Group 2 Innate Lymphoid Cells in Human Respiratory Disorders

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Abstract

Recent studies using animal models have generated profound insight into the functions of various subsets of innate lymphoid cells (ILCs). The group 2 ILC subset (ILC2) has been implicated in tissue homeostasis, defense responses against parasites, tissue repair, and immunopathology associated with type-2 immunity. In addition, progress has also been made in translating these findings from animal studies into a context of human immunity. Importantly, recent observations strongly support a role for ILC2s in several diseases of the human respiratory system. However, many aspects of human ILC2 biology are still unclear, including how these cells develop and which signals control their activity. As a result, the exact role played by ILCs in human health and disease remains poorly understood. Here, we summarize our current understanding of human ILC2 biology and focus on their potential involvement in various human respiratory disorders.

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Introduction

Innate lymphoid cells (ILCs) represent a relatively new family of lymphocytes that concentrate at barrier surfaces such as the skin, intestine, and lungs [1, 2]. Here, ILCs regulate tissue homeostasis by rapidly generating tailored responses to environmental signals. ILCs are involved in initiating, shaping and fine-tuning immune responses through the production of cytokines and other inflammatory mediators. Unlike lymphocytes of the adaptive immune system, ILCs do not express antigen-specific receptors, but they are instead activated by a wide range of signaling molecules produced by their microenvironment. Similar to T lymphocytes, ILCs are functionally diverse and can be grouped into cytotoxic and helper-like cells [2]. Cytotoxic ILCs consist of natural killer (NK) cells, whereas helper-like ILCs can be further subdivided into three subsets, i.e. group 1 ILCs (ILC1s), group 2 ILCs (ILC2s) and group 3 ILCs (ILC3s) [1, 2]. These subsets closely resemble T cell subsets in terms of the specific transcription factor (TF) network underlying their identity and the signature effector molecules they produce. Hence, NK cells, ILC1s, ILC2s and ILC3s can be viewed as innate counterparts of cytotoxic CD8⁺ T cells, T helper

(Th) 1, Th2 and Th17 cells, respectively. An innate equivalent of the regulatory T cell (Treg) was recently found in the intestine of both mice and humans, creating a fourth group of helper-like ILCs called regulatory ILCs (IL-Cregs) [3]. Analogous to T cells, a recent study showed that murine ILC2s can acquire memory-like characteristics for responding faster and more vigorously to unrelated secondary challenges [4]. The different groups of ILCs are divided based on their TF dependency and signature cytokine production [5]. NK cells depend on the TF eomesodermin (EOMES), secrete interferon-gamma (IFN- γ) and are involved in early protection against viral and bacterial infections as well as tumor immunosurveillance. ILC1s require T-bet expression (encoded by *TBX21*) and also produce IFN- γ to control intracellular viruses and bacteria. ILC2s are dependent on GATA3, a key activator of type-2 cytokine gene expression [6], and on retinoic acid receptor-related orphan nuclear receptor-alpha (ROR α). ILC2s mainly produce the signature type-2 cytokines interleukin (IL)-4, IL-5, IL-9, and IL-13 and play a crucial role in parasite expulsion and tissue repair. However, ILC2s have also been implicated in several diseases associated with aberrant type-2 immune responses (detailed below). The highly heterogeneous population of ILC3s relies on the TF ROR γ t (encoded by *RORC*) and includes lymphoid tissue inducer (LTi) cells and natural cytotoxicity receptor (NCR)-positive and NCR-negative ILC3s. ILC3 may produce IL-17 or IL-22 to promote epithelial immunity against extracellular pathogens and the LTi subset has the capacity to induce the formation of lymphoid organs during development.

This functional equivalency between T cells and ILCs has excited immunologists, as it questions the dominant role of the adaptive immune response in creating the specific local cytokine milieu typically seen during inflammation. Given their tissue localization and rapid activation kinetics, ILCs could act as first-responders with a potentially critical role as early cytokine producers. Since the formal discovery of helper-like ILCs in 2010, the field has rapidly expanded and made seminal discoveries concerning the development, differentiation, activation and biological functions of these innate immune cells. These studies have shown that ILCs can be essential players in various immunological processes, although the relative contributions of ILCs and T cells to the inflammatory cytokine milieu depend on the type of stimulus and the specific phase of the immune response (e.g. initiation, chronic phase) that was examined. Importantly, an increasing number of studies has implicated ILCs in various human (immune) disorders. We focus here on the biology of the

helper-like ILC2 subset and discuss the current state-of-the-art regarding their development, activation and potential role in human respiratory disorders – particularly in allergic asthma.

Development and Tissue Localization of Human ILC2s

The development of ILCs has been intensively studied in mice (for a comprehensive review, see Cherrier et al. [2]). Like other lymphocytes, ILCs are derived from multipotent hematopoietic stem cells (HSCs; Fig. 1, left part). HSCs first give rise to a common lymphoid progenitor (CLP) that in turn can differentiate into early T cell progenitors, early B cell precursors, and the earliest common ILC progenitors (referred to as CILPs) [7]. Murine CLP differentiation into CILPs is dependent on several TFs, including TCF1, NFIL3 and TOX [8]. CILPs give rise to a complex mixture of somewhat more restricted progenitor cells, including common helper ILC progenitors (CHILPs), PLZF⁺ ILC progenitors (ILCPs) and NK progenitors (NKPs). These progenitors rely on the TF ID2 and together give rise to NK cells, ILC1s, ILC2s, ILC3s and IL-Cregs. Finally, murine ID2⁺CHILPs and PLZF⁺ILCPs are thought to differentiate into mature ILC2s via a committed precursor (the ILC2 progenitor, or ILC2P) through the combined action of the TFs ROR α , GATA3, GFI1, ETS1 and BCL11B [2, 9–12].

