Neuro-immune Interactions in the Tissues

Coco Chu,1 David Artis,1,2,* and Isaac M. Chiu3,*
1Jill Roberts Institute for Research in Inflammatory Bowel Disease, Weill Cornell Medicine, Cornell University, New York, NY, USA
2Friedman Center for Nutrition and Inflammation, Joan and Sanford I. Weill Department of Medicine, Department of Microbiology and Immunology, Weill Cornell Medicine, Cornell University, New York, NY, USA
3Department of Immunology, Harvard Medical School, Boston, MA, USA
*Correspondence: dartis@med.cornell.edu (D.A.), isaac_chiu@hms.harvard.edu (I.M.C.)

https://doi.org/10.1016/j.immuni.2020.02.017

The ability of the nervous system to sense environmental stimuli and to relay these signals to immune cells via neurotransmitters and neuropeptides is indispensable for effective immunity and tissue homeostasis. Depending on the tissue microenvironment and distinct drivers of a certain immune response, the same neuronal populations and neuro-mediators can exert opposing effects, promoting or inhibiting tissue immu-

The immune system is composed of a diverse array of immune cells including innate and adaptive lymphocytes and myeloid cells. This system can directly sense internal and environ-

mental stimuli, and it participates in a wide variety of physi-

ological processes in tissues, including host defense against pathogens, interactions with the microbiota at barrier sur-

faces, and maintenance of tissue homeostasis (Belkaid and Hand, 2014; Rankin and Artis, 2018). However, excessive im-

mune responses can lead to chronic inflammation and autoim-

mune diseases (Pahwa et al., 2020; Rose and Mackay, 2019). Tissues and organs are also densely innervated by distinct branches of the nervous system that, like the immune system, directly sense and respond rapidly to environmental cues. The immune and nervous systems interact at various levels during embryonic development, in homeostasis, and in disease. For example, neurotransmitters and neuropeptides can directly impact immune cell function, including the regulation of immune responses to pathogens and tissue damage (Baral et al., 2019; Godinho-Silva et al., 2019a; Huh and Veiga-Fernandes, 2019; Klose and Artis, 2019; Veiga-Fernandes and Mucida, 2016). How neuro-immune interactions are estab-

lished and maintained in different tissues and the specific cellular interactions that underlie immune and homeostatic re-

sponses therein are important areas of investigation.

Recent technological developments, including novel trans-

genic mouse strains and in vivo neuronal ablation or activation techniques like optogenetics and chemogenetics, are enabling a deeper examination of the cellular and molecular mechanisms that underlie neuro-immune interactions in the context of health and disease. Here, we critically review recent advances in the understanding of neuronal regulation of host defense, inflamma-

tion, and homeostasis in peripheral tissues. We argue for the importance of considering infectious and inflammatory disease within a conceptual framework that integrates neuro-immune circuits both local and systemic, so as to better understand effective immunity and contexts of pathology and develop improved approaches to treat inflammation and disease.

The Peripheral Nervous System in Neuro-immune Crosstalk

The nervous system is organized as the central nervous system (CNS), composed of the brain and spinal cord, and the peripheral nervous system (PNS). The PNS is divided into the somatosen-

sory and autonomic systems. Every division of the PNS is able to communicate with immune cells, and immune cells express receptors for many classes of neurotransmitters, including cate-

cholamines, gamma-aminobutyric acid (GABA), acetylcholine, and neuropeptides (e.g., calcitonin gene-related peptide [CGRP], substance P [SP], vasoactive intestinal peptide [VIP], and neumedin U [NMU]) (Godinho-Silva et al., 2019a).

The somatosensory nervous system detects environmental and internal stimuli. The cell bodies of somatosensory neurons reside within the dorsal root ganglia (DRG) and trigeminal ganglia (TG), mediating touch, thermoception, proprioception, itch, and pain. Noceceptors are specialized somatosensory neurons that respond to noxious and/or injurious stimuli including intense heat, mechanical injury, and inflammatory mediators (Abraira and Ginty, 2013; Basbaum et al., 2009). Because nociceptors contain dense-core vesicles storing neuropeptides not only in their synaptic terminals at the CNS but also in their nerve endings within the peripheral tis-

sues, they are simultaneously equipped to inform the CNS about the presence of a noxious stimulus and to modulate immune cell responses at the tissue that is receiving the stimulus.

The autonomic system consists of the parasympathetic, sym-

pathetic, and enteric nervous systems. Parasympathetic neu-

rons mainly exit the brain through the vagus nerve, sending efferent signals to visceral organs including the heart, lungs, and intestine via the neurotransmitter acetylcholine (ACh). The vagus nerve is bi-directional and also carries sensory information from visceral organs to the CNS via the nodose and jugular ganglia (Chang et al., 2015; Umans and Liberles, 2018; Williams et al., 2016). Vagal afferent sensing of intestinal contents and activation of vagal efferent neurons that signal back to the intestine are key components of the “gut-brain axis.” Sympa-

thetic neurons mediate the body’s “fight or flight” response.
Sensory and autonomic neurons that mediate cough and sneezing are another example of the interactions between the nervous system and immune system. Neurons in the respiratory tract can detect bacterial and viral pathogens through the production of cytokines and chemokines. This activation of the immune system can lead to increased mucus production and coughing, which helps to clear the airways of pathogens.

In addition to the innate immune response, the nervous system also coordinates the adaptive immune response by communicating with lymphoid tissues. Neurons in the thoracic duct can detect bacterial and viral antigens and signal to lymphoid organs to mount an immune response. This coordination is critical for effective immunization and the development of memory immune responses.

