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Immunity
Review

Neuro-immune Interactions in the Tissues

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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 immunity. Here, we review the current understanding of the mechanisms that underlie the complex interactions between the immune and the nervous systems in different tissues and contexts. We outline current gaps in knowledge and 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 to develop improved approaches to treat inflammation and disease.

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 environmental stimuli, and it participates in a wide variety of physiological processes in tissues, including host defense against pathogens, interactions with the microbiota at barrier surfaces, and maintenance of tissue homeostasis (Belkaid and Hand, 2014; Rankin and Artis, 2018). However, excessive immune responses can lead to chronic inflammation and autoimmune 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 established and maintained in different tissues and the specific cellular interactions that underlie immune and homeostatic responses therein are important areas of investigation.

Recent technological developments, including novel transgenic 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, inflammation, 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 pain 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 somatosensory 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 catecholamines, gamma-aminobutyric acid (GABA), acetylcholine, and neuropeptides (e.g., calcitonin gene-related peptide [CGRP], substance P [SP], vasoactive intestinal peptide [VIP], and neuromedin 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. Nociceptors 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 tissues, 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, sympathetic, and enteric nervous systems. Parasympathetic neurons 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." Sympathetic neurons mediate the body's "fight or flight" response.

Box 1. Neural Regulation of Immunity in Non-mammalian Organisms

The evidence for communication between the nervous and immune systems extends to non-mammalian organisms, including the roundworm, *C. elegans*. As they feed on bacteria, *C. elegans* must quickly distinguish between pathogenic and beneficial microbes. In these worms, the sensory nervous system plays a key role in directly detecting bacterial pathogens and mediating avoidance behavior (Meisel et al., 2014; Zhang et al., 2005). Neurons also regulate immune responses at the cellular and molecular level in *C. elegans*. Specific sensory neuron subtypes expressing the GPCRs octopamine receptor-1 (OCTR-1) or neuropeptide receptor resemblance-1 (NPR-1) regulate immune defenses in *C. elegans* through modulation of innate immune signaling, microbial killing pathways, and the unfolded protein response (Styer et al., 2008; Sun et al., 2011). Neuro-endocrine signaling through an insulin-like neuropeptide INS7 also regulates clearance of bacterial infection (Kawli and Tan, 2008). In the skin, neuronal expression of a TGFβ homolog in *C. elegans* promotes antimicrobial peptide expression during fungal pathogen exposure (Zugasti and Ewbank, 2009). In the intestine of *C. elegans,* neural signaling through acetylcholine induces Wnt pathway genes that upregulate antimicrobial C-type lectins and lysozymes during bacterial infection (Labed et al., 2018). In Drosophila, sensory neuron signaling through Activin-β (a TGF-β family ligand) regulates proliferation and adhesion of hemocytes (Makhijani et al., 2017). In zebrafish, olfactory sensory neurons rapidly regulate $CD8^+$ T cell responses during rhabdovirus infections in a tropomyosin receptor kinase A (TrkA)-dependent manner (Sepahi et al., 2019). Therefore, the principles of neuron-immune signaling may have conserved elements across evolution, and investigation of the parallels between organisms could lead to new insights into crosstalk between these two ancient systems.

Preganglionic sympathetic neurons in the spinal cord project fibers to peripheral ganglia, where post-ganglionic neurons send efferent signals to the tissues via catecholamines (dopamine, norepinephrine, and epinephrine). The intrinsic enteric nervous system (ENS) is fully contained within the intestine, composed of sensory neurons that detect intestinal contents and motor neurons that drive secretory function and peristalsis (Furness, 2012, 2016). Enteric neurons are housed within the myenteric plexus (within the intestinal wall) or the submucosal plexus (beneath the lamina propria).

