REVIEW ARTICLE

Neuro-immune regulation of mucosal physiology

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Mucosal barriers constitute major body surfaces that are in constant contact with the external environment. Mucosal sites are densely populated by a myriad of distinct neurons and immune cell types that sense, integrate and respond to multiple environmental cues. In the recent past, neuro-immune interactions have been reported to play central roles in mucosal health and disease, including chronic inflammatory conditions, allergy and infectious diseases. Discrete neuro-immune cell units act as building blocks of this bidirectional multi-tissue cross-talk, ensuring mucosal tissue health and integrity. Herein, we will focus on reciprocal neuro-immune interactions in the airways and intestine. Such neuro-immune cross-talk maximizes sensing and integration of environmental aggressions, which can be considered an important paradigm shift in our current views of mucosal physiology and immune regulation.

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INTRODUCTION

Mucosal barriers are major interfaces with the external environment, playing central roles in organismal physiology and immune surveillance. At these sites, an integrated network of distinct tissues and cell types ensures mucosal homeostasis and host protection from environmental insults and infectious agents.¹ A major structural component of mucosal surfaces is the epithelial cell layer, which forms a physical barrier with the external environment and exerts dynamic responses that contribute to host defence. These processes encompass the production of mucus and inflammatory mediators, including chemokines and cytokines that recruit and regulate innate and adaptive immune cells.^{2,3} As such, a compromised barrier function is a prominent feature of mucosal inflammatory disorders, including those affecting the gastrointestinal (GI) tract (inflammatory bowel disease) and the respiratory system (asthma, allergy and chronic obstructive pulmonary disease (COPD)). Importantly, the worldwide incidence and prevalence of these diseases have increased drastically over the past 50 years,⁴ and understanding the onset and pathophysiology of such pathologies is an undisputed unmet need.

In the past few years, pioneer studies highlighted the importance of neuronal signals in regulating mucosal immune cells and mucosal barrier integrity.^{5–7} Therefore, understanding the communication strategies linking the nervous and immune systems may lead, in the near future, to novel therapies for multiple conditions, including chronic inflammation, cancer and metabolic disorders, amongst others. Herein, we review recent findings on how neuro-immune interactions shape the homeostasis of the GI and respiratory systems and will discuss how these interactions might be harnessed in health and disease.

MUCOSAL INNERVATION

Mucosal tissues are highly innervated, notably in the intestine, lung and nasal mucosae, where the nervous system exerts a

profound influence on digestive and respiratory processes, respectively. Importantly, these barriers also harbour a large set of lymphoid tissues and immune cell subsets, indicating that neuronal and immune cells may share similar anatomical compartments at mucosal sites.

Enteric innervation

The GI tract may be seen as a dynamic barrier that discriminates between beneficial commensals and pathogens, and that screens ingested material allowing for nutrients to be absorbed while excluding toxic substances.⁸ In addition, the digestive tract exerts a large range of functions, including digestion, absorption, motility, excretion, secretion and defence. These functions are partly regulated by signals arising from parasympathetic and sympathetic extrinsic neurons, which cell bodies are located within the brainstem and spinal cord, and by the intrinsic enteric nervous system (ENS), characterized by ganglionated plexus embedded in the lining of the GI wall^{5,7,9–12} (Fig. 1a). The ENS constitutes an extensive neuronal network with as many neurons as the spinal cord and it is therefore commonly called as the 'second brain'.⁵ The intrinsic innervation of the gut is arranged in two main ganglionated layers that contain numerous enteric neurons and enteric glial cells (ECGs): the myenteric plexus (or Auerbach's plexus), which is located between the circular and longitudinal muscle layers, and the submucosal plexus (or Meissner's plexus), which is placed in the gut submucosa.^{5,11,13,14} The myenteric plexus forms a continuous entity that extends from the upper oesophagus to the internal anal sphincter and receives inputs mainly from the vagus nerve to coordinate the contractions and relaxations of the gut smooth muscle and ensure the digestive tract motility and peristalsis.¹⁵ The submucosal plexus is found in the small and large intestine and contains two distinguishable ganglionated plexus in humans and large mammals. The inner submucosal plexus (closer to the intestinal lumen) mainly innervates the mucosa and the muscularis mucosae

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Fig. 1 Innervation of mucosal surfaces. **a** Neuronal network in the intestine. The gastrointestinal tract is innervated by intrinsic neurons (enteric) and by the axons of extrinsic sympathetic and parasympathetic neurons that project to the gut wall. IPANs intrinsic primary afferent sensory neurons, ENS enteric nervous system, CNS central nervous system. **b** The nervous system in the lungs. Vagal nerves supply all parasympathetic and most of the sensory neurons to the airways. Some sensory innervation originates from the dorsal root ganglia and run through spinal sympathetic nerves. ASM airway smooth muscle

