Book Chapter

Neural Regulation of Interactions between Group 2 Innate Lymphoid Cells and Pulmonary Immune Cells

Weiwei Chen^{1,2}, Qiang Shu² and Jie Fan^{1,3,4*}

¹Department of Surgery, University of Pittsburgh School of Medicine, USA

²The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, China

³Research and Development, Veterans Affairs Pittsburgh Healthcare System, USA

⁴McGowan Institute for Regenerative Medicine, University of Pittsburgh, USA

***Corresponding Author:** Jie Fan, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Published **January 18, 2021**

This Book Chapter is a republication of an article published by Jie Fan, et al. at Frontiers in Immunology in October 2020. (Chen W, Shu Q and Fan J (2020) Neural Regulation of Interactions Between Group 2 Innate Lymphoid Cells and Pulmonary Immune Cells. Front. Immunol. 11:576929. doi: 10.3389/fimmu.2020.576929)

How to cite this book chapter: Weiwei Chen, Qiang Shu, Jie Fan. Neural Regulation of Interactions between Group 2 Innate Lymphoid Cells and Pulmonary Immune Cells. In: Ajmal Khan, Ahmed Al-Harrasi, editors. Prime Archives in Immunology: 2nd Edition. Hyderabad, India: Vide Leaf. 2021.

© The Author(s) 2021. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License(<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Author Contributions: WC collected the data and drafted the manuscript. WC, QS, and JF conceived and designed the study. JF reviewed and finalized the manuscript. All authors contributed to the article and approved the submitted version.

Funding: This work was supported by the USA National Institutes of Health Grant R01-HL-079669 (JF), USA National Institutes of Health Grant R01HL076179 (JF), USA National Institutes of Health Grant R01-HL-139547 (JF), VA Merit Award 1I01BX002729 (JF), and VA BLR&D Award 1IK6BX004211 (JF).

Abstract

Emerging evidence supports the involvement of nervous system in the regulation of immune responses. Group 2 innate lymphoid cells (ILC2), which function as a crucial bridge between innate and adaptive immunity, are present in large numbers in barrier tissues. Neuropeptides and neurotransmitters have been found to participate in the regulation of ILC2, adding a new dimension to neuroimmunity. However, a comprehensive and detailed overview of the mechanisms of neural regulation of ILC2, associated with previous findings and prospects for future research, is still lacking. In this review, we compile existing information that supports neurons as yet poorly understood regulators of ILC2 in the field of lung innate and adaptive immunity, focusing on neural regulation of the interaction between ILC2 and pulmonary immune cells.

Keywords

Neuroimmunity; Adaptive Immunity; Innate Immunity; Lung; Group 2 Innate Lymphoid Cells (ILC2)

List of Abbreviations

$\alpha 7$ nAChR- $\alpha 7$ nicotinic acetylcholine receptor; Ach- Acetylcholine; AD- Atopic Dermatitis; Areg- Amphiregulin; CGRP- α -Calcitonin Gene-Related Peptide; CRTH2- Chemoattractant Receptor-homologous molecule expressed on Th2 cells; DCs- Dendritic Cells; PGD2- Prostaglandin D2; EGF- Epidermal Growth Factors; EGFR- EGF Receptor; HMGB1- High Mobility Group Box 1; ICOS- Inducible Costimulator; ILCs- Innate Lymphoid Cells; ILC2- Group 2 Innate Lymphoid Cells; KLRG1- Killer-cell Lectin Like Receptor G1; NMU- Neuromedin U; NMB- Neuromedin B; Nb- *Nippostrongylus brasiliensis*; PCTR1- Protectin Conjugates in Tissue Regeneration 1; PD-L1- Programmed Death-Ligand 1; PNECs- Pulmonary Neuroendocrine cells; PNS- Peripheral Nervous System; RAGE- Receptor for Advanced Glycation End products; RAMP1- Receptor Activity-Modifying Protein; TLRs- Toll-Like Receptors; Tregs- Regulatory T Cells; TSLP- Thymic Stromal Lymphopoietin; VIP- Vasoactive Intestinal Peptide

Introduction

The past decade has witnessed an unprecedented interest in the neural modulation of immunity [1–4]. The immune barrier consists of innate and adaptive components that adopt different strategies to perceive and respond to pathogen challenge. In the context of innate immunity, innate lymphoid cells (ILCs) have been demonstrated to be a crucial bridge between both immunity branches [5, 6].

ILCs are a heterogeneous family of lymphocytes that lack rearranged antigen receptors present on B and T cells. In earlier studies, lymphoid tissue inducer (LTi) cells and natural killer (NK) cells were initially identified as the subgroups of the ILCs [7–10]. In recent years, more subgroups of the ILCs were discovered and, based on the surface markers, cytokines, and transcription factors, categorized into three major types, ILC1, ILC2 and ILC3 [11,12]. These ILCs groups have distinct phenotypic, developmental, and functional properties. They are the innate counterparts of T lymphocytes: ILC1, ILC2, and ILC3

mirror CD4⁺ T helper (Th)1, Th2, and Th17 cells, respectively, based on cytokine secretion and transcription factor expression [11,13,14]. ILC1 consists of conventional NK cells and ILC1s. T-bet, a T-box transcription factor, is indispensable for the differentiation and interferon-gamma (IFN- γ) secretion ability of ILC1. ROR α and GATA3 are essential for the development of ILC2, which can be grouped into transient, circulating inflammatory ILC2 (iILC2) and tissue-resident natural ILC2 (nILC2) types [15,16]. ILC3 comprises the classical lymphoid inducer (LTi) cells and LTi-like ILC3 with or without natural cytotoxicity receptors (NCRs), all of which rely on the ROR γ t (transcription factor) for development and secrete IL-17 and/or IL-22. ILCs protect individuals against infectious agents, response to inflammatory stimuli, and orchestrate lymphoid organogenesis and tissue repair, at various tissues especially mucosal barriers [17–19].

Among all subsets, ILC2 are the center of numerous investigations. They are mainly localized at mucosal barriers, e.g. the small intestine, skin, and lung [19–21]. ILC2 are a master regulator of immune and inflammatory responses, but their own regulatory mechanisms remain largely elusive.

ILC2 are activated by host-derived alarmins such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), which are expressed during tissue injury [22–24]. Once activation takes place, ILC2 release large quantities of cytokines such as IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17, and amphiregulin [16,25–28]. Furthermore, ILC2 interact with other cells through surface-bound molecules, such as CD80/86, MHC class II, PD-L1, OX40L, and inducible costimulator ligand (ICOS-L), and participate in immune-regulatory functions [29–32]. ILC2 play critical roles in the regulation of inflammation, allergic immunity, metabolic homeostasis, parasite rejection, and tissue repair. Dysregulation of ILC2 contributes to inflammatory responses, including allergen-induced lung inflammation [33,34], airway hyperreactivity [35], and atopic dermatitis [36].

Currently, the nervous system is found to have complex dual functions to quickly stimulate or suppress immune cells to

defend the body against various inflammatory responses. There are continuing advances in our knowledge of neural regulation of ILC2, these brilliant results provide a new dimension of immune regulation [37–47]. Studies have shown that receptors for norepinephrine, acetylcholine (ACh), neuromedin U (NMU), neuromedin B (NMB), α -Calcitonin Gene-Related Peptide (CGRP), and other neurotransmitters are present on T cells, dendritic cells (DCs), macrophages, ILC2, and other immune cells [19,37,38,44,48–50], and pattern-recognition receptors (PRRs) and cytokine receptors are distributed on neurons [51–54]. Interestingly, immune cells are also able to synthesize and secrete catecholamines, acetylcholine, CGRP, and other neurotransmitters [39,48,49,55]. Moreover, ILC subtypes express the nicotinic and muscarinic cholinergic receptor for ACh, β 2-adrenergic receptor (β 2AR) for epinephrine and norepinephrine, calcitonin receptor-like (CALCRL) for CGRP, neuromedin U receptor 1 (NMUR1) for NMU, neuromedin B receptor (NMBR) for NMB, and VPAC1/2 (vasoactive intestinal peptide receptor) for vasoactive intestinal peptide (VIP) [19,39,44,50,56]. These findings suggest physical machinery for neuroimmune communications. Also, type I cytokines can also influence cells of the center nervous system (CNS) and mediate what is called “sickness behavior” [57,58]. In this review, we highlight existing information that describes neurons as novel regulators of ILC2 in the context of pulmonary innate and adaptive immunity.

Mechanisms Underlying ILC2 Interact with other Immune Cells

ILC2 function both as initiator of adaptive immunity or as responder to signals produced by B and T cells. Using ILC2-targeted models, investigations have shown multiple mechanisms by which ILC2 regulate innate and adaptive immune system.

Many previous studies showed that “crosstalk” exist between T cells and ILC2 (**Figure. 1**). For instance, ILC2 are the largest group of the cytokine-secreting leukocytes after ovalbumin or HDM treatment [59], and ILC2 activity is essential for the

efficient differentiation of Th2 cells [29,60–63]. In Rag2^{-/-} mice, in which T cells and B cells are depleted due to *Rag* deficiency, the numbers of ILC2 also markedly decreased after helminth infection, indicating that T cells advance the survival of ILC2 (64). Epithelial cells derived cytokines and alarmins activate ILC2, which can be main producer of type 2 cytokines. Moreover, ILC2 can activate CD4⁺T cells either in the priming phase or during the effector phase since they present major histocompatibility complex class II (MHCII) [65,66]. IL-33-activated ILC2 enhances DCs migration into cancer tissues via C-C motif chemokine ligand 5 (CCL5) and further improve CD8⁺ T cell-mediated tumor immunity [67]. Combination of anti-PD1 checkpoint blockade with rIL33 treatment collaborates to improve anti-tumor immunity by unleashing ILC2 activity [68]. Activated ILC2 further facilitate the polarization of the anti-inflammatory M2 macrophages, which in turn stimulate Foxp3 regulatory T cells (Tregs) [69]. Tregs are a subpopulation of T cells which modulate the adaptive immune responses through direct cell-cell interactions, as well as through the inhibitory functions of TGF- β and IL-10.

Through cytokines and interactions of ICOS with its ligand ICOS-L, ILC2 activate B cell to undergo isotype-switching, survival, and secrete IgG1 and IgE [64,70,71]. IgE produced by B cells, together with type 2 cytokines released by Th2 cells and ILC2, lead to further activation of smooth muscle contraction, mucus production, granulocyte effector cells, and, which in turn, result in the encapsulation or expulsion of inflammatory stimuli [72]. B cell-derived IgE is an important effector of type 2 immunity, and the recognition of allergens by IgE on mast cells is responsible for induction of the cardinal features of classic allergic responses, including anaphylactic shock [70]. ILC2-derived IL-5 is an important growth factor that contributes to B1 cell self-renewal [73,74]. ILC2 sorted from mesenteric fat-associated lymphoid clusters are able to increase IgA production by peritoneal B cells *in vitro* [74].

Multiple indirect (cytokines) and direct (surface-bound molecules) mechanisms are involved in interactions between ILC2 and other cells as summarized below (**Figure 1**) [29,75–77].

Figure 1: ILC2 interacts with other immune and non-immune cells through a variety of cytokines and cell surface mediators. (1) After activation with alarmins, ILC2 produce type II cytokines and mediators; (2) ILC2 interact with T cells via MHCII, CCL5, PD-1/PD-L1, OX40/OX40L, CD86, CD80, IL-4, IL-5, and IL-13; (3) ILC2 activate B cells to undergo isotype-switching, survival, self-renewal, and secrete antibodies via ICOS/ICOS-L, IL-5; (4) ILC2 stimulate Tregs via IL-5, IL-9, ICOS/ICOS-L, while ILC2-released IL-4 suppress Tregs. Tregs are capable of inhibiting ILC2; (5) ILC2 prime macrophages into a type 2 immune cell phenotype via IL-13; (6) Epithelial cells derived alarmins activate ILC2. ILC2-released IL-9 and Areg protect lung endothelial cells. (7) ILC2 increase eosinophils via IL-5, HMGB1. ILC2 inhibit eosinophils via IL-10. Eosinophil- or basophil-released IL-4 activate ILC2; (8) ILC2 activate DCs via IL-9, IL-13, HMGB1.