Whereas both neurons and immune cells can sense environmental stimuli, neuronal control of immunity, via the integrated nature of the neuronal response, enables increased reaction speed and physiological reach. Reaction to the stimuli and transmission of the signal perform on a timescale of milliseconds in neurons, rather than minutes to hours in the immune system, highlighting the selective advantage of co-evolved immune and neuronal systems (see Box 1). The impact of neuro-immune interactions also appears to be evolutionarily conserved. For example, *C. elegans* has evolved mechanisms by which neurons mediate both behavioral immunity and neuro-immune molecular signaling to protect against pathogens (Singh and Aballay, 2019; Wani et al., 2019). In mammals, 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 was 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.

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 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 hypertrophy (Chiu et al., 2013). *Streptococcus pyogenes* is another major cause of skin and soft tissue infections, including necrotizing fasciitis (flesh-eating disease), characterized by "pain out of proportion" with other manifestations. In a mouse model of necrotizing fasciitis, *S. pyogenes* exploits a pain-driven neuro-immune circuit to facilitate its survival (Pinho-Ribeiro et al., 2018). *S. pyogenes* secretes streptolysin S, a pore-forming toxin that activates $TRPV1^+$ neurons to produce pain. These neurons in turn release CGRP, which suppresses neutrophil recruitment and inhibits both human and mouse neutrophil killing of *S. pyogenes*. In *S. pyogenes* soft tissue infections, Botulinum neurotoxin A (BoNT/A) injections to block neuro-immune communication or administration of a CGRP receptor antagonist significantly improved the outcome of infection (Pinho-Ribeiro et al., 2018).

The lungs and respiratory tract are densely innervated by sensory and autonomic neurons that mediate cough and