In stark contrast, the development of human ILC2s from HSCs remains poorly understood (Fig. 1, right part). Human hematopoietic stem and progenitor cells – including CLP(-like) cells – reside within the CD34⁺ cell compartment [13]. Of note, most of the human CLP(-like) cells identified to date still possess some potential to differentiate into myeloid lineages, suggesting that an early and strict myeloid-lymphoid bifurcation, as documented in mice, might not exist in humans [14, 15]. Most studies into human ILC development have used CD34⁺ hematopoietic progenitor cells (HPCs) or CLP-like subfractions from various sources (e.g. bone marrow, fetal liver) to generate NK cells, ILC2s and ILC3s in vitro or in vivo after cell transfer into immunodeficient mice [reviewed by Juelke and Romagnani 15]. Initially, only unipotent human ILC precursor lineages were described, including an NK cell-restricted progenitor and a ROR γ t⁺ ILC3 progenitor [16, 17]. More recently, two studies reported the isolation of multipotent human ILCPs that were able to generate all cytotoxic and helper-like ILCs [18, 19]. While the CD34⁺ILCPs identified by Scoville et

CD5⁺ILC2s were unable to produce cytokines in vitro after stimulation, short-term culture and stimulation induced differentiation into CD5⁺CRTH2⁺ILC2s with the capacity to produce cytokines. Hence, a CD5⁺ human ILC2s might represent an ILC2 precursor analogous to the ILC2Ps described in mice [10, 11]. It is important to note here that peripheral blood CD5⁺CRTH2⁺ILC2s poorly respond to IL-25/IL-33 stimulation in vitro, indicating that circulating ILC2s also represent functionally immature or resting cells [21].

It remains unclear which signaling pathways and TFs promote human ILC2 differentiation from ILC precursors, although a strong resemblance with murine ILC2 development appears to exist. Human ILCPs express multiple TFs that have also been associated with murine ILC development, including *PLZF*, *ID2*, *GATA3*, *TOX* and *TCF7* (encoding TCF1) [18]. Furthermore, we recently generated genome-wide maps of active gene regulatory regions (marked by dimethylation of lysine 4 on histone H3 or “H3K4Me2”) in circulating human ILC2s from healthy individuals [22]. These analyses revealed chromatin regions with exceptionally high H3K4Me2 levels that represent super-enhancers (SEs). Across different cell types, genes associated with SEs are highly enriched for signature genes involved in shaping cell identity and function [23]. Human ILC2 SE-associated genes include *BCL11B*, *GATA3*, *RORA*, *ETS1*, and *TCF7*, each encoding TFs that are critical for ILC2 development in mice [22], again indicating that many parallels exist between human and murine ILC development. In mice, genetic inactivation of *Gata3* in hematopoietic precursors led to impaired generation of ILC2s [11, 24]. Furthermore, experiments using haploinsufficient and transgenic mice in which the *Gata3* gene copy number was increased revealed that ILC2 generation from CLPs is regulated by *Gata3* expression levels in a dose-dependent fashion [24]. Overexpression of *GATA3* in a human immature ILC population (Lin[−]CD127⁺CD117⁺NKp44[−]CRTH2[−]) provoked differentiation into ILC2s, while downregulation of *GATA3* impaired type-2 cytokine production by ILC2s [25]. Besides genes known from mouse studies, our epigenome analyses also implicated novel factors in human ILC2 development, including IRF1 and SMAD3 [22].

The cytokines used to induce differentiation of human ILC progenitors towards ILC2s – or any ILC subset – are different across studies. Common amongst these is the use of IL-2 and IL-7 [18–20], cytokines of broad importance for T cells and murine ILCs [2]. The proinflammatory cytokine IL-1 β was used to induce robust expansion

and differentiation of CD117⁺ILCPs to ILC2s and CD5⁺ILC2s to CD5⁺ILC2s [18, 20]. Together, these observations suggest that IL-2, IL-7, and IL-1 β signals are indispensable for ILCP to ILC2 differentiation. Notch signaling plays a crucial role in both murine T cell and ILC2 development [26, 27]. Differentiation of human thymic progenitors to either T cells or ILC2s in vitro seems to rely on Notch signal strength: a weak Notch signal induces T cell differentiation, while a strong Notch signal directs thymic progenitors towards the ILC2 fate [28]. Furthermore, single-cell RNA sequencing of ILC2s obtained from uninflamed human tonsil tissue support a role for Notch signaling in ILC2 function [29].

Besides in the peripheral blood and at barrier surfaces, ILC2s can also be detected in a wide range of tissues including cord blood, tonsils, liver, kidney, thymus and adipose tissue [28, 30–34]. ILC2s also reside in the lungs, where they are the dominant resident ILC subset at steady state [35]. Although their exact localization in the human lung remains largely unexplored, murine lung ILC2s reside in the vicinity of the epithelium, close to small conducting airways or in the alveolar space [reviewed in 36]. Upon challenge with cytokines, allergens or viruses, ILC2s accumulate in cellular infiltrates in the submucosa, near epithelial cells and T cells (but not near B cells) [37, 38]. Whether these ILC2s appear in such cellular foci due to local proliferation or through migration from more distal sites is currently unclear.