IL-4

IL-4 is expressed by activated ILC2 [78,79]. ILC2-released IL-4 participates in blocking the expansion of allergen-specific Tregs,

thus involving in food allergy [80]. ILC2-released IL-4 is also able to polarize Th2 cells during helminth infection [81].

On the other hand, eosinophil- or basophil-released IL-4 is found to affect ILC2 by enhancing ILC2 lineage proliferation, function and stability [82,83].

IL-5

ILC2 can coordinate adaptive and innate immune functions through IL-5. IL-5 is critical for B cell function [74] and eosinophil homeostasis (**Figure 1**) [84,85]. When splenic B cells co-culture with mesenteric ILC2, IL-5 from ILC2 plays a pivotal role in the release of IgA from B cells [74]. Besides, peripheral ILC2 in pulmonary, peritoneal cavity, and spleen are able to elevate the secretion of IgA, IgE, IgG1, and IgM by B cells in *ex vivo* co-cultures [86]. Furthermore, upon NP-Ficoll (primes for a high affinity IgM anti-NP response) treatment *in vivo*, IgM produced by B cells is selectively initiated by lung ILC2 in an IL-5-dependent pattern [86]. IL-5-producing ILC2 are also essential for the Th2 and Th9 cytokine responses against *Trichinella spiralis* infection [87]. In addition, lung ICOS⁺ILC2 act a protective factor in a bleomycin model in an IL-5-dependent manner [88]. Of note, the timing of IL-5 release by ILC2 seems important for the protective activity (88). Study showed that prostaglandin D₂ (PGD₂)-chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) signaling increases ILC2 and its production of IL-5, which promotes Tregs proliferation [89].

Our recent study discovered that high mobility group box 1 (HMGB1, a late mediator of sepsis) signals via receptor for advanced glycation end products (RAGE) to increase lung ILC2 by enhancing ILC2 proliferation and suppressing ILC2 death. The activated ILC2 increase type 2 cytokines production and eosinophil infiltration in the lungs, both of which improve haemorrhagic shock-induced acute lung injury [85]. Lung ILC2 activated by IL-33 secrete a large number of IL-5, which further up-regulate neutrophil and its IL-5 production [90].

IL-9

Price *et al*, reported in detail that ILC2 express IL-9 receptor [78]. Using IL-9 reporter and subsequently IL-9 fate mapping mice, two studies delineated the autocrine signaling mechanism of IL-9 in ILC2 that enhances IL-13 and IL-5 release (**Figure 1**) [66,91]. In papain-induced pulmonary inflammation model, IL-9 was secreted for a short period by ILC2, then ILC2 changed to release IL-13 and IL-5. Furthermore, IL-33, but not IL-25, increased IL-9-producing ILC2 [91]. Rauber *et al*, recently uncovered that IL-9 from ILC2 was necessary for Tregs activation and inflammation resolution in an arthritis model [92]. HMGB1-activated ILC2 also secrete IL-9, which increase DCs [93]. Moreover, our study discovered that ILC2-released IL-9 protects lung endothelial cells from pyroptosis by suppressing caspase-1 in a septic model [17].

IL-2 released by adaptive immune cells also play a crucial role in the IL-9 expression by ILC2, suggesting again the strong functional link between adaptive and innate lymphoid cells [94]. Besides, IL-2 functions as a costimulator to ILC2 and promotes cell proliferation and survival by activating NF- κ B pathway and gene transcription through p65 translocation [94]. IL-9 and IL-2 work synergistically to direct ILC2 biology, and increased IL-9 production is related to an asthma-like phenotype in humans and mice highlighting the key role of these cytokines [95-98].

IL-13

IL-13 also mediates the interaction between ILC2 and immune and non-immune cells (**Figure 1**). During infection of helminth in mice, IL-13 released from ILC2 is more abundant than that from Th2 cells for restricting worm expulsion and immune response [23,64,78]. IL-13 from ILC2 can induce goblet cell hyperplasia as well as mucus secretion [99]. Yet the precise mechanism of IL-13 receptors on pulmonary cells at different states remains to be fully elucidated.

Pulmonary IL-13⁺ ILC2 and CD4⁺ T cells cooperate to suppress *Nippostrongylus brasiliensis* (*Nb*) infection. Immune-damaged

larvae have a severe morphological defect that is due to the increase of CD4⁺ T cells and IL-13⁺ ILC2, as well as the activation of M2 macrophages [100]. Besides, alveolar macrophages can be primed by ILC2-derived IL-13 into a type 2 immune cell phenotype [101]. DCs are stimulated by IL-13 to convert to a type 2 chemokine-secreting phenotype. ILC2-derived IL-13 is also able to mediate DCs migration from the lungs to the LNs, thus impacting the differentiation of Th2 cell [102]. In addition, the number of IL-13⁺ ILC2 was reported to be markedly upregulated in patients with uncontrolled asthma, and it was significantly decreased when these patients had their symptoms controlled by treatment, suggesting an important role for ILC2-derived IL-13 in asthma [103]. In conclusion, ILC2-derived IL-13 can initiate and affect innate and adaptive type 2 immune responses.

Amphiregulin (Areg)

Areg is a member of the epidermal growth factors (EGF) family and acts via the EGF receptor (EGFR) [104]. Both hematopoietic and non-hematopoietic cells in the lung present EGFR [105]. ILC2 are a major cellular producer of Areg after activation with IL-33. ILC2-derived Areg is a critical component of effective pulmonary wound healing during influenza infection and restoring epithelial integrity and lung function [20]. The initiation of mucus secretion and wound healing can prevent or ameliorate some respiratory diseases, although enhanced and excessive mucus may play a detrimental role in diseases, such as asthma [106,107]. Pulmonary Tregs are also capable of producing Areg without TCR signaling (**Figure 1**) [108]. Thus, innate Areg released by ILC2 and Tregs is of high importance to restore tissue homeostasis and wound healing after pulmonary infection.

ICOS/ICOS-Ligand Interaction

Studies on helminth expulsion revealed ILC2-Tregs crosstalk [109]. Tregs and ILC2 colocalize to similar regions within the lung tissues and visceral adipose tissue under homeostatic and inflammatory conditions (**Figure 1**) [31]. Of note, ICOS⁺ Tregs

and ICOS-L⁺ ILC2 were reported to accumulate in tissues after *Nb* infection or IL-33 administration, while Tregs accumulation in ICOS-L knock-out mice or after administration with neutralizing monoclonal antibody against ICOS-L was reduced, indicating that ICOS-L⁺ ILC2 could improve Tregs expansion, thus establishing a pathway for Tregs to cooperate with ILC2 [31]. On the other hand, Tregs are capable of inhibiting ILC2 to restrict allergic airway inflammation [110]. Besides, ICOS/ICOS-L interaction on ILC2 affects STAT5 signaling, activating ILC2 function and proliferation in an allergic model [111].

PD-1/PD-L1 Interaction

ILC2 constitutively express the checkpoint inhibitor molecule programmed death-ligand 1 (PD-L1), which has been discovered to activate CD4⁺ Th2 cell responses during type 2 pulmonary responses (**Figure 1**) [30]. Conditional knockout of PD-L1 on ILC2 disrupted cytokine production and early Th2 polarization, resulting in delayed worm expulsion during infection with the gastrointestinal helminth *Nb* [30]. Nevertheless, ILC2 can also express PD-1, which was reported to be upregulated on activated ILC2, and depletion of these PD-1⁺ ILC2 resolves papain-induced lung inflammation [112].

E-cadherin/KLRG1 Interaction

E-cadherin, a cell adhesion molecule, interacts with the mature ILC2 marker, killer-cell lectin like receptor G1 (KLRG1). The finding that E-cadherin-KLRG1 ligation on human ILC2 reveals a significant decrease in GATA3 expression and type 2 cytokine (such as IL-5 and IL-13) release and the discovery that E-cadherin expression is suppressed in keratinocytes propose that inhibited E-cadherin expression may activate ILC2, promoting atopic dermatitis (AD) immunopathogenesis [28].

Other Interactions

OX40 ligand (OX40L) expression on ILC2 can be enhanced by IL-33. OX40-OX40L ligation has been reported to increase Th2

cell survival and number [32,113], thus promoting adaptive immunity [75].

A molecularly distinct subset of lung ILC2 can secrete IL-10 and suppress some pro-inflammatory genes. IL-2, IL-4, IL-27, IL-10, and NMU stimulate IL-10 production from ILC2 and are associated with decreased eosinophil recruitment to the lung, indicating that ILC2 have anti-inflammatory functions similar to Tregs (**Figure 1** and **3**) (26,114).

The expression of CD86, CD80, and MHCII by mouse ILC2 is also involved in ILC2 interactions with CD4⁺ T cells;

MHCII⁺ ILC2 can drive the differentiation of naive CD4⁺ T cells into T_H2 *in vitro*, whereas MHCII-deficient ILC2 upregulate Th2 cell-driven helminth expulsion *in vivo* [29,75].

Pulmonary Nervous System and its Regulatory Function

Lung is densely innervated by peripheral nervous system (PNS), which is divided into motor and somatosensory nervous systems (**Figure 2**).

Figure 2:The autonomic nervous system regulation of ILC2 function. Ach, Acetylcholine; α7nAChR, α7 nicotinic acetylcholine receptor; β₂AR, β₂-adrenergic receptor; NA, Norepinephrine.

Motor Nervous System

The motor nervous system consists of the autonomic (sympathetic, parasympathetic, and enteric) and somatic branches.

Autonomic nervous system serves to regulate involuntary functions. Sympathetic branch comes from the upper six thoracic spinal cord segments; the synapse together with the sympathetic ganglia, and postganglionic fibers then innervate the airways. The sympathetic nervous system participates in the body response to stress and regulates bronchodilation and the production of mucous.

The cholinergic parasympathetic branch comes from the vagal nuclei of the medulla; the superior and recurrent laryngeal vagal nerves synapse at the parasympathetic ganglia to innervate the lung [115]. The parasympathetic branch is mainly responsible for keeping homeostasis [116]. It regulates bronchoconstriction, carbon dioxide and oxygen levels, as well as neural reflexes including coughing.

Somatosensory Nervous System

The somatosensory nervous system delivers sensory stimuli, such as proprioception, touch, and pain. Somatosensory neurons are further divided into pruriceptors and nociceptors responsible for sensing itch-inducing or noxious stimuli, respectively. These neurons are important because their activation is closely related to inflammation and immunity (**Figure 2**).

All opponents of the PNS play a critical role in orchestrating immunity, inflammation, and tissue repair at host barrier tissues in response to stimuli and stressors. Primary evidence that neurotransmitters may regulate immune responses was that their release from nerves could lead to signaling through the surface receptors of lymphocyte cell [117]. Leukocytes have receptors for the neurotransmitters such as dopamine, serotonin and glutamate [117], and also produce neurotransmitters that work as paracrine or autocrine signals [118]. The neuronal reflex senses peripheral inflammation and coordinates the host response to injury and/or infection, regulating events within the initiation of inflammation [48,119]. Lung is heavily populated by resident immune cells, such as macrophages, DCs, $\gamma\delta$ T cells mast cells and ILCs. It allows fast, integrated reactions to pathogens and noxious stimuli [120].

Sympathetic Nervous System

Sympathetic nervous system helps the center nervous system to control innate immune responses between antiviral and pro-inflammatory actions (**Figure 2**) [121,122].