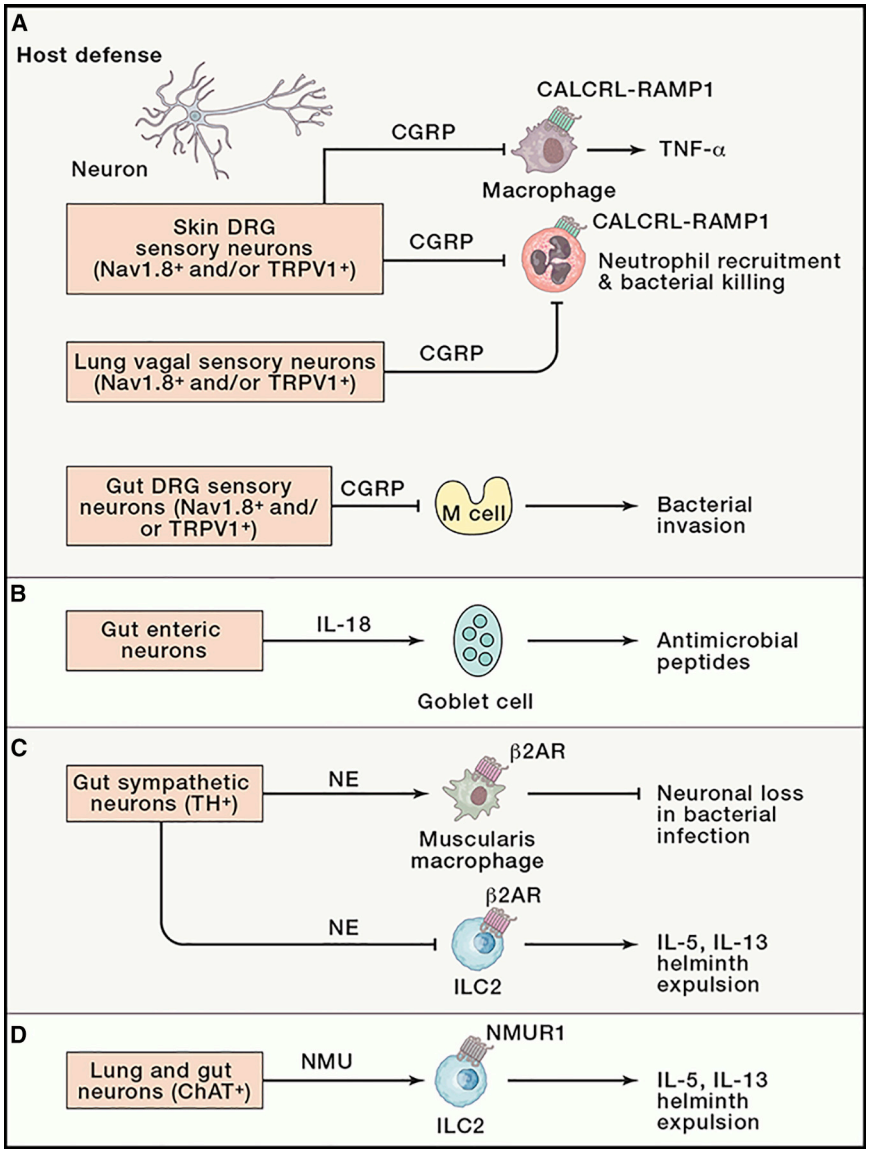

Figure 1. Neuro-immune Interactions in Host Defense

(A) Skin, lung, and gut-innervating Nav1.8$^+$ and/or TRPV1$^+$ nociceptor neurons release the neuropeptide calcitonin gene-related peptide (CGRP) from their nerve terminals during infection, which acts via the calcitonin receptor-like receptor/receptor activity-modifying protein 1 (CALCRL-RAMP1) receptor complex on immune cells. CGRP inhibits TNF-α expression and production by macrophages. It also modulates the ability of neutrophils to survey and kill bacterial pathogens. In the intestine, CGRP regulates Peyer's patch M cells to reduce bacterial invasion.

(B) Enteric neurons secrete the cytokine IL-18, which acts on goblet cells to promote the production of antimicrobial peptides and bacterial killing.

(C) Gut-innervating tyrosine hydroxylase (TH$^+$) sympathetic neurons secrete norepinephrine (NE), which acts via the beta-2 adrenergic receptor (β2AR) on muscularis macrophages to polarize a M2 phenotype, whose feedback to neurons 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.

(D) Lung and gut-innervating choline acetyltransferase positive (ChAT$^+$) neurons release neuropeptide neuromedin U (NMU), which acts via the neuromedin U receptor 1 (NMUR1) on ILC2s to promote type 2 cytokine production and helminth expulsion.

