulation of the splenic nerve sufficient to inhibit LPS-induced TNF-α production independent of α7R expression (92). This sympathetic inhibition of immune cell activity is also present in the GI tract, with stroke increasing sympathetic tone in the liver that reduced invariant natural killer T-cell crawling, IFN-γ, and IL-12 production, with increased IL-10 and IL-5 production (93). Confirming the role of sympathetic innervation, ablation of these neurons prevented immune suppression following stroke, while administration of NE alone recapitulated these effects (93). Thus, sympathetic innervation of the liver is capable of dictating immune cell activity and could have a significant impact on responses to pathogens. Highlighting the complexity of neuroimmune circuits in the GI tract, sympathetic neuronal activation is not always deleterious to the host production protective factors. Infection with *Salmonella* Typhimurium in the small intestine induced activation of extrinsic sympathetic neurons and NE-dependent reprogramming of muscularis macrophages from proinflammatory to a tissue-reparative phenotype (94). This signaling to muscularis macrophages was further found to protect against *S.* Typhimurium-delayed gastrointestinal transit caused by infection-induced loss of enteric neurons (Fig. 4) (95).

Despite these elegant experiments, it is uncertain what effect loss of this neuroimmune circuit would have on the host defense or overall immune response. In support of this host-protective role during systemic bacteremia, increased activation of sympathetic outflow reduced bacterial burden in the liver due to increased peritoneal macrophage and dendritic cell phagocytosis of *E. coli*, and increased monocyte and macrophage bactericidal activity, respectively (96). These studies reveal the compli-

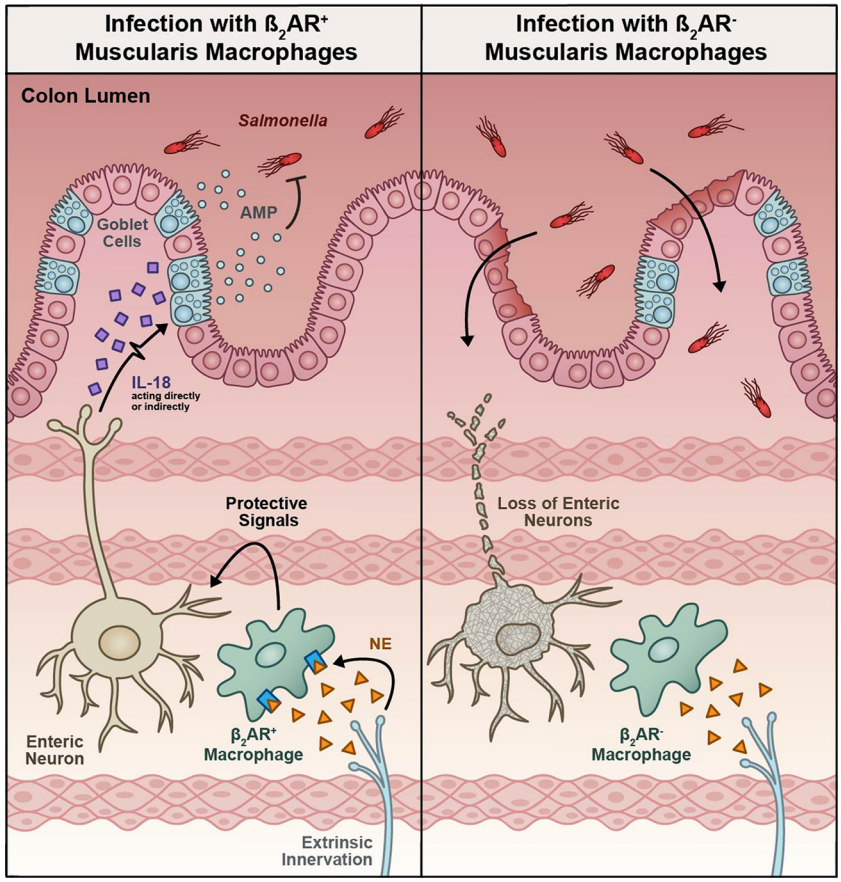

FIG 4 The enteric nervous system participates in the immune response to *S.* Typhimurium and can be altered by infection. Enteric infection can result in loss of enteric neurons that precipitates reduced or altered intestinal motility. Infection also induces activation of extrinsic sympathetic neurons that cause the release of norepinephrine to activate muscularis macrophages. These macrophages appear to have a unique role in reducing the depletion of enteric neurons (A). Production of IL-18 by the enteric nervous system can also induce antimicrobial peptide expression by goblet cells, directly aiding the killing of enteric bacterial pathogens (B).

cated nature of neuroimmune interactions while highlighting the importance of neural input in resolving certain infections. Perhaps it is not surprising that certain pathogens have been reported to detect the host through the release of these neurotransmitters. It has been reported that NE can act as a ligand for bacterial quorum-sensing proteins (97), inducing bacterial proliferation and expression of virulence genes (98, 99). Moreover, mice that lack the ability to produce NE were reported to have reduced *C. rodentium* colonization (98), highlighting that specific enteric bacterial pathogens could utilize host-derived signals to the benefit of the pathogen. This direct effect of NE on enteric bacterial pathogens demonstrates the need for carefully controlled experiments in which sympathetic neuronal ablation is performed to rule out the potential of enhanced virulence gene expression as opposed to reduced host defense capacity.