The nervous system is also involved in the regulation of the immune system through the production of cytokines. Neurons in the hypothalamus can produce cytokines that can modulate the immune response and influence immune cell function. This neuro-immune communication is critical for the regulation of immune responses and the maintenance of immune homeostasis.

In conclusion, the nervous system and immune system are closely integrated and work together to maintain homeostasis. The coordinated action of these two systems is critical for the detection and response to external threats, and the proper functioning of the immune system is essential for the proper functioning of the nervous system. Therefore, a deep understanding of the interactions between the nervous system and immune system is critical for the development of effective immunotherapies and the treatment of immune diseases.

---

**Neuro-immune Interactions in Host Defense**

The nervous system is poised to detect and respond to external threats, including invasion by bacterial, fungal, viral, and parasitic pathogens. Neurons can directly sense pathogenic ligands and rapidly communicate with macrophages, neutrophils, dendritic cells (DCs), and innate lymphoid cells (ILCs) to modulate antimicrobial responses (Figure 1). Neuro-immune interactions orchestrate the response to bacterial infections within several major barrier tissues, including the skin, the lung, and the intestinal tract.

In the skin, cutaneous nerves play an integral role in modulating bacterial host defenses. During Staphylococcus aureus skin infections, Nav1.8+ and TRPV1+ nociceptor neurons directly perceive bacterial host defenses. During Streptococcus pyogenes skin infections, Nav1.8+ and TRPV1+ nociceptor neurons directly sense bacteria through detection of N-formylated peptides and the pore-forming toxin α-hemolysin (Chiu et al., 2013). On the one hand, these nociceptors signal to the CNS to produce the unpleasant sensation of pain (Blake et al., 2018). Concurrently, nociceptors secrete neuropeptides from their peripheral nerve terminals that regulates immunity. In S. aureus infection, nociceptors release the neuropeptide CGRP in the skin, which inhibits macrophage production of tumor necrosis factor (TNF)-α, reduces monocyte influx, and suppresses draining lymph node hyper trophy (Chiu et al., 2013).

In the lungs, the sensory and autonomic systems are coordinated through neural reflex circuits that rapidly respond to changes and regulate immunity. A major neural circuit that modulates immune responses is discovered and coined the cholinergic “anti-inflammatory reflex” by Tracey and colleagues (Pavlov et al., 2018; Tracey, 2002). In this reflex, peripheral inflammation is sensed by vagal sensory afferent neurons, activating a brainstem circuit that leads to decreased peripheral cytokine production via vagal efferent neuron signaling. At a tissue level, nociceptor neurons utilize local axonal reflexes—reflexes in a single neuron, from one afferent nerve ending to an axon bifurcation and propagated to another nerve ending—to rapidly respond to danger and release neuropeptides that signal to the vasculature and to immune cells (Baral et al., 2019; Pinho-Ribeiro et al., 2017). Thus, the PNS can integrate responses to challenges at a tissue and systemic level and can coordinate with immune responses accordingly.
bronchoconstriction. Neuro-immune crosstalk also regulates pulmonary bacterial host defenses. Vagal nociceptor neurons suppress γδ T cell and neutrophil responses during lethal bacterial lung infections (Baral et al., 2018). In methicillin-resistant S. aureus (MRSA) pneumonia, ablation of TRPV1+ vagal sensory neurons leads to improved survival, core body temperature maintenance, and bacterial clearance. TRPV1+ neurons block the ability of neutrophils to infiltrate the lungs and survey the parenchyma for pathogens. TRPV1+ neurons also regulate lung homeostatic levels of γδ T cells, an important source of interleukin (IL)-17, to protect against MRSA infection. Treatment of mice with resiniferatoxin (RTX), a chemical that ablates TRPV1+ neurons, or blockade of CGRP signaling with a peptide antagonist significantly enhances survival and bacterial clearance. Therefore, nociceptor neurons suppress local immunity in respiratory tract infections, and targeting this signaling could lead to novel treatments for bacterial pneumonia.

The intestine is densely innervated and constantly exposed to microbial stimuli. Gut-innervating neurons and gut-resident enteric neurons also actively participate in host defense. Recent work has found that enteric neurons in the myenteric plexus are a major source of IL-18, which drives host protection against the enteric pathogen Salmonella enterica serovar Typhimurium (Jarret et al., 2020). Enteric neuron-derived IL-18 acts on intestinal goblet cells to induce the expression of antimicrobial peptides (AMPs) in the colon to protect against Salmonella infection. Gut-innervating nociceptor neurons (TRPV1+ Nav1.8+) also defend against Salmonella infection by crosstalk with epithelial cells and the intestinal microbiota (Lai et al., 2020). Salmonella invade the small intestine through intestinal microfold (M) cells, which are specialized epithelial cells within Peyer’s patch (PP) follicle-associated epithelium (FAE). Nociceptor neurons signal via CGRP to reduce the numbers of M cells, thus removing the gates of entry for these pathogens. Nociceptor signaling also maintains levels of segmented filamentous bacteria (SFB) in the small intestine—an intestine-resident microbe that provides resistance against Salmonella colonization and invasion. Gut-innervating tyrosine hydroxylase-expressing (TH+) sympathetic neurons also play a key role in host defense within the intestine. During homeostasis, TH+ neurons signal to resident muscularis macrophages (MMs) through the beta-2 adrenergic receptor (β2AR) to polarize the MMs toward a tissue-protective M2-like phenotype, which acts via the beta-2 adrenergic receptor (β2AR) on muscularis macrophages to polarize a M2 phenotype, which prevents cell loss in bacterial infection. Gut-innervating sympathetic neurons also act on ILC2 via β2AR to modulate type 2 cytokine production and helminth expulsion.