By surgically connecting the blood circulation of genetically distinguishable mice (CD45.1⁺ vs. CD45.2⁺), it was shown that during both steady-state and inflammatory conditions most of the tissue-resident ILCs were of host origin, even though complete chimerism was achieved in the blood and spleen (approximately 1 to 1 ratio of CD45.1⁺/CD45.2⁺) [39]. Therefore, ILCs are often considered as self-renewing tissue-resident cells. However, the extent to which ILCs are strictly tissue resident is currently under debate: Huang et al. [40] recently demonstrated migration of murine intestinal ILC2s to the lungs under inflammatory conditions using the same sphingosine 1-phosphate-mediated mechanism as described for T cells [40, 41]. The discovery of circulating ILCs in humans (mostly comprised of ILC2s and multipotent progenitors [18]) further underscores that ILCs are not strictly tissue resident and that active recruitment of circulating (immature) cells could contribute to tissue ILC numbers. Of note, nasal allergen challenge in allergic rhinitis (AR) patients increased peripheral blood ILC2 numbers already within 4 h, indicative of active ILC2 recruitment to the circulation [42]. Although mechanistic

Fig. 2. ILC2 interactions within the lung. ILC2s engage in extensive crosstalk with various other cell types in the lung, including immune cells, structural cells and neuronal cells. ILC2s integrate signals from their microenvironment by expressing a diverse set of activating and inhibitory receptors (see Fig. 3 for a detailed overview) while they communicate with other cells mainly via the production of IL-5, IL-13, IL-9 and amphiregulin (Areg), as well as through cell-to-cell interactions. See text for references and additional details.

cally unclear, this rapid increase in ILC2 numbers might involve prostaglandin D2 (PGD2), which can be induced within minutes after allergen challenge [43] and can activate ILC2s that highly express the PGD2 receptor CRTH2 (see below). Thus, while ILCs clearly exhibit a tissue-resident nature, they do appear to have the capacity to migrate under specific inflammatory conditions.

Cytokine Regulation of ILC2 Activity and Phenotypic Plasticity

Upon activation, ILC2s engage in the production of type-2 cytokines and other inflammatory mediators [44] (Fig. 2, 3). The “alarmin” cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) are at the core of regulating ILC2 activity. IL-25 is a member of the IL-17 family that binds and activates the IL-17RB receptor. IL-33 belongs to the IL-1 family and is a ligand for the ST2 receptor (encoded by *IL1RL1*). TSLP is an IL-2 family member that signals through the TSLPR/IL-7Ra receptor complex [45]. Although epithelial cells are the main pro-

ducers of IL-25, IL-33 and TSLP, other cells (e.g., endothelial cells, eosinophils, basophils, Th2 cells, mast cells, macrophages) also secrete these alarmins [44]. Based on the recent finding that epithelial chemosensory tuft cells are the main producers of IL-25 in the intestine and in the human sinonasal epithelium [46–48], it is conceivable that this is also the case in the lung epithelium. IL-2 derived from various sources is important for ILC2 survival and can also synergize with alarmins such as IL-33 to stimulate the production of type-2 cytokines. In addition to T cells and mast cells [49–51], ILC3 can be a potent source of IL-2 in pulmonary inflammation, based on findings in eosinophilic crystalline pneumonia in mice [52]. Hence, ILC2s engage in a dynamic crosstalk with many other cell types in tissues like the lung (Fig. 2).

In mice, two further subsets of ILC2s have been described: “natural ILC2s” (nILC2s) and “inflammatory ILC2s” (iILC2s) [53]. nILC2s were found in the lungs of naïve mice and express high levels of ST2 and low levels of IL-17RB. In contrast, iILC2s are not present in the lungs of naïve mice but accumulate upon treatment with IL-25. These cells do not express ST2 but strongly upreg-

costimulation resulted in increased transcription of *IL-17RB*, *IL1RL1* and *CRLF2*, which encode subunits of the IL-25, IL-33 and TSLP receptors, respectively. This indicates that IL-1 β , in combination with IL-2, acts as a potent priming signal upstream of IL-25, IL-33 and TSLP. Paradoxically, IL-1 β in combination with IL-12 induces up-regulation of *TBX21*, *STAT1* and *NFIL3*, as well as down-regulation of *RORA*, *CRTH2*, *GATA3* and *BCL11B* in human ILC2s, thus establishing an “ILC1-like” phenotype [21, 55–57] (Fig. 3). This ILC2 $\rightarrow$ ILC1 transition was marked by the simultaneous establishment of an active chromatin signature at both the *IFNG* and *IL5/IL13* loci [55]. Such “ex-ILC2s” started to abundantly produce IFN- γ instead of IL-5 and IL-13, demonstrating functional plasticity [55, 56]. Importantly, ILC2 $\rightarrow$ ILC1 plasticity is potentially clinically relevant given that this conversion seems to occur in the lungs of chronic obstructive pulmonary disease (COPD) patients [56, 57], as well as in the intestine of Crohn’s disease patients [21]. More generally, evidence is accumulating that the ILC2 phenotype is highly dynamic and heterogeneous, as the expression of activating (cytokine) receptors is determined by tissue-specific signals [22, 37, 58].