The nerves of the sympathetic nervous system distribute the neurotransmitter catecholamines into tissue microenvironments in which immune response gene transcription occurs, including all lymphoid organs, most musculoskeletal structures and visceral organs, and the vasculature and perivascular tissues [123].

Recent reports have revealed a sympathetic nervous system-mediated steering of innate immune response programs, which include enhanced transcription of pro-inflammatory cytokine genes (such as *Il6*, *tnf*, and *Il1β*) [121,124] and inhibition of type I IFN-mediated antiviral responses [122].

Stimulation of the sympathetic nervous system has also been shown to change the production and trafficking of innate immune cells, for instance, through the upregulation of myelopoiesis and the mobilization of monocytes, splenic neutrophils, natural killer cells, and haematopoietic stem cells [123]. A current study showed that the sympathetic nervous system stimulates IL-33 and then ILC2 in adipose tissue. Cold exposure stimulates IL-33 expression, ILC2 and eosinophils in adipose tissue. Furthermore, sympathetic denervation induced by 6-hydroxydopamine (6-OHDA) cancels this effect [125].

Catecholamines are monoamine neurotransmitters which are mainly released by the postganglionic fibers of the sympathetic nervous system and the chromaffin cells of the adrenal medulla. Included among catecholamines are dopamine, epinephrine (adrenaline), and norepinephrine (noradrenaline) (**Table 1** and **Figure 2**). Release of the epinephrine and norepinephrine from the adrenal glands and adrenergic nerves is part of the fight-or-flight response.

Table 1. Sources, receptors on ILC2, and relationships with ILC2 of several neurotransmitters.

Neurotransmitters		Sources		Relevant receptors on ILC2	Relationships with ILC2
Catecholamines	Epinephrine (Adrenaline)	Autonomic (Involuntary)	Sympathetic nervous system;	β_2 AR	<ul style="list-style-type: none">● Adrenergic neurons colocalize with ILC2;● β_2AR agonist administration impairs ILC2 responses and reduces inflammation;● Mediator of the “anti-inflammatory reflex”;
	Norepinephrine		Parasympathetic nervous system; Also released by tuft cells;	α_7 nAChR	<ul style="list-style-type: none">● α_7nAChR agonist administration inhibits the proliferation of ILC2, but does not alter the death of ILC2;● α_7nAChR agonist administration inhibits type 2 cytokine production from ILC2 and ameliorates ILC2-mediated lung inflammation;● Mediator of the “anti-inflammatory reflex”;
Acetylcholine					
CGRP		Somatic (Voluntary, afferent and efferent neurons)	Sensory neurons; Also released by PNECs and ILC2;	RAMP1 and CALCRL	<ul style="list-style-type: none">● CGRP-secreting PNECs locate in close proximity to ILC2 near airway branch points;● ILC2 express both CGRP and its receptor CGRPR;● CGRP stimulates ILC2 proliferation;● CGRP suppresses KLRG1⁺ILC2s proliferation but promotes IL-5 expression;● CGRP alone does not increase cytokine production from ILC2, a combination of (NMU + IL-33 + CGRP) stimulates IL-5 but limits IL-13 production and ILC2 proliferation;
VIP			Sensory neurons;	VPAC1 and VPAC2	<ul style="list-style-type: none">● VIP stimulates IL-5 from ILC2, ILC2-derived IL-5 activates nociceptors on sensory neurons and upregulates the release of VIP, which in return acts via VPAC2 and leads ILC2 and subsequently T cells to release more IL-5 and thereby forming a type 2 inflammatory positive feedback loop mainly based on the neuro-immune axis;
NMU			Sensory neurons (released by cholinergic sensory neurons originating from DRG); Also secreted by some APCs (including monocytes, B cells, and dendritic cells);	NMUR1	<ul style="list-style-type: none">● NMU-expressing neurons locate in close vicinity to ILC2;● NMU elevates ILC2 proliferation;● Stimulation of ILC2 with NMU leads to strong and immediate production of tissue protection and innate inflammatory cytokines in a NMUR1-dependent manner;● NMU increases IL-10 production in activated ILC2, IL-10 further stimulates IL-10 production in ILC2 through a positive feedback loop;● ILC2 activated by NMU increase the number of lung eosinophils and mast cells;● IL-13 enhance NMU production in DRG neurons, thus indicating the existence of a reciprocal neuron–ILC2 regulatory loop via ILC2-derived IL-13 and neuronal NMU expression;
NMB		CNS (olfactory bulb, dentate gyrus, amygdala, basal ganglia, brainstem); PNS (Gastrointestinal tract; Trigeminal and dorsal root ganglia (DRG));		NMBR	<ul style="list-style-type: none">● Treatment with NMB inhibits ILC2 responses, eosinophilia and mucus production;● Basophils prime ILC2 for NMB-mediated inhibition;

Catecholamines exert their effects via two classes of adrenergic receptors, α and β . Both groups could be functionally divided into subgroups (α_1 and α_2 ; β_1 , β_2 , and β_3).

Norepinephrine regulates leukocyte gene expression through β -adrenergic receptors [123]. β -adrenergic receptor is expressed on most immune cells, such as B cells, T cells, and other innate cells [40,126-128]. It was initially thought to regulate adaptive immune responses by suppressing the expression of TH1-type genes, such as *Il12 β* and *Ifn γ* , and activating the transcription of TH2-type cytokine genes, i.e. *Il4* and *Il5* [129-131].

Interestingly, β_2 AR has an inhibitory effect on innate immune responses. β_2 -agonists suppress cardiodepressant and inflammatory factors, including HMGB1 and TNF. Recently, ILC2 from the lung and the gut-related tissues (small intestinal lamina propria, colonic lamina propria and mesenteric lymph nodes) were found to express β_2 AR. ILC2 were also shown to colocalize with adrenergic neurons in the mouse intestine [40].

β_2 AR deficiency led to enhanced ILC2 proliferation and subsequent type 2 cytokine production in lung and intestinal tissues after infection with *Nb*. Lung eosinophilia was observed following enhanced IL-5 production from ILC2 in β_2 AR-deficient mice. On the other aspect, β_2 AR agonist administration disrupted ILC2 responses and suppressed inflammation *in vivo*. By using conditional β_2 AR-deficient mice, or by transferring ILC2 progenitors from wild-type mice or β_2 AR-deficient mice into ILC-deficient mice, the group generated ILC2-specific β_2 AR-deficient mice and ensured that the β_2 AR negatively controls ILC2 and type 2 inflammation [40]. This study provides another evidence of neuronal regulatory circuit that regulates ILC2-dependent type 2 inflammation. β_2 AR-agonists are the most effective medications for the treatment of asthma. β_2 AR-mediated ILC2 regulation could be one of the pathways of β_2 AR-agonists effect in asthma [33,38,44,110,132,133]. On the other hand, β_2 AR is the first adrenergic receptor documented to participate in the “anti-inflammatory reflex” of the parasympathetic system which will be discussed in the next section (**Table 1** and **Figure 2**).

Parasympathetic Nervous System

Parasympathetic and sympathetic systems are usually considered to work in opposition to maintain physiological homeostasis. While current literatures suggested that both branches work together to restrain systemic inflammation in life-threatening illnesses, including arthritis, inflammatory bowel disease, sepsis and endotoxemia [46,48,134-138].

Nerve fibers of the parasympathetic nervous system arise from three primary areas: cranial nerves (facial nerve, oculomotor nerve, and glossopharyngeal nerve), vagus nerve, and pelvic splanchnic nerves (three spinal nerves in the sacrum, S2-4).

The parasympathetic nervous system mainly utilizes acetylcholine (ACh) as its neurotransmitter (**Table 1** and **Figure 2**). Tuft cells, capable of secreting the ILC2 activator IL-25, also secrete ACh [139,140]. The ACh has two kinds of receptors, the nicotinic and muscarinic cholinergic receptors. $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR), one of the nicotinic acetylcholine receptors, is expressed by ILC2 at steady state, and this expression is further increased following alarmin-induced activation such as IL-33 [44]. The $\alpha 7$ nAChR is also present on B cells [141], T cells [142,143], macrophages [144] and ILC3 [41].

$\alpha 7$ nAChR^{-/-} mice were more susceptible to severe lung injury and higher mortality than $\alpha 7$ nAChR^{+/+} mice. Increased $\alpha 7$ nAChR⁺ alveolar neutrophils and macrophages were observed in the mice injured lungs. The immunomodulatory cholinergic $\alpha 7$ nAChR pathway of alveolar neutrophils and macrophages alleviated E. coli- and LPS-induced acute lung injury by inhibiting transalveolar neutrophil migration and chemokine production.

It was reported that the expression of HMGB1 protein was suppressed by $\alpha 7$ nAChR agonist nicotine and the survival of post-sepsis acute lung injury was improved [145]. In addition, administration of $\alpha 7$ nAChR agonist inhibits type 2 cytokine production from ILC2 and ameliorates ILC2-mediated lung inflammation induced via IL-33 stimulation or Alternaria

alternata inhalation. Mechanistically, $\alpha 7$ nAChR agonist is reported to inhibit cellular markers for proliferation in ILC2 (Ki67, NF- κ B and GATA3 signaling pathways) [27,44,146,147] (**Table 1**). These findings indicate that $\alpha 7$ nAChR may be a potential therapeutic target for acute lung injury [120].

On the other aspect, Bcl-2, an anti-apoptotic factor of ILC2, was unchanged by $\alpha 7$ nAChR agonist treatment. These studies indicate that parasympathetic nervous system modulates ILC2 proliferation, but not death. The suppressive effects of $\alpha 7$ nAChR on ILC2 may serve as the mechanism underlying the observed reduced pulmonary allergic inflammation induced by nicotine treatment [148]. Moreover, in cancer immunity, nicotine treatment stimulates tumor growth by suppressing apoptosis and promoting cell proliferation [149-152].

Anti-Inflammatory Reflex

The vagus nerve is the main parasympathetic nerve connecting between the central nervous system and peripheral organs [128,153]. Pharmacological or electrical stimulation of the vagus nerve can restrain the systemic inflammation response, organ damage, and mortality in different experimental hemorrhage and resuscitation (43), pancreatitis (154), ischemia and reperfusion [43,155,156], colitis [157], endotoxemia [42,155,158,159] and sepsis [145,160].

Mechanically, the motor and sensory vagus nerve form a complex neural reflex circuit termed the “anti-inflammatory reflex” which control spleen cytokine production through splenic nerve [42,161-166]. ACh released by the vagus nerve in the celiac mesenteric ganglia stimulates postsynaptic $\alpha 7$ nAChR of splenic nerve [46]. The adrenergic splenic nerve release norepinephrine to activate a discrete subset of spleen lymphocytes via β_2 AR. Activated lymphocytes then release Ach [48,167]. Lymphocyte-derived Ach downregulates macrophage cytokine release, and switches them toward a tissue-protective, M2 anti-inflammatory phenotype. $\alpha 7$ nAChR mediates Ach-induced signal transduction in macrophages and monocytes [144]. $\alpha 7$ nAChR inhibits the inflammasome activity [168],

enhances the JAK2-STAT3 pathway [169], stabilizes mitochondrial membranes, and suppresses the nuclear translocation of NF- κ B [119,145,168-170].

The eventual influence of inflammatory reflex on the spleen is the inhibition of cytokine release by spleen macrophages, which produce over 90% of the IL-1 and TNF during acute endotoxemia [159,167]. The anti-inflammatory reflex is a special instance of a functional network between the efferent parasympathetic vagus nerve, the splenic nerve (termed sympathetic) and T cells relaying neural signals. In depicting this cooperation, the use of the classical sympathetic-versus-parasympathetic neuronal designation should be modified.