The intestine is densely innervated and constantly exposed to microbial stimuli. Gut-innervating neurons and gut-resident enteric neurons also actively participate in host defense. Recent work has found that enteric neurons in the myenteric plexus are a major source of IL-18, which drives host protection against the enteric pathogen Salmonella enterica serovar Typhimurium (Jarret et al., 2020). Enteric neuron-derived IL-18 acts on intestinal goblet cells to induce the expression of antimicrobial peptides (AMPs) in the colon to protect against Salmonella infection. Gut-innervating nociceptor neurons (TRPV1+ Nav1.8+) also defend against Salmonella infection by crosstalk with epithelial cells and the intestinal microbiota (Lai et al., 2020). Salmonella invade the small intestine through intestinal microfold (M) cells, which are specialized epithelial cells within Peyer’s patch (PP) follicle-associated epithelium (FAE). Nociceptor neurons signal via CGRP to reduce the numbers of M cells, thus removing the gates of entry for these pathogens. Nociceptor signaling also maintains levels of segmented filamentous bacteria (SFB) in the small intestine—an intestine-resident microbe that provides resistance against Salmonella colonization and invasion. Gut-innervating tyrosine hydroxylase-expressing (TH+) sympathetic neurons also play a key role in host defense within the intestine. During homeostasis, TH+ neurons signal to resident muscularis macrophages (MMs) through the beta-2 adrenergic receptor (β2AR) to polarize the MMs toward a tissue-protective M2-like phenotype, which acts via the beta-2 adrenergic receptor (β2AR) on muscularis macrophages to polarize a M2 phenotype, which prevents cell loss in bacterial infection. Gut-innervating sympathetic neurons also act on ILC2 via β2AR to modulate type 2 cytokine production and helminth expulsion.

The intestine is densely innervated and constantly exposed to microbial stimuli. Gut-innervating neurons and gut-resident enteric neurons also actively participate in host defense. Recent work has found that enteric neurons in the myenteric plexus are a major source of IL-18, which drives host protection against the enteric pathogen Salmonella enterica serovar Typhimurium (Jarret et al., 2020). Enteric neuron-derived IL-18 acts on intestinal goblet cells to induce the expression of antimicrobial peptides (AMPs) in the colon to protect against Salmonella infection. Gut-innervating nociceptor neurons (TRPV1+ Nav1.8+) also defend against Salmonella infection by crosstalk with epithelial cells and the intestinal microbiota (Lai et al., 2020). Salmonella invade the small intestine through intestinal microfold (M) cells, which are specialized epithelial cells within Peyer’s patch (PP) follicle-associated epithelium (FAE). Nociceptor neurons signal via CGRP to reduce the numbers of M cells, thus removing the gates of entry for these pathogens. Nociceptor signaling also maintains levels of segmented filamentous bacteria (SFB) in the small intestine—an intestine-resident microbe that provides resistance against Salmonella colonization and invasion. Gut-innervating tyrosine hydroxylase-expressing (TH+) sympathetic neurons also play a key role in host defense within the intestine. During homeostasis, TH+ neurons signal to resident muscularis macrophages (MMs) through the beta-2 adrenergic receptor (β2AR) to polarize the MMs toward a tissue-protective M2-like phenotype, which acts via the beta-2 adrenergic receptor (β2AR) on muscularis macrophages to polarize a M2 phenotype, which prevents cell loss in bacterial infection. Gut-innervating sympathetic neurons also act on ILC2 via β2AR to modulate type 2 cytokine production and helminth expulsion.

The intestine is densely innervated and constantly exposed to microbial stimuli. Gut-innervating neurons and gut-resident enteric neurons also actively participate in host defense. Recent work has found that enteric neurons in the myenteric plexus are a major source of IL-18, which drives host protection against the enteric pathogen Salmonella enterica serovar Typhimurium (Jarret et al., 2020). Enteric neuron-derived IL-18 acts on intestinal goblet cells to induce the expression of antimicrobial peptides (AMPs) in the colon to protect against Salmonella infection. Gut-innervating nociceptor neurons (TRPV1+ Nav1.8+) also defend against Salmonella infection by crosstalk with epithelial cells and the intestinal microbiota (Lai et al., 2020). Salmonella invade the small intestine through intestinal microfold (M) cells, which are specialized epithelial cells within Peyer’s patch (PP) follicle-associated epithelium (FAE). Nociceptor neurons signal via CGRP to reduce the numbers of M cells, thus removing the gates of entry for these pathogens. Nociceptor signaling also maintains levels of segmented filamentous bacteria (SFB) in the small intestine—an intestine-resident microbe that provides resistance against Salmonella colonization and invasion. Gut-innervating tyrosine hydroxylase-expressing (TH+) sympathetic neurons also play a key role in host defense within the intestine. During homeostasis, TH+ neurons signal to resident muscularis macrophages (MMs) through the beta-2 adrenergic receptor (β2AR) to polarize the MMs toward a tissue-protective M2-like phenotype, which acts via the beta-2 adrenergic receptor (β2AR) on muscularis macrophages to polarize a M2 phenotype, which prevents cell loss in bacterial infection. Gut-innervating sympathetic neurons also act on ILC2 via β2AR to modulate type 2 cytokine production and helminth expulsion.