and is mostly involved in the sensing and control of the luminal environment, regulating the GI blood flow and epithelial cell functions.¹⁵ The outer submucosal plexus (closer to the circular muscle layer) plays a significant role in intestinal motility.¹⁵ In addition, scarce and small ganglia can be found in the mucosa, close to the muscularis mucosae.¹⁶ The ENS also contains abundant EGCs and neuronal terminal varicosities that are in close proximity with lymphoid tissues and resident immune cells in the lamina propria.^{5,17} Enteric neurons can be divided into three main classes: (i) intrinsic primary afferent neurons, which are sensitive to chemical and mechanical stimuli; (ii) interneurons that connect to other interneurons and to motor neurons; (iii) motor neurons that act on different effector cells, notably muscle cells, blood vessels, epithelial cells and haematopoietic cells in the intestinal lamina propria.^{5,9,12,18,19} While the ENS activity is largely autonomous, its functions can be affected and modulated by external neural inputs provided by the Central nervous system (CNS) via vagal, spinal, pelvic and sympathetic innervation.⁵ Extrinsic neuronal inputs do not control discrete gut motility patterns, but they can enhance or inhibit ENS activities.^{8,9} Reciprocally, the ENS can also project axons outwards to the periphery through vagal, spinal, pelvic and sympathetic

pathways.^{9,19,20} Notably, 90% of the vagal fibres that transit between the gut and the brain are of afferent origin, suggesting that the brain may be more of a receiver than a transmitter in the so-called gut-brain axis.¹⁹

Pulmonary innervation

The nervous system in the respiratory tract plays an important role in the maintenance of lung homeostasis, via regulation of mucus secretion, vascular permeability, airway smooth muscle tone and blood flow.^{21,22} The pulmonary intrinsic ganglia, mostly comprised of neurons and glia cells, are a recognized component of the developing lung. The lung buds develop as an outgrowth of the foregut, which contains migrating neural crest cells that ultimately give rise to the ENS.^{23,24} The innervation of the lung consists of afferent and efferent neurons whose axons reach the airways by both vagal and sympathetic nerves²² (Fig. 1b). Vagal nerves supply all parasympathetic and most of the sensory neuronal fibres in the airways. Pulmonary afferent signals are transmitted by vagal sensory axons originating in the lamina propria, which cell bodies reside in the jugular and nodose ganglia and then project into the medulla²⁵ (Fig. 1b). Some additional afferent sensory signals originate in the dorsal root ganglia that connect with thoracic

ganglia to supply sympathetic innervation to blood vessels.²¹ The afferent innervation of the lungs encompasses sensory receptors, mechano-sensors and chemosensors, which have been extensively studied and reviewed elsewhere.²² Nociceptive C-fibres represent 80% of the vagal afferents nerves, and the former are promptly activated by noxious chemical and inflammatory molecules. Mechano-sensory nerves are activated by respiratory mechanical forces and are normally less sensitive to inflammatory stimuli.^{22,26,27} Lung afferent and efferent neurons connect via the brainstem. Efferent neuronal activation can be classified into sympathetic nerves, cholinergic parasympathetic nerves, non-adrenergic non-cholinergic parasympathetic nerves and the efferent sensory nerves.²⁸ The efferent nerves arising from the thoracic and superior sympathetic ganglia are mainly involved in the relaxation of airway smooth muscles via activation of β -adrenoceptors.²⁸ Mounting evidence indicates that activation of peripheral neurons results in neurotransmitter and neuropeptide release that can lead to neurogenic inflammation and enhanced central reflex activity through the CNS; notably, it was reported that altered innervation can contribute to respiratory diseases such as asthma, rhinitis and COPD.²²

ENTERIC NEURO-IMMUNE INTERACTIONS

The expression of neurotransmitter and neuropeptide receptors by distinct immune cell subsets has been suggesting that neuronal-derived signals may regulate immune cell function.^{5,7,29} Reciprocally, neurons can also express cytokine receptors to detect and respond to inflammation.³⁰ This shared molecular repertoire supports an integrated neuro-immune interaction that is likely to contribute to organ homeostasis. Neuro-immune communications are of particular significance in the intestine, where the diversity and complexity of neuronal and immune cell types is required to sense, react and adapt to ever changing conditions.³¹

Neuronal–mast cell cross-talk

Mast cells were shown to lie in close juxtaposition with intestinal substance P (SP-) and/or calcitonin gene-related peptide (CGRP-) producing neurons, both in rats and humans.^{32,33} Activation by external stimuli induces mast cell degranulation with consequent release of inflammatory granule components, such as histamine and mast cell proteases.³⁴ Importantly, mast cell-derived mediators can act on enteric neuron receptors.³⁵ Notably, histamine can activate neurons in the myenteric plexus of the small intestine,

Fig. 2 Intestinal neuro-immune interactions. **a** Microbial commensals tune intestinal peristalsis by shaping the bidirectional communication among CSF1-producing enteric neurons and BMP2-producing MMs. Activation of extrinsic sympathetic ganglia by bacteria drives the polarization of β 2AR-expressing MMs into a tissue-protective phenotype. **b** EGC sense microbiota-derived cues to control GFLs expression. EGC-derived GFLs activate RET-expressing ILC3s, which induces IL-22 that ultimately controls intestinal homeostasis. **c** Cholinergic enteric neurons sense and respond to worm products by producing the neuropeptide NMU. Neuronal-derived NMU induces an immediate activation of NMUR1-expressing ILC2s that contributes to a protective immune response against parasitic infections. CSF1 colony-stimulating factor 1, Mo monocyte, BMP2, bone morphogenetic protein 2, NE norepinephrine, *Arg1* encodes the arginase 1 protein, *Chi3l3* encodes the chitinase-like protein 3, GFLs, glial cell line-derived neurotrophic factor family ligands, IL interleukin, ILC innate lymphoid cell, NMU neuromedin U

