Author/s:
Trevizan-Bau, P;Mazzone, SB

Title:
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Date:
2023-11-01

Citation:
Trevizan-Bau, P. & Mazzone, S. B. (2023). Neuroimmune pathways regulating airway inflammation. Annals of Allergy Asthma and Immunology, 131 (5), pp.550-560. https://doi.org/10.1016/j.anai.2023.07.020.

Persistent Link:
https://hdl.handle.net/11343/337337

Neuroimmune Pathways Regulating Airway Inflammation

Pedro Trevizan-Baú, PhD[1,2] and Stuart B. Mazzone, PhD[3*]

1 Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria, Australia

2 Department of Microbiology and Immunology, University of Melbourne, Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia

3 Department of Anatomy and Physiology, University of Melbourne, Victoria, Australia

*** Corresponding Author:** Professor Stuart B. Mazzone, Department of Anatomy and Physiology, University of Melbourne, Victoria, Australia; +61 3 83446457; stuart.mazzone@unimelb.edu.au

Key words:

Sensory neurons; Sympathetic; Parasympathetic; Vagal; Anti-inflammatory reflex; Vagal; Neuropeptides; Neuroinflammation

Abbreviations:

α7nAChR, alpha 7 subtype nicotinic acetylcholine receptor

ATP, Adenosine Triphosphate

CGRP, Calcitonin gene-related peptide

COPD, Chronic obstructive pulmonary disease

DAMPs, Danger-associated molecular patterns

HMGB1, High Mobility Group Box-1

IFNAR, Type I interferon receptor

IFNGR, Type 2 interferon receptor

IgE, Immunoglobulin E

IL-, Interleukin

ILC2, Type 2 innate lymphoid cells

LPS, Lipopolysaccharide

Mrgprs, Mas-related G protein-coupled receptors

NEBs, Neuroepithelial bodies

P2X, ATP-gated receptor cation channel family

RAGE, Receptor for advanced glycation end-products

Th2, Type 2 helper T

TLR, Toll-like receptor

TNFα, Tumor Necrosis Factor alpha

TRP, Transient Receptor Potential

VIP, Vasoactive intestinal peptide

Glossary of terms

Afferent and efferent: In the context of this review, afferent refers to sensory function of nerve fibers while efferent refers to their motor function.

Alarmins: Early mediators of inflammation that can act as danger signals recognized by the immune system. Examples include HMGB1 and ATP.

Anti-inflammatory reflex: A neural circuit involving sympathetic innervation to the spleen and other abdominal viscera which serves to dampen system inflammation.

Bitter taste receptors: A class of seven transmembrane G-protein coupled receptors (known as Tas2rs) responsive to chemicals with a bitter flavor and normally found in taste buds on the tongue. Tas2Rs are also expressed by airway brush cells.

Brush cells: Specialized chemosensory airway epithelial cells that express bitter taste receptors, receive innervation from the nervous system and are involved in immune regulation.

Ganglia: A component of the peripheral nervous system where the cell bodies of sensory or autonomic neurons reside. Examples include the vagal sensory ganglia, dorsal root sensory ganglia and sympathetic pre-vertebral ganglia.

Neuroendocrine cells: Specialized chemosensory airway epithelial cells that possess features like both nerve cells and endocrine cells. They receive innervation from the nervous system and make and secrete mediators in response to chemical stimuli.

Neurogenic inflammation: Inflammation mediated through neural mechanisms.

Neuroimmune synapse: Direct signaling between nerve fibers and immune cells may occur when both reside in close spatial proximity.

Neuroinflammation: Inflammation within the peripheral and/ or central nervous system.

Plasticity: This term is used to describe the changes that can occur in neurons, or the neuroimmune/ neuroendocrine processes discussed in the review.

Targeted lung denervation: Radio frequency energy delivered via bronchoscopy to achieve full circumferential nerve ablation in the main bronchi of each lung.

Funding statement: SBM is supported by grants from the National Health and Medical Research Council of Australia (NHMRC; grant ID 1078943), and the Australian Research Council (ARC; grant ID DP210103740).

Clinical Trial Registration: Not applicable

Conflict of Interest: SBM declares honoraria from Merck, NeRRe Therapeutics, Reckitt Benckiser and Bellus Health and grant support from Merck and Bellus Health. None of the disclosed entities above had any involvement in the conceptualization, design, data collection, analysis, decision to publish, or preparation of the manuscript.

Word count: 4399

Figures: 4

Tables: 1

INTRODUCTION

Airways inflammation is a common feature of pulmonary morbidity and defining the specific nature of inflammation has been central to understanding the different pathophysiology of diseases like asthma, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, bronchiectasis, and others. A major focus of airway research has been to resolve the identity of the cellular players and chemical mediators involved in a particular pathology with the notion that these cells and processes impact the normal functioning of airway tissues and therefore represent the drivers of the hallmark symptoms displayed by patients. With respect to the nervous system, a substantive body of literature confirms that inflammatory processes can alter the structure and function of airway nerves leading to symptoms such as coughing, sneezing, dyspnea and reflex changes in bronchomotor tone, mucus secretion and blood flow, reviewed in[1]. However, a more up-to-date perspective positions the nervous system as an active player in immune regulation, rather than simply a passive target of inflammation[2,3]. In this regard, neural and immune processes may be bidirectionally intertwined, such that altered neural activity as a result of inflammation is also involved in the activation, coordination and regulation of the immune system, both of which contribute to the clinical presentation of disease. This article will focus on recent preclinical discoveries that support neuroimmune crosstalk and discuss considerations for clinical translation of these discoveries to improve the management of patients with airways disease.

FRAMEWORKS SUPPORTING CROSSTALK BETWEEN NEURONS AND IMMUNE CELLS

The airways, from the nose to the lung parenchyma, and from the epithelial surface to the deepest layers and adventitia, have a rich supply of nerve fibers mostly derived from cranial (trigeminal, glossopharyngeal and vagal) and sympathetic nerve branches[4] (Figure 1). These fibers are broadly classified as either sensory (afferent) or motor (efferent) in action, although functionally and molecularly

defined subsets of each of these broad divisions exist (reviewed in detail elsewhere)[4]. Sensory fibers arise from the cranial nerve sensory ganglia (majority) and the spinal dorsal root ganglia (minority) and are responsive to a wide variety of chemical and physical stimuli. Motor fibers are either parasympathetic or sympathetic in nature and regulate diverse airway end organs, including glands, airway and vascular smooth muscle, and other specialized intrinsic airway cells. Both sensory and motor fibre types are influenced by inflammation leading to altered activity, sensitivity and/ or nerve fibre density. Additionally, available evidence suggests that each fibre type contributes to the regulation of a variety of immune cells, including mast cells, dendritic cells, type 2 T helper (Th2) cells, B cells and innate lymphoid type 2 cells (ILC2s)[2,3].

Signaling frameworks for neuroimmune crosstalk

The nervous and immune systems each rely on chemical signaling molecules and receptors to communicate between cells and interact with the local environment. This not only allows for within system communication but also communication between the two systems. Thus, airway neurons are equipped with an array of receptors that bind cytokines, tissue alarmins and other molecules known to orchestrate inflammatory responses (Figure 2). Examples include receptors for tumor necrosis factor alpha (TNFα), prostaglandins, leukotrienes, interferons, interleukins, and adenosine triphosphate (ATP), each of which provides a means for the immune system to alter airway neuron structure and function, common in airways disease[4-10]. Importantly, resident and infiltrating immune cells often express receptors for neurotransmitters, enabling immune cells to be responsive to a range of neuropeptides, acetylcholine, noradrenaline, serotonin and dopamine[11-13] (Figure 2). A classic example of this is the neuropeptide substance P which has long been known to be a mediator of the neurogenic flare and itch response in human skin, in part through its ability to degranulate mast cells[14].