The intestine is densely innervated and constantly exposed to microbial stimuli. Gut-innervating neurons and gut-resident enteric neurons also actively participate in host defense. Recent work has found that enteric neurons in the myenteric plexus are a major source of IL-18, which drives host protection against the enteric pathogen Salmonella enterica serovar Typhimurium (Jarret et al., 2020). Enteric neuron-derived IL-18 acts on intestinal goblet cells to induce the expression of antimicrobial peptides (AMPs) in the colon to protect against Salmonella infection. Gut-innervating nociceptor neurons (TRPV1+ Nav1.8+) also defend against Salmonella infection by crosstalk with epithelial cells and the intestinal microbiota (Lai et al., 2020). Salmonella invade the small intestine through intestinal microfold (M) cells, which are specialized epithelial cells within Peyer’s patch (PP) follicle-associated epithelium (FAE). Nociceptor neurons signal via CGRP to reduce the numbers of M cells, thus removing the gates of entry for these pathogens. Nociceptor signaling also maintains levels of segmented filamentous bacteria (SFB) in the small intestine—an intestine-resident microbe that provides resistance against Salmonella colonization and invasion. Gut-innervating tyrosine hydroxylase-expressing (TH+) sympathetic neurons also play a key role in host defense within the intestine. During homeostasis, TH+ neurons signal to resident muscularis macrophages (MMs) through the beta-2 adrenergic receptor (β2AR) to polarize the MMs toward a tissue-protective M2-like phenotype, which acts via the beta-2 adrenergic receptor (β2AR) on muscularis macrophages to polarize a M2 phenotype, which prevents cell loss in bacterial infection. Gut-innervating sympathetic neurons also act on ILC2 via β2AR to modulate type 2 cytokine production and helminth expulsion.
phenotype (Gabanyi et al., 2016). During gastrointestinal infection caused by *Salmonella* and other enteric pathogens, these macrophages protect enteric neurons from caspase-11-dependent death through their expression of arginase and protective polyamines (Matheis et al., 2020). Therefore, neuro-immune crosstalk is a major component of host immunity and protection against enteric pathogen invasion.

Nociceptor neurons drive protective skin immunity against fungal pathogens. In *Candida albicans* skin infections, TRPV1+ nociceptors drive IL-23 production by CD301b+ dermal dendritic cells, which induces γδ T cell production of IL-17 and protective immunity against *C. albicans* (Kashem et al., 2015). This protective response is mediated by CGRP, as its injection was sufficient to restore IL-23 and IL-17 responses in nociceptor ablated mice (Figure 2). Nociceptor neurons respond to fungi by detection of *C. albicans*-derived β-glucan through Dectin-1 (Maruyama et al., 2018). Sequential optogenetic stimulation of TRPV1+ neurons drives anticipatory host defense against *C. albicans* and *S. aureus* infections by inducing dendritic cell and γδ T cell responses (Cohen et al., 2019). Therefore, it is possible that activating a local neuro-immune circuit prior to infection could protect against subsequent infections.

Parasitic pathogens introduce complexities to host immunity because they possess host evasion mechanisms that are not susceptible to antibody or cellular antimicrobial mechanisms. The nervous system and its reflexes can orchestrate both protective type 2 immunity and clearance of parasites though “weep and sweep” mechanisms like cough, diarrhea, and mucus production. In the context of helminth infections like *Nippostrongylus brasiliensis* that migrates through the lung and colonizes the small intestine, the neuropeptide NMU directly activates type 2 innate lymphoid cells (ILC2) through its cognate receptor NMUR1 to drive anti-parasitic immunity at both barrier sites. In the intestine, a subset of enteric neurons express NMU...
Box 2. Neuro-mediator Signaling within Immune Cells

Depending on the stimulus, interactions between a neuro-mediator and its receptor signaling pathway within immune cells could lead to very different outcomes. For example, while the neuropeptide NMU potently drives ILC2 activation and helminth expulsion (Cardoso et al., 2017; Klose et al., 2017; Wallrapp et al., 2017), CGRP and β2-adrenergic agonists inhibit ILC2 activation and anti-helminth defense (Moriyama et al., 2018; Nagashima et al., 2019; Wallrapp et al., 2019; Xu et al., 2019). The neuropeptide NMU signals through the Gαq-coupled receptor NMUR1, which leads to calcium influx and NFAT signaling. β2-adrenergic agonists and CGRP, by contrast, signal through Gαs coupled receptors, which induces cAMP levels. cAMP-induced signaling via protein kinase A (PKA) and inducible cAMP early repressor (ICER) potently inhibits TNF-α expression and induces IL-10 (Harzenetter et al., 2007; Holzmann, 2013). CGRP is pleiotropic in nature, both inhibiting macrophage TNF production and neutrophil killing of bacteria while inducing IL-23 expression by dendritic cells. How this neuropeptide signals within immune cells to affect downstream responses is not fully defined, and therefore, more work is needed to elucidate its role in different inflammatory contexts and in immune function. Overall, more mechanistic studies investigating how specific neuro-mediators regulate transcription and function in distinct immune cells are necessary. Many neuro-mediators also signal via ion channels and not GPCRs. One example is the nicotinic acetylcholine receptor, which inhibits macrophage activity through downstream adenylylate cyclase 6 signaling to drive the vagal cholinergic “anti-inflammatory reflex” (Tarnawski et al., 2018). A major area of future research is to determine how neuro-mediator signaling pathways modulate transcription and protein level changes in immune cells and how these signaling pathways interact with traditional cytokine signaling.