inducing neuronal hyperexcitability via neuronal H₂ receptors.³⁶ Additionally, mast cell-derived tryptase can trigger proteinase-activated receptor 2 (PAR2) on submucosal neurons to causes acute and long-term neuronal hyperexcitability³⁷ and release of CGRP and SP neuromodulators.³⁸ Interestingly, PAR2 activation was reported to induce neurogenic colitis, and treatment with SP and CGRP receptor antagonists ameliorates PAR2-induced colitis in mice.³⁹ Reciprocally, peptidergic neurons were also shown to modulate mast cell function via secretion of SP, CGRP and vasoactive intestinal peptide (VIP).³⁵ Human mast cells respond to SP and VIP stimulation, which leads to mast cell degranulation and cytokine production.⁴⁰ In rodents, enteric mast cells isolated from rats infected with *Nippostrongylus brasiliensis* secrete histamine after stimulation with SP.⁴¹ Notably, mast cell proteases were described to enzymatically cleave neuron-derived SP and VIP,⁴² suggesting that mast cells may limit neuronal activation.³⁵ While there is no clear evidence of a functional mast cell–neuron interaction regulating enteric pathophysiology, some studies have correlated mast cell activation with irritable bowel syndrome (IBS) pathogenesis. Notably, supernatants from colonic biopsies of IBS patients display higher levels of histamine and tryptase, when compared with healthy controls,^{43–45} and increased mast cell numbers are found in the vicinity of enteric neurons from IBS patients.^{43,44} Interestingly, supernatants of colonic biopsies from IBS patients were shown to increase the firing rates of mesenteric afferent nerves and dorsal root ganglia nociceptive neurons in rats⁴⁴ and human submucosal neurons.⁴⁵ Altogether, these findings suggest that mast cell-derived molecules activate adjacent peptidergic neurons, which, in turn, may regulate mast cell function in a bidirectional positive feedback-loop that might be significant in the context of neurogenic inflammatory disorders.

Neuronal-macrophage cross-talk

The intestine also contains a vast reservoir of macrophages, most of which locate in the lamina propria and in the muscularis externa.⁴⁶ Recently, two independent studies reported that muscularis macrophages (MMs) can establish a bidirectional interplay with mucosal neurons^{47,48} (Fig. 2a). Muller et al.⁴⁸ demonstrated that MMs can regulate homeostatic GI motility. Blockage of colony stimulatory factor 1 receptor (CSF1R) signalling, an essential molecule for macrophage development, induces MM depletion and subsequent GI dysfunction.⁴⁸ In addition, MMs in close proximity to enteric neurons secrete bone morphogenetic protein 2 (BMP2) that control peristalsis.⁴⁸ In agreement, treatment with a BMP receptor inhibitor phenocopies MM-depleted mice, while ex vivo administration of BMP2 restores colonic peristalsis.⁴⁸ Interestingly, enteric neurons in the muscularis are the main source of CSF1, suggesting a reciprocal neuronal regulation of MMs.⁴⁸ Remarkably, the production of CSF1 and BMP2 rely on luminal microbes, demonstrating that commensal signals can shape neuronal–MM interaction and GI activity.⁴⁸ Thus, an intricate bidirectional interplay between MMs and enteric neurons is regulated by microbial cues to control intestinal peristalsis. More recently, Gabanyi et al.⁴⁷ demonstrated that intestinal lamina propria macrophages display a pro-inflammatory phenotype, while MMs show a tissue-protective profile.⁴⁷ Remarkably, MMs differentially express β_2 adrenergic receptor (β_2 AR), which is required for norepinephrine signalling. The expression of β_2 AR together with a close proximity to neurons suggested a molecular and anatomic basis for a neuron–MM interaction. In agreement, infection with *Salmonella typhimurium* activates extrinsic tyrosine hydroxylase-expressing neurons in the sympathetic ganglia, with consequent norepinephrine release in the intestinal muscularis.⁴⁷ In turn, norepinephrine activation of β_2 AR-expressing MMs drives their polarization towards a tissue-protective phenotype⁴⁷ (Fig. 2a).

Neuronal-innate lymphoid cell cross-talk

Group 2 innate lymphoid cells (ILC2s) are abundant at mucosal barriers.⁴⁹ ILC2s contribute to anti-helminth immunity, regulation of allergic responses and tissue repair.⁴⁹ Akin to other ILC subsets, ILC2s do not recognize specific antigen or pathogen-associated molecular patterns (PAMPs).^{49,50} ILC2s rather sense barrier disruption by integrating locally produced host-derived signals, including alarmins, lipid mediators and neuropeptides.^{49,50} Three recent independent studies reported that mucosal neurons can directly regulate innate type 2 immunity via neuromedin U (NMU)^{51–53} (Fig. 2c). Analysis of the ILC transcriptome revealed that ILC2s selectively express NMU receptor 1 (*Nmur1*), which encodes the peripheral receptor of the neuropeptide NMU.^{51–53} NMU is a highly conserved neuropeptide, mostly expressed in the GI tract and the CNS.⁵⁴ Intestinal ILC2s were found in close proximity to NMU-expressing cholinergic neurons^{51,52} (Fig. 2c). Remarkably, activation of ILC2s with NMU leads to a uniquely fast and potent production of type 2 cytokines, including interleukin-5 (IL-5), IL-13 and amphiregulin in a *Nmur1*-dependent manner.^{51,52} Accordingly, in vivo NMU treatment induces a selective increase of innate type 2 cytokine production, while their adaptive counterparts are unaffected. Interestingly, infection with *N. brasiliensis* induces a strong increase of *Nmu* expression in infected tissues, suggesting that NMU–NMUR1 axis may orchestrate barrier defence.^{51,52} In agreement, in vivo administration of NMU to *N. brasiliensis*-infected mice results in a selective increase of innate type 2 responses, reduced tissue damage and reduced infection burden.^{51,52} Sensing molecules, such as toll-like receptors (TLRs), contribute for the initiation of type 2 immunity.⁵⁰ Using neurosphere-derived neuronal organoids, Cardoso et al.⁵¹ revealed that cholinergic neurons upregulate *Nmu* expression in response to *N. brasiliensis* excretory/secretory products in a myeloid differentiation primary response 88 (MYD88)-dependent manner.⁵¹ Taken together, these studies uncover a novel layer of homeostatic regulation whereby mucosal cholinergic neurons sense pathogen-derived molecules that induce NMU production and consequent activation of ILC2s (Fig. 2c).