ENTERIC NERVOUS SYSTEM

The ENS is comprised of an estimated 200 to 600 million neurons that reside in the intestinal wall from the oral to the anal cavity. These neurons are found within two layers: the myenteric plexus (MP) that is between the outer longitudinal muscle and the inner circular muscle and the submucosal plexus that is found between the circular muscle and the mucosa. Neurons in the myenteric plexus can be found in discrete ganglia, modulate GI motility, and are closely associated with muscularis macrophages, while submucosal neurons regulate ion secretion, epithelial permeability, and blood flow. Although early experiments demonstrated that the ENS can control GI physiology

in the complete absence of extrinsic innervation, both the myenteric and submucosal plexus receive input from the CNS (1, 100). As it is beyond the scope of this review to detail the nearly 20 neuronal cell types identified, reflexes, and all the mechanisms that serve to control GI function, the interested reader is directed to the excellent comprehensive reviews on this subject in references 1 and 101. In addition to the host-protective functions of increased water secretion and contractility to expel pathogens or their toxins, the ENS can drive localized antibacterial responses. Recently, conditional deletion of IL-18 in ENS neurons significantly increased bacterial burden and morbidity during *S.* Typhimurium infection (102). This ENS-derived IL-18 was proposed to increase goblet cell antimicrobial peptide expression in the proximal colon during infection but not at steady state (Fig. 4) (102). It is uncertain if this effect of ENS-derived IL-18 has similar effects on Paneth or goblet cells in the small intestine, another site of *S.* Typhimurium entry. Finally, while IL-18 expression by ChAT$^-$ neuronal nitric oxide synthase$^+$ (nNOS) and ChAT$^-$ nNOS$^-$ myenteric plexus neurons was observed, the mucosal IL-18$^+$ innervation was revealed by the general neuronal marker βIII tubulin (102). This makes it difficult to ascertain if these neurons are part of the intrinsic or extrinsic innervation, or if they are part of the ENS and originate in either the MP or submucosal plexus. The types of neurons in the ENS that project from the MP to the mucosa are limited to intrinsic primary afferent neurons, and are typically cholinergic, expressing ChAT and not nNOS (103). Whether this IL-18 expression in the ENS represents a novel neuronal type, or this ability to induce AMP is a conserved feature of neurons in both the myenteric and submucosal plexus is unknown. In addition, although conditional ablation studies were performed using Cre recombinase expression under the heart and neural crest derivatives expressed 2 (Hand2) promoter, to provide ENS-selective ablation, the specificity of this promoter is uncertain. Single-cell sequencing data identify Hand2 expression in the ENS and in sympathetic neurons, meaning that extrinsic sympathetic innervation could equally have been affected using this ablation strategy (104). Despite these concerns, these exciting data demonstrate the complexity and numerous layers of neuronal control of host defenses to enteric bacterial pathogens.

This multitude of neuroimmune circuits in the GI tract is further illustrated by recent reports demonstrating that enteric neurons producing vasoactive intestinal peptide (VIP) can affect select aspects of host immune function. These VIP$^+$ neurons in the mucosa have been found in close association with tertiary lymphoid tissue in the intestine that contain ILC3 (105, 106). Although these ILC3 were found to express high levels of the VIP receptor 2 (VIPR2), the functional ramifications are contentious, with activation of VIPR2 *in vitro* reducing (105) and increasing IL-22 expression (106). To decipher the role of VIP *in vivo* during an infection, a chemogenetic approach was used (107, 108), whereby controlled selective expression of an activating designer receptor exclusively activated by a designer drugs (DREADD) receptor was achieved in VIP$^+$ cells using Cre recombinase under the control of the VIP promoter (105). Mice infected with *C. rodentium* and administered the DREADD ligand clozapine-*N*-oxide (CNO) to induce VIP$^+$ cell activation had fewer IL-22-producing ILC3s. This was accompanied by significantly increased *C. rodentium* translocation to the liver and spleen, and consequent mortality. These findings were confirmed using expression of an inhibitory DREADD whereby blockade of VIP$^+$ cell activity reduced bacterial translocation to the spleen and liver. These intriguing studies leave much to be determined in understanding the contribution of this pathway to the regulation of mucosal immunity. In particular, there are at least four discrete types of neurons in the ENS that produce VIP, each with a unique functionality (1). While microscopy suggests that these VIP-expressing neurons are secretor motor neurons adjacent to tertiary lymphoid organs, it is not clear that DREADD-based activation or inhibition occurred in the GI tract. Neurons expressing VIP can be found in tissues ranging from the pancreas, where VIP exerts control over exocrine functions (109), to the suprachiasmatic nucleus in the brain that functions to entrain circadian rhythm (110, 111). Despite these concerns, these data illustrate that a number of neuroimmune circuits exist that exert control over host defenses, and

demonstrate the need for further development of techniques to provide targeted stimulation of discrete neurons in the periphery. This need for precise identification and control of cell types while dissecting a presumed neuroimmune circuit is critically important, as neuron-associated cells such as glial cells have also been shown to be part of an immune regulatory network in the colon. Glial-derived neurotrophic factors, which induce development of the ENS, also induce IL-22 expression in ILC3s (112). These studies demonstrate the complex integrative physiology of neuroimmune circuits in the GI tract and will require unique and highly selective tools to identify the contributions of each component.

Conclusions. The crosstalk between the immune system and the nervous system is incredibly complex, and we have only begun to illuminate how the nervous system detects bacterial pathogens and modulates immune cell function. Neural immune communication has thus far been revealed to induce both host-protective and immune-inhibitory pathways that are exploited by pathogens. The sheer number and types of neurons in the ENS, and connections to extrinsic innervation, suggest that there are likely a number of neuroimmune circuits that have yet to be discovered. These pathways will have significant implications and present opportunities in the development of new treatment modalities for infections.