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

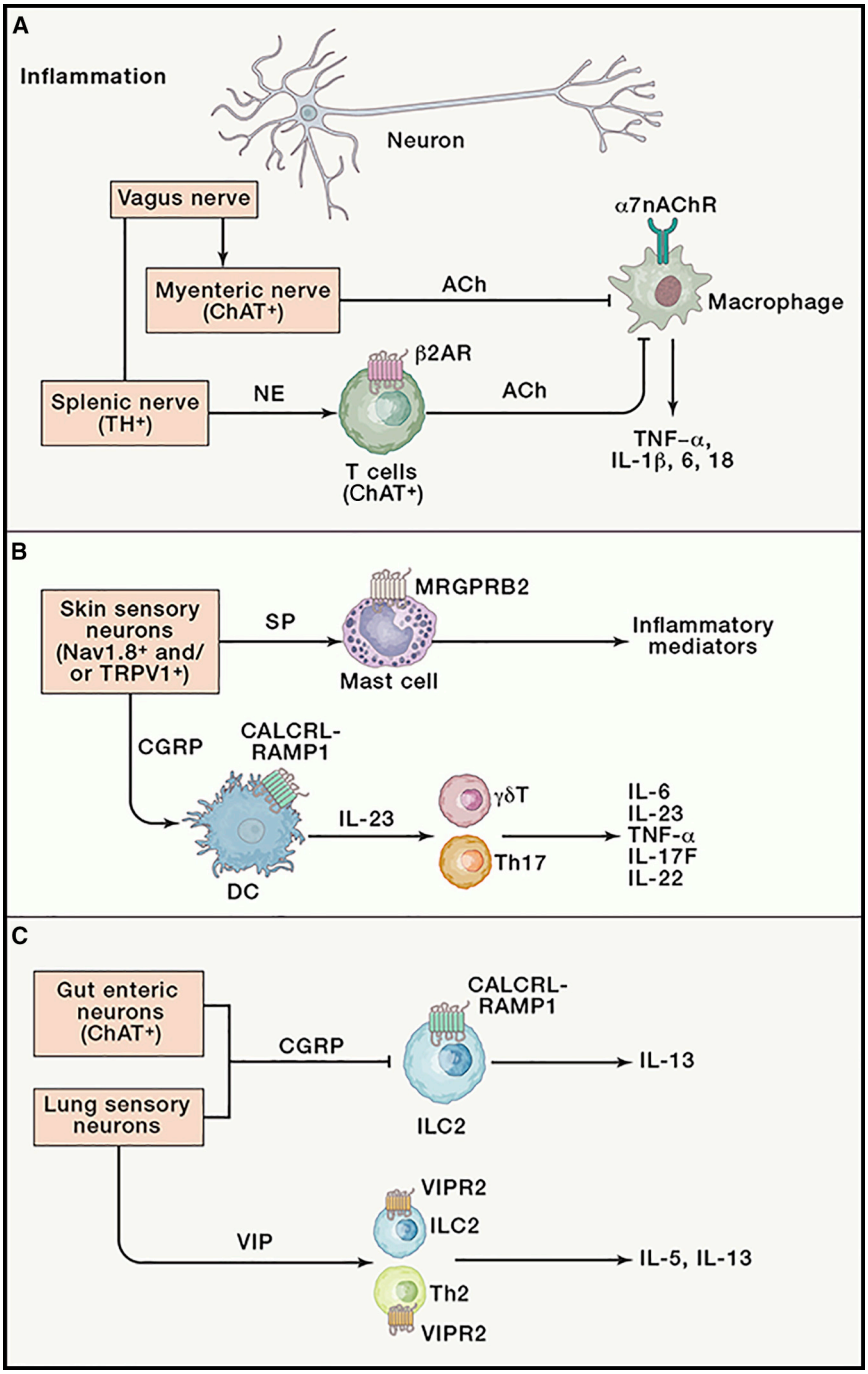

Figure 2. Neuro-immune Interactions in Inflammation

(A) The cholinergic "anti-inflammatory reflex" circuit. The vagus nerve signals to TH$^+$ splenic nerves, which secrete NE to activate ChAT$^+$ T cells to produce acetylcholine (ACh), which in turn signals to macrophages via the α7 nictonic acetylcholine receptor to reduce pro-inflammatory cytokine production. The vagus nerve also directly activates ChAT$^+$ myenteric nerves to drive its ACh production to reduce pro-inflammatory cytokines from macrophages in the intestine.

(B) Skin-innervating nociceptors release the neuropeptides substance P (SP) and CGRP. SP acts via the Mas-related G-protein coupled receptor member B2 (MRGPRB2) on mast cells to promote the release of inflammatory mediators. CGRP acts via the CALCRL-RAMP1 receptor complex on DCs to induce IL-23 from DCs, which in turn leads to γδT cell and Th17 cell activation and pro-inflammatory cytokine and type 17 cytokine production.

(C) Enteric ChAT$^+$ neurons and lung-innervating sensory neurons release neuropeptide CGRP, inhibiting ILC2 proliferation and IL-13 production. Lung sensory neurons also release neuropeptide vasoactive intestinal peptide (VIP), which acts via the vasoactive intestinal peptide receptor 2 (VIPR2) on ILC2 and Th2 cells, increasing the production of type 2 cytokines.

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 adenylate 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.

(Cardoso et al., 2017; Klose et al., 2017) and colocalize with ILC2s. *Nmur1*$^{-/-}$ mice exhibit significantly impaired helminth clearance (Cardoso et al., 2017; Klose et al., 2017), and *in vivo* administration of NMU leads to enhanced ILC2 activation, eosinophil recruitment, and accelerated expulsion of the parasite. NMU induces ILC2 proliferation and production of type 2 cytokines, including IL-5, IL-9, and IL-13 (Figure 1). The neuropeptide CGRP, in contrast to NMU, restrains ILC2 activation and inhibits anti-helminth responses (Nagashima et al., 2019). CGRP can be expressed by nociceptor neurons as well as ILC2 themselves as part of a negative feedback loop following infection. Sympathetic neurons also inhibit ILC2 responses and helminth clearance through the activation of β2AR, which are expressed by ILC2 (Moriyama et al., 2018). Therefore, in the case of helminth infections, different branches of the PNS either promote or inhibit protective type 2 immunity, illustrating the complexities of neuro-immune communication and the need to better elucidate how the nervous system is integrated into host defense (also see Box 2).