Indirect studies suggested that anti-inflammatory reflex is involved in the regulation of ILC. Dalli *et al*, reported that dissection of the right vagus downregulated the number of peritoneal ILC3 and changed peritoneal macrophage responses. Right vagotomy led to decreased peritoneal levels of pro-resolving mediators, which include the protective immunoresolvent protectin conjugates in tissue regeneration 1 (PCTR1), as well as increased inflammation-initiating eicosanoids. Ach restored the PCTR1 production from ILC3. Treatment of PCTR1 or ILC3 repaired tissue and ameliorated *E. coli* infections in vagotomized mice [41]. Another group studied the regulation of ILC2 using coding $\alpha 7$ nAChR (Chrna7) knockout mice, pulmonary C fibre (PCF, which releases ACh and neuropeptides) degeneration mice, and vagotomized mice. Knockout of Chrna7 enhanced resident ILC2s and trafficking iILC2s in the lung, worsened allergic inflammation. However, PCF degeneration and vagotomy significantly reduced these two types of ILC2 and attenuated asthma responses [171]. Although there is no direct evidence suggests that “anti-inflammatory reflex” regulates ILC2, it is promising to investigate since the number of ILC2 is significantly increased in spleen during inflammation or infection [172].

In sum, parasympathetic nervous system participates in the pathogenesis of various diseases, with a different role in each disease.

Sensory Neurons

The lung is innervated by a dense network of sensory neurons that mainly comes from vagal afferents whose cell bodies reside in the vagal ganglia (jugular and nodose ganglia); while other sensory nerve innervation originates from the dorsal root ganglion (DRG) (**Figure 3**) [115,173-175]. Nociceptive receptors are richly expressed in sensory neurons endings, which are abundantly present in the lung parenchyma and near the airways; this poises them to act as the first wall for host defense and these neurons interact directly with inflammatory stimuli such as ATP, pathogens, allergens, protons, heat, mechanical injury and chemical irritants like immune cells such as APCs, macrophages, and other phagocytes [116,176-178].

Figure 3: The somatic nervous system regulation of ILC2 function. CGRP, α -calcitonin gene-related peptide; DRG, Dorsal root ganglion; NMB, neuromedin B; NMU, neuromedin U; PNECs, Pulmonary neuroendocrine cells; VIP, Vasoactive Intestinal Peptide.

Sensory neuronal action potentials evoked by this interaction are then transmitted into the CNS within milliseconds of the detection of inflammation or invasion. This action-potential

signaling mechanism is significantly faster than immune cell. Once activated, nociceptive receptors induce coughing, pain and bronchoconstriction [173,178-180]. Neuropeptides emanating from nociceptor nerve terminals also participate in the nociceptors crosstalk with immune cells [173,181-183].

Excitation of nociceptors increases the release of multiple neuropeptides, such as substance P, VIP and CGRP which regulate both innate and adaptive immune cells [184] (**Table 1** and **Figure 3**).

CGRP

CGRP, encoded by *Calca*, is a member of the calcitonin family peptides that not only secreted by peripheral nociceptive neurons but also found in central neurons [45,185]. CGRP binds to a heteromeric receptor composed of a receptor activity-modifying protein (RAMP1) and a G-protein coupled receptor termed CALCRL. CGRP via these receptors stimulates AC, which results in cAMP and PKA pathway activation and leads to the phosphorylation of several downstream pathways including NOS, MAPK, and CREB pathways [186].

In skin bacterial infection, lymph node hypertrophy and TNF- α production are found to be suppressed by CGRP [183,187]. CGRP levels markedly increased in the bronchoalveolar lavage fluid (BALF) after *Staphylococcus aureus* infection. *S. aureus* also increases cultured neuronal production of CGRP *in vitro*. CGRP could alleviate the symptoms of *S. aureus*-induced pneumonia by suppressing TNF- α , CXCL1, $\gamma\delta$ T cells and neutrophils [188].

A recent study discovered the relationship between CGRP and pulmonary neuroendocrine cells (PNECs), which comprise ~1% of the airway cell population [189,190]. PNECs (locate in close proximity to ILC2 near airway branch points) could secrete CGRP, which aggravates allergen-induced asthma in mice by stimulating ILC2 proliferation and the secretion of IL-5 from ILC2 [133]. On the other hand, *Il5*^{hi}ILC2 produce both CGRP and its receptor CGRPR following *Nb* infection. CGRP

treatment alone does not increase cytokine production from ILC2, a combination of neuromedin U (NMU) + IL-33 with CGRP stimulates IL-5 but limits IL-13 production and ILC2 proliferation. Worm expulsion and ILC2 responses are augmented without CGRP signaling [39]. Interestingly, Xu *et al*, reported that OVA-induced inflammation increased the expression of *Calca* in KLRG1⁺ILC2. CGRP suppressed KLRG1⁺ILC2s proliferation but promoted IL-5 expression [191]. Collectively, these paradoxes point to both pro- and anti-inflammatory properties of CGRP on immune responses in the lung and warrant further investigation (**Table 1** and **Figure 3**).

VIP

The neuropeptide VIP also involves in the regulation of ILC2. It has been firstly characterized as a polypeptide isolated from the small intestine with multiple impacts on different systems such as respiratory and cardiovascular systems [192]. VIP can be perceived by VIP receptor type 1 (VPAC1) or VIP receptor type 2 (VPAC2), which are differentially regulated according to cell type and activity conditions [193,194]. Similar to CGRP, VIP enhances the AC/cAMP/PKA pathway and phospholipase C, which causes the accumulation of intracellular Ca²⁺ [195].

Of note, pulmonary and intestinal ILC2 express VPAC1 and VPAC2 and produce IL-5 when they are incubated with IL-7 and VIP- or VPAC2-specific agonist [84]. Talbot *et al*, discovered a critical relationship between ILC2, VIP, T cells, and nociceptive neurons [182]. Reciprocally, ILC2-derived IL-5 activates nociceptors on afferent neurons and upregulates the release of VIP, which in return acts via VPAC2 and leads ILC2 and subsequently T cells to release more IL-5 and thereby forming a type 2 inflammatory positive feedback loop mainly based on the neuro-immune axis (**Table 1** and **Figure 3**) [182]. Since the levels of blood eosinophils and type 2 cytokine release from ILC2 are regulated by circadian rhythm and food intake, this suggests that VIP might influence blood eosinophils via upregulation of ILC2 [84].

NMU

NMU is a neuropeptide mainly released by cholinergic sensory neurons originating from DRG, but not parasympathetic neurons in the vagal ganglion [196]. The initial biological functions ascribed to NMU were food intake and body weight reduction, smooth muscle contraction of the uterus, pronociceptive effects promotion and circadian rhythm regulation [197,198]. In addition, NMU is also occasionally secreted by some APCs, including monocytes, B cells, and dendritic cells [199]. Thus, it is suggested to play an important role in the regulation of adaptive and innate immunity. In an allergen-induced asthma model, airway eosinophilia was shown to decrease in *nmu*^{-/-} mice. NMU directly stimulated extracellular/signal-regulated kinase phosphorylation and Ca²⁺ mobilization. NMU also induced cell adhesion to components of the extracellular matrix, and chemotaxis *in vitro* [200].

Recent studies reported that NMU from lamina propria play a regulatory role in mice type 2 innate immunity through binding to the neuromedin U receptor 1 (Nmur1), which is selectively enriched in ILC2. Consistent with this idea, NMU-expressing neurons have been discovered in close vicinity to ILC2 in the lungs [19,37,38]. Lung ILC2 present NMUR1 at steady state and upon IL-25 stimulation, however, NMUR1 was inhibited upon IL-33 exposure [38].

In a mice model of worm infection in the lungs and intestine, stimulation of ILC2 with NMU led to strong and immediate production of tissue protection and innate inflammatory cytokines in a NMUR1-dependent manner, thereby alleviating worm burden [37].

In a model of airway allergy, ILC2 were activated by NMU *in vitro*, and *in vivo* co-treatment of NMU with IL-25 significantly increased lung histopathology. Disruption of NMU-NMUR1 pathway decreased ILC2 number and effector function, and changed transcriptional programs following *in vivo* allergen exposure [38]. NMU elevates pulmonary ILC2 proliferation and a selectively potent secretion of innate IL-5, IL-13 and

amphiregulin [19,37,38]. Furthermore, ILC2 activated by NMU increase the number of lung eosinophils and mast cells, thus alleviating antihelminth responses [19,37,38]. Interestingly, IL-13 enhance NMU production in DRG neurons, thus indicating the existence of a reciprocal neuron–ILC2 regulatory loop via ILC2-derived IL-13 and neuronal NMU expression [38]. IL-10, primarily secreted by Tregs, was increased by NMU in activated intestinal ILC2. IL-10 further stimulated IL-10 production in ILC2 through a positive feedback loop (**Table 1** and **Figure 3**) [114]. These findings suggested that NMU treatment enhance inflammation-induced damage in the lungs and pointed to a double-edged sword of NMU-NMUR1 signaling.

NMB

NMB belongs to the neuromedin family that includes NMB, NMC, NMK, NML, NMN, NMU and NMS [201,202]. It is expressed widely in the CNS (olfactory bulb, dentate gyrus, amygdala, basal ganglia, and brainstem) and the PNS (gastrointestinal tract, trigeminal and dorsal root ganglia (DRG)) [203,204].

NMB controls cell growth, blood glucose, body temperature, emotion, energy homeostasis, exocrine and endocrine secretion, food intake, grooming and scratching, nociception and smooth muscle contraction. Inclan-Rico, Juan M *et al*, found that administration of NMB suppressed ILC2 responses via NMU receptor (NMBR), eosinophilia and mucus production after *Nb* infection in the lung. In consistent with *in vivo* results, *in vitro* treatment of NMB inhibited the growth of sorted ILC2 (**Table 1** and **Figure 3**). Of note, ILC2 sorted from basophil-depleted mice were unchanged by NMB stimulation, indicating that basophils are indispensable for the inhibitory effects of NMB on ILC2 [50].

Promising Directions for Research on the Neural Regulation of Ilc2 in the Lung

The discoveries of neural control of ILC2 have added a new dimension to neuroimmunity. All previously known findings of ILC2 in the lung could be re-examined from this perspective.

Previous studies on the lung ILC2 have been mainly performed in models of allergic disease [205-208], helminth infection [16,50,209,210], and septic lung injury [17,85,211,212]. Multiple neural pathways have been reported to be involved in these disease models [4,213,214]. Although recent studies have found some clues, the relationship between nervous system and ILC2 still remains contradictory and inconclusive. For example, CGRP shows opposite effects on different ILC2 subtypes. NMU is able to enhance the pro-inflammatory function of ILC2 as well as its anti-inflammatory function. Does the nervous system or neural mediators play ameliorating or worsening roles in these diseases by regulating ILC2? If future studies can prove this hypothesis, then ILC2 will not only be a bridge between innate and adaptive immunity, but also between the nervous system and the immune system.

The second promising research direction will be the effects of neural-regulated ILC2 on nervous system. Type I cytokines and their receptors (such as IL-1, IL-6 and TNFs) are expressed widely in CNS cells and are important for the development and function of the CNS [58]. However, the impacts of ILC2-released type II cytokines and mediators on nervous system remains to be elucidated. Currently, a reciprocal DRG–ILC2 regulatory loop via ILC2-derived IL-13 and neuronal NMU expression has been found [38]. Besides, ILC2-derived IL-5 activates nociceptors on afferent neurons and upregulates the release of VIP, which in return, acting via VPAC2 leads to ILC2 and T cells to release more IL-5 and, thereby, forming a type 2 inflammatory positive feedback loop [182]. It would be important to explore unidentified neuron-ILC2 positive/negative regulatory loops.