Adding to the neuronal regulation of ILC2 responses, catecholaminergic neurons, a sympathetic nervous system component, were shown to impair innate type 2 responses.⁵⁵ Catecholaminergic neurons are a source of norepinephrine that binds the β_2 AR.⁵ β_2 AR deficiency leads to increased ILC2 responses, while β_2 AR agonist treatment led to deficient ILC2 responses and impaired inflammation, indicating that neuronal-derived signals can limit type 2 inflammation⁵⁵ (Fig. 2c).

Interestingly, while norepinephrine regulates enteric MM polarization towards an anti-inflammatory protective M2 phenotype,⁴⁷ adipose tissue sympathetic neuron-associated macrophages, which also sense norepinephrine, exhibit a pro-inflammatory profile.⁵⁶ In addition, IL-13-dependent and IL-4-dependent mechanisms are essential to maintain M2 macrophage homeostasis.⁵⁷ These observations suggest that tissue-resident macrophages by differently integrating neuronal-derived and microenvironment-derived cues might be able to rapidly adapt to local settings. Since ILC2s, that also share β_2 AR expression, are an important source of IL-13, it would be interesting to dissect how norepinephrine levels differently regulate mucosal ILC2 and macrophage pools and how the balance between these two immune subsets may modulate immunity in the context of helminth infections, allergic inflammation and metabolic disorders.

ENTERIC GLIAL–IMMUNE CELL INTERACTIONS

In addition to the cues provided by intrinsic and extrinsic enteric neurons, EGCs were also shown to be important players in the neuro-immune cross-talk.^{7,58} EGCs represent a key cellular element of the ENS and they have been historically described as providers of neuronal structural support.⁵⁸ Nevertheless, it is

Fig. 3 Pulmonary neuro-immune interactions. **a** Sensory neurons and pulmonary neuroendocrine cells sense and respond to allergens by secreting VIP and CGRP, respectively. VIP activates ILC2s and differentiated Th2 cells, via VIPR2 signalling, to release IL-5. ILC2-derived and Th2-derived IL-5 stimulates IL-5R α -expressing sensory neurons inducing further VIP production that supports a positive feedforward loop; CGRP activates ILC2s to produce IL-5, which in turn promotes eosinophil recruitment. **b** Sensory neurons modulate lung immunity by producing CGRP that suppresses protective neutrophil and $\gamma\delta$ T cell responses. **c** Sensory neurons sense and respond to worm products and allergens by producing NMU. Neuronal-derived NMU induces an immediate activation of NMUR1-expressing ILC2s that shape host immunity and allergic inflammation. VIP vasoactive intestinal peptide, CGRP calcitonin gene-related peptide, IL interleukin, NMU neuromedin U

becoming clear that EGCs are more broadly important to GI homeostasis.⁵⁸ As an example, EGCs were shown to promote neuronal survival to oxidative stress damage.^{59,60} Earlier studies employing two independent models (*Gfap*-HSVtk and *Gfap*-HA-CL4-TCR)^{61,62} indicated that ablation of glial fibrillary acidic protein (GFAP)-expressing EGCs led to fatal intestinal inflammation, accompanied by disrupted intestinal epithelial barrier.^{61,62} Nevertheless, recent studies revisited this issue to demonstrate that EGCs are a morphologically and functionally heterogeneous populations, and that GFAP expression is limited to a particular EGC subset.⁶³ Notably, the proteolipid protein 1 (PLP1) was found to be broadly expressed by EGCs.⁶³ In contrast to earlier observations, inducible ablation of EGCs using *Plp1*^{CreER}.*Rosa26*^{DTA} fail to induce intestinal inflammation and disrupted intestinal barrier permeability.⁶⁴ The distinct outcomes of these studies may rely on non-cell-autonomous expression of different transgenic lines or to insufficient efficiency in deleting glial cell. Nevertheless, it has been established that during acute intestinal inflammation EGCs can cause intestinal neurodegeneration through secretion of inflammatory mediators that drive neuronal cell death and intestinal dysmotility.^{65,66} Thus, deficits of glial neuroprotective functions or activation of neurotoxic glial properties can lead to GI

derangements. Nonetheless, additional studies with EGC-autonomous genetic models are still required to clearly elucidate the role of EGCs in intestinal homeostasis.