The “Anti-inflammatory Reflex”

Inflammation is a process marked by the four cardinal signs—pain, redness, swelling, and heat. Immune cells are recruited, and cytokines, which aid in pathogen clearance and tissue repair, are secreted during the inflammatory process. However, exaggerated acute inflammatory responses and persistent chronic inflammation are drivers of many diseases, including septic shock, allergy and asthma, arthritis, and inflammatory bowel diseases (Angus and van der Poll, 2013; Galli et al., 2008; Murdoch and Lloyd, 2010; Rubin et al., 2012). The nervous system actively modulates immune responses in the context of acute inflammation and chronic inflammatory diseases, either playing a pro- or anti-inflammatory role, depending on the circumstances.

Seminal studies revealed a cholinergic “anti-inflammatory reflex” that regulates systemic (Rosas-Ballina et al., 2008; Rosas-Ballina et al., 2011; Wang et al., 2003) and local immune activation (Matteoli et al., 2014). This neural reflex was first discovered in the context of endotoxemia and toxic shock (Borovikova et al., 2000; Wang et al., 2003). Macrophages sense endotoxins including lipopolysaccharide (LPS) from bacterial pathogens, become activated, and secrete pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-18. Excessive production of these pro-inflammatory cytokines—a cytokine storm—can affect multiple organs and cause septic shock, which is potentially lethal (Angus and van der Poll, 2013). The nervous system can act as a brake on this process. Sensory afferents in the vagus nerve detect cytokines, including TNF and IL-1β (Zanos et al., 2018), which in turn activate a brainstem circuit that signals via the efferent vagus back to the periphery to shut down cytokine production. In this circuit, efferent vagal fibers signal to the celiac mesenteric ganglia (CMG) via ACh to postsynaptic α7 nicotinic acetylcholine receptor (α7nAChR) on post-ganglionic neurons (Vida et al., 2011). These neurons then signal via the splenic nerve, which releases norepinephrine (NE) in the spleen (Rosas-Ballina et al., 2008; Vida et al., 2011). A subpopulation of splenic T cells express choline acetyltransferase (ChAT) and produce ACh in response to this NE, relaying the neuronal signals to the splenic α7nAChR-expressing...
macrophages (Rosas-Ballina et al., 2011) (Figure 2). Both vagus nerve stimulation and splenic nerve stimulation can inhibit LPS-induced TNF synthesis by splenic macrophages and constrict systemic NF levels (Borovikova et al., 2000; Rosas-Ballina et al., 2008; Rosas-Ballina et al., 2011; Vida et al., 2011). Additionally, vagotomy will increase systemic TNF levels in response to LPS administration (Borovikova et al., 2000; Vida et al., 2011). A similar “anti-inflammatory reflex” was also discovered in the intestine. Stimulation of the vagus nerve leads to an efferent signal to cholinergic enteric neurons in the myenteric plexus, which in turn regulates intestinal muscularis-resident macrophage phenotypes and reduces intestinal inflammation (Matteoli et al., 2014). Administration of α7 nicotinic agonists potently decreases TNF-α and HMGB1 release by macrophages in endotoxin shock (Borovikova et al., 2000; Wang et al., 2003). It is possible that targeted activation of the “anti-inflammatory reflex” could protect against many inflammatory diseases. Because the vagus nerve innervates many peripheral tissues, this reflex could be a fundamental way for the body to modulate inflammation.

Employment of the method of vagus nerve stimulation (VNS) to treat chronic inflammation was first pioneered in animal models of inflammatory diseases and conditions, including endotoxemia, hypovolemic shock, postoperative ileus, inflammatory bowel disease (IBD), rheumatoid arthritis (RA), and kidney ischemia-reperfusion injury (Borovikova et al., 2000; de Jonge et al., 2005; Guarini et al., 2003; Inoue et al., 2016; Levine et al., 2014; Meregnani et al., 2011). Currently, VNS is at the forefront of approaches that use electronic stimulation of nerves to treat disease, a field within bioelectronic medicine. The anti-inflammatory and disease-ameliorating efficacy of VNS in these animal studies has led to human clinical trials in IBD and RA—both of the diseases show depressed vagus nerve activity (Bruchfeld et al., 2010; Lindgren et al., 1993). In a trial in IBD, seven patients with active Crohn’s disease underwent VNS via implanted cuff electrodes and were followed up for 6 months. Five out of seven patients exhibited reduced disease activity and improved biochemical and endoscopic indices, along with restored vagus nerve activity (Bonaz et al., 2016). In an 84-day open trial of VNS in RA, seventeen patients who were not responsive to conventional methotrexate treatment showed decreased TNF production and alleviated disease severity after VNS (Koopman et al., 2016). Although further investigation with larger longitudinal cohorts of patients as well as randomized double-blinded control studies are needed, along with tests of changes in pro-inflammatory cytokines and other constituents of the “anti-inflammatory reflex,” this preliminary clinical evidence supports that targeting the “anti-inflammatory reflex” by VNS is a potential therapeutic method for IBD, RA, and other autoimmune diseases that are caused by excessive pro-inflammatory cytokines.