The third area of interest will be the neural regulation of ILC1 and ILC3. Recent studies have shown that three ILCs are functionally plastic. For instance, plastic iILC2 can coproduce both type-2 cytokines and the ILC3-characteristic cytokine (IL-17A) [215]. Under certain conditions, c-Kit⁺ILC2 can convert to ILC3-like cells [216]. Besides, IL-12 and IL-18 converted ILC2 into ILC1 in patients with chronic obstructive pulmonary disease (COPD) [217]. Does neuromodulation affect the interconversion of ILC2 and two other cell subtypes? If these plastic properties can be elucidated, we can gain a comprehensive understanding of the relationship between ILC as a cell type and the nervous system.

Least but not last, these results have great therapeutic implications for precision medicine. For example, NMUR1 is selectively expressed by ILC2, while receptors for classical ILC2 activators, i.e. IL-25, IL-33 and TSLP, are widely expressed by various cell types [19,37,38]. Meanwhile, researchers have developed many methods to selectively stimulate and inhibit neurons [218,219]. Combining these advances will allow us to identify more effective clinical targets.

Conclusion

Emerging evidence from *in vivo* animal models, human studies, and *in vitro* experiments indicates that neuropeptides and neurotransmitters released from various neurons and non-neuronal cells are critical in regulation of immune responses in different tissues including the lung. This review article provides a comprehensive overview of the effects of novel neural mediators and pathways on ILC2 and underlying mechanisms as well as the insights into the direct and indirect interactions between ILC2 and other immune cells, highlighting ILC2 as the bridge between innate and adaptive immunity. However, the research in neuro-immune area is, in general, in a premature status, and numerous questions remain to be addressed. For examples, the most of signaling pathways that mediate neural regulation of ILC2 are yet clear; and the mechanisms, by which ILC2 selectively respond to neural and non-neural signaling need to be elucidated as well. In addition, translational and

clinical investigations are required to promote the application resulted from the studies in this area.

References

1. Chavan SS, Tracey KJ. Essential Neuroscience in Immunology. *J Immunol.* 2017; 198: 3389–3397.
2. Irwin MR, Cole SW. Reciprocal regulation of the neural and innate immune systems. *Nat Rev Immunol.* 2011; 11: 625–632.
3. Hoffman C, Aballay A. Role of neurons in the control of immune defense. *Curr Opin Immunol.* 2019; 60: 30–36.
4. Zhai Q, Lai D, Cui P, Zhou R, Chen Q, et al. Selective Activation of Basal Forebrain Cholinergic Neurons Attenuates Polymicrobial Sepsis-Induced Inflammation via the Cholinergic Anti-Inflammatory Pathway. *Crit Care Med.* 2017; 45: e1075–1082.
5. Zhong C, Zhu J. Transcriptional regulators dictate innate lymphoid cell fates. *Protein Cell.* 2017; 8: 242–254.
6. Schuijs MJ, Halim TYF. Group 2 innate lymphocytes at the interface between innate and adaptive immunity. *Ann N Y Acad Sci.* 2018; 1417: 87–103.
7. Kiessling R, Klein E, Wigzell H. “Natural” killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur J Immunol.* 1975; 5: 112–117.
8. Pross HF, Jondal M. Cytotoxic lymphocytes from normal donors. A functional marker of human non-T lymphocytes. *Clin Exp Immunol.* 1975; 21: 226–235.
9. Kelly KA, Scollay R. Seeding of neonatal lymph nodes by T cells and identification of a novel population of CD3-CD4+ cells. *Eur J Immunol.* 1992; 22: 329–334.
10. Mebius RE, Rennert P, Weissman IL. Developing lymph nodes collect CD4+CD3- LTbeta+ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. *Immunity.* 1997; 7: 493–504.
11. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, et al. Innate lymphoid cells—a proposal for uniform nomenclature. *Nat Rev Immunol.* 2013; 13: 145–149.

12. Vivier E, Artis D, Colonna M, Diefenbach A, Di Santo JP, et al. Innate Lymphoid Cells: 10 Years On. *Cell*. 2018; 174: 1054–1066.
13. Eberl G, Colonna M, Di Santo JP, McKenzie AN. Innate lymphoid cells. Innate lymphoid cells: a new paradigm in immunology. *Science*. 2015; 348: aaa6566.
14. Artis D, Spits H. The biology of innate lymphoid cells. *Nature*. 2015; 517: 293–301.
15. Wong SH, Walker JA, Jolin HE, Drynan LF, Hams E, et al. Transcription factor RORalpha is critical for nuocyte development. *Nat Immunol*. 2012; 13: 229–236.
16. Huang Y, Guo L, Qiu J, Chen X, Hu-Li J, et al. IL-25-responsive, lineage-negative KLRG1hi; cells are multipotential ‘inflammatory’ type 2 innate lymphoid cells. *Nat Immunol*. 2015; 16: 161–169.
17. Lai D, Tang J, Chen L, Fan EK, Scott MJ, et al. Group 2 innate lymphoid cells protect lung endothelial cells from pyroptosis in sepsis. *Cell Death Dis*. 2018; 9: 369.
18. Hammad H, Lambrecht BN. Barrier Epithelial Cells and the Control of Type 2 Immunity. *Immunity*. 2015; 43: 29–40.
19. Klose CSN, Mahlaköiv T, Moeller JB, Rankin LC, Flamar AL, et al. The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. *Nature*. 2017; 549: 282–286.
20. Monticelli LA, Sonnenberg GF, Abt MC, Alenghat T, Ziegler CG, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat Immunol*. 2011; 12: 1045–1054.
21. Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity*. 2012; 36: 451–463.
22. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity*. 2001; 15: 985–995.
23. Fallon PG, Ballantyne SJ, Mangan NE, Barlow JL, Dasvarma A, et al. Identification of an interleukin IL-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J Exp Med*. 2006; 203: 1105–1116.

24. Cording S, Medvedovic J, Aychek T, Eberl G. Innate lymphoid cells in defense, immunopathology and immunotherapy. *Nat Immunol.* 2016; 17: 755–757.
25. Monticelli LA, Osborne LC, Noti M, Tran SV, Zaiss DM, et al. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. *Proc Natl Acad Sci U S A.* 2015; 112: 10762–10767.
26. Seehus CR, Kadavallore A, Torre B, Yeckes AR, Wang Y, et al. Alternative activation generates IL-10 producing type 2 innatelymphoid cells. *Nat Commun.* 2017; 8: 1900.
27. Mjosberg J, Bernink J, Golebski K, Karrich JJ, Peters CP, et al. The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. *Immunity.* 2012; 37: 649–659.
28. Salimi M, Barlow JL, Saunders SP, Xue L, Gutowska-Owsiak D, et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med.* 2013; 210: 2939–2950.
29. Oliphant CJ, Hwang YY, Walker JA, Salimi M, Wong SH, et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4⁺ T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity.* 2014; 41: 283–295.
30. Schwartz C, Khan AR, Floudas A, Saunders SP, Hams E, et al. ILC2s regulate adaptive Th2 cell functions via PD-L1 checkpoint control. *J Exp Med.* 2017; 214: 2507–2521.
31. Molofsky AB, Van Gool F, Liang HE, Van Dyken SJ, Nussbaum JC, et al. Interleukin-33 and Interferon- γ Counter-Regulate Group 2 Innate Lymphoid Cell Activation during Immune Perturbation. *Immunity.* 2015; 43: 161–174.
32. Webb GJ, Hirschfield GM, Lane PJ. OX40, OX40L and Autoimmunity: a Comprehensive Review. *Clin Rev Allergy Immunol.* 2016; 50: 312–332.
33. Chang YJ, DeKruyff RH, Umetsu DT. The role of type 2 innate lymphoid cells in asthma. *J Leukoc Biol.* 2013; 94: 933–940.
34. Barlow JL, Bellosi A, Hardman CS, Drynan LF, Wong SH, et al. Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways

- hyperreactivity. *J Allergy Clin Immunol*. 2012; 129: 191–198.e191-194.
35. Kim HY, Chang YJ, Subramanian S, Lee HH, Albacker LA, et al. Innate lymphoid cells responding to IL-33 mediate airway hyperreactivity independently of adaptive immunity. *J Allergy Clin Immunol*. 2012; 129: 216–227.e211-216.
36. Imai Y, Yasuda K, Sakaguchi Y, Haneda T, Mizutani H, et al. Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. *Proc Natl Acad Sci U S A*. 2013; 110: 13921–13926.
37. Cardoso V, Chesné J, Ribeiro H, García-Cassani B, Carvalho T, et al. Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature*. 2017; 549: 277–281.
38. Wallrapp A, Riesenfeld SJ, Burkett PR, Abdulnour RE, Nyman J, et al. The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation. *Nature*. 2017; 549: 351–356.
39. Nagashima H, Mahlaköiv T, Shih HY, Davis FP, Meylan F, et al. Neuropeptide CGRP Limits Group 2 Innate Lymphoid Cell Responses and Constrains Type 2 Inflammation. *Immunity*. 2019; 51: 682-695.e6.
40. Moriyama S, Brestoff JR, Flamar AL, Moeller JB, Klose CSN, et al. β 2-adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses. *Science*. 2018; 359: 1056–1061.
41. Dalli J, Colas RA, Arnardottir H, Serhan CN. Vagal Regulation of Group 3 Innate Lymphoid Cells and the Immunoresolvent PCTR1 Controls Infection Resolution. *Immunity*. 2017; 46: 92–105.
42. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature*. 2000; 405: 458–462.
43. Cai B, Chen F, Ji Y, Kiss L, de Jonge WJ, et al. Alpha7 cholinergic-agonist prevents systemic inflammation and improves survival during resuscitation. *J Cell Mol Med*. 2009; 13: 3774–3785.

44. Galle-Treger L, Suzuki Y, Patel N, Sankaranarayanan I, Aron JL, et al. Nicotinic acetylcholine receptor agonist attenuates ILC2-dependent airway hyperreactivity. *Nat Commun.* 2016; 7: 13202.
45. Holzmann B. Modulation of immune responses by the neuropeptide CGRP. *Amino Acids.* 2013; 45: 1–7.
46. Vida G, Peña G, Deitch EA, Ulloa L. $\alpha 7$ -cholinergic receptor mediates vagal induction of splenic norepinephrine. *J Immunol.* 2011; 186: 4340–4346.
47. Vida G, Peña G, Kanashiro A, Thompson-Bonilla Mdel R, Palange D, Deitch EA, et al. $\beta 2$ -Adrenoreceptors of regulatory lymphocytes are essential for vagal neuromodulation of the innate immune system. *FASEB J.* 2011; 25: 4476–4485.
48. Rosas-Ballina M, Olofsson PS, Ochani M, Valdés-Ferrer SI, Levine YA, et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science.* 2011; 334: 98–101.
49. Kawashima K, Fujii T, Moriwaki Y, Misawa H. Critical roles of acetylcholine and the muscarinic and nicotinic acetylcholine receptors in the regulation of immune function. *Life Sci.* 2012; 91: 1027–1032.
50. Inclan-Rico JM, Ponessa JJ, Valero-Pacheco N, Hernandez CM, Sy CB, et al. Basophils prime group 2 innate lymphoid cells for neuropeptide-mediated inhibition. *Nat Immunol.* 2020; 21: 1181–1193.
51. Hosoi T, Okuma Y, Matsuda T, Nomura Y. Novel pathway for LPS-induced afferent vagus nerve activation: possible role of nodose ganglion. *Auton Neurosci.* 2005; 120: 104–107.
52. de Lartigue G, Barbier de la Serre C, Espero E, Lee J, Raybould HE. Diet-induced obesity leads to the development of leptin resistance in vagal afferent neurons. *Am J Physiol Endocrinol Metab.* 2011; 301: E187–195.
53. Ma F, Zhang L, Westlund KN. Reactive oxygen species mediate TNFR1 increase after TRPV1 activation in mouse DRG neurons. *Mol Pain.* 2009; 5: 31.
54. Li M, Shi J, Tang JR, Chen D, Ai B, et al. Effects of complete Freund's adjuvant on immunohistochemical