EGCs are the main producers of neurotrophic factors including glial cell line-derived neurotrophic factor (GDNF) family of ligands (GFL), including GDNF, neurturin (NRTN), artemin (ARTN) and persephin (PSPN), all of which can modulate enteric neuronal function.³¹ As an example, *Nrtn*-deficient mice exhibit reduced myenteric plexus innervation density and altered GI motility, while *Gdnf*-knockout mice display intestinal aganglionosis.^{67–69} GFL signalling involves the shared signalling-transducing element RET (rearranged during transfection) and one of four GDNF family receptor- α (GFR α) members.⁷⁰ GFLs form a binary complex with a specific co-receptor GFR α that in turn binds to and activates the tyrosine kinase RET. RET signals have been reported to have major roles in development and maintenance of neurons, kidney development and spermatogonial stem cells.⁷⁰ In the ENS, RET expression drives proliferation, survival and migration of the neural crest-derived precursors of enteric neurons and EGCs in the foetal gut.^{71,72} In agreement, humans with hypomorphic RET mutations develop Hirschsprung's disease, which is characterized by intestinal aganglionosis.⁷⁰ Interestingly, RET signals were also

shown to modulate haematopoietic cell functions. RET signals are required for intestinal Peyer's patches development through control of lymphoid tissue initiator cells,⁷³ and RET activation also controls T cell-derived cytokines *in vitro*⁷⁴ and haematopoietic stem cell survival and function.⁷⁵ Strikingly, intestinal group 3 ILC3s in the adult intestine were shown to express high levels of the neuroregulator RET.⁷⁶ RET-deficient ILC3s have an impaired capacity to produce IL-22, a critical cytokine for epithelial barrier integrity. In agreement, mice with RET-deficient ILC3s displayed a poor epithelial reactivity that was associated with exacerbated dextran sulphate sodium-induced intestinal inflammation and increased susceptibility to *Citrobacter rodentium* infection.⁷⁶ GFL-producing EGCs were found in close association with ILC3s, suggesting a paracrine EGC–ILC3 interaction⁷⁶ (Fig. 2b). Astonishingly, microbial products and host-derived alarmins regulate glial-derived GFL expression in an MYD88-dependent manner.⁷⁶ Notably, glial cell-specific deletion of MYD88 signalling impaired GFL and innate IL-22 expression and increased susceptibility to intestinal inflammation and infection.⁷⁶ In conclusion, glial cells translate microbial and host-derived alarmin cues into GFL production, which then target neuronal and ILC3 function to promote intestinal homeostasis (Fig. 2b).

PULMONARY NEURO-IMMUNE INTERACTIONS

The airway epithelium is the first line of contact with inhaled agents, including allergens and pathogens. Allergic lung diseases are accompanied by bronchial hyperreactivity, cough, spasm and inflammation.^{77–79} These features can be explained by an impaired airway innervation and/or dysregulated immune responses,⁸⁰ putting forward a pivotal role of neuro-immune interactions in lung homeostasis and defence. While multiple studies described spatial interaction between airway nerves and infiltrating immune cells, few reports adequately defined the nerve fibres and/or the immune cell populations that may underlie neuro-immune interactions. Nevertheless, it is now established that inflammatory cells and airway nerves may be found in close proximity, notably during airway inflammation.^{22,81}

Neuronal-granulocyte cross-talk

Mast cells and eosinophils, which are key players in allergic inflammation, appear to cluster around airway nerves in asthmatic patients and allergen-challenged animals.^{82–86} In agreement, parasympathetic and postganglionic neurons express intercellular adhesion molecule-1 and/or eotaxin that mediate eosinophil adhesion and recruitment, respectively.^{87–89} Similarly, eosinophil-derived molecules activate vagal pulmonary neurons to secrete VIP, SP and CGRP that induces eosinophil chemotaxis.^{80,90–92} Interestingly, VIP and SP promotes the release of mast cell-derived allergic mediators such as histamine, leukotriene C4, prostaglandin D2, IL-6 and tumour necrosis factor (TNF).^{40,85,93}

Neuronal-dendritic cell cross-talk

Airway dendritic cells (DCs) can be found in close proximity to CGRP-expressing nociceptors in the airways of allergic mice.^{94–97} Interestingly, upon allergic airway inflammation, T cell activation and proliferation preferentially occurs close to DC–neuron clusters, suggesting that neurons may influence DC–T cell interactions.⁹⁷ Nevertheless, this topic remains a matter of controversy. While some reports showed increased T-helper type 2 (Th2) responses in response to CGRP-exposed DCs upon allergen stimulation,^{98–100} other works revealed CGRP as a negative regulator of DC function. Notably, CGRP-treated DCs decrease antigen-specific T cell responses and increase regulatory T cells numbers, while adoptive transfer of CGRP-treated DCs reduces allergic airway inflammation.^{80,101} Interestingly, animals subjected to C-fibre denervation display reduced DC numbers and immune cell influx in the

lung.¹⁰² In line with these findings, stimulation of pulmonary nociceptor neurons with capsaicin increases both neuropeptide release and immune cell infiltration.¹⁰³

Neuronal-ILC2 cross-talk

Recent studies interrogated the role of the neuropeptide VIP on the regulation of adaptive and innate type 2 responses.^{80,104} Intestinal and lung ILC2s express VIP receptor 2 (VIPR2) and their activation with VIP-specific or VIPR2-specific agonist promotes IL-5 production and eosinophilia¹⁰⁵ (Fig. 3a). These findings suggested a role of VIP/VIPR2 axis in mucosal homeostasis and allergic inflammation. In agreement, Talbot et al.¹⁰³ elegantly demonstrated that IL-5 activates lung nociceptors to release VIP, which, in turn, stimulates lung-resident ILC2 and Th2 cells leading to bronchial hyper-responsiveness (Fig. 3a). Importantly, the authors further showed that pharmacological silencing of local sensory neurons abolished the pro-inflammatory signalling loop leading to the resolution of allergic inflammation.¹⁰³