IL-4 and IL-9 possibly activate ILC2s in an autocrine fashion, as ILC2s secrete IL-4 and IL-9 but also express both the IL-4 receptor and IL-9 receptor [56, 59]. By itself, IL-4 does not induce proliferation or cytokine secretion in cultured ILC2. However, coculture with IL-33 leads to a synergistic increase in ILC2 proliferation and IL-5 and IL-13 production [56]. Because eosinophils – which can be induced by ILC2-derived IL-5 – are a major source of IL-4, crosstalk between ILC2 and eosinophils was proposed to amplify type-2 inflammation in chronic rhinosinusitis (CRS) [56]. IL-4 also suppresses the plasticity of ILC2s to ILC1s and is capable of restoring CRTH2 expression on “ex-ILC2s” [56]. In mice, autocrine IL-9 signaling increased IL-13 and IL-5 production by ILC2s and promoted ILC2 survival via an enhanced expression of the anti-apoptotic protein BCL3 [59, 60]. The TL1A cytokine, a ligand for the DR3 receptor that is secreted by macrophages and dendritic cells, was shown to induce IL-5 and IL-13 production in both murine and human ILC2s in vitro. Furthermore, the DR3 receptor is expressed on murine and human ILC2s and is required for the expansion, survival, and activation of ILC2s in several mouse models of allergic lung inflammation [61, 62].

Other cytokines are capable of suppressing ILC2 activity (Fig. 3). In mice, IL-10 and TGF- β secreted by Tregs were shown to suppress ILC2 activity [63]. A second study confirmed the inhibitory function of IL-10 and

TGF- β on human ILC2s [64]. IL-10 almost completely blocked production of IL-4, IL-5, IL-9 and IL-13 by ILC2s. TGF- β also inhibited IL-4, IL-5 and IL-13 production, but enhanced IL-9 production [64]. In contrast, epithelial cell-derived TGF- β specifically enhanced murine ILC2 activity during allergen-induced airway inflammation [65], indicating that TGF- β signaling can play a dual or context-specific role in regulating ILC2s. Interestingly, ILC2 activation by IL-33 in mice also induces IL-10 production [66], suggesting autocrine ILC2 suppression via IL-10 signaling. The Th1-associated cytokines IL-12 and IL-27 are also able to inhibit mouse and human ILC2 responses [21, 55, 67]. Moreover, ILC2s express type I and type II interferon receptors on their surface. Addition of type I (IFN- α and IFN- β) or type II (IFN- γ) interferons significantly impaired ILC2 activation and cytokine production in vitro and in vivo [68–70]. Of note, neither type I nor type II interferons alone induce ILC2 plasticity [68].

Control of ILC2 Activity by Lipid Mediators and Cell-to-Cell Interactions

Besides regulation by cytokines, ILC2s also respond to lipid mediators such as prostaglandins and cysteinyl leukotrienes (cysLTs; Fig. 3). Contrary to cytokine stimulation, stimulation with lipids causes robust IL-4 production by ILC2s [71]. One of the prostaglandins capable of activating ILC2s is PGD2. PGD2 is a ligand for the signature ILC2 surface marker CRTH2 and is mainly secreted by mast cells but also by eosinophils [72] (Fig. 2). PGD2 stimulation mediates human ILC2 chemotaxis, induces type-2 cytokine production (with a quantitatively similar strength as IL-25 and IL-33 combined) and the secretion of other cytokines, including IL-3, IL-8, IL-21, GM-CSF and CSF-1 [73]. Similar to IL-1 β and IL-2, exposure of ILC2s to PGD2 leads to upregulation of the IL-33 receptor, suggesting that PGD2 acts as a priming signal for subsequent alarmin-driven activation [73]. In contrast, PGI2 and PGE2 inhibit IL-5 and IL-13 production by ILC2s [74, 75]. PGE2 binds the E-type prostanoid receptors 2 and 4 present on ILC2s, evoking the downregulation of GATA3 and CD25 and decreased IL-5 and IL-13 production [74].

Human ILC2s also express leukotriene receptors CysLT₁ and CysLT₂ [76]. Addition of the cysLTs LTC₄, LTD₄ or LTE₄ to cultured ILC2s reduced apoptosis and promoted cell migration as well as cytokine production, including type-2 cytokines, amphiregulin (Areg), and proinflammatory cytokines such as IL-8, GM-CSF and

TNF α . Similar ILC2 responses were observed to endogenous cysLTs produced by activated human mast cells. ILC2s from atopic dermatitis patients express higher levels of CysLT₁ than ILC2s from healthy subjects. Furthermore, increased levels of LTE₄ were detected in sputa from patients with asthma, indicating a potential pathogenic role for cysLTs in the activation of ILC2s [77]. The natural proresolving mediator lipoxin A₄ (LXA₄) on the other hand, inhibited IL-13 production by ILC2s by binding to the ALX/FPR2 receptor [77].