Signaling pathways and molecular machinery that have been traditionally ascribed to the nervous system are increasingly identified in immune cells, while functions thought to be immune cell specific have been shown to be present in airway neurons. Semaphorins, through actions on neuropilin receptors, are best known for their role in neuronal axon guidance, but more recently members of this family have been identified as stabilizers of regulatory T cell (Treg) activity during allergic lung inflammation[15-17]. Similarly, an array of ion channels commonly associated with stimulus transduction and electrical events in the excitable membranes of airway neurons have been identified in immune cells, including members of the transient receptor potential (TRP) family of cation channels and voltage gated sodium channels. TRPA1 channels, most notable for their expression by airway sensory neurons, appear to be upregulated in Th2 cells in murine models of asthma, and genetic deletion or pharmacological inhibition of TRPA1 in animal models improves disease severity[18]. Conversely, airway neurons express toll-like receptors (TLRs) and other receptors involved in respiratory pathogen recognition, as well as inflammatory signaling molecules such as the alarmin High Mobility Group Box-1 (HMGB1), a key molecule coordinating airways inflammation and currently under clinical trial exploration as a therapeutic target in patients with airways diseases[8,19]. Other examples of regulatory systems shared by neurons and immune cells include members of the Mas-related G protein-coupled receptor (Mrgprs) family, which play a role in communication between the nervous and immune systems and are important regulators of chronic inflammation[20,21]. This ever-growing list of shared signaling pathways might suggest that these two fundamental communication systems, the nervous and immune systems, are more closely related than previously considered.

Anatomical frameworks for neuroimmune crosstalk

Airway nerve fibers and resident or recruited airway immune cells frequently exhibit a close spatial proximity to one another, which has been posited as evidence of 'neuroimmune synapses' serving to facilitate crosstalk between these two systems. For example, in animal models of allergic asthma and in airway samples from human asthma patients, eosinophils are commonly observed to be enriched in number around airway nerve fibers and their products, including major basic protein, have been shown to regulate nerve fibre activity and density[22,23]. Similar associations between airway nerve fibers and pulmonary macrophages and mast cells have been identified[24], while sensory nerve terminals are most dense along the airway mucosa[4], where mucosal immune processes are essential for barrier defense. Direct neural innervation of specialized intrinsic airway immune tissues, including bronchial associated lymphoid tissue[25], has also been reported and neural innervation additionally extends to extrinsic immune tissues and organs, such as the spleen[26], providing an important framework for neural control of the systemic components of inflammation that contribute to many airways diseases.

The interaction between the nervous and immune systems need not be through direct neuroimmune contacts. Many signaling mediators can diffuse from their site of release and influence distant cells. Additionally, an increasing number of specialized, and often rare, airway cell types, including airway neuroendocrine cells, neuroepithelial bodies (NEBs) and brush cells, are innervated by the nervous system and play important roles in inflammatory control[27-30] (Figure 2). In this regard, such cells may serve as intermediaries, relaying signals between airway nerves and classic immune cells, and in doing so coordinate neuroinflammatory processes. An interesting example of this are the tracheal brush cells which may act as early responders to pathogens in the respiratory system. Brush cells express bitter taste receptors, known as Tas2Rs and more commonly associated with sensing bitter tastes in lingual taste buds, but can also be activated by molecules expressed by certain pathogens, including gram negative and positive bacteria[29]. The resultant signaling events in brush cells lead to the release of mediators including acetylcholine, cysteinyl-leukotrienes and ATP which in turn stimulates adjacent

airway nerve fibers to release neuropeptides resulting in plasma extravasation and neutrophil recruitment, along with elevated levels of complement system components and other mediators accompanying inflammation[29,31]. Comparable cells are also present in the upper airways[32]. Airway neuroendocrine cells, similar to brush cells, display molecular characteristics of both neuronal and endocrine cell types[33]. They are often found clustered as 5-20 cell NEBs, an airway organelle that is densely innervated by sensory nerve fibers and additionally contains Clara-like cells, collectively forming a unique NEB microenvironment[33]. NEBs are concentrated at airway branch points, ideally positioned to sense aerosolized particles which concentrate at these locations. Consistent with this, NEBs are putatively involved in generating type 2 immune responses to aeroallergens[34]. As neuroendocrine cells have a neuronal phenotype and NEBs are directly innervated by sensory nerve fibers, a signaling nexus between NEBs, nerves and airway immune cells, notably ILC2s, likely exists. Interestingly, NEB prevalence and cell composition is regulated over the lifespan and in disease, suggesting an important role for NEBs in both the development and repair of the airways[35].

SENSORY NEURON-IMMUNE CELL CROSSTALK

Specialized subsets of airway sensory neurons known as nociceptors monitor the airway environment for potentially noxious or harmful stimuli, including a wide range of chemical irritants, pathogens and the products of inflammation[4]. The most fundamental role of airway nociceptors is an afferent function, needed for the initiation of defensive reflexes that both trap and limit exposure to irritants (mucous secretion and bronchoconstriction) and facilitate irritant clearance (cough). In this regard, nociceptors are equipped with receptors and ion channels for detecting diverse physical and chemical stimuli and transducing these stimuli into centrally propagating action potentials needed for initiating reflex events[4] (Figure 3).

Recent studies suggest that nociceptors not only respond to traditional inflammatory stimuli, such as cytokines, prostaglandins, leukotrienes and bradykinin, but they are also responsive to a range of danger-associated molecular patterns (DAMPs) or early inflammatory alarmins, and even pathogens themselves[5,6,8-10]. For example, airway sensory neurons express the receptor for advanced glycation end-products (RAGE) and TLR4, receptors for the alarmin HMGB1, a key epithelial and inflammatory cell mediator involved in the pathogenesis of airway infections and chronic lung diseases such as asthma[8]. HMGB1 applied to airway nociceptors evokes action potentials and promotes sensory neuron growth, dependent on RAGE expression, suggesting that this signaling axis may be one mechanism that leads to reflex hypersensitivities and sensory neuron hyperinnervation (Figure 3). Other novel inflammatory mediators regulating airway sensory neurons include the shingolipid metabolite, shingosine-1-phosphate via S1PR3 receptors[10], the purine ATP notably via P2X3 homomeric or P2X2/3 heteromeric receptors[36], and interferons via and type I (IFNAR1) and type 2 (IFNGR1 and IFNGR2) interferon receptors[5]. Interestingly, some pathogens may generate molecules that directly promote sensory neuron activation independent of inflammation. Mycobacterium tuberculosis was recently shown to activate airway nociceptors via the cell wall derived glycolipid, sulfolipid-1. Notably, sulfolipid-1 is an effective activator of the cough reflex, suggesting that this may represent a mechanism to facilitate bacterial transmission[37].

In addition to afferent functions, nociceptors also display efferent functions mediated by their ability to release neuropeptides, such as substance P, CGRP and vasoactive intestinal peptide (VIP), from their peripheral nerve terminals in the airways. Sensory neuron derived neuropeptides regulate the recruitment and activation of diverse inflammatory cell types, blood flow and vascular permeability, and mucous secretion – so called neurogenic inflammation[4]. For example, in murine models of allergic airway inflammation, sensory neuron activation and VIP release was shown to amplify ILC2 and Th2 cell activation while substance P promoted mucous cell metaplasia[38,39]. Intriguingly, sensory neuron

expression of the high affinity IgE receptor (FcεR1) was shown to directly respond to allergen complexed with IgE to amplify Th2 cell immunity, a novel mode of sensory neuron activation leading to the release of substance P[7]. Indeed, the silencing of sensory neurons has therapeutic benefit in these preclinical models of allergy by reducing inflammation[39]. Efferent functions of nociceptors may extend to modulating adaptive and humoral immunity, as airway nociceptor activation and substance P release was shown to help trigger the formation of antibody-secreting B cells and their release of IgE[40].