Akin to intestinal ILC2s, lung ILC2s also express *Nmur1* and these innate immune cells are in close contact with NMU-cholinergic pulmonary neurons^{51–53} (Fig. 3c). Noteworthy, Wall-rapp et al.⁵³ also observed that *in vitro* treatment with IL-13 increased *Nmu* expression in dorsal root ganglia neurons, thus suggesting a putative reciprocal neuron–ILC2 regulation. *In vitro* and *in vivo* NMU treatment induces lung ILC2 proliferation and production of type 2 inflammatory and tissue repair cytokines.^{51–53} Consistently, *in vivo* activation of the NMU/NMUR1 axis potentiates type 2 innate responses in the context of parasite infection and allergic reactions. Notably, *Nmur1*-deficient mice or chimeric mice reconstituted with *Nmur1*-deficient ILC2s displayed reduced ILC2 cytokine production and increased susceptibility to *N. brasiliensis* pulmonary infection.⁵¹ Moreover, loss of NMU–NMUR1 signalling altered ILC2 transcriptional programs following allergen challenge *in vivo*.⁵³

Finally, another independent study further revealed the involvement of cholinergic pathways on innate immune responses. Galle-Treger et al.¹⁰⁶ showed that ILC2s express $\alpha 7$ -nicotinic acetylcholine receptor ($\alpha 7$ nAChR), which is known to downregulate airway inflammation. Using a translational approach, the authors observed that engagement of $\alpha 7$ nAChR results in impaired human ILC2 cytokine production and reduced airway hyper-responsiveness (AHR) in a humanized ILC2 mouse model.¹⁰⁶

Pulmonary neuroendocrine cells are often found in association with peptidergic nerves and are increased in asthmatic patients and in close proximity with ILC2s.¹⁰⁷ Pulmonary neuroendocrine cells were shown to regulate goblet cell hyperplasia and ILC2 function via γ -aminobutyric acid (GABA) and CGRP production, respectively.¹⁰⁷ Notably, CGRP and GABA co-administration to ovalbumin-challenged pulmonary neuroendocrine cell-deficient mice restored asthma-associated inflammation characterized by goblet cell hyperplasia, type 2 cytokine production and inflammatory cell infiltrates¹⁰⁷ (Fig. 3a). Therefore, it is conceivable that pulmonary neuroendocrine–ILC2 interactions cooperate to amplify allergic asthma responses.¹⁰⁷ Additionally, it has been found that pulmonary nociceptor neurons repress $\gamma \delta$ T cell and neutrophil responses via CGRP release, impairing protective immunity against lethal *Staphylococcus aureus* pneumonia¹⁰⁸ (Fig. 3b). Similarly, *Streptococcus pyogenes* skin infection activates nociceptors to produce CGRP, which in turn suppresses protective neutrophil responses.¹⁰⁹ Notably, botulinum neurotoxin A or CGRP antagonists abrogate neuron-mediated immune suppression and improve defence to *S. pyogenes* infection.¹⁰⁹

Altogether, these studies shed light on the central role of neuro-immune signalling in the pathogenesis of lung diseases. Nevertheless, despite major recent advances in this topic, a better understanding of the cellular and molecular mechanisms underlying lung neuro-immune cross-talk is still required.

MUCOSAL NEURONS AS SENSORS OF ENVIRONMENTAL SIGNALS

Host defence comprises avoidance behaviour of harmful challenges driven by neuronal activity, recognition and response to an invading pathogen, toxin or allergen, and induction of tolerance mechanisms coordinated by the immune system.¹¹⁰ These strategies are particularly important at mucosal sites, where a tightly balanced response needs to be coordinated to induce tolerance to commensals and dietary elements, but also to mount an effective protective response against foreign agents and toxic products.^{111,112}

Mounting evidence indicates that the nervous system can sense environmental cues to instruct immune cells ensuring an integrated neuro-immune response that promotes host homeostasis.^{7,80} For example, the recognition of immunoglobulin E-mediated mast cell degranulation by adjacent neurons may act as a primordial inflammatory cue that drives avoidance behaviour to discrete antigens.¹¹³ An additional example may be provided by food poisoning, where vomit and diarrhoea illustrate the coordinated action of mucosal neurons to eliminate deleterious antigen from the GI tract.¹¹⁴ Thus, neurons are equipped to sense local environmental cues, including noxious stimuli, microbial products, immune-derived mediators and alarmins, and respond to those changes through the release of neurotransmitters and neuropeptides, or by triggering or altering their action potential firing patterns.