Besides through soluble factors, ILC2s can also be regulated via signals received through cell-to-cell contact (Fig. 2, 3). The costimulatory molecule ICOS and its ligand ICOS-L are both expressed on ILC2s and this ICOS:ICOS-L interaction is important for ILC2 survival, IL-5/IL-13 production and required for allergen-induced airway hyperreactivity [78]. In contrast, coculture of ILC2s with induced Tregs resulted in decreased production of IL-5 and IL-13, which was dependent on ICOS:ICOS-L interaction [63]. Similar to ICOS:ICOS-L stimulation, signaling via GITR:GITR-L interaction is important for the proliferation, survival and function of both murine and human ILC2s. GITR-deficient mice displayed reduced airway inflammation upon allergen or IL-33 treatment [79]. In addition, OX40:OX40L costimulatory signaling might also be relevant for ILC2 activation. One study in mice showed that OX40L expression was strongly upregulated in ILC2s upon IL-33 stimulation, which was then used by ILC2s to promote T cell responses [80]. Although these authors did not find evidence for autocrine OX40:OX40-L stimulation of ILC2 activity, we found that both OX40 and OX40-L are expressed in lung ILC2s in mice treated with IL-33 [22]. Furthermore, IL-33/IL-25-stimulated human ILC2s displayed active chromatin around the *TNFRSF4* gene encoding OX40 [22], suggestive of active gene transcription. Additional studies will reveal whether autocrine OX40:OX40-L stimulation is relevant for ILC2 activation.

Suppression of ILC2 activity through cell-to-cell contact signaling can occur via KLRG1-E-cadherin ligation. E-cadherin is expressed on lung epithelial cells [81] and the binding of E-cadherin to KLRG1 on ILC2s results in downregulation of GATA3, decreased production of type-2 cytokines and reduced proliferation [82]. Both mouse and human ILC2s were shown to express the co-inhibitory receptor PD-1. Interaction of PD-1 with its ligand PD-L1 negatively regulated ILC2 activity, as blocking PD-1:PD-L1 signaling increased IL-13 production by ILC2s [83].

Neuronal and Metabolic Control of ILC2s

Recently, several research groups presented functional crosstalk between neuronal and immune cells at specific anatomical sites [84]. The first neuropeptide shown to modulate murine ILC2 activation was vasoactive intestinal peptide (VIP; Fig. 2, 3). VIP receptor type 1 (VPAC1) and type 2 (VPAC2) expression was detected in mouse intestinal and lung ILC2s, and in vivo treatment with a VPAC2 antagonist led to decreased IL-13 and ST2 expression by ILC2s [85, 86]. VIP is secreted by sensory neurons upon IL-5 stimulation, which in turn leads to IL-5 production by activated ILC2s (aILC2s), creating a positive feedback loop during allergic lung inflammation in mice [86]. Secondly, in 2017 three groups reported that the neuropeptide neuromedin U (NMU) produced by cholinergic neurons has the capacity to activate murine ILC2s [87–89]. Expression of NMUR1, a receptor of NMU, was found on murine lung and intestinal ILC2s, while NMUR1 expression could not be detected in other lymphoid or myeloid cells [87, 88]. In vitro (and to a lesser extent in vivo), exposure of ILC2s to NMU stimulated proliferation and IL-5/IL-13 cytokine production [87, 88]. Thirdly, calcitonin gene-related peptide (CGRP) secreted by pulmonary neuroendocrine cells (PNECs), located in close proximity to ILC2s, promotes ILC2 activation [90]. In combination with IL-33 or IL-25, CGRP enhanced IL-5 and IL-6 secretion by ILC2s. This PNEC-ILC2 signaling axis might be clinically relevant, as both PNEC and ILC2 numbers are increased in the lungs of asthmatic patients [90]. In contrast to the activating roles of NMU, VIP, and CGRP, signaling through the β_2 -adrenergic receptor (β_2 AR) has been implicated to negatively impact ILC2 activity [91]. Although β_2 AR-deficient mice did not exhibit altered steady-state ILC2 biology, these mice showed exaggerated ILC2 proliferation and cytokine production during type-2 inflammation. Furthermore, β_2 AR-agonist treatment reduced IL-5⁺ and IL-13⁺ ILC2 numbers and dampened the type-2 immune response. Although receptors for these neuropeptides have been detected on human peripheral blood and tissue ILC2s, the relevance of ILC2-neuronal crosstalk for human immunity remains to be elucidated. Future studies should reveal the importance of the ILC2-neuronal crosstalk as one of the neurogenic components involved in bronchial hyperreactivity in asthma.

Finally, metabolites and steroid hormones also modulate ILC2 activity (Fig. 3). More specifically, ILC2 activity is suppressed by the vitamin A metabolite retinoic acid [92], anti-inflammatory corticosteroids [93] and the male

sex hormone testosterone [94, 95]. Intriguingly, the latter phenomenon might contribute to the higher prevalence of asthma in women than in men. ILC2s and the cytokines they produce are also critical for maintaining metabolic homeostasis in adipose tissue, thereby preventing insulin resistance and limiting obesity [96–98].

Our recent epigenome profiling study of healthy human ILC2s included cells directly processed from peripheral blood (naïve ILC2s) or activated in vitro using IL-25 and IL-33 (activated ILC2s), allowing us to model human ILC2 activation and investigate its molecular underpinnings [22]. Upon activation of naïve ILC2s, various genes involved in lipid metabolism and (cytokine/chemokine-mediated) signaling pathways acquired an active chromatin status (H3K4Me2⁺) in activated ILC2, suggesting these genes were upregulated upon ILC2 activation. Besides known signal transduction routes (e.g., IL-2 signaling), these analyses also revealed pathways not previously linked to human ILC activation (e.g., PDGFR- β signaling) [22], providing a valuable resource to explore in future studies.