### Neuro-immune Interactions in Inflammation at Barrier Surfaces

As outlined above, the sensory nervous system communicates closely with the immune system at mucosal barrier surfaces, including the skin, lung, and intestine. The skin is the largest barrier organ consistently exposed to environmental stimuli. Cutaneous sensory neurons can sense a wide variety of stimuli, such as heat, acidity, chemicals, mechanical stimulation, inflammatory cytokines, and microbial products (Baral et al., 2019; Szallasi et al., 2007). A population of cutaneous sensory neurons express the ion channels TRPV1 and/or Nav1.8 and are responsible for nociception and pain production. This population of neurons secrete CGRP, SP, and other neuropeptides that play a pivotal role in regulating immune responses and inflammation (Baral et al., 2019; Pinho-Ribeiro et al., 2017). Multiple immune cell populations, such as mast cells, macrophages, DCs, γδ T cells, CD4+ T cells, and ILCs, localize in close proximity with sensory neurons and express receptors for sensory neuropeptides (Baral et al., 2019; Pinho-Ribeiro et al., 2017) (Figure 2).

Nociceptive sensory neurons play a crucial role in driving Th17 cell- and IL-17-associated immune responses in the skin. In the imiquimod (IMQ)-induced murine model of psoriasis, nociceptive neurons regulate the activation of dermal dendritic cells (dDCs), which serve as the principal source of IL-23, and downstream induction of IL-17 production by T cells that drive skin inflammation (Riol-Blanco et al., 2014). Ablation of TRPV1+ or Nav1.8+ neurons led to decreased IL-23 production from the dDCs and subsequently alleviated type 17 inflammation. Sensory neurons are poised to respond to noxious stimuli quickly, and this raises the question of whether neuronal activation alone is sufficient to initiate immune responses and elicit IL-17-associated inflammation. In this context, a recent study employed optogenetic stimulation of TRPV1-Cre-Ai32 mice, which express the light-sensitive channelrhodopsin-2 in TRPV1+ neurons, and found that repeated photoactivation of the TRPV1+ neurons alone resulted in inflammation in the ear—including increased ear thickness, erythema, and scaling skin—that was reminiscent of IMQ-induced psoriasis-like dermatitis, with γδ T cell, CD4+ T cell and neutrophil infiltration and elevated IL-17 production (Cohen et al., 2019). CGRP was crucial in driving the Th17 cell response, as TRPV1+ neurons secreted CGRP after photoactivation, and application of CGRP-Rα (a CGRP antagonist) reduced IL-6 and IL-23 levels following optogenetic stimulation. Considering that a neuronal response to a stimulus signals through axonal reflexes to affect neighboring regions of skin, this neuro-immune circuit may contribute to anticipatory immune responses in adjacent areas of the stimulus. In this context, optogenetic activation of the neurons led to clearance of infections in adjacent areas through axonal reflexes (Cohen et al., 2019).

In contrast to its pro-inflammatory role in IL-17-associated inflammation, the neuropeptide CGRP is anti-inflammatory in type 2 inflammation, in which ILC2s play an important role in initiating and amplifying the inflammatory response (Figure 2). A subpopulation of ILC2s express CALCRL-RAMP1, the receptor complex that detects CGRP (Nagashima et al., 2019; Wallrapp et al., 2019). In models of IL-33- or ovalbumin (OVA)-induced allergic asthma, CGRP suppresses inflammation through inhibiting ILC2 proliferation and IL-13 production, leading to decreased eosinophil recruitment and reduced tissue damage (Nagashima et al., 2019; Wallrapp et al., 2019). Similarly, in the models of IL-25- or OVA-induced allergic inflammation in the small intestine, CGRP antagonizes ILC2 activation and proliferation, leading to a reduction in mast cells, the presence of which is a phenotypic marker of allergic inflammation (Xu et al., 2019). In addition, CGRP may also regulate adaptive immune responses in allergic asthma by inhibiting DC maturation and function. Adding CGRP to in vitro cultured bone-marrow-derived DCs reduces the
expression of co-stimulatory molecules CD40 and CD86. When co-cultured with T cells in vitro, CGRP-stimulated DCs suppress the activation and proliferation of OVA-specific T cells and induce more Foxp3+ regulatory T cells; when adoptively transferred to mice, these DCs help control OVA-induced allergic responses and airway inflammation (Rochlitzer et al., 2011).

It is unclear how the cellular source of CGRP impacts the influence of this pleiotropic neuropeptide in immune responses. TRPV1+ neurons (Lai et al., 2020; Pinho-Ribeiro et al., 2018), pulmonary neuroendocrine cells (PNECs, a type of specialized epithelial cells) (Sui et al., 2018), and ChAT+ enteric neurons can produce CGRP (Xu et al., 2019). Moreover, ILC2 immune cells also express CGRP during inflammation (Nagashima et al., 2019; Wallrapp et al., 2019; Xu et al., 2019). Defining the contribution of individual sources of CGRP at different stages and in distinct niches of type 2 inflammation will require targeted genetic tools and spatio-temporally precise techniques (e.g., opogenetic targeting of neurons). Given the important role of CGRP in regulating inflammation, targeting the CGRP pathway using monoclonal antibodies (Godsbys et al., 2017; Silberstein et al., 2017) or small molecule receptor antagonists (Olesen et al., 2004), which are currently being used to treat chronic migraines, is a potential therapeutic opportunity to control immune responses at barrier surfaces.