Neuronal sensing of noxious stimuli

Sensory neurons detect noxious stimuli from mechanical, thermal and chemical origin via engagement of sensor receptors such as the transient receptor potential (TRP) ion channels.¹¹⁵ The TRP vanilloid 1 and ankyrin 1 (TRPV1 and TRPA1, respectively) channels are the most studied members of the TRP family. For instance, capsaizipine, a TRPV1 antagonist, ameliorates acid-evoked hyperaemia, suggesting that sensory neurons in the oesophageal and duodenal mucosa are activated by gastric acid.¹¹⁶ Furthermore, genetic ablation of TRPA1 or treatment with TRPA1 antagonists reduces allergic-driven immune cell infiltration, cytokine and mucus production and ameliorates AHR.¹¹⁷ Similarly, TRPV1-expressing pulmonary vagal sensory neurons can also mediate AHR. In fact, genetic ablation or silencing of TRPV1 neurons abrogate bronchial hyperreactivity, while chemical or optogenetic stimulation of TRPV1 neurons aggravates AHR.¹¹⁸ Thus, TRPV1-expressing and TRPA1-expressing pulmonary vagal neurons can integrate chemical and mechanical stimuli that are transmitted to the CNS to coordinate a neuronal response that leads to protective airway reflexes such as coughing, sneezing and bronchoconstriction.¹¹⁹

Neuronal and neuroendocrine sensing of microbial products

Immune responses can be initiated by the activation of pattern recognition receptors (PRRs), including TLRs, which recognize highly conserved microbial structures, known as PAMPs.¹²⁰ Microbial cues were shown to be recognized by PRRs expressed by the ENS.¹²¹ Human and murine enteric neurons and EGCs express TLR2, -3, -4 and -7^{122–125} and TLR9 expression was also reported for enteric neurons in the rat colon.¹²⁶ While the importance of microbial cues to neuronal function remains largely unknown, increasing evidence suggest that an interplay between mucosal neurons and the microbiome influences mucosal homeostasis.¹²⁷ *Tlr2*-deficient mice display ENS structural alterations, decreased levels of GDNF, GI dysmotility and increased susceptibility to colitis.¹²⁴ Interestingly, germ-free (GF) mice phenocopy TLR2 deficiency and administration of a TLR2 ligand to these mice partially restores GI alterations.¹²⁴ Similarly, TLR4-deficient and MYD88-deficient mice or mice harbouring MYD88 signalling disruption on neural crest cells displayed decreased number of enteric neurons and GI motility.¹²⁸ In line with these findings, mice

treated with antibiotics or GF mice show lower enteric neuronal density, GI motility and neuronal excitability.^{128–130} In addition, such mice were also reported to exhibit altered TLR expression in the ENS, decreased Peyer's patches counts and mild intestinal inflammation.¹³¹ Microbiota signals were also shown to modulate EGC homeostasis. EGC differentiation and migration from the intestinal muscularis to the lamina propria is determined by microbial cues.¹³² Furthermore, invading or symbiotic bacteria differently regulate TLR, S100 calcium-binding protein- β expression and nitric oxide production by human EGCs.¹²⁵ Finally, it has been suggested that maternal microbiota signals might modulate the nervous and immune systems of their progeny.^{133,134} Altogether, these reports indicate that commensal microbial sensing by the ENS controls GI activity and potentially modulates neuro-immune interactions.

In addition to the recognition of commensal microbiota by neuronal cells, the latter are also able to integrate cues from pathogenic microbes.¹³⁵ Notably, while the capacity of nociceptor neurons to sense fungal and bacterial products has been mostly established in skin,^{136,137} this sensing ability is likely to be conserved in mucosal neurons. In the context of *Staphylococcus aureus* infection in the skin, bacterial components, such as *N*-formylated peptides and the pore-forming toxin α -hemolysin, can directly activate calcium mobilization and CGRP release in dorsal root ganglia sensory neurons, thus modulating infection-induced hyperalgesia.¹³⁶ Similarly, during *Candida albicans* infection, sensory neurons in the dorsal root ganglia are activated upon stimulation with heat-killed *C. albicans* or zymosan, as revealed by increased neuronal calcium flux and release of CGRP.¹³⁷ Interestingly, ablation of sensory neurons leads to an impaired control of *C. albicans* in the skin.¹³⁷ Multiple studies have now demonstrated that microbial pathogen product are sensed by the ENS. Stimulation of EGCs with lipopolysaccharide (LPS) induces IL-1 β production,¹³⁸ while TNF is induced after LPS activation of enteric neurons.¹³⁹ The enteric pathogen *Campylobacter jejuni* was shown to stimulate afferent neurons that innervate the GI tract and spread information to the CNS.¹⁴⁰ Furthermore, toxin B of *Clostridium difficile* activates VIP-positive submucosal neurons.¹⁴¹ An unresolved question relates to the mechanisms by which neuronal cells sense microbial aggressions. It was recently demonstrated that enteric neurons and EGC sense microbial products and parasites, via MYD88 signalling, to produce neuroregulatory molecules that, in turn, activate innate lymphocytes.^{51,76} Nevertheless, whether this sensing property is broadly used by neuronal cells throughout the body, and whether other sensing molecules might be employed by neurons to instruct immune cells remains elusive.