- distribution of IL-1beta and IL-1R I in neurons and glia cells of dorsal root ganglion. *Acta Pharmacol Sin.* 2005; 26: 192–198.
55. Cosentino M, Bombelli R, Ferrari M, Marino F, Rasini E, et al. HPLC-ED measurement of endogenous catecholamines in human immune cells and hematopoietic cell lines. *Life Sci.* 2000; 68: 283–295.
56. Quatrini L, Vivier E, Ugolini S. Neuroendocrine regulation of innate lymphoid cells. *Immunol Rev.* 2018; 286: 120–136.
57. Schedlowski M, Engler H, Grigoleit JS. Endotoxin-induced experimental systemic inflammation in humans: a model to disentangle immune-to-brain communication. *Brain Behav Immun.* 2014; 35: 1–8.
58. Mousa A, Bakhiet M. Role of cytokine signaling during nervous system development. *Int J Mol Sci.* 2013; 14: 13931–13957.
59. Klein Wolterink RG, Kleinjan A, van Nimwegen M, Bergen I, de Bruijn M, et al. Pulmonary innate lymphoid cells are major producers of IL-5 and IL-13 in murine models of allergic asthma. *Eur J Immunol.* 2012; 42: 1106–1116.
60. Van Dyken SJ, Nussbaum JC, Lee J, Molofsky AB, Liang HE, et al. A tissue checkpoint regulates type 2 immunity. *Nat Immunol.* 2016; 17: 1381–1387.
61. Guo L, Huang Y, Chen X, Hu-Li J, Urban JF Jr, et al. Innate immunological function of TH2 cells in vivo. *Nat Immunol.* 2015; 16: 1051–1059.
62. Halim TY, MacLaren A, Romanish MT, Gold MJ, McNagny KM, et al. Retinoic-acid-receptor-related orphan nuclear receptor alpha is required for natural helper cell development and allergic inflammation. *Immunity.* 2012; 37: 463–474.
63. Gold MJ. Group 2 innate lymphoid cells facilitate sensitization to local, but not systemic, TH2-inducing allergen exposures. *J Allergy Clin Immunol.* 2014; 133: 1142–1148.
64. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature.* 2010; 464: 1367–1370.
65. Saenz SA, Siracusa MC, Perrigoue JG, Spencer SP, Urban JF Jr, et al. IL25 elicits a multipotent progenitor cell

- population that promotes TH₂ cytokine responses. *Nature*. 2010; 464: 1362–1366.
66. Wilhelm C, Hirota K, Stieglitz B, Van Snick J, Tolaini M, et al. An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. *Nat Immunol*. 2011; 12: 1071–1077.
 67. Mattioli I, Diefenbach A. Enabling anti-tumor immunity by unleashing ILC2. *Cell Res*. 2020; 30: 461–462.
 68. Moral JA, Leung J, Rojas LA, Ruan J, Zhao J, et al. ILC2s amplify PD-1 blockade by activating tissue-specific cancer immunity. *Nature*. 2020; 579: 130–135.
 69. Besnard AG, Guabiraba R, Niedbala W, Palomo J, Reverchon F, et al. IL-33-mediated protection against experimental cerebral malaria is linked to induction of type 2 innate lymphoid cells, M2 macrophages and regulatory T cells. *PLoS Pathog*. 2015; 11: e1004607.
 70. Licona-Limon P, Kim LK, Palm NW, Flavell RA. TH₂, allergy and group 2 innate lymphoid cells. *Nat Immunol*. 2013; 14: 536–542.
 71. Fukuoka A, Futatsugi-Yumikura S, Takahashi S, Kazama H, Iyoda T, et al. Identification of a novel type 2 innate immunocyte with the ability to enhance IgE production. *Int Immunol*. 2013; 25: 373–382.
 72. Moriyama S, Artis D. Neuronal regulation of group 2 innate lymphoid cells and type 2 inflammation. *Adv Immunol*. 2019; 143: 1–9.
 73. Erickson LD, Foy TM, Waldschmidt TJ. Murine B1 B cells require IL-5 for optimal T cell-dependent activation. *J Immunol*. 2001; 166: 1531–1539.
 74. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, et al. Innate production of TH₂ cytokines by adipose tissue-associated c-Kit⁺;Sca-1⁺; lymphoid cells. *Nature*. 2010; 463: 540–544.
 75. Drake LY, Iijima K, Kita H. Group 2 innate lymphoid cells and CD4⁺ T cells cooperate to mediate type 2 immune response in mice. *Allergy*. 2014; 69: 1300–1307.
 76. Mirchandani AS, Besnard AG, Yip E, Scott C, Bain CC, et al. Type 2 innate lymphoid cells drive CD4⁺ Th2 cell responses. *J Immunol*. 2014; 192: 2442–2448.

77. von Burg N, Turchinovich G, Finke D. Maintenance of Immune Homeostasis through ILC/T Cell Interactions. *Front Immunol.* 2015; 6: 416.
78. Price AE, Liang HE, Sullivan BM, Reinhardt RL, Eisley CJ, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc Natl Acad Sci U S A.* 2010; 107: 11489–11494.
79. Doherty TA, Khorram N, Lund S, Mehta AK, Croft M, et al. Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production. *J Allergy Clin Immunol.* 2013; 132: 205–213.
80. Noval Rivas M, Burton OT, Oettgen HC, Chatila T. IL-4 production by group 2 innate lymphoid cells promotes food allergy by blocking regulatory T-cell function. *J Allergy Clin Immunol.* 2016; 138: 801–811.e809.
81. Pelly VS, Kannan Y, Coomes SM, Entwistle LJ, Rückerl D, et al. IL-4-producing ILC2s are required for the differentiation of T. *Mucosal Immunol.* 2016; 9: 1407–1417.
82. Bal SM, Bernink JH, Nagasawa M, Groot J, Shikhagaie MM, et al. IL-1 β , IL-4 and IL-12 control the fate of group 2 innate lymphoid cells in human airway inflammation in the lungs. *Nat Immunol.* 2016; 17: 636–645.
83. Motomura Y, Morita H, Moro K, Nakae S, Artis D, et al. Basophil-derived interleukin-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation. *Immunity.* 2014; 40: 758–771.
84. Nussbaum JC, Van Dyken SJ, von Moltke J, Cheng LE, Mohapatra A, et al. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature.* 2013; 502: 245–248.
85. Zhang K, Jin Y, Lai D, Wang J, Wang Y, et al. RAGE-induced ILC2 expansion in acute lung injury due to haemorrhagic shock. *Thorax.* 2020; 75: 209–219.
86. Drake LY, Iijima K, Bartemes K, Kita H. Group 2 Innate Lymphoid Cells Promote an Early Antibody Response to a Respiratory Antigen in Mice. *J Immunol.* 2016; 197: 1335–1342.
87. Angkasekwinai P, Sodthawon W, Jeerawattanawart S, Hansakon A, Pattanapanyasat K, et al. ILC2s activated by IL-25 promote antigen-specific Th2 and Th9 functions that

- contribute to the control of *Trichinella spiralis* infection. PLoS One. 2017; 12: e0184684.
88. Hrusch CL, Manns ST, Bryazka D, Casaos J, Bonham CA, et al. ICOS protects against mortality from acute lung injury through activation of IL-5. Mucosal Immunol. 2018; 11: 61–70.
89. Wu L, Lin Q, Ma Z, Chowdhury FA, Mazumder MHH, et al. Mesenchymal PGD2 activates an ILC2-Treg axis to promote proliferation of normal and malignant HSPCs. Leukemia. 2020; 360: eaa8546.
90. Xu J, Guardado J, Hoffman R, Xu H, Namas R, et al. IL33-mediated ILC2 activation and neutrophil IL5 production in the lung response after severe trauma: A reverse translation study from a human cohort to a mouse trauma model. PLoS Med. 2017; 14: e1002365.
91. Turner JE, Morrison PJ, Wilhelm C, Wilson M, Ahlfors H, et al. IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. J Exp Med. 2013; 210: 2951–2965.
92. Rauber S, Lubber M, Weber S, Maul L, Soare A, et al. Resolution of inflammation by interleukin-9-producing type 2 innatelymphoid cells. Nat Med. 2017; 23: 938–944.
93. Wan J, Huang L, Ji X, Yao S, Hamed Abdelaziz M, et al. HMGB1-induced ILC2s activate dendritic cells by producing IL-9 in asthmatic mouse model. Cell Immunol. 2020; 352: 104085.
94. Roediger B, Kyle R, Tay SS, Mitchell AJ, Bolton HA, et al. IL-2 is a critical regulator of group 2 innate lymphoid cell function during pulmonary inflammation. J Allergy Clin Immunol. 2015; 136: 1653–1663.e1657.
95. Erpenbeck VJ, Hohlfeld JM, Volkmann B, Hagenberg A, Geldmacher H, et al. Segmental allergen challenge in patients with atopic asthma leads to increased IL-9 expression in bronchoalveolar lavage fluid lymphocytes. J Allergy Clin Immunol. 2003; 111: 1319–1327.
96. Temann UA, Geba GP, Rankin JA, Flavell RA. Expression of interleukin 9 in the lungs of transgenic mice causes airway inflammation, mast cell hyperplasia, and bronchial hyperresponsiveness. J Exp Med. 1998; 188: 1307–1320.

97. Temann UA, Laouar Y, Eynon EE, Homer R, Flavell RA. IL9 leads to airway inflammation by inducing IL13 expression in airway epithelial cells. *Int Immunol.* 2007; 19: 1–10.
98. Shimbara A, Christodouloupoulos P, Soussi-Gounni A, Olivenstein R, Nakamura Y, et al. IL-9 and its receptor in allergic and nonallergic lung disease: increased expression in asthma. *J Allergy Clin Immunol.* 2000; 105: 108–115.
99. Sugita K, Steer CA, Martinez-Gonzalez I, Altunbulakli C, Morita H, et al. Type 2 innate lymphoid cells disrupt bronchial epithelial barrier integrity by targeting tight junctions through IL-13 in asthmatic patients. *J Allergy Clin Immunol.* 2018; 141: 300–310.e311.
100. Bouchery T, Kyle R, Camberis M, Shepherd A, Filbey K, et al. ILC2s and T cells cooperate to ensure maintenance of M2 macrophages for lung immunity against hookworms. *Nat Commun.* 2015; 6: 6970.
101. Saluzzo S, Gorki AD, Rana BMJ, Martins R, Scanlon S, et al. First-Breath-Induced Type 2 Pathways Shape the Lung Immune Environment. *Cell Rep.* 2017; 18: 1893–1905.
102. Halim TY, Steer CA, Mathä L, Gold MJ, Martinez-Gonzalez I, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity.* 2014; 40: 425–435.
103. Jia Y, Fang X, Zhu X, Bai C, Zhu L, et al. IL-13. *Am J Respir Cell Mol Biol.* 2016; 55: 675–683.
104. Zaiss DMW, Gause WC, Osborne LC, Artis D. Emerging functions of amphiregulin in orchestrating immunity, inflammation, and tissue repair. *Immunity.* 2015; 42: 216–226.
105. Zaiss DM, van Loosdregt J, Gorlani A, Bekker CP, Gröne A, et al. Amphiregulin enhances regulatory T cell-suppressive function via the epidermal growth factor receptor. *Immunity.* 2013; 38: 275–284.
106. Crosby LM, Waters CM. Epithelial repair mechanisms in the lung. *Am J Physiol Lung Cell Mol Physiol.* 2010; 298: L715–731.
107. Puddicombe SM, Polosa R, Richter A, Krishna MT, Howarth PH, et al. Involvement of the epidermal growth