REFERENCES

1. Furness JB, Callaghan BP, Rivera LR, Cho H-J. 2014. The enteric nervous system and gastrointestinal innervation: integrated local and central control, p 39–71. In Lyte M, Cryan JF (ed), Microbial endocrinology: the microbiota-gut-brain axis in health and disease. Springer New York, New York, NY. https://doi.org/10.1007/978-1-4939-0897-4_3.
2. Dubin AE, Patapoutian A. 2010. Nociceptors: the sensors of the pain pathway. J Clin Invest 120:3760–3772. https://doi.org/10.1172/JCI42843.
3. Chiu IM, Heesters BA, Ghasemlou N, Von Hehn CA, Zhao F, Tran J, Wainger B, Strominger A, Muralidharan S, Horswill AR, Wardenburg JB, Hwang SW, Carroll MC, Woolf CJ. 2013. Bacteria activate sensory neurons that modulate pain and inflammation. Nature 501:52–57. https://doi.org/10.1038/nature12479.
4. Pinho-Ribeiro FA, Baddal B, Haarsma R, O'Seaghdha M, Yang NJ, Blake KJ, Portley M, Verri WA, Dale JB, Wessels MR, Chiu IM. 2018. Blocking neuronal signaling to immune cells treats streptococcal invasive infection. Cell 173:1083–1097.e22. https://doi.org/10.1016/j.cell.2018.04.006.
5. Baral P, Umans BD, Li L, Wallrapp A, Bist M, Kirschbaum T, Wei Y, Zhou Y, Kuchroo VK, Burkett PR, Yipp BG, Liberles SD, Chiu IM. 2018. Nociceptor sensory neurons suppress neutrophil and gammadelta T cell responses in bacterial lung infections and lethal pneumonia. Nat Med 24:417–426. https://doi.org/10.1038/nm.4501.
6. Berthoud HR, Carlson NR, Powley TL. 1991. Topography of efferent vagal innervation of the rat gastrointestinal tract. Am J Physiol 260:R200–7. https://doi.org/10.1152/ajpregu.1991.260.1.R200.
7. Kupari J, Haring M, Agirre E, Castelo-Branco G, Ernfors P. 2019. An atlas of vagal sensory neurons and their molecular specialization. Cell Rep 27:2508–2523.e4. https://doi.org/10.1016/j.celrep.2019.04.096.
8. van der Zanden EP, Snoek SA, Heinsbroek SE, Stanisor OI, Verseijden C, Boeckxstaens GE, Peppelenbosch MP, Greaves DR, Gordon S, De Jonge WJ. 2009. Vagus nerve activity augments intestinal macrophage phagocytosis via nicotinic acetylcholine receptor $\alpha4\beta2$. Gastroenterology 137:1029–1039.e4. https://doi.org/10.1053/j.gastro.2009.04.057.
9. Halliez MCM, Buret AG. 2015. Gastrointestinal parasites and the neural control of gut functions. Front Cell Neurosci 9:452. https://doi.org/10.3389/fncel.2015.00452.
10. Klose CSN, Mahlakoiv T, Moeller JB, Rankin LC, Flamar AL, Kabata H, Monticelli LA, Moriyama S, Putzel GG, Rakhilin N, Shen X, Kostenis E, Konig GM, Senda T, Carpenter D, Farber DL, Artis D. 2017. The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. Nature 549:282–286. https://doi.org/10.1038/nature23676.
11. Cardoso V, Chesné J, Ribeiro H, García-Cassani B, Carvalho T, Bouchery T, Shah K, Barbosa-Morais NL, Harris N, Veiga-Fernandes H. 2017. Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. Nature 549:277–281. https://doi.org/10.1038/nature23469.
12. Moriyama S, Brestoff JR, Flamar A-L, Moeller JB, Klose CSN, Rankin LC, Yudanin NA, Monticelli LA, Putzel GG, Rodewald H-R, Artis D. 2018. β_2-adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses. Science 359:1056–1061. https://doi.org/10.1126/science.aan4829.
13. Nagashima H, Mahlakõiv T, Shih H-Y, Davis FP, Meylan F, Huang Y, Harrison OJ, Yao C, Mikami Y, Urban JF, Caron KM, Belkaid Y, Kanno Y, Artis D, O'Shea JJ. 2019. Neuropeptide CGRP limits group 2 innate lymphoid cell responses and constrains type 2 inflammation. Immunity 51:682–695.e6. https://doi.org/10.1016/j.immuni.2019.06.009.
14. Julius D. 2013. TRP channels and pain. Annu Rev Cell Dev Biol 29:355–384. https://doi.org/10.1146/annurev-cellbio-101011-155833.
15. Robinson DR, McNaughton PA, Evans ML, Hicks GA. 2004. Characterization of the primary spinal afferent innervation of the mouse colon using retrograde labelling. Neurogastroenterol Motil 16:113–124. https://doi.org/10.1046/j.1365-2982.2003.00456.x.
16. Sugiuar T, Bielefeldt K, Gebhart GF. 2004. TRPV1 function in mouse colon sensory neurons is enhanced by metabotropic 5-hydroxytryptamine receptor activation. J Neurosci 24:9521–9530. https://doi.org/10.1523/JNEUROSCI.2639-04.2004.
17. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D. 2000. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288:306–313. https://doi.org/10.1126/science.288.5464.306.
18. Chiu IM, von Hehn CA, Woolf CJ. 2012. Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. Nat Neurosci 15:1063–1067. https://doi.org/10.1038/nn.3144.
19. Richardson JD, Vasko MR. 2002. Cellular mechanisms of neurogenic inflammation. J Pharmacol Exp Ther 302:839–845. https://doi.org/10.1124/jpet.102.032797.
20. Malin SA, Christianson JA, Bielefeldt K, Davis BM. 2009. TRPV1 expression defines functionally distinct pelvic colon afferents. J Neurosci 29:743–752. https://doi.org/10.1523/JNEUROSCI.3791-08.2009.
21. Jones RCW, Xu L, Gebhart GF. 2005. The mechanosensitivity of mouse colon afferent fibers and their sensitization by inflammatory mediators require transient receptor potential vanilloid 1 and acid-sensing ion channel 3. J Neurosci 25:10981–10989. https://doi.org/10.1523/JNEUROSCI.0703-05.2005.
22. Hockley JRF, Taylor TS, Callejo G, Wilbrey AL, Gutteridge A, Bach K, Winchester WJ, Bulmer DC, McMurray G, Smith E. 2019. Single-cell RNAseq reveals seven classes of colonic sensory neuron. Gut 68:633–644. https://doi.org/10.1136/gutjnl-2017-315631.
23. Ruff MR, Wahl SM, Pert CB. 1985. Substance P receptor-mediated chemotaxis of human monocytes. Peptides 6(Suppl 2):107–111. https://doi.org/10.1016/0196-9781(85)90142-1.
24. Saria A. 1984. Substance P in sensory nerve fibres contributes to the development of oedema in the rat hind paw after thermal injury. Br