Microbial cues also modify mucosal neuronal activity in an indirect manner. For example, intestinal enteroendocrine cells can sense the luminal environment and convey information to neurons via release of hormones.¹⁴² Commensal spore-forming bacteria induce serotonin synthesis in colonic enteroendocrine cells that in turn act on myenteric neurons to control GI motility.¹⁴³ More recently, it was also suggested that a direct cell-to-cell contact through cytoplasmic processes (neuropods) might mediate enteroendocrine–neuron interactions.¹⁴⁴ Chemosensory serotonin-expressing enteroendocrine cells were found to establish synaptic connections with serotonin-responding neurons, allowing for direct recognition of luminal changes and immediate neuromodulation of intestinal physiologic responses.¹⁴⁵ Noteworthy, pulmonary neuroendocrine cells, which are found in the vicinity of pulmonary neurons, were also shown to promptly induce lung immune responses by releasing neuropeptides.^{107,146}

Enteric neuronal and glial cell sensing of inflammation

Sensory vagus neurons express receptors for pro-inflammatory molecules, such as TNF and IL-1 β , and administration of TNF and

IL-1 β activates firing in the mouse cervical vagus nerve. In turn, vagus stimulation can also regulate the production of immunomodulators by immune cells.¹⁴⁷ In the airway mucosa, release of pro-inflammatory mediators from resident immune cells, including histamine, nerve growth factor, brain-derived neurotrophic factor, IL-1 β , TNF and IL-5, directly affects pulmonary neuronal activity.^{86,89,103,119} In turn, activated neurons release neuropeptides (SP, VIP and CGRP) that promote further activation and recruitment of immune cells.^{80,81} Similarly, enteric neurons and EGC were shown to sense host-derived alarmins, in an MYD88-dependent manner, to produce neuroregulatory molecules that, in turn, activate ILCs.^{51,76} Altogether, these studies indicate that mucosal innervation is able to sense inflammatory conditions and alter its neuronal activity, providing a mechanism for reciprocal neuro-immune regulation.

CONCLUSION AND PERSPECTIVES

The cross-talk between the nervous and immune systems at mucosal barrier has seen major breakthroughs in the recent past. These pioneering studies uncovered cellular and molecular missing links between the two major sensing systems in the body, and explored how these neuro-immune interactions contribute to tissue homeostasis. As an example, there is solid mounting evidence that mucosal-protective immunity can be regulated by neuronal cells,^{47,48,51,52,76} while neuro-immune interactions can be also detrimental in defined mucosal pathological scenarios.^{53,103} Nevertheless, our understanding of how neuronal and immune cells interact and respond to ever changing physiological and pathological conditions is still very limited and has been hampered by conceptual and technical limitations.

Using genetic mouse models or in vitro studies, it has been shown that distinct immune cells can integrate signals from a wide range of neuromodulatory molecules.^{5,7} However, since multiple non-neuronal cells can also secrete molecules historically ascribed to be of neuronal origin, whether the observed effects are underlined by neuro-immune interactions remains unclear. Another major challenge in interrogating and deciphering novel neuro-immune cell units (NICUs) relates to our poor understanding of mucosal neuronal circuit architecture and their molecular fingerprints. Nevertheless, the use of combined recent technological advances might allow to address these unmet needs in the future. A clear example may be provided by the use of mouse models with RiboTag targeted mutation of the ribosomal protein L22 locus associated with a *Cre* driver mouse line, which allows for immunoprecipitation of actively translated ribosome-associated mRNAs from specific neuronal cells.^{47,148} In addition, technologies that interrogate the neuronal anatomical mapping and its functional connectivity, such as noninvasive gene delivery using engineered viral tracing,¹⁴⁹ may also help shed light into mucosal neuronal circuits. Classically established methods to functionally address neuronal activities, such as physical lesions, pharmacological or chemical inhibitors and electrical stimulation, can provided useful information on how neuronal circuits shape immunity. Nevertheless, most of these methods are not tractable, and fail to provide tissue-autonomous information at cellular and molecular level. The development of opto- and chemo-genetics allows to manipulate neuronal activity in a tractable manner and, therefore, these approaches will possibly establish causal relationships between defined neuronal activity and discrete immune response.⁵ As an example, the use of opto-genetic approaches was recently used to reveal macrophage-neuron cell units in the adipose tissue.⁵⁶ Nevertheless, while these novel experimental approaches are likely to advance our understanding of mucosal neural activity, there are some pitfalls that should be taken in consideration. Notably, despite the existence of a wide range of transgenic mouse lines driving *Cre* recombinase expression, none

of these lines specifically target mucosal neurons since the latter share development and molecular pathways with other central and peripheral neurons. Other limitations can also occur when using chemo- and opto-genetic approaches. For example, when using designer receptors exclusively activated by designer drugs (DREADDs), spatial specificity is only possible with high-resolution delivery of the modulatory drug, which can be an obstacle in the GI tract.¹⁵⁰ The implementation of opto-genetic techniques at mucosal sites may also be hampered by the further need to develop a portable and stable micro device that allow normal behaviour in mice.¹⁵⁰ Nevertheless, the more recent development of a transparent graphene sensor with a customized abdominal window may allow, in the future, to harness opto-genetics to study neuro-immune interactions at mucosal sites.¹⁵¹

A deeper understanding of how neuro-immune interactions regulate tissue and organ physiology is a major challenge in the future. The interrogation of cellular and molecular players of mucosal NICUs will shed light into the neuro-immune scaffolds that underlie tissue homeostasis. In addition, these efforts will likely lead to the identification of novel targets that may be used in the therapy of mucosal inflammatory, infectious and cancer conditions. In conclusion, the mucosal nervous system partners with immune cells to ensure local tissue health. Since the pressure of pathogens and other environmental cues is an important evolutionary force, it is possible that the establishment of functional NICUs may have occurred very early in evolution. Finally, this new paradigm of coordinated multi-tissue physiology may also exist beyond mucosal tissues, raising the possibility that neuro-immune sensory units may also regulate physiology and homeostasis at an organismal level.

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ADDITIONAL INFORMATION

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