The Role of ILC2s in Human Immunity and Respiratory Diseases

Many studies in animal models have implicated ILC2s in type-2 immune responses: ILC2s are essential for clearing helminth infections [99] and play key roles in tissue repair after influenza infection [59] or during wound healing [100]. Additionally, ILC2s were shown to play a major role in the induction of allergic airway inflammation upon treatment with model allergens such as papain, the *Alternaria alternata* fungus, chitin and house dust mite [101–104]. Importantly, upon pulmonary exposure to the epithelial alarmins IL-33, IL-25 and TSLP, ILC2s rapidly produce IL-5 and IL-13 to induce eosinophilia – independently of T cells [103, 105–109]. Together, these data indicate that ILC2s might also be crucial for human tissue homeostasis and type-2 immunity. Indeed, recent studies implicated ILC2s in the pathophysiology of several human diseases, including a number of respiratory disorders [110].

Nevertheless, the importance of ILCs for general human homeostasis and immunity was recently questioned [111]. In this study, many children with severe combined immune deficiency that received hematopoietic cell transplantation showed an incomplete reconstitution or absence of ILCs and NK cells. Despite a lack of circulating ILCs, these patients did not seem more susceptible to dis-

ease, making the authors conclude that ILCs are dispensable for human immunity [111]. However, these patients received antibiotics and immunoglobulin replacement therapy to prevent infections and the status of ILCs in tissues was not assessed. Hence, additional studies are needed to reach general conclusions regarding the importance of ILCs in the control of human disease. Importantly, the possibility that ILCs are redundant for safeguarding human tissue homeostasis does not exclude a critical role for these cells in host defense against specific pathogens such as helminths or in human pathologies. This may be supported by the finding that circulating ILC2s are reduced in patients with common variable immunodeficiency [112]. Nevertheless, a more minor role for ILCs in everyday human health would make the ideal backdrop for strategies aimed at drugging ILCs without adverse effects to general human immunity.

AR and CRS

AR is a heterogeneous IgE-mediated reaction to an inhaled and generally innocent antigen, e.g. pollen and animal dander, leading to a type-2 inflammation of the nasal mucosa. Conversely, CRS is characterized by prolonged inflammation of the nose and paranasal sinuses. CRS can be caused by many factors and the precise mechanisms of inflammation remain poorly understood [113]. CRS endotypes can be subdivided into CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP), the latter displaying a type-2 inflammatory environment. Even though there is no clear causal link between AR and rhinosinusitis, a shared pathophysiological relationship has been proposed [114].

The increased presence of circulating ILC2s in patients with AR remains somewhat controversial. Bartemes et al. [115] showed that peripheral blood ILC2 numbers were not increased in AR patients, whereas Fan et al. [116] and Zhong et al. [117] reported a significant increase in peripheral blood ILC2 percentages in AR patients compared to healthy controls. The latter two studies also found a strong correlation between ILC2 percentages and IL-13 (but not IL-5) levels, and ILC2s from AR patients produced higher levels of type-2 cytokines upon stimulation compared to cells from healthy controls. Importantly, ILC2 numbers in peripheral blood of AR patients increased upon allergen exposure [42, 118]. This increase in peripheral blood ILC2s appeared to be antigen-specific, as ILC2 numbers were higher in house dust mite-sensitive AR patients than in mugwort-sensitive AR patients, implying that different antigens perhaps lead to immune responses with different ILC2 contributions [119]. To-

gether, these studies indicate increased ILC2 differentiation and activation in the blood circulation of AR patients.

CRSwNP patients exhibited higher ST2⁺ILC2 percentages in inflamed sinonasal mucosa as compared to CRSs-NP patients and healthy controls [120, 121]. Moreover, TSLP, IL-25 and IL-33 expression was increased in nasal polyps of CRSwNP patients, indicative of a tissue micro-environment that promotes ILC2 activation [122–124]. Indeed, compared to cells from the blood or noninflamed tissue, ILC2s from nasal polyps expressed higher levels of the activation marker ICOS, produced more IL-13 in response to IL-33 stimulation or even spontaneously secreted IL-5 and IL-13 in vitro [125]. Overall, these findings strongly suggest that ILC2s are key contributors to the inflammation seen in CRSwNP patients.

Chronic Obstructive Pulmonary Disease

COPD is a heterogeneous condition characterized by a progressive and irreversible loss of lung function, primarily caused by long-term exposure to cigarette smoke [126]. Some COPD patients suffer from regular acute exacerbations that are often triggered by respiratory tract infections, leading to worsening of symptoms. COPD is associated with a type-1 immune response, as reflected by increased IL-12 and IFN- γ expression in bronchial biopsies and bronchoalveolar lavage (BAL) samples [56]. While peripheral blood from COPD patients exhibits higher ILC1 frequencies [57], it appears that these ILC1s represent ex-ILC2s that have acquired an ILC1 phenotype due to exposure to high levels of IL-12 (Fig. 3) [56]. Consistent with this hypothesis, circulating ILC2 numbers were reduced in blood from COPD patients in a manner that correlated with increased ILC1 counts and disease severity [57]. As cigarette smoke exposure induces a cytokine environment (i.e., IL-1, IL-18 and IL-33) that augments ILC2 plasticity [57], it is conceivable that continuous ILC2 $\rightarrow$ ILC1 conversion is an important driver of COPD. In contrast, one study reported increased ILC2 percentages in peripheral blood from COPD patients [127].