J Pharmacol 82:217–222. https://doi.org/10.1111/j.1476-5381.1984.tb16461.x.
25. Brain SD, Williams TJ. 1989. Interactions between the tachykinins and calcitonin gene-related peptide lead to the modulation of oedema formation and blood flow in rat skin. Br J Pharmacol 97:77–82. https://doi.org/10.1111/j.1476-5381.1989.tb11926.x.
26. Brain SD. 1997. Sensory neuropeptides: their role in inflammation and wound healing. Immunopharmacology 37:133–152. https://doi.org/10.1016/s0162-3109(97)00055-6.
27. Brain SD, Williams TJ. 1985. Inflammatory oedema induced by synergism between calcitonin gene-related peptide (CGRP) and mediators of increased vascular permeability. Br J Pharmacol 86:855–860. https://doi.org/10.1111/j.1476-5381.1985.tb11107.x.
28. Buckley TL, Brain SD, Rampart M, Williams TJ. 1991. Time-dependent synergistic interactions between the vasodilator neuropeptide, calcitonin gene-related peptide (CGRP) and mediators of inflammation. Br J Pharmacol 103:1515–1519. https://doi.org/10.1111/j.1476-5381.1991.tb09819.x.
29. Boudard F, Bastide M. 1991. Inhibition of mouse T-cell proliferation by CGRP and VIP: effects of these neuropeptides on IL-2 production and cAMP synthesis. J Neurosci Res 29:29–41. https://doi.org/10.1002/jnr.490290104.
30. Qi J, Buzas K, Fan H, Cohen JI, Wang K, Mont E, Klinman D, Oppenheim JJ, Howard O. 2011. Painful pathways induced by TLR stimulation of dorsal root ganglion neurons. J Immunol 186:6417–6426. https://doi.org/10.4049/jimmunol.1001241.
31. Schaible HG. 2014. Nociceptive neurons detect cytokines in arthritis. Arthritis Res Ther 16:470. https://doi.org/10.1186/s13075-014-0470-8.
32. Oetjen LK, Mack MR, Feng J, Whelan TM, Niu H, Guo CJ, Chen S, Trier AM, Xu AZ, Tripathi SV, Luo J, Gao X, Yang L, Hamilton SL, Wang PL, Brestoff JR, Council ML, Brasington R, Schaffer A, Brombacher F, Hsieh CS, Gereau RWt, Miller MJ, Chen ZF, Hu H, Davidson S, Liu Q, Kim BS. 2017. Sensory neurons co-opt classical immune signaling pathways to mediate chronic itch. Cell 171:217–228.e13. https://doi.org/10.1016/j.cell.2017.08.006.
33. Cevikbas F, Wang X, Akiyama T, Kempkes C, Savinko T, Antal A, Kukova G, Buhl T, Ikoma A, Buddenkotte J, Soumelis V, Feld M, Alenius H, Dillon SR, Carstens E, Homey B, Basbaum A, Steinhoff M. 2014. A sensory neuron–expressed IL-31 receptor mediates T helper cell–dependent itch: involvement of TRPV1 and TRPA1. J Allergy Clin Immunol 133:448–460.e7. https://doi.org/10.1016/j.jaci.2013.10.048.
34. Boonen B, Alpizar YA, Sanchez A, López-Requena A, Voets T, Talavera K. 2018. Differential effects of lipopolysaccharide on mouse sensory TRP channels. Cell Calcium 73:72–81. https://doi.org/10.1016/j.ceca.2018.04.004.
35. Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, Miller JD, Davies SN, Geppetti P, Walker JM, Di Marzo V. 2002. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc Natl Acad Sci U S A 99:8400–8405. https://doi.org/10.1073/pnas.122196999.
36. Cao E, Cordero-Morales JF, Liu B, Qin F, Julius D. 2013. TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. Neuron 77:667–679. https://doi.org/10.1016/j.neuron.2012.12.016.
37. Chen J, Hamers AJP, Finsterbusch M, Massimo G, Zafar M, Corder R, Colas RA, Dalli J, Thiemermann C, Ahluwalia A. 2018. Endogenously generated arachidonate-derived ligands for TRPV1 induce cardiac protection in sepsis. FASEB J 32:3816–3831. https://doi.org/10.1096/fj.201701303R.
38. Borbiro I, Badheka D, Rohacs T. 2015. Activation of TRPV1 channels inhibits mechanosensitive Piezo channel activity by depleting membrane phosphoinositides. Sci Signal 8:ra15. https://doi.org/10.1126/scisignal.2005667.
39. Bertin S, Aoki-Nonaka Y, de Jong PR, Nohara LL, Xu H, Stanwood SR, Srikanth S, Lee J, To K, Abramson L, Yu T, Han T, Touma R, Li X, Gonzalez-Navajas JM, Herdman S, Corr M, Fu G, Dong H, Gwack Y, Franco A, Jefferies WA, Raz E. 2014. The ion channel TRPV1 regulates the activation and proinflammatory properties of CD4(+) T cells. Nat Immunol 15:1055–1063. https://doi.org/10.1038/ni.3009.
40. de Jong PR, Takahashi N, Harris AR, Lee J, Bertin S, Jeffries J, Jung M, Duong J, Triano AI, Lee J, Niv Y, Herdman DS, Taniguchi K, Kim C-W, Dong H, Eckmann L, Stanford SM, Bottini N, Corr M, Raz E. 2014. Ion channel TRPV1-dependent activation of PTP1B suppresses EGFR-associated intestinal tumorigenesis. J Clin Invest 124:3793–3806. https://doi.org/10.1172/JCI72340.
41. Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A. 2004. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41:849–857. https://doi.org/10.1016/s0896-6273(04)00150-3.
42. Macpherson LJ, Xiao B, Kwan KY, Petrus MJ, Dubin AE, Hwang S, Cravatt B, Corey DP, Patapoutian A. 2007. An ion channel essential for sensing chemical damage. J Neurosci 27:11412–11415. https://doi.org/10.1523/JNEUROSCI.3600-07.2007.
43. Trevisani M, Siemens J, Materazzi S, Bautista DM, Nassini R, Campi B, Imamachi N, Andrè E, Patacchini R, Cottrell GS, Gatti R, Basbaum AI, Bunnett NW, Julius D, Geppetti P. 2007. 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. Proc Natl Acad Sci U S A 104:13519–13524. https://doi.org/10.1073/pnas.0705923104.
44. Meseguer V, Alpizar YA, Luis E, Tajada S, Denlinger B, Fajardo O, Manenschijn J-A, Fernández-Peña C, Talavera A, Kichko T, Navia B, Sánchez A, Señarís R, Reeh P, Pérez-García MT, López-López JR, Voets T, Belmonte C, Talavera K, Viana F. 2014. TRPA1 channels mediate acute neurogenic inflammation and pain produced by bacterial endotoxins. Nat Commun 5:3125. https://doi.org/10.1038/ncomms4125.
45. Kamei J, Aizawa N, Nakagawa T, Kaneko S, Kume H, Homma Y, Igawa Y. 2018. Attenuated lipopolysaccharide-induced inflammatory bladder hypersensitivity in mice deficient of transient receptor potential ankilin1. Sci Rep 8:15622–15622. https://doi.org/10.1038/s41598-018-33967-x.
46. Caceres AI, Brackmann M, Elia MD, Bessac BF, del Camino D, D'Amours M, Witek JS, Fanger CM, Chong JA, Hayward NJ, Homer RJ, Cohn L, Huang X, Moran MM, Jordt S-E. 2009. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. Proc Natl Acad Sci U S A 106:9099–9104. https://doi.org/10.1073/pnas.0900591106.
47. Ramachandran R, Hyun E, Zhao L, Lapointe TK, Chapman K, Hirota CL, Ghosh S, McKemy DD, Vergnolle N, Beck PL, Altier C, Hollenberg MD. 2013. TRPM8 activation attenuates inflammatory responses in mouse models of colitis. Proc Natl Acad Sci U S A 110:7476–7481. https://doi.org/10.1073/pnas.1217431110.
48. Khalil M, Babes A, Lakra R, Försch S, Reeh PW, Wirtz S, Becker C, Neurath MF, Engel MA. 2016. Transient receptor potential melastatin 8 ion channel in macrophages modulates colitis through a balance-shift in TNF-alpha and interleukin-10 production. Mucosal Immunol 9:1500–1513. https://doi.org/10.1038/mi.2016.16.
49. Szolcsanyi J, Szallasi A, Szallasi Z, Joo F, Blumberg PM. 1990. Resiniferatoxin: an ultrapotent selective modulator of capsaicin-sensitive primary afferent neurons. J Pharmacol Exp Ther 255:923–928.
50. Blake KJ, Baral P, Voisin T, Lubkin A, Pinho-Ribeiro FA, Adams KL, Roberson DP, Ma YC, Otto M, Woolf CJ, Torres VJ, Chiu IM. 2018. Staphylococcus aureus produces pain through pore-forming toxins and neuronal TRPV1 that is silenced by QX-314. Nat Commun 9:37. https://doi.org/10.1038/s41467-017-02448-6.
51. Stucchi AF, Shofer S, Leeman S, Materne O, Beer E, McClung J, Shebani K, Moore F, O'Brien M, Becker JM. 2000. NK-1 antagonist reduces colonic inflammation and oxidative stress in dextran sulfate-induced colitis in rats. Am J Physiol Gastrointest Liver Physiol 279:G1298–G1306. https://doi.org/10.1152/ajpgi.2000.279.6.G1298.
52. Weinstock JV, Blum A, Metwali A, Elliott D, Bunnett N, Arsenescu R. 2003. Substance P regulates Th1-type colitis in IL-10 knockout mice. J Immunol 171:3762–3767. https://doi.org/10.4049/jimmunol.171.7.3762.
53. Kincy-Cain T, Bost KL. 1996. Increased susceptibility of mice to Salmonella infection following in vivo treatment with the substance P antagonist, spantide II. J Immunol 157:255–264.
54. Ho WZ, Lai JP, Zhu XH, Uvaydova M, Douglas SD. 1997. Human monocytes and macrophages express substance P and neurokinin-1 receptor. J Immunol 159:5654–5660.
55. Castagliuolo I, Keates AC, Qiu B, Kelly CP, Nikulasson S, Leeman SE, Pothoulakis C. 1997. Increased substance P responses in dorsal root ganglia and intestinal macrophages during Clostridium difficile toxin A enteritis in rats. Proc Natl Acad Sci U S A 94:4788–4793. https://doi.org/10.1073/pnas.94.9.4788.
56. Lai JP, Douglas SD, Ho WZ. 1998. Human lymphocytes express substance P and its receptor. J Neuroimmunol 86:80–86. https://doi.org/10.1016/s0165-5728(98)00025-3.
57. Lai NY, Musser MA, Pinho-Ribeiro FA, Baral P, Jacobson A, Ma P, Potts DE, Chen Z, Paik D, Soualhi S, Yan Y, Misra A, Goldstein K, Lagomarsino