In conclusion, the sensory and autonomic nervous systems play an active and critical role in driving host defenses or immunopathology during bacterial, fungal, and parasitic infections. The type of neuro-immune modulation depends on the type of neuron involved, the neuropeptide or neuro-mediator, and the context of the inflammation. Targeting neuronal signaling using pharmacological, genetic, or electrical modulation could be repurposed to treat infection. Prior to this, however, a deeper understanding of how the nervous system senses pathogen infection and how different branches of the PNS coordinate their signals to modulate immunity is necessary. The nervous system also coordinates other major physiological functions like food intake, metabolism, and tissue repair, which play integral roles in infection and host defenses, but how these processes are integrated with direct neuro-immune crosstalk is not yet known. Overall, investigation of the role of the nervous system and how it signals to the immune system during pathogen invasion could reveal basic mechanisms of neuro-immune crosstalk that could transform our understanding of local and systemic inflammation.

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 TNF 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 endotoxic 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-alleviating 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, $\gamma\delta$ 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 $\gamma\delta$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 $CGRP_{8-37}$ (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., optogenetic targeting of neurons). Given the important role of CGRP in regulating inflammation, targeting the CGRP pathway using monoclonal antibodies (Goadsby 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 capsaicin—a selective TRPV1 agonist—aggravates immune cell infiltration and airway inflammation, whereas ablating Nav1.8$^+$ neurons or silencing them with QX-134—a nociceptor-specific inhibitor—reduces immune cell infiltration and alleviates inflammation (Talbot et al., 2015).

Nociceptive neurons also potently activate mast cells by release of the neuropeptide SP, which acts via the Mas-related G-protein coupled receptor member B2 (MRGPRB2) on mast cells to induce degranulation (McNeil et al., 2015). This signaling plays a key role in acute inflammatory conditions. SP injection into the skin is sufficient to induce MRGPRB2-dependent mast cell degranulation, leading to acute swelling and recruitment of neutrophils (Green et al., 2019). In HDM-induced skin inflammation, the cysteine protease Der p1 activates TRPV1$^+$ neurons to release SP, which in turn acts on mast cells via MRGPRB2 to induce degranulation, cytokine production, and inflammation (Serhan et al., 2019).

ChAT$^+$ neurons regulate type 2 immune responses against parasitic infections in the intestine and also regulate type 2 inflammation in the lung via the activation of ILC2 responses through NMU-NMUR1 interactions (Klose et al., 2017; Wallrapp et al., 2017). In contrast, TH$^+$ neurons inhibit ILC2 responses and suppress type 2 inflammation through NE-β2AR interactions (Moriyama et al., 2018). In addition, other neuropeptides like neuropeptide Y (NPY) and corticotropin-releasing hormone (CRH) regulate inflammatory immune responses (Cao et al., 2005; Wheway et al., 2005). Most of the studies of these neuropeptides are based on their effects in *in vitro* immune cell cultures or on experiments utilizing mouse models in which neuropeptide receptors are deleted in a non-conditional manner. Directed *in vivo* studies are required to elucidate the functional significance of these pathways.

In conclusion, 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 also 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 (Seillet 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*$^{-/-}\gamma c^{-/-}$ mice (which lack adaptive immune cells and ILCs) protected the mice from DSS-induced tissue damage (Seillet et al., 2020). In contrast, use of *Rorc*^{cre} *Vipr2*$^{fl/fl}$ 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.