Many similar examples of sensory neuron-immune interactions have been reported in other tissues, including the skin and gastrointestinal tract, leading to suggestions that nociceptors may subserve the role of a master regulator of skin and mucosal immunity[2]. However, the immune regulation mediated by airway sensory neuron activation may be more nuanced than simply promoting inflammation. For example, CGRP has emerged as a potential negative regulator of ILC2s in allergic asthma, although whether this is neuronally derived is unclear[41,42]. Similarly, in preclinical models of lethal Staphylococcus aureus pneumonia, nociceptor activity and CGRP release was shown to suppress the recruitment and surveillance of neutrophils and lung γδ T cells, both of which are necessary for bacterial immunity[43]. Consequently, targeted ablation of airway sensory neurons increased γδ T cell– and neutrophil-mediated host defense in response to Staphylococcus aureus pneumonia, promoting lung bacterial clearance and survival[43].

Despite these interesting and powerful preclinical studies noted above, evidence for meaningful sensory neuron mediated modulation of inflammation in airways disease in humans is lacking. Surprisingly, even though the highly accessible nasal airway environment provides an opportunity to address this, there have been relatively few studies with no conclusive evidence for neural regulation of inflammation in human clinical conditions, reviewed in[44]. Whilst older studies have shown that the sensory neuron stimulant capsaicin produces rhinorrhea, pain and inflammatory cell recruitment in the nose[45], whether inflammatory responses are due to specific actions of sensory neurons is unclear.

Humans with various airways diseases do show signs that nociceptors become sensitized and undergo plasticity resulting in altered neuronal phenotypes and nerve terminal density in the airways, processes that are thought to contribute to disease pathogenesis[4]. A common example of this is in patients with chronic cough, where the underlying features are suggestive of a sensory hypersensitivity disorder characterized by an increase responsiveness to a range of sensory stimuli[46]. Hypersensitivity is evident upon cough challenge testing employing sensory neuron stimuli and presents clinically as excessive coughing in response to relatively innocuous odors, changes in air temperature or activities, such as laughing. In addition, biopsies taken from the airways of chronic cough patients have shown evidence of hyperinnervation, where the average nerve length and number of branching points were significantly increased in epithelium, but not subepithelium, compared to nerve fibers in healthy airways[47]. These functional and structural changes in airway sensory neurons are believed to be linked in part to neuroinflammatory events, whereby the extrinsic peripheral nerves and associated sensory ganglia that supply the airways and lungs develop an inflammatory phenotype (Figure 3). Indeed, in preclinical studies, vagal sensory ganglia display elevated numbers of dendritic cells and macrophages during airways inflammation associated with severe influenza infection[48], allergen sensitization[49] and challenge or exposure to the bacterial cell wall endotoxin lipopolysaccharide[50]. Accompanying this elevation in ganglia inflammatory cells is the nuclear-to-cytoplasm translocation of neuronal HMGB1, a sign of potential neuronal injury and a known mediator of immune cell recruitment and expansion[8]. Consistent with this, both respiratory viral infection and the bacterial cell wall endotoxin lipopolysaccharide (LPS) exposure dramatically change the transcriptional landscape in the vagal ganglia with evidence of an upregulation of a range of pro-inflammatory and injury associated genes[48,50].

The cause of neuroinflammation is not entirely clear. In some circumstances, airway nerves may be directly impacted by pathogens which can infect nerves and promote neuroinflammation. This has been shown to occur for some respiratory viruses, including influenza and human meta pneumovirus, and has

been proposed as a mechanism contributing to the neurological impacts of Sars-CoV2 in COVID-19[51]. Neuroinflammation may also arise from aberrant and excessive airway sensory neuron signaling generated by the sensory neuron nerve terminals located in the airway inflammatory environment. In this regard, the peripheral inflammatory events triggered by infection and disease may establish a secondary pathology in the nerve supply to the airways which contributes to overall disease pathogenesis though promoting disordered sensory neuron function, phenotype and structure. This secondary pathology may extend on occasions into the central nervous system, as evidenced in preclinical models of pulmonary fibrosis and disease exhaust particle exposure where markers of inflammation are elevated in brainstem regions receiving inputs from airway sensory neurons[52,53]. The mechanisms underlying the development of central neuroinflammation are poorly described for airways diseases, although similar events are seen in other conditions of sensory hypersensitivity, including chronic pain, where central nervous system inflammatory processes are thought to have a significant contribution to clinical disease presentation. Whether human airway nerve supplies or their upstream brain circuits also display neuroinflammatory phenotypes in airways disease is not clear. However, the clinical presentation of patients can often be consistent with neuropathic changes, for example in the vagus nerve in patients with chronic cough and laryngeal dysfunction[54].

AUTONOMIC NEURON-IMMUNE CELL CROSSTALK

Both sympathetic and parasympathetic neural outflows from the central nervous system may contribute to the regulation of inflammation. This is supported by clinical observations suggesting that autonomic imbalances may contribute to aberrant inflammatory processes, including in systemic autoimmune diseases (e.g., rheumatoid arthritis or systemic lupus) and localized diseases such as those impacting the pulmonary system (discussed below). Furthermore, therapies targeting autonomic

processes which are commonly used in treating airways diseases, such as β-adrenergic agonists and anticholinergics, may have effects on the inflammation accompanying disease[55,56]. Although this interaction between the autonomic nervous system and immune system has long been recognized, the specific mechanisms involved have only recently begun to be elucidated. Much of the experimental work in this regard has focused on understanding how autonomic neural activity influences immune organs, such as the spleen, thereby regulating the systemic contributions to inflammatory disease. However, local tissue interactions between autonomic nerves and immune cells have also been described.

Neuroanatomical studies have revealed that sympathetic autonomic neurons pools in the celiac and splanchnic prevertebral ganglia densely innervate immune tissues in the spleen and at other abdominal locations[57] (Figure 4). These peripheral sympathetic neurons are in turn regulated by a dedicated immune-specific subset of sympathetic neurons arising from the spinal cord which respond to an immune challenge and increase output to peripheral sympathetic ganglia, notably via the splanchnic nerves[26,58]. Surgical resection of the splanchnic nerve, or modulation of its activity, results in substantive measurable effects on splenic mediated immune processes. For example, experimental studies have demonstrated increased splanchnic nerve activity during systemic LPS inflammation, suppresses the plasma levels of pro-inflammatory cytokines TNFα, interferon gamma and interleukin (IL-6), which concomitantly enhance the levels of circulating anti-inflammatory cytokines (e.g., IL-10). This autonomic mediated response has been dubbed 'the anti-inflammatory reflex' and demonstrated to be recruitable in studies using rats[59], mice[60], and sheep[60]. Recently, it was found that pro- and anti-inflammatory cytokines are controlled by distinct sympathetic efferent pathways, but still within the splanchnic nerves[61]. Furthermore, the extent of sympathetic neural regulation of inflammation by the splanchnic nerves may be distributed across immune cell pools in multiple abdominal organs such as the spleen, adrenals, intestine, liver, stomach, and pancreas[26].

Although the mechanisms leading to the activation of the splanchnic sympathetic outflow are not fully characterized, it seems likely that sensory neuron activation (as described above) leading to reflex regulation of central sympathetic pathways may be involved. Interestingly, in an experimental rat model of intestinal inflammation, abdominal vagus nerve stimulation was shown to be a safe and effective treatment for reducing inflammation severity and improving intestinal function[62] suggesting therapeutic potential of activating the anti-inflammatory reflex via abdominal vagal sensory neuron stimulation. Consequently, human clinical trials are currently underway to investigate the anti-inflammatory actions of vagus nerve stimulation via implantable nerve stimulators in patients with Crohn's disease (trial: NCT05469607), which if successful may pave the way for future trials in patients with a variety of chronic inflammatory conditions.