VN, Nordstrom A, Sivanathan KN, Wallrapp A, Kuchroo VK, Nowarski R, Starnbach MN, Shi H, Surana NK, An D, Wu C, Huh JR, Rao M, Chiu IM. 2020. Gut-innervating nociceptor neurons regulate Peyer's patch microfold cells and SFB levels to mediate Salmonella host defense. Cell 180:33–49.e22. https://doi.org/10.1016/j.cell.2019.11.014.
58. Mabbott NA, Donaldson DS, Ohno H, Williams IR, Mahajan A. 2013. Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium. Mucosal Immunol 6:666–677. https://doi.org/10.1038/mi.2013.30.
59. Katz DM, Karten HJ. 1980. Substance P in the vagal sensory ganglia: localization in cell bodies and pericellular arborizations. J Comp Neurol 193:549–564. https://doi.org/10.1002/cne.901930216.
60. Hayakawa T, Kuwahara-Otani S, Maeda S, Tanaka K, Seki M. 2011. Projections of calcitonin gene-related peptide immunoreactive neurons in the vagal ganglia of the rat. J Chem Neuroanat 41:55–62. https://doi.org/10.1016/j.jchemneu.2010.11.003.
61. Mulderry PK, Ghatei MA, Spokes RA, Jones PM, Pierson AM, Hamid QA, Kanse S, Amara SG, Burrin JM, Legon S. 1988. Differential expression of alpha-CGRP and beta-CGRP by primary sensory neurons and enteric autonomic neurons of the rat. Neuroscience 25:195–205. https://doi.org/10.1016/0306-4522(88)90018-8.
62. Ramirez VT, Sladek J, Godinez DR, Rude KM, Chicco P, Murray K, Brust-Mascher I, Gareau MG, Reardon C. 2020. Sensory nociceptive neurons contribute to host protection during enteric infection with Citrobacter rodentium. J Infect Dis https://doi.org/10.1093/infdis/jiaa014.
63. Doods H, Hallermayer G, Wu D, Entzeroth M, Rudolf K, Engel W, Eberlein W. 2000. Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. Br J Pharmacol 129:420–423. https://doi.org/10.1038/sj.bjp.0703110.
64. Glowka TR, Steinebach A, Stein K, Schwandt T, Lysson M, Holzmann B, Tsujikawa K, de Jonge WJ, Kalff JC, Wehner S. 2015. The novel CGRP receptor antagonist BIBN4096BS alleviates a postoperative intestinal inflammation and prevents postoperative ileus. Neurogastroenterol Motil 27:1038–1049. https://doi.org/10.1111/nmo.12584.
65. O'Hara JR, Skinn AC, MacNaughton WK, Sherman PM, Sharkey KA. 2006. Consequences of Citrobacter rodentium infection on enteroendocrine cells and the enteric nervous system in the mouse colon. Cell Microbiol 8:646–660. https://doi.org/10.1111/j.1462-5822.2005.00657.x.
66. Chakraborty S, Nepiyushchikh Z, Davis MJ, Zawieja DC, Muthuchamy M. 2011. Substance P activates both contractile and inflammatory pathways in lymphatics through the neurokinin receptors NK1R and NK3R. Microcirculation 18:24–35. https://doi.org/10.1111/j.1549-8719.2010.00064.x.
67. Rayner SE, Van Helden DF. 1997. Evidence that the substance P-induced enhancement of pacemaking in lymphatics of the guinea-pig mesentery occurs through endothelial release of thromboxane A2. Br J Pharmacol 121:1589–1596. https://doi.org/10.1038/sj.bjp.0701306.
68. Moore TC, Lami JL, Spruck CH. 1989. Substance P increases lymphocyte traffic and lymph flow through peripheral lymph nodes of sheep. Immunology 67:109–114.
69. Hanes WM, Olofsson PS, Talbot S, Tsaava T, Ochani M, Imperato GH, Levine YA, Roth J, Pascal MA, Foster SL, Wang P, Woolf C, Chavan SS, Tracey KJ. 2016. Neuronal circuits modulate antigen flow through lymph nodes. Bioelectron Med 3:18–28. https://doi.org/10.15424/bioelectronmed.2016.00001.
70. Hukkanen M, Konttinen Y, Terenghi G, Polak JM. 1992. Peptide-containing innervation of rat femoral lymphatic vessels. Microvasc Res 43:7–19. https://doi.org/10.1016/0026-2862(92)90003-8.
71. Hosaka K, Rayner SE, von der Weid PY, Zhao J, Imtiaz MS, van Helden DF. 2006. Calcitonin gene-related peptide activates different signaling pathways in mesenteric lymphatics of guinea pigs. Am J Physiol Heart Circ Physiol 290:H813–22. https://doi.org/10.1152/ajpheart.00543.2005.
72. Kurkowski R, Kummer W, Heym C. 1990. Substance P-immunoreactive nerve fibers in tracheobronchial lymph nodes of the guinea pig: origin, ultrastructure and coexistence with other peptides. Peptides 11:13–20. https://doi.org/10.1016/0196-9781(90)90103-c.
73. Lorton D, Bellinger DL, Felten SY, Felten DL. 1991. Substance P innervation of spleen in rats: nerve fibers associate with lymphocytes and macrophages in specific compartments of the spleen. Brain Behav Immun 5:29–40. https://doi.org/10.1016/0889-1591(91)90005-u.
74. Fink T, Weihe E. 1988. Multiple neuropeptides in nerves supplying mammalian lymph nodes: messenger candidates for sensory and autonomic neuroimmunomodulation? Neurosci Lett 90:39–44. https://doi.org/10.1016/0304-3940(88)90783-5.