Pulmonary or Cystic Fibrosis

Fibrosis in the lungs can arise when an insult to the epithelium leads to exaggerated scar formation and epithelial barrier disruption. Studies in mouse models have implicated the type-2 cytokine IL-13 as a profibrotic factor [128]. Indeed, patients suffering from idiopathic pulmonary fibrosis had increased levels of IL-13 in their BAL fluid [129]. Strikingly, IL-25 levels as well as ILC2 num-

bers were also elevated in the BAL fluid of these patients, suggesting ILC2s might represent a major source of IL-13 in pulmonary fibrosis [129]. ILC2s were also elevated in patients with systemic sclerosis, which correlated with the extent of skin fibrosis and the presence of interstitial lung disease, further supporting a profibrotic function for ILC2s [130].

Cystic fibrosis (CF) is a genetic disorder caused by a mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, leading to an overproduction of mucus in the lungs and frequent airway infections. In CF patients, levels of IL-33 are increased in BAL fluid, suggesting a possible increased activation of ILC2s [51, 131]. Infection with *Aspergillus fumigatus* in a mouse model for CF induced greater expansion of lung ILC2s as compared to wild-type mice, indeed suggesting increased ILC2 activation in CF lungs [51]. While these observations are intriguing, systematic studies of ILC2 prevalence and phenotype in the lungs of CF patients are required to confirm a possible contribution of ILC2s (or converted ex-ILC2s) to the local inflammatory environment.

Allergic Asthma

Asthma is a heterogeneous and chronic disease characterized by reversible airway obstruction, airway hyperresponsiveness and inflammation of the airways, causing symptoms such as coughing, wheezing and shortness of breath. Over 50% of asthma patients exhibit an allergic eosinophilic airway inflammation driven by an excessive type-2 immune response against inhaled allergens [132]. Over the past couple of years, several research groups have studied the role of ILC2s in the development and maintenance of asthma in humans (summarized in Table 1). Besides increased proportions or absolute numbers of ILC2s in both peripheral blood and lung material (sputum and/or BAL fluid) [133–135], ILC2s were shown to be more active and to produce more IL-5 and IL-13 in asthma patients [115, 127, 136, 137]. In this context, it is crucial that ILC2s are in close proximity to the epithelial barrier. This enables ILC2s to rapidly respond to airway allergens through the production of IL-13, which targets tight junctions and thereby disrupts bronchial epithelial barrier integrity in asthmatic patients [138] (Fig. 2). Women with severe asthma showed a more pronounced increase in circulating ILC2s as compared to male asthmatics, likely due to testosterone-mediated ILC2 suppression [95]. In one cohort of patients with severe asthma, ILC2s even represented the dominant producers of the type-2 cyto-

Table 1. Summary of key studies addressing the role of human ILC2s in asthma

Study population	Tissues	Goal of the study	Key findings	Ref.
Allergic asthma and allergic rhinitis patients	Peripheral blood	To examine whether ILC2s are involved in allergic diseases	Increased ILC2 numbers and responsiveness to IL-25 and IL-33 in allergic asthma patients	115
Asthma patients	BAL fluid	To investigate the role of ILC2s in the persistence of asthma	Increased ILC2 numbers and IL-33 levels in asthma patients, which positively correlated with disease severity	140
Patients with mild to moderate asthma	Peripheral blood and sputum	Identify biomarkers to predict eosinophilic airway inflammation in asthma patients	ILC2 counts positively correlated with eosinophilic inflammation in asthma patients	133
Severe eosinophilic asthma and steroid-naïve mild atopic asthma patients	Peripheral blood and sputum	To investigate the role of ILC2s in chronic airway eosinophilia	Increased numbers and cytokine production by ILC2s in blood and sputum of patients with severe asthma compared to mild asthma	136
Children with severe therapy-resistant asthma	Peripheral blood, BAL fluid and sputum	To determine the presence of ILC2s in the airways of children with severe asthma	Increased ILC2 numbers in BAL fluid and sputum of children with asthma	134
Asthma patients subdivided into controlled, partially controlled and uncontrolled disease status	Peripheral blood	To analyse clinical manifestations of ILC2s in a diverse group of asthma patients	IL-13 ⁺ ILC2 numbers were increased in patients with uncontrolled and partially controlled asthma; IL-13 ⁺ ILC2 decreased after treatment	137
Allergic asthma patients	Peripheral blood and BAL fluid	To investigate the mechanisms of steroid resistance	TSLP induces steroid resistance in ILC2s, elevated TSLP levels in BAL fluid correlated with steroid resistance	141
Patients with mild and stable asthma	Peripheral blood, sputum and bone marrow	To study the role of ILC2s in allergen-induced eosinophilic airway inflammation	Increased ILC2 numbers and activation in sputum and decreased circulating ILC2 numbers 24 h after allergen challenge	139
Asthma patients and patients with both asthma and allergic rhinitis	Peripheral blood	To explore the relationship between ILC2s and clinical characteristics of asthma	Increased ILC2 numbers in both patient groups, but no correlation with lung function	135
Patients with moderate to severe asthma	Peripheral blood	To determine differences in ILC2 prevalence between the two sexes	Increased ILC2 numbers and cytokine production in asthma patients, with strongest increases seen in asthmatic women	95