Autonomic neural pathways have the potential to modify inflammation because many immune cells are responsive to autonomic neurotransmitters, due to their expression of adrenergic and cholinergic receptors. Indeed, a large literature supports a role for sympathetic nerves modulating airway immune responses via noradrenaline mediated activation of β2 adrenergic receptors on immune cells[12,63-65]. However, other presumed sympathetic neurotransmitters, such as dopamine, may have similar actions, such as alleviating allergen induced ILC2 responses and airway inflammation[13]. Notably Liu and colleagues[65] showed that both surgical sympathetic denervation and chemical ablation of sympathetic nerves in the lung enhance airway inflammation after LPS immune challenge, indicative of a meaningful role for ongoing sympathetic nerve activity in controlling airway inflammation severity. Some actions of parasympathetic neural pathways may also be beneficial in airways disease, despite the association of cholinergic neural processes with the development of airways hyperreactivity and excessive mucus production[4]. For example, vagotomy (severing the preganglionic parasympathetic input to the lungs) worsened Escherichia coli and influenza-induced acute lung injury[66,67], perhaps by reducing vagally-mediated anti-inflammatory responses. Similarly, an alpha 7 nicotinic acetylcholine receptor (α7nAChR)

agonist attenuated hyperoxia-induced lung injury by reducing the accumulation of pulmonary and circulating HMGB1[68], and enhanced lung stem cell proliferation and transdifferentiation promoting lung repair during LPS-induced lung injury[69]. Whether these responses are due to local or systemic (e.g., via the spleen) actions of the vagus nerve is unclear and appear specific for nicotinic cholinergic receptor mechanisms, as the effects of muscarinic acetylcholine receptor activation are not beneficial in airways disease[56].

β- adrenergic agonists and anticholinergics drugs that target autonomic neural processes have been a mainstay of airways disease symptom management for decades and whilst anti-inflammatory properties of such agents are predicted, and in some cases demonstrated in experimental studies, detailed clinical evidence has yet to be fully collected. In a recent systematic review of the anti-inflammatory properties of muscarinic receptor antagonists revealed current evidence is limited to studies of tiotropium, demonstrating an impact on cytokine and other inflammatory mediator production, and inflammatory cell counts induced by different pro-inflammatory stimuli[70]. Limited data exist for other inflammatory endpoint measures and for other anticholinergic agents used commonly in the clinic. Similarly, despite the preclinical evidence supporting an anti-inflammatory action of beta-adrenergic agonists, demonstrating this in patients with airways disease is complicated by the fact that many patients receiving β-agonists also concomitantly receive corticosteroid therapy. Whether these drugs impact the sequela of airways inflammation, for example sensory ganglia neuroinflammation and resultant hypersensitivity and neuronal remodeling, is unclear although preclinical studies in rodents suggest at least tiotropium may have direct sensory neuron modulating effects[71].

CENTRAL NEURAL PROCESSES

The central nervous system (CNS) represents an important link between visceral sensory and autonomic motor activity. The role of the CNS in visceral inflammatory disease has perhaps been best studied with respect to the gastro-intestinal system, where the importance of the Gut-Brain axis is well established, reviewed in[72]. In this regard, the local gastrointestinal environment, established by gut microbiota, neuroendocrine and immune signaling, plays a critical role in shaping neural activity flowing to and from the CNS. The ramification of this signaling is widespread, not only influencing gut function but also impacting other organ systems, including neurological processes controlling anxiety, mood and cognition. Although less developed in concept, a lung-brain axis exists, with the local lung environment contributing to sensory signaling to the brain and impacting neurological function. Consequently, lung inflammation and disease can have profound impacts on mood and cognitive function. For example, in preclinical animal studies, airway allergic inflammation has been shown to influence emotional-like behaviors through 'bottom-up' pathways impacting the prefrontal cortex and amygdala[73,74]. In patients, these pathways may be responsible for diverse neurological manifestations, including the development of anxiety, depression and attention impairment, common in patients with asthma or other chronic lung diseases, and perhaps even long-COVID syndrome [75-77].

Preclinical and clinical studies have mapped the neural circuitry through which airway sensory neuron inputs reach higher brain centers[78,79], including in patient populations[80,81], providing the neuroanatomical substrates for the lung-brain axis. Notably, the lung-brain axis is unlikely to be unidirectional. Indeed, emotive processes in the brain may contribute to disease severity in asthmatic patients through 'top-down' processes[82,83]. For example, a populational study showed that emotional disorders may drive asthma exacerbations[84], and experimentally, it has been demonstrated that higher brain activity associated with emotion, such as in the amygdala, can regulate airway resistance and bronchoconstriction in anxiety-like behavior, reviewed in[85]. Interestingly, recent studies have also identified a role for the higher brain in storing immunological memory[86]. In these preclinical studies,

ensembles of neurons in the insula cortex, a region of the brain known for interoceptive and exteroceptive sensory processing, were shown to acquire and retrieve specific immunological information and provide powerful immunological neural drives to peripheral tissues. This is somewhat reminiscent of much older studies demonstrating Pavlovian-style conditioning of immune cell activity[87], presumably though higher brain-dependent processing. How and when these higher order control systems are engaged, their interrelationship with the peripheral neural and immunological processes more commonly studied and their true contribution to disease pathology in humans awaits further investigations.

EMERGING CLINICAL CONSIDERATIONS

Understanding the complexities of neuroimmune crosstalk in different airways diseases may help improve the delivery and efficacy of existing and emerging treatments and identify opportunities for novel therapy development. For example, the potential role of the airway nervous system in regulating immune cell function is intriguing considering recent advancements towards the use of targeted lung denervation in the treatment of airways diseases, notably COPD and asthma[88,89]. In a double-blind, randomized, sham-controlled study targeted lung denervation in patients with moderate to severe COPD, patients in the active arm demonstrated a significantly lengthened time-to-first severe COPD exacerbation at 2 years post-denervation which remained largely stable over a total of 3 years of follow up[90,91]. However, no effects on markers of inflammation were assessed. In two patients with severe asthma, targeted lung denervation improved cough in both patients, and one patient reported a marked reduction in rescue medication use at 6 months, although lung function was unaltered in both and again measures on inflammation were not collected[88]. In preclinical studies in sheep, circumferential targeted denervation of the mainstem bronchi produced a stable reduction in airway efferent nerves at and distal

to the denervation site, as evidenced by a reduction in nerve fibre profiles expressing a marker for acetylcholine[92]. This may argue a potential effect on measures of airways inflammation in disease, similar to that expected for anticholinergic therapy.

Recent years has seen a rapid progression of airway sensory nerve targeting drugs entering into clinical trials for treating the symptoms of airways diseases (notably chronic cough) [46,93], including agents targeting purinergic (P2X3) signaling, various TRP channels (TRPV1, TRPA1, TRPV4, TRPM8), and sensory nerve specific voltage gated sodium channels which serve as the master regulator of action potential formation and propagation. Although the agents have shown mixed efficacy on the primary endpoint under investigation, the impacts on disease associated inflammation have largely not been recorded or reported. Given the strong preclinical evidence for the role of sensory neuron derived neuropeptides in regulating inflammation, assessment of this as an endpoint in clinical trials may assist with unpicking the relative importance of this mechanism regulating human disease. Indeed, histological studies suggest that neuropeptide expression in human airway tissues is significantly less abundant compared to rodents[47], raising questions as to the translatability of the preclinical studies. Conversely, lavage samples taken from patients with existing airways diseases frequently measure elevated levels of substance P and CGRP, complicating the interpretation of histological findings. Specific endpoint measures in clinical studies may help shed light on these conflicting observations.