75. Gautron L, Sakata I, Udit S, Zigman JM, Wood JN, Elmquist JK. 2011. Genetic tracing of Nav1.8-expressing vagal afferents in the mouse. J Comp Neurol 519:3085–3101. https://doi.org/10.1002/cne.22667.
76. Rodda LB, Lu E, Bennett ML, Sokol CL, Wang X, Luther SA, Barres BA, Luster AD, Ye CJ, Cyster JG. 2018. Single-cell RNA sequencing of lymph node stromal cells reveals niche-associated heterogeneity. Immunity 48:1014–1028.e6. https://doi.org/10.1016/j.immuni.2018.04.006.
77. Riley TP, Neal-McKinney JM, Buelow DR, Konkel ME, Simasko SM. 2013. Capsaicin-sensitive vagal afferent neurons contribute to the detection of pathogenic bacterial colonization in the gut. J Neuroimmunol 257:36–45. https://doi.org/10.1016/j.jneuroim.2013.01.009.
78. Worthington JJ, Reimann F, Gribble FM. 2018. Enteroendocrine cells—sensory sentinels of the intestinal environment and orchestrators of mucosal immunity. Mucosal Immunol 11:3–20. https://doi.org/10.1038/mi.2017.73.
79. Reardon C, Murray K, Lomax AE. 2018. Neuroimmune communication in health and disease. Physiol Rev 98:2287–2316. https://doi.org/10.1152/physrev.00035.2017.
80. Matteoli G, Gomez-Pinilla PJ, Nemethova A, Di Giovangiulio M, Cailotto C, van Bree SH, Michel K, Tracey KJ, Schemann M, Boesmans W, Vanden Berghe P, Boeckxstaens GE. 2014. A distinct vagal anti-inflammatory pathway modulates intestinal muscularis resident macrophages independent of the spleen. Gut 63:938–948. https://doi.org/10.1136/gutjnl-2013-304676.
81. Murray K, Barboza M, Rude KM, Brust-Mascher I, Reardon C. 2019. Functional circuitry of neuro-immune communication in the mesenteric lymph node and spleen. Brain Behav Immun 82:214–223. https://doi.org/10.1016/j.bbi.2019.08.188.
82. Rosas-Ballina M, Olofsson PS, Ochani M, Valdés-Ferrer SI, Levine YA, Reardon C, Tusche MW, Pavlov VA, Andersson U, Chavan S, Mak TW, Tracey KJ. 2011. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science 334:98–101. https://doi.org/10.1126/science.1209985.
83. Dhawan S, De Palma G, Willemze RA, Hilbers F, Verseijden C, Luyer M, Nuding S, Wehkamp J, Souwer Y, de Jong EC, Seppen J, van den Wijngaard RM, Wehner S, Verdu EF, Bercik P, de Jonge WJ. 2016. Acetylcholine producing T-cells in the intestine affect antimicrobial peptide expression and microbial diversity. Am J Physiol Gastrointest Liver Physiol 311:G920–G933. https://doi.org/10.1152/ajpgi.00114.2016.
84. Ramirez VT, Godinez DR, Brust-Mascher I, Nonnecke EB, Castillo PA, Gardner MB, Tu D, Sladek JA, Miller EN, Lebrilla CB, Bevins CL, Gareau MG, Reardon C. 2019. T-cell derived acetylcholine aids host defenses during enteric bacterial infection with Citrobacter rodentium. PLoS Pathog 15:e1007719. https://doi.org/10.1371/journal.ppat.1007719.
85. Serhan CN. 2014. Pro-resolving lipid mediators are leads for resolution physiology. Nature 510:92–101. https://doi.org/10.1038/nature13479.
86. Basil MC, Levy BD. 2016. Specialized pro-resolving mediators: endogenous regulators of infection and inflammation. Nat Rev Immunol 16:51–67. https://doi.org/10.1038/nri.2015.4.
87. Dalli J, Colas RA, Arnardottir H, Serhan CN. 2017. Vagal regulation of group 3 innate lymphoid cells and the immunoresolvent PCTR1 controls infection resolution. Immunity 46:92–105. https://doi.org/10.1016/j.immuni.2016.12.009.
88. Ramon S, Dalli J, Sanger JM, Winkler JW, Aursnes M, Tungen JE, Hansen TV, Serhan CN. 2016. The protectin PCTR1 is produced by human M2 macrophages and enhances resolution of infectious inflammation. Am J Pathol 186:962–973. https://doi.org/10.1016/j.ajpath.2015.12.012.
89. Rankin LC, Girard-Madoux MJ, Seillet C, Mielke LA, Kerdiles Y, Fenis A, Wieduwild E, Putoczki T, Mondot S, Lantz O, Demon D, Papenfuss AT, Smyth GK, Lamkanfi M, Carotta S, Renauld JC, Shi W, Carpentier S, Soos T, Arendt C, Ugolini S, Huntington ND, Belz GT, Vivier E. 2016. Complementarity and redundancy of IL-22-producing innate lymphoid cells. Nat Immunol 17:179–186. https://doi.org/10.1038/ni.3332.
90. Reardon C, Duncan GS, Brustle A, Brenner D, Tusche MW, Olofsson P, Rosas-Ballina M, Tracey KJ, Mak TW. 2013. Lymphocyte-derived ACh regulates local innate but not adaptive immunity. Proc Natl Acad Sci U S A 110:1410–1415. https://doi.org/10.1073/pnas.1221655110.
91. Komegae EN, Farmer DGS, Brooks VL, McKinley MJ, McAllen RM, Martelli D. 2018. Vagal afferent activation suppresses systemic inflammation via the splanchnic anti-inflammatory pathway. Brain Behav Immun 73:441–449. https://doi.org/10.1016/j.bbi.2018.06.005.
92. Vida G, Pena G, Deitch EA, Ulloa L. 2011. alpha7-cholinergic receptor mediates vagal induction of splenic norepinephrine. J Immunol 186:4340–4346. https://doi.org/10.4049/jimmunol.1003722.
93. Wong CHY, Jenne CN, Lee W-Y, Léger C, Kubes P. 2011. Functional