kines that define the type-2 inflammatory environment [136]. In addition to higher ILC2 numbers in sputum of asthmatic patients during steady-state asthma conditions, absolute numbers of IL-5⁺ and IL13⁺ ILC2s were further increased in vivo 24 h – but no longer at 48 h – after allergen challenge. Instead, absolute numbers of ILC2s in peripheral blood decreased 24 h after challenge, suggesting active recruitment of circulating blood ILC2s to the airways [139]. Cytokines known to activate ILC2s, such as IL-33 and TSLP, are increased in BAL fluid of asthmatic patients, resulting in enhanced ILC2 activity [140, 141]. Besides ILC2 activation, TSLP also induces corticosteroid therapy resistance in ILC2s and not in Th2 cells, suggesting that ILC2s might mediate the development of corticosteroid resistance in asthma patients [141]. It is also important to note that several stud-

ies did not observe significantly increased ILC2 numbers in peripheral blood of asthma patients or strong correlations between ILC2 prevalence and disease severity [77, 134, 137]. In the context of obesity-linked asthma, ILC2s may actually play both beneficial and detrimental roles: while potent ILC2 activity in adipose tissue represents a possible protective mechanism by suppressing obesity [97, 98, 142], aberrant ILC2 activation in the lung might contribute to triggering airway inflammation in asthma. Of note, obesity-linked airway hyperactivity in mice was shown to be facilitated by IL-17-producing ILC3s rather than ILC2s [143].

Recently, Kotas and Locksley [144] proposed a thought-provoking model in which ILCs represent the dominant tissue effector lymphocytes during fetal development and childhood, which are then progressively re-

placed as we age by adaptive lymphocytes such as tissue-resident T cells. They furthermore argue that an incorrect establishment of the innate lymphoid niche in tissues at the early stages of life, for example an imbalance in the ILC composition of the lung lymphoid niche, may increase susceptibility to childhood asthma later in life [144]. In support of this hypothesis, asthma often develops early in human life and neonatal mice appear particularly susceptible to allergic sensitization due to hyperactivity of the IL-33/ILC2 axis in the developing lungs [145].

Only few studies have explored a possible role of ILC2s in virus-induced asthma exacerbations. Although it is largely unknown how classic type-1 inflammatory triggers, such as respiratory infection with rhinovirus or influenza virus, induce asthma exacerbation, IL-33 and TSLP are thought to be key mechanistic links. It was recently shown that these cytokines can be strongly induced by rhinovirus infection of airway epithelium, both in vivo in mice and in vitro in human bronchial epithelial cell cultures [146, 147]. Thus, rhinovirus has the capacity to induce ILC2 activation and type-2 cytokine production as a consequence.

Large genome-wide association studies have linked hundreds of genetics variants and numerous genes to asthma susceptibility and pathophysiology [148]. Several of these genes, such as *IL33*, *IL1RL1* (encoding the IL-33 receptor), *RORA*, and *IL5-IL13*, have obvious links with ILC2 biology. However, the underlying mechanisms and the cell types involved remain poorly understood. Our group has recently shown that there is a strong link between the epigenetic landscape (or “epigenome”) in human ILC2s and the genetic basis of asthma [22]. Most asthma-associated genes resided near H3K4Me2⁺ active gene regulatory regions in ILC2 and a total of 308 asthma-associated single nucleotide polymorphisms from genome-wide association studies localized to these H3K4Me2⁺ active gene regulatory elements in ILC2s. These findings suggest that genetic variation linked to asthma pathophysiology could disturb gene regulatory processes in ILC2s. While the epigenome of Th2 cells was also linked to asthma genetics, substantial cell type-specific differences existed between the variants that associate with either ILC2s or Th2 cells [22]. Future studies will have to uncover how other relevant cell types (e.g. mast cells, dendritic cells, epithelial cells) connect to asthma disease genetics and how these genetic variants impact gene expression programs, alter cell identity and deregulate type-2 immunity.

Outlook

Despite impressive progress in understanding ILC biology, many aspects of how ILCs fit in the bigger picture of human immunity under normal and pathological conditions remain controversial or unanswered. For example, we still have a limited understanding of how human ILC2s develop, what mechanisms control their activity in vivo and whether ILC2s represent drivers or bystanders of the various diseases they have recently been associated with. In-depth characterization of ILC2s, including their progenitors, in various human tissues and disease settings is likely to provide answers to at least some of these questions in the near future. Particularly intriguing aspects of ILC2 biology are their adaptability to different tissue microenvironments, as is reflected by the impressive diversity of signals they can interpret (Fig. 2, 3) and their striking plasticity – features that are likely to be relevant for the role of ILC2s in many human respiratory diseases. Along those lines, studying ILC2 steady-state heterogeneity (e.g. using multidimensional single-cell profiling approaches) and how this evolves under inflammatory conditions might reveal surprising new aspects of ILC2 function. Therapeutic targeting of type-2 immunity to treat allergic airway disease has yielded some promising results (e.g. antibodies targeting type-2 cytokines, IL-4R signaling or TSLP [149]), although overall success has been modest. A need for improved ways to therapeutically control type-2 immune responses therefore continues to exist. Importantly, future studies should aim at further unravelling the unique aspects of ILC2 cell identity in comparison to their adaptive Th2 counterparts, which will be especially relevant for the development of therapeutic strategies to specifically target ILC2s in human disease.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

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