TRPV1 and/or Nav1.8+ neurons in the lung secrete the neuropeptide VIP, which binds to VIP receptor type 2 (VIPR2) or VPAC2 expressed on immune cells, and this can drive allergic inflammation (Nussbaum et al., 2013; Talbot et al., 2015). In models of OVA- or house dust mite (HDM)-induced allergic asthma, IL-5 produced by activated immune cells directly acts on Nav1.8+ neurons to induce VIP secretion. VIP then stimulates ILC2s and TH2 cells, creating a positive feedback loop that accelerates type 2 inflammation. Intranasal administration of CGRP-stimulated DCs suppress type 2 inflammation through NE- and VIPR2 (VIP receptor type 2) expression on immune cells, and this can drive allergic inflammation (Nussbaum et al., 2013; Talbot et al., 2015). In models of OVA- or house dust mite (HDM)-induced allergic asthma, IL-5 produced by activated immune cells directly acts on Nav1.8+ neurons to induce VIP secretion. VIP then stimulates ILC2s and TH2 cells, creating a positive feedback loop that accelerates type 2 inflammation. Intranasal administration of CGRP-stimulated DCs suppress type 2 inflammation through NE- and VIPR2 (VIP receptor type 2) expression on immune cells, and this can drive allergic inflammation (Nussbaum et al., 2013; Talbot et al., 2015). In models of OVA- or house dust mite (HDM)-induced allergic asthma, IL-5 produced by activated immune cells directly acts on Nav1.8+ neurons to induce VIP secretion. VIP then stimulates ILC2s and TH2 cells, creating a positive feedback loop that accelerates type 2 inflammation. Intranasal administration of CGRP-stimulated DCs suppress type 2 inflammation through NE- and VIPR2 (VIP receptor type 2) expression on immune cells, and this can drive allergic inflammation (Nussbaum et al., 2013; Talbot et al., 2015).

Noxious sensory and autonomic neurons play a pivotal role in modulating inflammatory immune responses at mucosal barrier surfaces. The effects of the relevant neuropeptides are varied and appear to depend on the cellular sources and targets as well as on the inflammatory context. It is clear that immune cells can feed back into these regulatory circuits. Although there is limited understanding of how these signals are varied and integrated in distinct contexts, it is clear that the nervous system controls inflammation at barrier tissues via sophisticated regulatory interactions. There is already evidence that targeting these circuits by specifically interfering with neuropeptide signaling can control inflammation. More precise and comprehensive understanding of the interactions between neurons and immune cells in different tissues and inflammatory settings is indispensable for significant therapeutic progress.

Neuro-ILC3 Interactions in Intestinal Homeostasis and Tissue Repair

In addition to regulating host defenses and inflammatory responses, neuro-immune interactions are also important to tissue homeostasis and tissue repair (Figure 3). It should be noted that neural pathways also regulate hematopoiesis and in this way impact immunity and immune-contributions to physiology (reviewed in Godinho-Silva et al. [2019a]). Enteric neuron-derived VIP, which is induced by food intake and suppressed by fasting, binds its receptor VIPR2 on ILC3s and stimulates production of IL-22, a cytokine that works predominantly on non-lymphoid cells (e.g., epithelial cells) and is critical for the maintenance of homeostasis at barrier surfaces, particularly the intestinal tract (Seiillet et al., 2020). During the recovery phase following intestinal damage caused by dextran sulfate sodium (DSS) treatment, VIPr2−/− mice exhibited a decreased frequency of IL-22-producing ILC3s, along with more severe signs of colitis. Adoptive transfer of WT but not VIPr2−/− ILC3s into Rag2−/−γc−/− mice (which lack adaptive immune cells and ILCs) protected the mice from DSS-induced tissue damage (Seiillet et al., 2020). In contrast, use of Rorc−/−VIPr2−/− mice Talbot et al. (2020) found that VIPergic neurons reduced IL-22 production by CCR6+ILC3s through VIPR2. Chemogenetic activation of enteric VIPergic neurons led to increased bacterial load and decreased survival of mice after Citrobacter rodentium infection, a setting in which ILC3-mediated IL-22 production is essential for host protective immunity, and chemogenetic inhibition of VIPergic neurons helped protect against bacterial dissemination to the spleen and liver (Talbot et al., 2020). The contrasting results between the studies could be partially due to use of targeted conditional ILC-targeted ablation of VIPR2 in the second study compared to total VIPR2 deficiency in the first study. That VIP expression can be induced by food intake implies an anticipatory neuroimmune coordination that is beneficial to the organism, given that food is non-self and could contain components that are potentially harmful and induce tissue damage.
Enteric ILC3 responses are also regulated by brain circadian circuits and environmental light cues. IL-22 production by ILC3s oscillates with light-dark cycles (Godinho-Silva et al., 2019b; Seillet et al., 2020; Talbot et al., 2020; Teng et al., 2019; Wang et al., 2019). The suprachiasmatic nuclei (SCN) in the hypothalamus integrates environmental light signals and control the circadian rhythm of an organism (Bernard et al., 2007). Surgical ablation of the SCN by electrolytic lesion or conditional deletion of the circadian clock gene *Arntl* in SCN and forebrain pyramidal neurons disrupts ILC3 circadian rhythms and function in the intestine (Godinho-Silva et al., 2019b). Therefore, circadian circuits in the brain regulate peripheral intestinal homeostasis and tissue repair via ILC3 responses, adding another layer of complexity in the dialog between the nervous system and the immune system, with implications for our understanding of how lifestyle could influence intestinal homeostasis and subsequent susceptibility to diseases like IBD.

**Neuro-macrophage Interactions in Intestinal and Adipose Tissue Homeostasis**

Neuron-macrophage interactions play an indispensable role in intestinal and adipose tissue homeostasis (Figure 3). The development of muscularis macrophages (MMs) requires colony stimulatory factor (CSF1), which is secreted by neighboring enteric neurons. MMs localize along enteric nerve fibers and secrete bone morphogenetic protein 2 (BMP2)—a soluble factor that belongs to the transforming growth factor β (TGF-β) superfamily. BMP2 activates enteric neurons via BMPR—a transmembrane serine kinase that consists of 2 subunits (Muller et al., 2014). Selective transient deletion of the MMs leads to uncoordinated enteric smooth muscle contractions and decreased intestinal peristalsis, which are controlled by enteric neurons, and can be partially rescued by injecting BMP2 (Muller et al., 2014).