innervation of hepatic iNKT cells is immunosuppressive following stroke. Science 334:101–105. https://doi.org/10.1126/science.1210301.
94. Gabanyi I, Muller PA, Feighery L, Oliveira Thiago Y, Costa-Pinto Frederico A, Mucida D. 2016. Neuro-immune interactions drive tissue programming in intestinal macrophages. Cell 164:378–391. https://doi.org/10.1016/j.cell.2015.12.023.
95. Matheis F, Muller PA, Graves CL, Gabanyi I, Kerner ZJ, Costa-Borges D, Ahrends T, Rosenstiel P, Mucida D. 2020. Adrenergic signaling in muscularis macrophages limits infection-induced neuronal loss. Cell 180:64–78.e16. https://doi.org/10.1016/j.cell.2019.12.002.
96. Ben-Shaanan TL, Azulay-Debby H, Dubovik T, Starosvetsky E, Korin B, Schiller M, Green NL, Admon Y, Hakim F, Shen-Orr SS, Rolls A. 2016. Activation of the reward system boosts innate and adaptive immunity. Nat Med 22:940–944. https://doi.org/10.1038/nm.4133.
97. Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V. 2006. The QseC sensor kinase: a bacterial adrenergic receptor. Proc Natl Acad Sci U S A 103:10420–10425. https://doi.org/10.1073/pnas.0604343103.
98. Moreira CG, Russell R, Mishra AA, Narayanan S, Ritchie JM, Waldor MK, Curtis MM, Winter SE, Weinshenker D, Sperandio V. 2016. Bacterial adrenergic sensors regulate virulence of enteric pathogens in the gut. mBio 7:e00826-16. https://doi.org/10.1128/mBio.00826-16.
99. Lyte M, Arulanandam B, Nguyen K, Frank C, Erickson A, Francis D. 1997. Norepinephrine induced growth and expression of virulence associated factors in enterotoxigenic and enterohemorrhagic strains of Escherichia coli. Adv Exp Med Biol 412:331–339. https://doi.org/10.1007/978-1-4899-1828-4_54.
100. Furness JB. 2012. The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol 9:286–294. https://doi.org/10.1038/nrgastro.2012.32.
101. Schneider S, Wright CM, Heuckeroth RO. 2019. Unexpected roles for the second brain: enteric nervous system as master regulator of bowel function. Annu Rev Physiol 81:235–259. https://doi.org/10.1146/annurev-physiol-021317-121515.
102. Jarret A, Jackson R, Duizer C, Healy ME, Zhao J, Rone JM, Bielecki P, Sefik E, Roulis M, Rice T, Sivanathan KN, Zhou T, Solis AG, Honcharova-Biletska H, Vélez K, Hartner S, Low JS, Qu R, de Zoete MR, Palm NW, Ring AM, Weber A, Moor AE, Kluger Y, Nowarski R, Flavell RA. 2020. Enteric nervous system-derived IL-18 orchestrates mucosal barrier immunity. Cell 180:50–63.e12. https://doi.org/10.1016/j.cell.2019.12.016.
103. Furness JB, Jones C, Nurgali K, Clerc N. 2004. Intrinsic primary afferent neurons and nerve circuits within the intestine. Prog Neurobiol 72:143–164. https://doi.org/10.1016/j.pneurobio.2003.12.004.
104. Zeisel A, Hochgerner H, Lönnerberg P, Johnsson A, Memic F, van der Zwan J, Häring M, Braun E, Borm LE, La Manno G, Codeluppi S, Furlan A, Lee K, Skene N, Harris KD, Hjerling-Leffler J, Arenas E, Ernfors P, Marklund U, Linnarsson S. 2018. Molecular architecture of the mouse nervous system. Cell 174:999–1014.e22. https://doi.org/10.1016/j.cell.2018.06.021.
105. Talbot J, Hahn P, Kroehling L, Nguyen H, Li D, Littman DR. 2020. Feeding-dependent VIP neuron–ILC3 circuit regulates the intestinal barrier. Nature 579:575–580. https://doi.org/10.1038/s41586-020-2039-9.
106. Seillet C, Luong K, Tellier J, Jacquelot N, Shen RD, Hickey P, Wimmer VC, Whitehead L, Rogers K, Smyth GK, Garnham AL, Ritchie ME, Belz GT. 2020. The neuropeptide VIP confers anticipatory mucosal immunity by regulating ILC3 activity. Nat Immunol 21:168–177. https://doi.org/10.1038/s41590-019-0567-y.
107. Roth BL. 2016. DREADDs for neuroscientists. Neuron 89:683–694. https://doi.org/10.1016/j.neuron.2016.01.040.
108. Urban DJ, Roth BL. 2015. DREADDs (designer receptors exclusively activated by designer drugs): chemogenetic tools with therapeutic utility. Annu Rev Pharmacol Toxicol 55:399–417. https://doi.org/10.1146/annurev-pharmtox-010814-124803.
109. Sawmiller DR, Henning RJ. 2006. Vasoactive intestinal peptide, p 1215–1222. *In* Kastin AJ (ed), Handbook of biologically active peptides Academic Press, Burlington, MA.
110. Liu D, Stowie A, de Zavalia N, Leise T, Pathak SS, Drewes LR, Davidson AJ, Amir S, Sonenberg N, Cao R. 2018. mTOR signaling in VIP neurons regulates circadian clock synchrony and olfaction. Proc Natl Acad Sci U S A 115:E3296–E3304. https://doi.org/10.1073/pnas.1721578115.
111. Haspel JA, Anafi R, Brown MK, Cermakian N, Depner C, Desplats P, Gelman AE, Haack M, Jelic S, Kim BS, Laposky AD, Lee YC, Mongodin E, Prather AA, Prendergast BJ, Reardon C, Shaw AC, Sengupta S, Szentirmai É, Thakkar M, Walker WE, Solt LA. 2020. Perfect timing: circadian rhythms, sleep, and immunity—an NIH workshop summary. JCI Insight 5:e131487. https://doi.org/10.1172/jci.insight.131487.
112. Ibiza S, García-Cassani B, Ribeiro H, Carvalho T, Almeida L, Marques R, Misic AM, Bartow-McKenney C, Larson DM, Pavan WJ, Eberl G, Grice EA, Veiga-Fernandes H. 2016. Glial-cell-derived neuroregulators control type 3 innate lymphoid cells and gut defence. Nature 535:440–443. https://doi.org/10.1038/nature18644.