- factor receptor in epithelial repair in asthma. *FASEB J.* 2000; 14: 1362–1374.
108. Arpaia N, Green JA, Moltedo B, Arvey A, Hemmers S, et al. A Distinct Function of Regulatory T Cells in Tissue Protection. *Cell.* 2015; 162: 1078–1089.
109. Smith KA, Filbey KJ, Reynolds LA, Hewitson JP, Marcus Y, et al. Low-level regulatory T-cell activity is essential for functional type-2 effector immunity to expel gastrointestinal helminths. *Mucosal Immunol.* 2016; 9: 428–443.
110. Rigas D, Lewis G, Aron JL, Wang B, Banie H, et al. Type 2 innate lymphoid cell suppression by regulatory T cells attenuates airway hyperreactivity and requires inducible T-cell costimulator-inducible T-cell costimulator ligand interaction. *J Allergy Clin Immunol.* 2017; 139: 1468–1477.e1462.
111. Maazi H, Patel N, Sankaranarayanan I, Suzuki Y, Rigas D, et al. ICOS:ICOS-ligand interaction is required for type 2 innate lymphoid cell function, homeostasis, and induction of airway hyperreactivity. *Immunity.* 2015; 42: 538–551.
112. Yu Y, Tsang JC, Wang C, Clare S, Wang J, et al. Single-cell RNA-seq identifies a PD-1. *Nature.* 2016; 539: 102–106.
113. Croft M. Control of immunity by the TNFR-related molecule OX40 CD134;. *Annu Rev Immunol.* 2010; 28: 57–78.
114. Bando JK, Gilfillan S, Di Luccia B, Fachi JL, Sécca C, et al. ILC2s are the predominant source of intestinal ILC-derived IL-10. *J Exp Med.* 2020; 21: e20191520.
115. Belvisi MG. Overview of the innervation of the lung. *Curr Opin Pharmacol.* 2002; 2: 211–215.
116. McMahon SB, La Russa F, Bennett DL. Crosstalk between the nociceptive and immune systems in host defence and disease. *Nat Rev Neurosci.* 2015; 16: 389–402.
117. Franco R, Schoneveld OJ, Pappa A, Panayiotidis M II. The central role of glutathione in the pathophysiology of human diseases. *Arch Physiol Biochem.* 2007; 113: 234–258.
118. Papa I, Saliba D, Ponzoni M, Bustamante S, Canete PF, et al. TFH-derived dopamine accelerates productive synapses in germinal centres. *Nature.* 2017; 547: 318–323.

119. Pavlov VA, Tracey KJ. The vagus nerve and the inflammatory reflex—linking immunity and metabolism. *Nat Rev Endocrinol.* 2012; 8: 743–754.
120. Su X, Matthay MA, Malik AB. Requisite role of the cholinergic $\alpha 7$ nicotinic acetylcholine receptor pathway in suppressing Gram-negative sepsis-induced acute lung inflammatory injury. *J Immunol.* 2010; 184: 401–410.
121. Cole SW, Arevalo JM, Takahashi R, Sloan EK, Lutgendorf SK, et al. Computational identification of gene-social environment interaction at the human IL6 locus. *Proc Natl Acad Sci U S A.* 2010; 107: 5681–5686.
122. Collado-Hidalgo A, Sung C, Cole S. Adrenergic inhibition of innate anti-viral response: PKA blockade of Type I interferon gene transcription mediates catecholamine support for HIV-1 replication. *Brain Behav Immun.* 2006; 20: 552–563.
123. Nance DM, Sanders VM. Autonomic innervation and regulation of the immune system 1987-2007;. *Brain Behav Immun.* 2007; 21: 736–745.
124. Grebe KM, Takeda K, Hickman HD, Bailey AL, Embry AC, et al. Cutting edge: Sympathetic nervous system increases proinflammatory cytokines and exacerbates influenza A virus pathogenesis. *J Immunol.* 2010; 184: 540–544.
125. Ding X, Luo Y, Zhang X, Zheng H, Yang X, et al. IL-33-driven ILC2/eosinophil axis in fat is induced by sympathetic tone and suppressed by obesity. *J Endocrinol.* 2016; 231: 35–48.
126. Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve—an integrative interface between twosupersystems: the brain and the immune system. *Pharmacol Rev.* 2000; 52: 595–638.
127. Muller PA, Koscsó B, Rajani GM, Stevanovic K, Berres ML, et al. Crosstalk between Muscularis Macrophages and Enteric Neurons Regulates Gastrointestinal Motility. *Cell.* 2014; 158: 1210.
128. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. *Nat Rev Immunol.* 2006; 6: 318–328.

129. Lee HJ, Takemoto N, Kurata H, Kamogawa Y, Miyatake S, et al. GATA-3 induces T helper cell type 2 Th2; cytokine expression and chromatin remodeling in committed Th1 cells. *J Exp Med*. 2000; 192: 105–115.
130. Panina-Bordignon P, Mazzeo D, Lucia PD, D'Ambrosio D, Lang R, et al. Beta2-agonists prevent Th1 development by selective inhibition of interleukin 12. *J Clin Invest*. 1997; 100: 1513–1519.
131. Cole SW, Korin YD, Fahey JL, Zack JA. Norepinephrine accelerates HIV replication via protein kinase A-dependent effects on cytokine production. *J Immunol*. 1998; 161: 610–616.
132. Cai T, Qiu J, Ji Y, Li W, Ding Z, et al. IL-17-producing ST2. *J Allergy Clin Immunol*. 2019; 143: 229–244.e229.
133. Sui P, Wiesner DL, Xu J, Zhang Y, Lee J, et al. Pulmonary neuroendocrine cells amplify allergic asthma responses. *Science*. 2018; 360: eaan8546.
134. Pavlov VA, Ochani M, Gallowitsch-Puerta M, Ochani K, Huston JM, et al. Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. *Proc Natl Acad Sci U S A*. 2006; 103: 5219–5223.
135. Rosas-Ballina M, Valdés-Ferrer SI, Dancho ME, Ochani M, Katz D, et al. Xanomeline suppresses excessive pro-inflammatory cytokine responses through neural signal-mediated pathways and improves survival in lethal inflammation. *Brain Behav Immun*. 2015; 44: 19–27.
136. Ji H, Rabbi MF, Labis B, Pavlov VA, Tracey KJ, et al. Central cholinergic activation of a vagus nerve-to-spleen circuit alleviates experimental colitis. *Mucosal Immunol*. 2014; 7: 335–347.
137. Miksa M, Das P, Zhou M, Wu R, Dong W, et al. Pivotal role of the alpha2A;-adrenoceptor in producing inflammation and organ injury in a rat model of sepsis. *PLoS One*. 2009; 4: e5504.
138. Levine YA, Koopman FA, Faltys M, Caravaca A, Bendele A, et al. Neurostimulation of the cholinergic anti-inflammatory pathway ameliorates disease in rat collagen-induced arthritis. *PLoS One*. 2014; 9: e104530.

139. von Moltke J, Ji M, Liang HE, Locksley RM. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature*. 2016; 529: 221–225.
140. Schneider C, O’Leary CE, Locksley RM. Regulation of immune responses by tuft cells. *Nat Rev Immunol*. 2019; 19: 584–593.
141. Skok MV, Grailhe R, Agenes F, Changeux JP. The role of nicotinic receptors in B-lymphocyte development and activation. *Life Sci*. 2007; 80: 2334–2336.
142. Nizri E, Hamra-Amitay Y, Sicsic C, Lavon I, Brenner T. Anti-inflammatory properties of cholinergic up-regulation: A new role for acetylcholinesterase inhibitors. *Neuropharmacology*. 2006; 50: 540–547.
143. Nizri E, Irony-Tur-Sinai M, Lory O, Orr-Urtreger A, Lavi E, et al. Activation of the cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via suppression of Th1 and Th17 responses. *J Immunol*. 2009; 183: 6681–6688.
144. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, et al. Nicotinic acetylcholine receptor $\alpha 7$ subunit is an essential regulator of inflammation. *Nature*. 2003; 421: 384–388.
145. Wang H, Liao H, Ochani M, Justiniani M, Lin X, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat Med*. 2004; 10: 1216–1221.
146. Hoyler T, Klose CS, Souabni A, Turqueti-Neves A, Pfeifer D, et al. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity*. 2012; 37: 634–648.
147. Klein Wolterink RG, Serafini N, van Nimwegen M, Vosshenrich CA, de Bruijn MJ, et al. Essential, dose-dependent role for the transcription factor Gata3 in the development of IL-5+ and IL-13+ type 2 innate lymphoid cells. *Proc Natl Acad Sci U S A*. 2013; 110: 10240–10245.
148. Mishra NC, Rir-Sima-Ah J, Langley RJ, Singh SP, Peña-Philippides JC, et al. Nicotine primarily suppresses lung Th2 but not goblet cell and muscle cell responses to allergens. *J Immunol*. 2008; 180: 7655–7663.

149. Cesario A, Russo P, Nastrucci C, Granone P. Is alpha7-nAChR a possible target for lung cancer and malignant pleural mesothelioma treatment? *Curr Drug Targets*. 2012; 13: 688–694.
150. West KA, Brognard J, Clark AS, Linnoila IR, Yang X, et al. Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. *J Clin Invest*. 2003; 111: 81–90.
151. Dasgupta P, Kinkade R, Joshi B, Decook C, Haura E, et al. Nicotine inhibits apoptosis induced by chemotherapeutic drugs by up-regulating XIAP and survivin. *Proc Natl Acad Sci U S A*. 2006; 103: 6332–6337.
152. Schaal C, Chellappan SP. Nicotine-mediated cell proliferation and tumor progression in smoking-related cancers. *Mol Cancer Res*. 2014; 12: 14–23.
153. Steinman L. Elaborate interactions between the immune and nervous systems. *Nat Immunol*. 2004; 5: 575–581.
154. van Westerloo DJ, Giebelen IA, Florquin S, Bruno MJ, Larosa GJ, et al. The vagus nerve and nicotinic receptors modulate experimental pancreatitis severity in mice. *Gastroenterology*. 2006; 130: 1822–1830.
155. Bernik TR, Friedman SG, Ochani M, DiRaimo R, Susarla S, et al. Cholinergic antiinflammatory pathway inhibition of tumor necrosis factor during ischemia reperfusion. *J Vasc Surg*. 2002; 36: 1231–1236.
156. Altavilla D, Guarini S, Bitto A, Mioni C, Giuliani D, et al. Activation of the cholinergic anti-inflammatory pathway reduces NF-kappaB activation, blunts TNF-alpha production, and protects against splanchnic artery occlusion shock. *Shock*. 2006; 25: 500–506.
157. Pullan RD, Rhodes J, Ganesh S, Mani V, Morris JS, et al. Transdermal nicotine for active ulcerative colitis. *N Engl J Med*. 1994; 330: 811–815.
158. Bernik TR, Friedman SG, Ochani M, DiRaimo R, Ulloa LR, et al. Pharmacological stimulation of the cholinergic antiinflammatory pathway. *J Exp Med*. 2002; 195: 781–788.
159. Huston JM, Ochani M, Rosas-Ballina M, Liao H, Ochani K, et al. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. *J Exp Med*. 2006; 203: 1623–1628.