CONCLUSIONS

In preclinical studies, a nexus between neural and immune signaling pathways has been demonstrated, involving the interplay between different neural and immune cell types, supported by common signaling pathways, close anatomical relationships, dedicated reflex pathways and specialized airway structural cells. Plasticity in the signaling pathways contributing to this neuroimmune crosstalk

could involve changes in neural, immune or endocrine systems that in turn could have meaningful impacts on disease severity and patient morbidity. The implications of these emerging preclinical findings consolidate the notion that the nervous and immune systems are intimately intertwined in performing the roles of airways protection, injury responses, and tissue remodeling and repair. Even though translating these preclinical findings into improved management of patients with an airways inflammatory disease has proved challenging, the strong preclinical frameworks supporting their importance provide impetus for further work to be dedicated towards this outcome.

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Figure Legends

Figure 1: Neural innervation to the airways. The airways receive sensory and motor neural innervation carried by cranial (vagus) and spinal nerves. Sensory neurons arise from the vagal nodose, vagal jugular and spinal dorsal root ganglia and can display both afferent and efferent signaling, while brainstem-derived vagal motor neurons and spinal cord derived motor neurons are efferent only, representing the parasympathetic and sympathetic innervation respectively. Each of these pathways can be impacted by inflammation as well as act to regulate immune cell function.

Figure 2: Modes of sensory neuroimmune crosstalk in airways disease. The airway mucosa represents an ideal location for neuroimmune interactions as airway epithelia, sensory nerve terminals and immune cells share close spatial proximity. (a) Sensory neurons may directly interact with immune cells through neuroimmune 'synapses' or (b) a functional interaction may be mediated by specialized epithelia cells, including brush and neuroendocrine cells (NEBs).

Figure 3: Neuroinflammatory mechanisms in airways disease. Inflammation in the lungs has been shown to promote the development of inflammation within the nervous system (neuroinflammation). The mechanisms are not fully understood, but likely involve excessive inflammatory driven sensory neuron signaling, leading to release of proinflammatory mediators (including HMGB1 and ATP) in the sensory ganglia. Neuroinflammation leads to altered sensory neuron growth and sprouting (structure), neurochemistry (phenotype) and sensitivity (excitability), contributing to the morbidity associated with airways disease.

Figure 4: Anti-inflammatory reflex mechanisms in airways disease. Spinal sympathetic outflows via the splanchnic nerve reach immune cell pools in multiple abdominal organs, including the spleen, stomach, liver and intestines. Activation of these sympathetic pathways during systemic inflammation inhibits the production of pro-inflammatory mediators and promotes the production of anti-inflammatory mediators. Loss of the anti-inflammatory reflex results in dysregulated inflammation while mimicking it can be therapeutic in airways disease.

731 **Table 1: Important concepts in neuroimmune crosstalk**

Concept	Example(s)
Neuroimmune frameworks	
Neurons and immune cells share common and complimentary signaling processes	• Immune cells can express receptors for classic neurotransmitters, including neuropeptides, acetylcholine and noradrenaline • Nerve fibers express receptors for classic inflammatory mediators, including interferons, prostaglandins and cytokines
Close spatial proximity between immune cells and airway nerve fibers facilitates bidirectional communication	• Eosinophils shown to concentrate around airway nerves in asthmatic patients • Sensory neuropeptides modulate immune cells in airway mucosa
Specialized airway epithelial cells can facilitate neuroimmune interactions	• Airway brush cells sense bacterial stimuli and activate neurons via ATP and other mediators • Neuroendocrine cells forming neuroepithelial bodies (NEBs) positioned at airway branch sites and help regulate allergic inflammation via neuronal signaling
Sensory neural pathways	
Sensory neuropeptides can facilitate or inhibit inflammatory cell actions	• Substance P and vasoactive intestinal peptide promote allergic inflammation • Calcitonin gene-related peptide negatively regulates ILC2s and neutrophils
Sensory neurons can respond to and become sensitized by tissue alarmins and pathogen-products	• ATP Binding to P2X receptors and HMGB1 binding to RAGE have actions on airway sensory neurons • Mycobacterium tuberculosis activates sensory neurons via cell wall derived glycolipid, sulfolipid-1
Sensory ganglia can become inflamed during airway pathologies	• Influenza infection shown to promote inflammatory cell recruitment and transcriptional changes in vagal ganglia • Patients with laryngeal dysfunction can show signs of vagal neuropathy
Sensory hypersensitivity contributes to disease morbidity	• Chronic cough is associated with cough hypersensitivity syndrome, characterized by heightened sensitivity to a broad range of triggers • Sensory innervation to the airways is increased in patients with asthma and chronic cough
Autonomic neural pathways	
An anti-inflammatory reflex regulates systemic components of inflammation	• The sympathetic nervous system suppresses systemic inflammation via splanchnic nerve

	innervation of the spleen and abdominal viscera • The anti-inflammatory reflex can be initiated through vagal sensory neuron stimulation
Common therapies targeting autonomic neural processes can modify inflammation	• The muscarinic receptor antagonist tiotropium reduces cytokine and inflammatory mediator production/ cell recruitment induced by different pro-inflammatory stimuli • Endogenous noradrenaline and dopamine, as well as beta-adrenergic therapies, suppress immune cells • alpha 7 nicotinic acetylcholine receptor (α7nAChR) agonists attenuate hyperoxia-induced lung injury
Central nervous system	
A lung-brain axis may regulate neuroimmune interactions	• Dedicated CNS circuits encode sensory inputs from the airways • CNS neuroinflammation can occur in models of airways disease • High brain processes for regulating immunological memory have been described • Mood and cognitive comorbidities are common in patients with airways disease

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CME Questions/Answers/Rationale

Learning Objectives: At the conclusion of this activity, participants should be able to:

- Describe the preclinical discoveries that form frameworks supporting neuroimmune interactions and the regulation of airways inflammation.
- Discuss clinical implications of neuroimmune interactions in the presentation and management of airways disease.

Q1. Which of the following is NOT correct about neuroimmune interactions in the airways?

a. Immune cell products can activate airway nerve fibers because neurons can express many different receptors for inflammatory mediators.
b. Immune cells need to bind to airway nerve fibers before any interactions can occur.
c. Signaling mechanisms once thought to be specific for neurons have been identified in immune cells and vice versa.
d. Sensory and motor neurons in the airways can both have efferent functions that serve to modify immune cell activity.

Q1 ANS: b. *Immune cells need to bind to airway nerve fibers before any interactions can occur.*

Rationale: Whilst direct contact between nerve fibers and immune cells in the airways has been reported, such interactions are not necessary for neuroimmune crosstalk. Close spatial relationships can allow for neuroimmune 'synapse' formation, diffusible mediators may allow for signaling to occur over larger distances and specialized intermediary cells, such as brush and neuroendocrine cells, may support neuroimmune cross talk in airways.

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Q2. Which of the following is NOT correct about neuroepithelial bodies (NEBs)?

a. NEBs are often composed of clusters of 5-20 neuroendocrine cells concentrated at airway branch points.
b. NEBs have been shown to be involved in generating type 2 immune responses to aeroallergen inhalation.
c. NEBs express bitter taste receptors.
d. NEBs express markers commonly found in neurons and are densely innervated by vagal sensory nerve fibers.

Q2 ANS: C. *NEBs express bitter taste receptors.*

Rationale: NEBs are comprised of neuroendocrine cells, Clara-like cells and sensory nerve fibers. They therefore display features of neurons, but additionally have been shown to play active roles in co-ordinating type 2 inflammatory responses during allergen exposure. The number of NEBs is highest during development but expansion of cells within the NEB microenvironment during airway injury might suggest a role for NEBs in injury repair. Other specialized airways cells involved in neuroimmune interactions, namely brush cells, are known for their expression of bitter taste receptors.

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Q3. Which of the following is evidence that sensory neurons can regulate immune function?

a. Sensory neurons become hyperexcitable during inflammation.
b. Sensory neurons can respond directly to pathogens as they express receptors for other pathogen-derived molecules.
c. Sensory neuron terminal density is increased in the airways in many inflammatory diseases.
d. Sensory neurons can release neuropeptides or other signaling molecules which have immunomodulatory actions.