Thus, communication between enteric neurons and the MMs regulates intestinal motility and homeostasis at steady state. Given that chronic disruption of intestinal peristalsis is a common symptom of irritable bowel syndrome (IBS) (Lind, 1991), targeting neuro-macrophage interactions could be a potential therapeutic strategy to improve the quality of life of people who suffer from this disease.

As shown in Figure 3, enteric neurons release neuropeptide VIP, which acts on VIPR2 expressed by ILC3s to modulate IL-22 production (increase or decrease). Enteric neurons also secrete macrophage colony-stimulating factor 1 (CSF1), which binds to its receptor CSF1R on muscularis macrophages to regulate the production of bone morphogenetic protein 2 (BMP2) from muscularis macrophages and gastrointestinal motility.

Enteric ILC3 responses are also regulated by brain circadian circuits and environmental light cues. IL-22 production by ILC3s oscillates with light-dark cycles (Godinho-Silva et al., 2019b; Seillet et al., 2020; Talbot et al., 2020; Teng et al., 2019; Wang et al., 2019). The suprachiasmatic nuclei (SCN) in the hypothalamus integrates environmental light signals and control the circadian rhythm of an organism (Bernard et al., 2007). Surgical ablation of the SCN by electrolytic lesion or conditional deletion of the circadian clock gene *Arntl* in SCN and forebrain pyramidal neurons disrupts ILC3 circadian rhythms and function in the intestine (Godinho-Silva et al., 2019b). Therefore, circadian circuits in the brain regulate peripheral intestinal homeostasis and tissue repair via ILC3 responses, adding another layer of complexity in the dialog between the nervous system and the immune system, with implications for our understanding of how lifestyle could influence intestinal homeostasis and subsequent susceptibility to diseases like IBD.

**Neuro-macrophage Interactions in Intestinal and Adipose Tissue Homeostasis**

Neuron-macrophage interactions play an indispensable role in intestinal and adipose tissue homeostasis (Figure 3). The development of muscularis macrophages (MMs) requires colony stimulatory factor (CSF1), which is secreted by neighboring enteric neurons. MMs localize along enteric nerve fibers and secrete bone morphogenetic protein 2 (BMP2)—a soluble factor that belongs to the transforming growth factor β (TGF-β) superfamily. BMP2 activates enteric neurons via BMPR—a transmembrane serine kinase that consists of 2 subunits (Muller et al., 2014). Selective transient deletion of the MMs leads to uncoordinated enteric smooth muscle contractions and decreased intestinal peristalsis, which are controlled by enteric neurons, and can be partially rescued by injecting BMP2 (Muller et al., 2014).

Thus, communication between enteric neurons and the MMs regulates intestinal motility and homeostasis at steady state. Given that chronic disruption of intestinal peristalsis is a common symptom of irritable bowel syndrome (IBS) (Lind, 1991), targeting neuro-macrophage interactions could be a potential therapeutic strategy to improve the quality of life of people who suffer from this disease.

Civilizations and solutions to this disease.
role in a specific homeostatic or disease setting is essential for developing such therapeutics.

Concluding Remarks

The nervous system contributes to host defense, inflammation, tissue homeostasis, and tissue repair through communicating with the immune system via neurotransmitters and neuropeptides. It is becoming clear that the nervous system plays a pivotal role in regulating tissue immunity. Depending on the tissue microenvironment and distinct drivers of a certain immune response, the same neuronal populations and even the same specific neuro-mediators can exert opposing effects on immune cells, promoting or inhibiting tissue immunity. Given its rapid reactions and reflex circuits, the ability of the nervous system to sense environmental stimuli and to relay the signals to the immune system is indispensable for efficient and effective anticipatory immune responses. Further investigation on neuro-mediator receptor signaling pathways and development of new genetic tools to determine and to manipulate cellular sources of neuro-mediators in the context of immunity and inflammation will greatly increase our understanding of neuroimmune interactions in the context of health and disease. Identification of neuronal mediators beyond traditional neurotransmitters that regulate immunity is also another area of future research. Mapping the specific peripheral neural circuits involved in signaling to the immune system could lead to anatomically targeted approaches to regulate immune responses. However, our current knowledge of the sophisticated dialog between the nervous and immune systems is not sufficiently advanced. Addressing these gaps in knowledge will inform the development of novel and targeted therapeutic strategies to treat infection, chronic inflammation, and tissue damage.

ACKNOWLEDGMENTS

We thank the members of the Artis lab and the Chiu lab for critical reading of the manuscript. Research in the Artis lab is supported by the National Institutes of Health (AI074878, AI095466, AI095608, and AI102942), the Burroughs Wellcome Fund, and the Chan Zuckerberg Initiative.

DECLARATION OF INTERESTS

D.A. has contributed to scientific advisory boards at FARE, Genentech, KRF, Pfizer, and Takeda in the last twelve months. I.M.C. receives sponsored research support from GSK and Allergan Pharmaceuticals and is a member of scientific advisory boards for GSK and Kintsa pharmaceuticals.

REFERENCES


Jocken, J.W., and Blaak, E.E. (2018). Intestinal Epithelial Wnt Signaling Mediates Acetylcholine-Trig-