160. van Westerloo DJ, Giebelen IA, Florquin S, Daalhuisen J, Bruno MJ, et al. The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis. *J Infect Dis.* 2005; 191: 2138–2148.
161. Pavlov VA, Wang H, Czura CJ, Friedman SG, Tracey KJ. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. *Mol Med.* 2003; 9: 125–134.
162. Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. *J Clin Invest.* 2007; 117: 289–296.
163. Tracey KJ. The inflammatory reflex. *Nature.* 2002; 420: 853–859.
164. Tracey KJ. Reflex control of immunity. *Nat Rev Immunol.* 2009; 9: 418–428.
165. Andersson U, Tracey KJ. Reflex principles of immunological homeostasis. *Annu Rev Immunol.* 2012; 30: 313–335.
166. Oke SL, Tracey KJ. From CNI-1493 to the immunological homunculus: physiology of the inflammatory reflex. *J Leukoc Biol.* 2008; 83: 512–517.
167. Rosas-Ballina M, Ochani M, Parrish WR, Ochani K, Harris YT, et al. Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. *Proc Natl Acad Sci U S A.* 2008; 105: 11008–11013.
168. Lu B, Kwan K, Levine YA, Olofsson PS, Yang H, et al. alpha7 nicotinic acetylcholine receptor signaling inhibits inflammasome activation by preventing mitochondrial DNA release. *Mol Med.* 2014; 20: 350–358.
169. de Jonge WJ, van der Zanden EP, The FO, Bijlsma MF, van Westerloo DJ, et al. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. *Nat Immunol.* 2005; 6: 844–851.
170. Guarini S, Altavilla D, Cainazzo MM, Giuliani D, Bigiani A, et al. Efferent vagal fibre stimulation blunts nuclear factor-kappaB activation and protects against hypovolemic hemorrhagic shock. *Circulation.* 2003; 107: 1189–1194.

171. Feng X, Li L, Feng J, He W, Li N, et al. Vagal- $\alpha 7$ nAChR signalling attenuates allergic asthma responses and facilitates asthma tolerance by regulating inflammatory group 2 innate lymphoid cells. *Immunol Cell Biol* 2020.
172. Zhou L. Striking similarity: GATA-3 regulates ILC2 and Th2 cells. *Immunity*. 2012; 37: 589–591.
173. Trankner D, Hahne N, Sugino K, Hoon MA, Zuker C. Population of sensory neurons essential for asthmatic hyperreactivity of inflamed airways. *Proc Natl Acad Sci U S A*. 2014; 111: 11515–11520.
174. Chang RB, Storchlic DE, Williams EK, Umans BD, Liberles SD. Vagal Sensory Neuron Subtypes that Differentially Control Breathing. *Cell*. 2015; 161: 622–633.
175. Mazzone SB, Undem BJ. Vagal Afferent Innervation of the Airways in Health and Disease. *Physiol Rev*. 2016; 96: 975–1024.
176. Kollarik M, Ru F, Brozmanova M. Vagal afferent nerves with the properties of nociceptors. *Auton Neurosci*. 2010; 153: 12–20.
177. Ni D, Gu Q, Hu HZ, Gao N, Zhu MX, et al. Thermal sensitivity of isolated vagal pulmonary sensory neurons: role of transient receptor potential vanilloid receptors. *Am J Physiol Regul Integr Comp Physiol*. 2006; 291: R541–550.
178. Basbaum A, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. *Cell*. 2009; 139: 267–284.
179. Canning BJ, Mori N, Mazzone SB. Vagal afferent nerves regulating the cough reflex. *Respir Physiol Neurobiol*. 2006; 152: 223–242.
180. Dubin AE, Patapoutian A. Nociceptors: the sensors of the pain pathway. *J Clin Invest*. 2010; 120: 3760–3772.
181. Caceres AI, Brackmann M, Elia MD, Bessac BF, del Camino D, et al. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Proc Natl Acad Sci U S A*. 2009; 106: 9099–9104.
182. Talbot S, Abdunnour RE, Burkett PR, Lee S, Cronin SJ, et al. Silencing Nociceptor Neurons Reduces Allergic Airway Inflammation. *Neuron*. 2015; 87: 341–354.

183. Pinho-Ribeiro FA, Verri WA, Chiu IM. Nociceptor Sensory Neuron-Immune Interactions in Pain and Inflammation. *Trends Immunol.* 2017; 38: 5–19.
184. Blake KJ, Jiang XR, Chiu IM. Neuronal Regulation of Immunity in the Skin and Lungs. *Trends Neurosci.* 2019; 42: 537–551.
185. Russell FA, King R, Smillie SJ, Kodji X, Brain SD. Calcitonin gene-related peptide: physiology and pathophysiology. *Physiol Rev.* 2014; 94: 1099–1142.
186. Walker CS, Conner AC, Poyner DR, Hay DL. Regulation of signal transduction by calcitonin gene-related peptide receptors. *Trends Pharmacol Sci.* 2010; 31: 476–483.
187. Chiu IM, Heesters BA, Ghasemlou N, Von Hehn CA, Zhao F, et al. Bacteria activate sensory neurons that modulate pain and inflammation. *Nature.* 2013; 501: 52–57.
188. Baral P, Umans BD, Li L, Wallrapp A, Bist M, Kirschbaum T, et al. Nociceptor sensory neurons suppress neutrophil and $\gamma\delta$ T cell responses in bacterial lung infections and lethal pneumonia. *Nat Med.* 2018; 24: 417–426.
189. Kuo CS, Krasnow MA. Formation of a Neurosensory Organ by Epithelial Cell Slithering. *Cell.* 2015; 163: 394–405.
190. Noguchi M, Sumiyama K, Morimoto M. Directed Migration of Pulmonary Neuroendocrine Cells toward Airway Branches Organizes the Stereotypic Location of Neuroepithelial Bodies. *Cell Rep.* 2015; 13: 2679–2686.
191. Xu H, Ding J, Porter CBM, Wallrapp A, Tabaka M, et al. Transcriptional Atlas of Intestinal Immune Cells Reveals that Neuropeptide α -CGRP Modulates Group 2 Innate Lymphoid Cell Responses. *Immunity.* 2019; 51: 696–708.e699.
192. Said S, Mutt V. Polypeptide with broad biological activity: isolation from small intestine. *Science.* 1970; 169: 1217–1218.
193. Lara-Marquez M, O'Dorisio M, O'Dorisio T, Shah M, Karacay B. Selective gene expression and activation-dependent regulation of vasoactive intestinal peptide receptor type 1 and type 2 in human T cells. *J Immunol.* 2001; 166: 2522–2530.

194. Calvo JR, Pozo D, Guerrero JM. Functional and molecular characterization of VIP receptors and signal transduction in human and rodent immune systems. *Adv Neuroimmunol*. 1996; 6: 39–47.
195. Dickson L, Finlayson K. VPAC and PAC receptors: From ligands to function. *Pharmacol Ther*. 2009; 121: 294–316.
196. Moriyama M, Furue H, Katafuchi T, Teranishi H, Sato T, et al. Presynaptic modulation by neuromedin U of sensory synaptic transmission in rat spinal dorsal horn neurones. *J Physiol*. 2004; 559: 707–713.
197. Howard AD, Wang R, Pong SS, Mellin TN, Strack A, et al. Identification of receptors for neuromedin U and its role in feeding. *Nature*. 2000; 406: 70–74.
198. Nakahara K, Kojima M, Hanada R, Egi Y, Ida T, et al. Neuromedin U is involved in nociceptive reflexes and adaptation to environmental stimuli in mice. *Biochem Biophys Res Commun*. 2004; 323: 615–620.
199. Hedrick JA, Morse K, Shan L, Qiao X, Pang L, et al. Identification of a human gastrointestinal tract and immune system receptor for the peptide neuromedin U. *Mol Pharmacol*. 2000; 58: 870–875.
200. Moriyama M, Fukuyama S, Inoue H, Matsumoto T, Sato T, et al. The neuropeptide neuromedin U activates eosinophils and is involved in allergen-induced eosinophilia. *Am J Physiol Lung Cell Mol Physiol*. 2006; 290: L971–977.
201. Ohki-Hamazaki H, Neuromedin B. *Prog Neurobiol*. 2000; 62: 297–312.
202. Gajjar S, Patel BM. Neuromedin: An insight into its types, receptors and therapeutic opportunities. *Pharmacol Rep*. 2017; 69: 438–447.
203. Krane IM, Naylor SL, Helin-Davis D, Chin WW, Spindel ER. Molecular cloning of cDNAs encoding the human bombesin-like peptideneuromedin B. Chromosomal localization and comparison to cDNAs encoding its amphibian homolog ranatensin. *J Biol Chem*. 1988; 263: 13317–13323.
204. Wada E, Way J, Lebacqz-Verheyden AM, Battey JF, Neuromedin B. and gastrin-releasing peptide mRNAs are

- differentially distributed in the rat nervous system. *J Neurosci.* 1990; 10: 2917–2930.
205. Khumalo J, Kirstein F, Hadebe S, Brombacher F. IL-4R α signalling in CD4+CD25+FoxP3+ T regulatory cells restrain airway inflammation via limiting local tissue IL-33. *JCI Insight.* 2020; 5: 136206.
 206. Howard E, Lewis G, Galle-Treger L, Hurrell BP, Helou DG, et al. IL-10 production by ILC2s requires Blimp-1 and cMaf, modulates cellular metabolism and ameliorates airway hyperreactivity. *J Allergy Clin Immunol.* 2020; 6: S0091-674920;31234-3.
 207. Xiao Q, He J, Lei A, Xu H, Zhang L, et al. PPAR γ enhances ILC2 function during allergic airway inflammation via transcription regulation of ST2. *Mucosal Immunol.* 2020.
 208. Helou DG, Shafiei-Jahani P, Lo R, Howard E, Hurrell BP, et al. PD-1 pathway regulates ILC2 metabolism and PD-1 agonist treatment ameliorates airway hyperreactivity. *Nat Commun.* 2020; 11: 3998.
 209. Miller MM, Patel PS, Bao K, Danhorn T, O'Connor BP, et al. BATF acts as an essential regulator of IL-25-responsive migratory ILC2 cell fate and function. *Sci Immunol.* 2020; 5: eaay3994.
 210. Huang Y, Mao K, Chen X, Sun MA, Kawabe T, et al. S1P-dependent interorgan trafficking of group 2 innate lymphoid cells supports host defense. *Science.* 2018; 359: 114–119.
 211. Akama Y, Park EJ, Satoh-Takayama N, Gaowa A, Ito A, et al. Sepsis Induces Dereglulation of IL-13 Production and PD-1 Expression in Lung Group 2 Innate Lymphoid Cells. *Shock.* 2020.
 212. Xu H, Xu J, Xu L, Jin S, Turnquist HR, et al. Interleukin-33 contributes to ILC2 activation and early inflammation-associated lung injury during abdominal sepsis. *Immunol Cell Biol.* 2018; 96: 935–947.
 213. Wang W, Cohen JA, Wallrapp A, Trieu KG, Barrios J, et al. Age-Related Dopaminergic Innervation Augments T Helper 2-Type Allergic Inflammation in the Postnatal Lung. *Immunity.* 2019; 51: 1102–1118.e1107.
 214. O'Leary CE, Schneider C, Locksley RM. Tuft Cells- Systemically Dispersed Sensory Epithelia Integrating

- Immune and Neural Circuitry. *Annu Rev Immunol.* 2019; 37: 47–72.
215. Zhang K, Xu X, Pasha MA, Siebel CW, Costello A, et al. Cutting Edge: Notch Signaling Promotes the Plasticity of Group-2 Innate Lymphoid Cells. *J Immunol.* 2017; 198: 1798–1803.
216. Bernink JH, Ohne Y, Teunissen MBM, Wang J, Wu J, et al. c-Kit-positive ILC2s exhibit an ILC3-like signature that may contribute to IL-17-mediated pathologies. *Nat Immunol.* 2019; 20: 992–1003.
217. Silver JS, Kearley J, Copenhaver AM, Sanden C, Mori M, et al. Inflammatory triggers associated with exacerbations of COPD orchestrate plasticity of group 2 innate lymphoid cells in the lungs. *Nat Immunol.* 2016; 17: 626–635.
218. Wiegert JS, Mahn M, Prigge M, Printz Y, Yizhar O. Silencing Neurons: Tools, Applications, and Experimental Constraints. *Neuron.* 2017; 95: 504–529.
219. Fenno L, Yizhar O, Deisseroth K. The development and application of optogenetics. *Annu Rev Neurosci.* 2011; 34: 389–412.