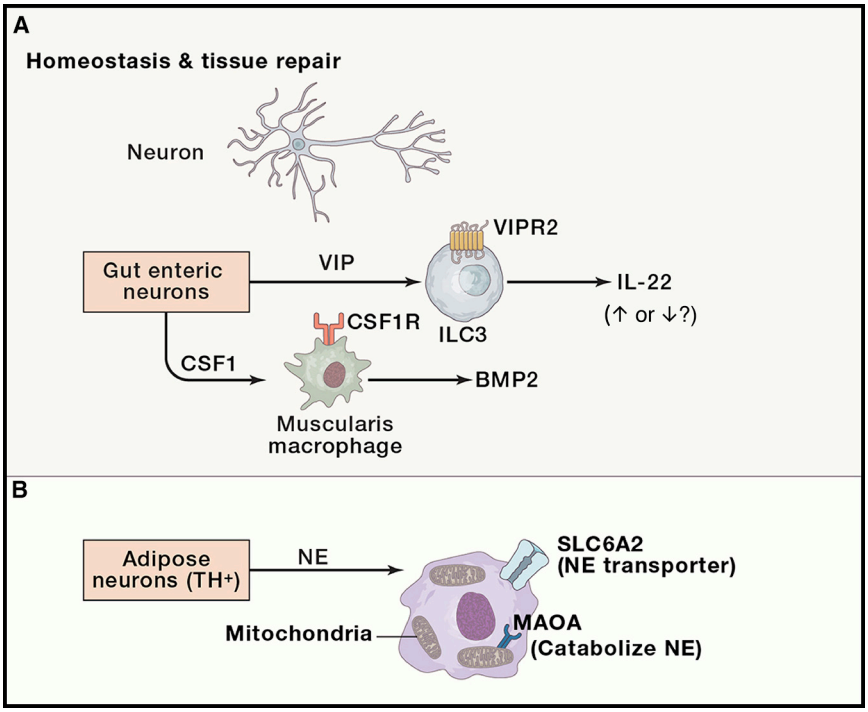

Figure 3. Neuro-immune Interactions in Tissue Repair and Homeostasis

(A) 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.

(B) Adipose tissue TH$^+$ neuron-derived NE can be taken up and catabolized by tissue resident macrophages via the sodium-dependent noradrenaline transporter SLC6A2 and monoamine oxidase A (MAOA), respectively.

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.

TH$^+$ neurons in the adipose tissue serve as the source of norepinephrine (NE), which induces lipolysis (Camell et al., 2017; Jocken and Blaak, 2008; Pirzgalska et al., 2017). Adipose tissue macrophages (ATMs) express solute carrier family 6 member 2 (SLC6A2) on the cell membrane and monoamine oxidase A (MAOA) on the mitochondrial membrane, which import and metabolize NE, respectively (Camell et al., 2017; Pirzgalska et al., 2017). ATMs increase the expression of NE catabolic machinery like MAOA during aging, consume more NE, and impair lipolysis. Defects in adipose tissue metabolism and thermogenesis in aging are associated with increased visceral adipose tissue and failure to maintain body temperature during cold stress (Camell et al., 2017). In this context, treatment of aged mice with the MAOA inhibitor clorgyline restored NE level and lipolysis in visceral adipose tissue (Camell et al., 2017), indicating that targeting neuron-macrophage interactions in adipose tissues may offer new therapeutic approaches to control conditions that are associated with disrupted adipose tissue homeostasis.

Taken together, these studies indicate that the nervous system continually communicates with tissue-resident cells, including ILCs, and macrophages, to mediate tissue protection and homeostasis. In this context, one could consider the possibility of therapeutically targeting neuronal responses to control physiological processes like intestinal homeostasis and lipid metabolism in adipose tissues, highlighting the need to better define the molecular basis of neuro-immune cell communication. Given that the PNS participates in the regulation of a broad spectrum of immune responses across multiple tissues and organs, methods like optogenetics have the potential to provide spatio-temporal control of manipulation of these circuits with improved therapeutic efficacy and reduced side effects compared to conventional therapeutics. Thus, comprehensive understanding of which branches of the nervous system play a

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 neuromediators 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 mediatiors 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.

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