Q3 ANS: d. *Sensory neurons can release neuropeptides or other signaling molecules which have immunomodulatory actions.*

Rationale: Whilst sensory neuron excitability and terminal field density can change during airways inflammation and some pathogens may be able to directly interact with sensory neurons, for example Mycobacterium tuberculosis via sulfolipid-1, these are not requisite processes for sensory neurons to

modulate immune cell function. Rather, the release of sensory neuron derived signaling molecules is one mechanism via which sensory neurons mediate immunomodulation. For example, the neuropeptide CGRP has been shown to act as a negative regulator of ILC2-mediated inflammation.

References:

1. Mazzone SB, Undem BJ. Vagal Afferent Innervation of the Airways in Health and Disease. Physiol Rev 2016; 96(3): 975-1024.
2. Wang J, Kollarik M, Ru F, et al. Distinct and common expression of receptors for inflammatory mediators in vagal nodose versus jugular capsaicin-sensitive/TRPV1-positive neurons detected by low input RNA sequencing. PLoS One 2017; 12(10): e0185985.
3. Talbot S, Abdulnour RE, Burkett PR, et al. Silencing Nociceptor Neurons Reduces Allergic Airway Inflammation. Neuron 2015; 87(2): 341-54.

Q4. What is the preclinical evidence that supports a role of autonomic nerves in immune cell modulation:

a. The anti-inflammatory effects of beta 2 (β2)-adrenergic receptor agonists and muscarinic acetylcholine receptor antagonists.
b. The anti-inflammatory effects of nicotinic acetylcholine receptor agonists.
c. The observation that sympathectomy exacerbates inflammation.
d. All of the above.

Q4 ANS: d. *All of the above*

Rationale: β-agonists and muscarinic antagonists, notably tiotropium, have demonstrable effects on airways inflammation. Many immune cells express adrenergic receptors, and in general sympathetic

derived noradrenaline is anti-inflammatory through a β2-adrenergic mechanism. Hence, denervating the sympathetic supply to the airways exacerbates inflammation while a β2-agonist is beneficial. Cholinergic signaling may be either pro-inflammatory or anti-inflammatory, depending on the expression of muscarinic or nicotinic receptors, respectively. Preclinical studies suggest nicotinic receptor agonists have anti-inflammatory effects.

References:

1. Moriyama S, Brestoff JR, Flamar AL, et al. β(2)-adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses. Science 2018; 359(6379): 1056-61.
2. Calzetta L, Coppola A, Ritondo BL, Matino M, Chetta A, Rogliani P. The Impact of Muscarinic Receptor Antagonists on Airway Inflammation: A Systematic Review. Int J Chron Obstruct Pulmon Dis 2021; 16: 257-79.
3. Gao ZW, Li L, Huang YY, et al. Vagal-α7nAChR signaling is required for airway anti-inflammatory responses and arginase 1 expression during an influenza infection. Acta Pharmacol Sin 2021; 42(10): 1642-52.

Q5. Neuroimmune interactions may not be confined to the airways in inflammatory airways disease. Which of the following statements supports this assertion?

a. Sympathetic innervation to the spleen and other abdominal organs is part of a generalized anti-inflammatory system that regulates systemic inflammation.
b. Pre-clinical studies have provided evidence of inflammation within the nerves (neuroinflammation) innervating the airways in models of airways disease.
c. The brain may contribute to immunological responses through dedicated ‘top-down’ pathways.
d. All of the above.

Q5 ANS: *d. All of the above*

Rationale: The anti-inflammatory reflex involves immune-specific subset of sympathetic neurons arising from the spinal cord that innervate sympathetic autonomic neurons pools in the celiac and splanchnic prevertebral ganglia. Activation of this pathway suppresses the plasma levels of pro-inflammatory cytokines TNFα, interferon gamma and interleukin (IL-6), while enhancing the levels of circulating anti-inflammatory cytokines (e.g., IL-10). Lung inflammation during viral, allergen and bacterial exposure is accompanied by inflammatory cell infiltration into the vagal sensory ganglia, a potential alterative site where neuroimmune interactions become important in disease. The insula cortex in the brain may contribute to generalized immunological responses by storing immunological memories.

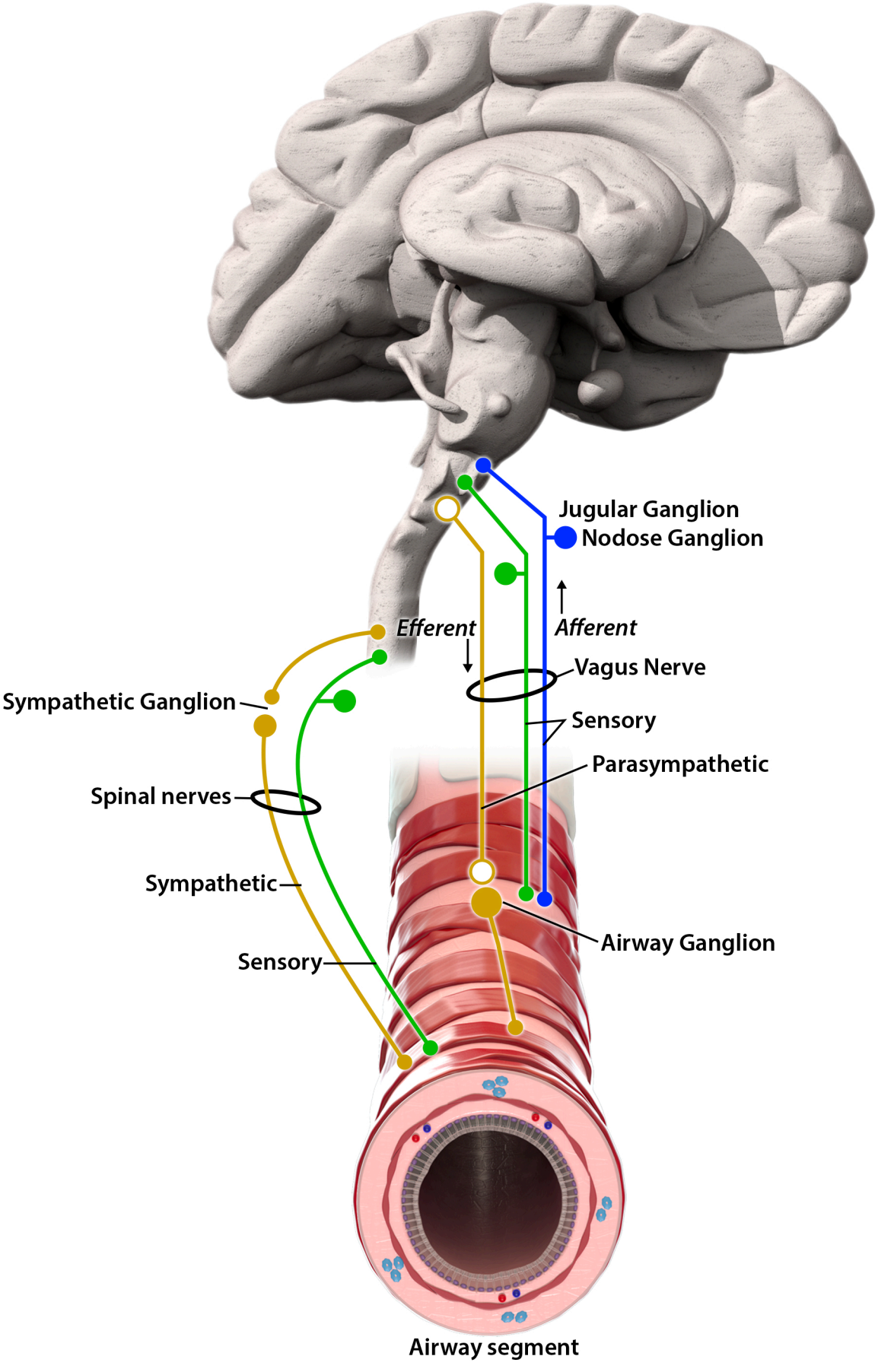
References:

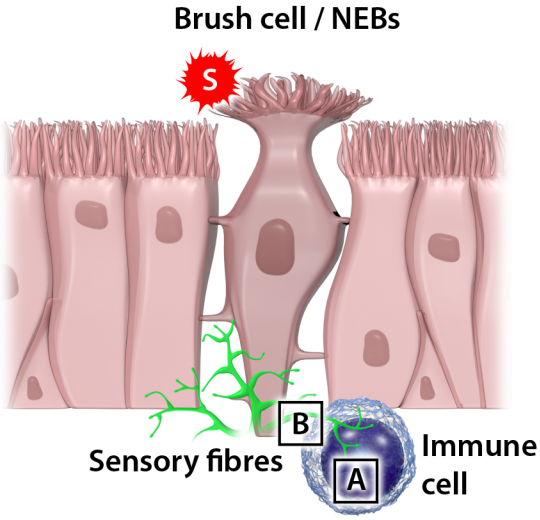
1. Lankadeva YR, May CN, McKinley MJ, et al. Sympathetic nerves control bacterial clearance. Sci Rep 2020; 10(1): 15009.
2. Martelli D, Yao ST, McKinley MJ, McAllen RM. Reflex control of inflammation by sympathetic nerves, not the vagus. J Physiol 2014; 592(7): 1677-86.

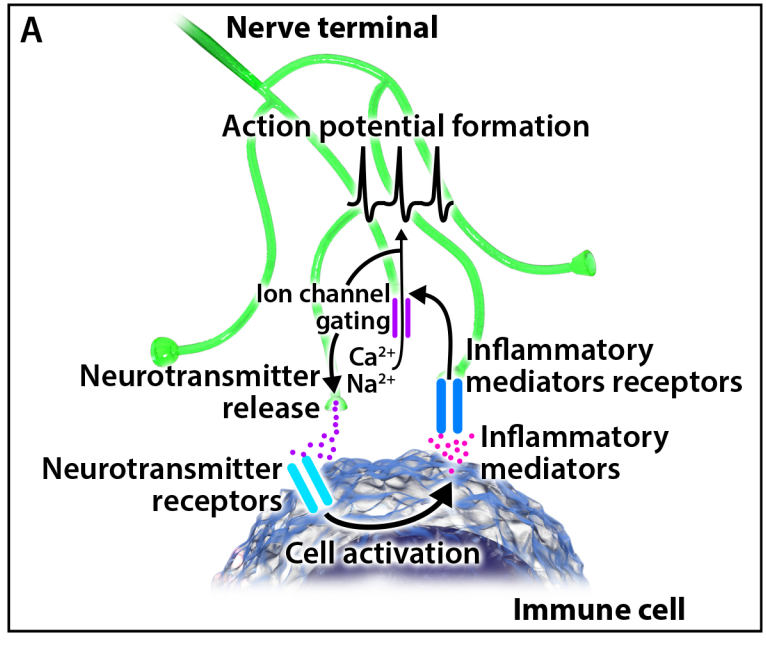
Key Messages

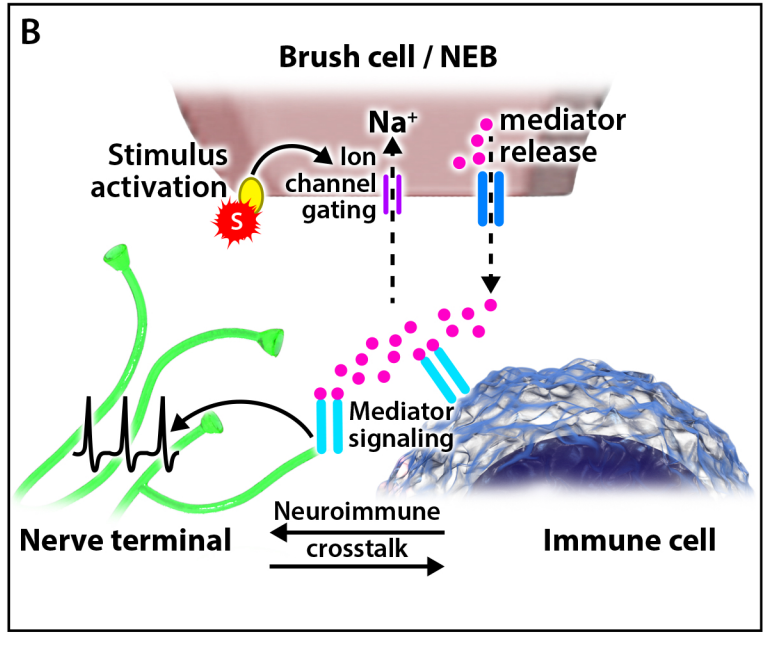
- The airways are densely innervated by sensory and motor nerves fibers which arise from cranial and spinal nerve origins.
- Bidirectional interactions between nerve fibers and immune cells in the airways can occur because of shared and complimentary signaling processes and close spatial relationships.

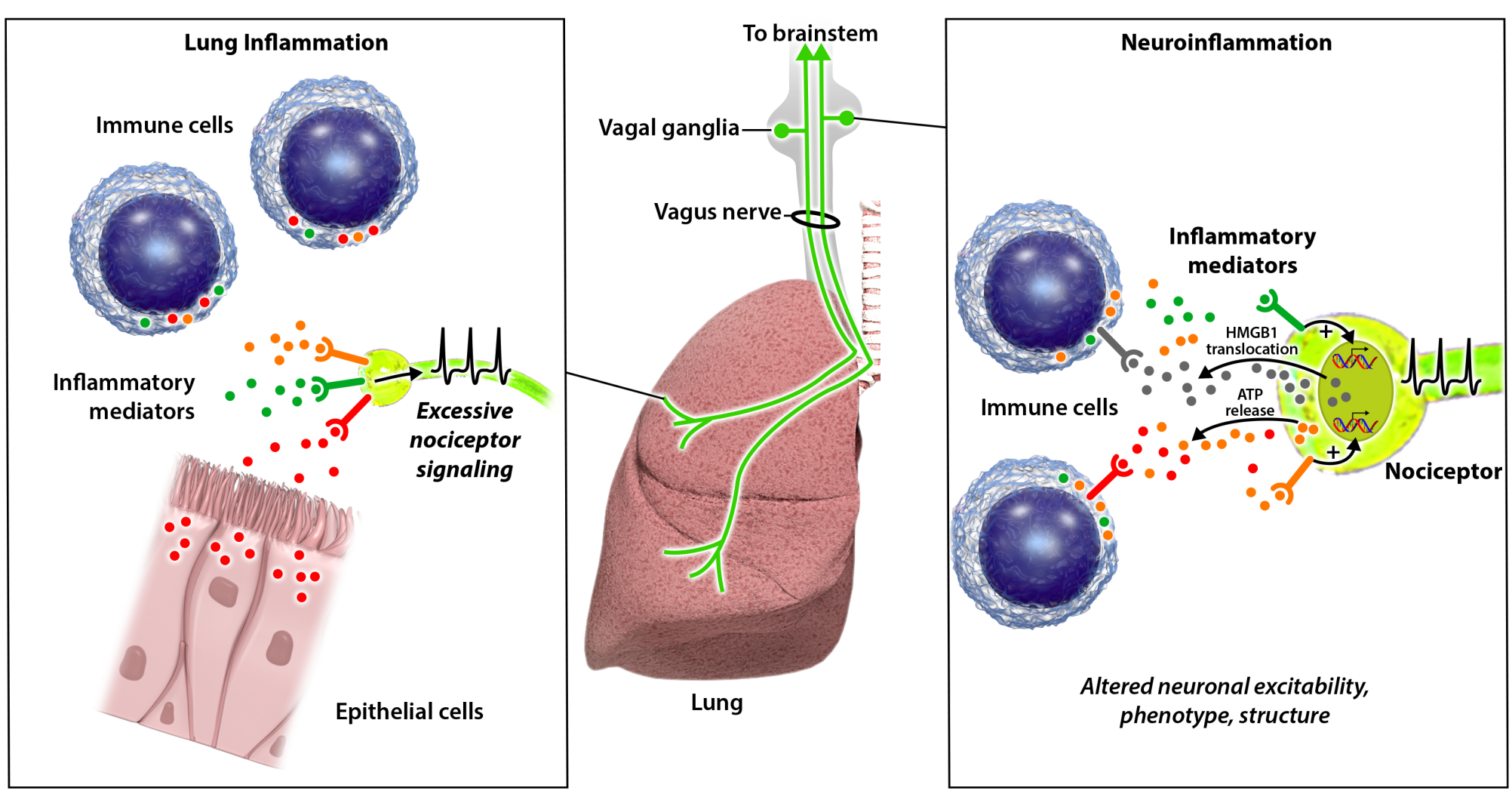
- Interactions between nerve fibers and immune cells in the airways can also be co-ordinated by specialized chemosensory airway cells and dedicated lung-brain reflex circuits.
- Airways inflammation can lead to neuroinflammation whereby the nerves supplying the airways become inflamed resulting in structural, phenotypic and functional neuroplasticity.
- Therapies targeting neural signaling may modify both the neurological symptoms of disease and the associated airways inflammation, although the clinical evidence for the latter is presently limited.

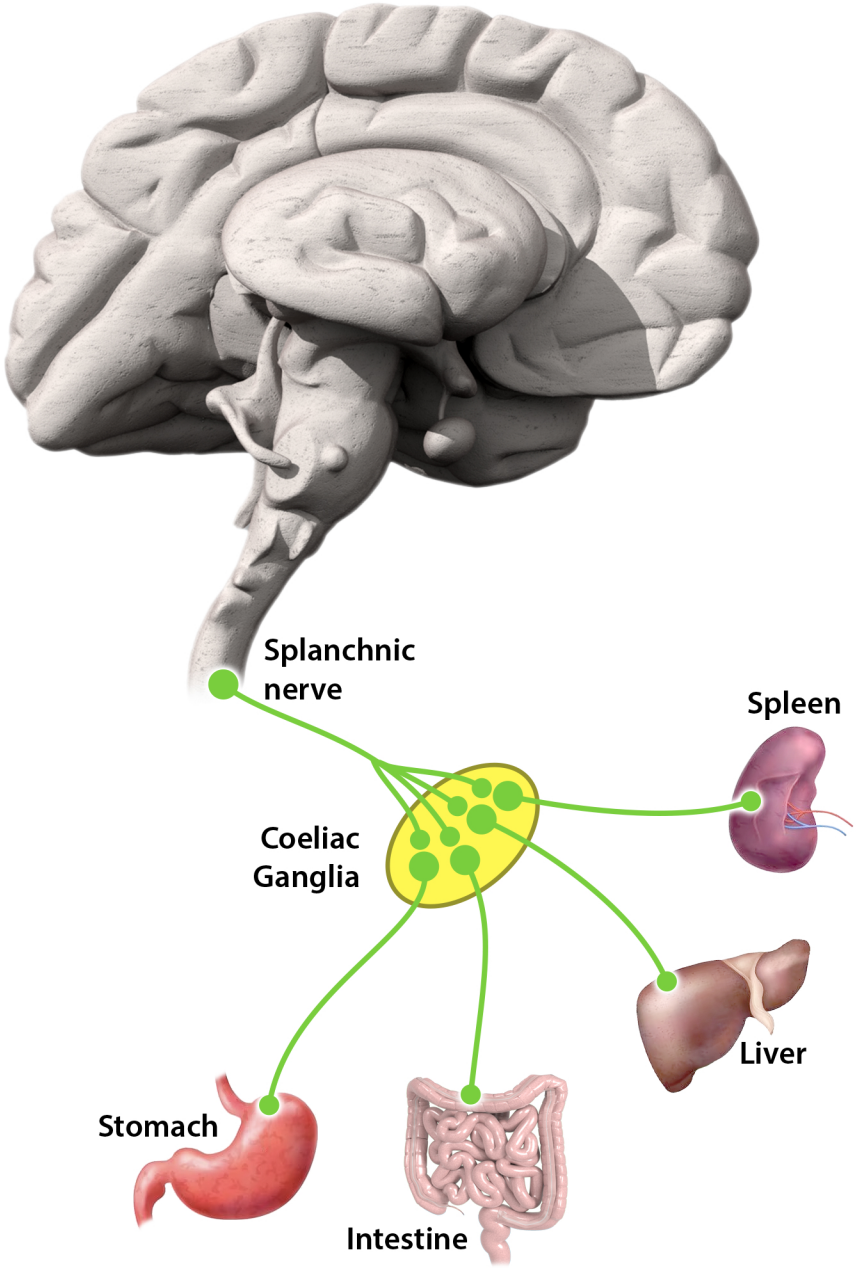

Jugular Ganglion
Nodose Ganglion
Efferent
Afferent
Vagus Nerve
Sympathetic Ganglion
Sensory
Parasympathetic
Spinal nerves
Sympathetic
Airway Ganglion
Sensory
Airway segment

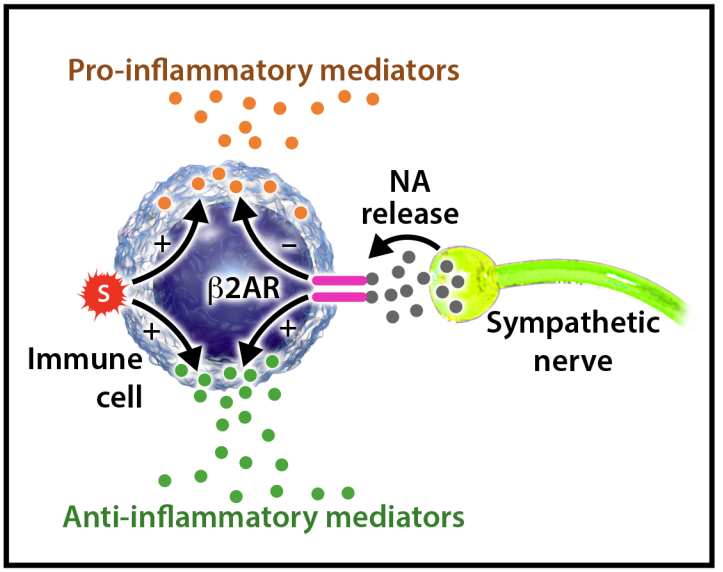

Brush cell / NEBs
S
B
A
Sensory fibres
Immune cell


A
Nerve terminal
Action potential formation
Ion channel gating
Ca2+
Na2+
Neurotransmitter release
Inflammatory mediators receptors
Inflammatory mediators
Neurotransmitter receptors
Cell activation
Immune cell


B
Brush cell / NEB
Na+
mediator release
Stimulus activation
Ion channel gating
S
Mediator signaling
Neuroimmune crosstalk
Nerve terminal
Immune cell


Lung Inflammation
Immune cells
Inflammatory mediators
Excessive nociceptor signaling
Epithelial cells
To brainstem
Vagal ganglia
Vagus nerve
Lung
Neuroinflammation
Inflammatory mediators
HMGB1 translocation
ATP release
Immune cells
Nociceptor
Altered neuronal excitability, phenotype, structure


Splanchnic nerve
Spleen
Coeliac Ganglia
Liver
Stomach
Intestine


Pro-inflammatory mediators
NA release
+
−
S
β2AR
+
+
Immune cell
Sympathetic nerve
Anti-inflammatory mediators