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Pulmonary Group 2 Innate Lymphoid Cells: Surprises and Challenges

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SUMMARY

Group 2 innate lymphoid cells (ILC2s) are a recently described subset of innate lymphocytes with important immune and homeostatic functions at multiple tissue sites, especially the lung. These cells expand locally after birth and during postnatal lung maturation and are resident in the lung and other peripheral organs. They are modified by a variety of processes and mediate inflammatory responses to respiratory pathogens, inhaled allergens and noxious particles. Here, we review the emerging roles of ILC2s in pulmonary homeostasis and discuss recent and surprising advances in our understanding of how circadian rhythms, hormones, age, neurotransmitters, environmental challenges, and infection influence ILC2s. We also review how these responses may underpin the development, progression and severity of pulmonary inflammation and chronic lung diseases and highlight some of the remaining challenges for ILC2 biology.

INTRODUCTION

Innate lymphoid cells (ILCs) are a heterogeneous family of cells that include group 1 ILCs (ILC1) characterized by their production of interferon-γ (IFN-γ), ILC2s that predominantly express IL-5 and IL-13, and ILC3s that secrete IL-22 and/or IL-17 [1-3]. ILCs are important in maintaining tissue homeostasis by regulating lymphoid tissue development (ILC3 [4]), tissue repair (ILC2 [5] and (ILC3 [6, 7]) fat metabolism (ILC2 [8]). Collectively, they protect the body against a multitude of organisms including intracellular pathogens (ILC1), bacteria (ILC1 and ILC3) [9, 10 11], parasitic worms (ILC2) and fungi (ILC3) [12]. Nevertheless, when dysregulated they can drive chronic inflammation such as occurs in chronic obstructive pulmonary disease (COPD) (ILC1 and ILC3), allergy and asthma (ILC2), and cancer (ILC3) as well as autoimmune diseases including inflammatory bowel disease (ILC1, ILC2, ILC3), atopic dermatitis (ILC2), multiple sclerosis (ILC2 and ILC3) and psoriasis (ILC3) [13-16]. Here, we

review recent advances in our understanding of the contribution of ILC2s in inflammation and immunity, with a particular focus on the lung, and the challenges in understanding their key roles in maintaining immune homeostasis and the implications for respiratory diseases and therapeutic intervention. We place this in the context of recent and surprising findings that have been enabled by the development of a wide range of molecular tools (**Table 1**) and ILC modulators (**Table 2**) and highlight some of the remaining challenges in ILC2 biology.

Transcriptional Blueprint Regulating ILC2 Development

ILC2s are not a uniform population and there are inconsistencies in the markers they express. Nevertheless, ILC2s are delineated into at least two subsets: natural ILC2 (nILC2) that respond mainly to IL-33, and; inflammatory ILC2 (iILC2) that are highly responsive to IL-25 [17] and have the capacity to migrate between mucosal sites during inflammation in response to chemotactic signals [18]. nILC2s are generally recognized by their expression of the IL-33 receptor (IL-33R, also known as ST2). iILC2s express the activation marker KLRG1 and the IL-25R but strikingly, do not express ST2 which raises questions about the universal use of this receptor to mark ILC2s [19]. It is proposed that iILC2s are highly responsive precursors that are mobilized by inflammatory stimuli but ultimately adopt an nILC2-like, or ILC3-like phenotype [17].

ILC2s are characterized by their expression of Gata binding protein-3 (Gata3) and their production of cytokines including IL-4, IL-5, IL-9 and IL-13. They arise from the common lymphoid progenitor (CLP) in the bone marrow which give rise to the more restricted ILC2 lineage-specific progenitor, ILC2p. The development of this progenitor relies on Gata3 [20, 21] and the transcription factor retinoic acid receptor-related orphan nuclear receptor-α (RORα encoded by *Rora* gene) [22]. A tight transcriptional network involving factors such as inhibitor of DNA binding-2 (Id2), Notch [23], nuclear factor interleukin-3 (Nfil3) [24, 25], promyelocytic

leukemia zinc finger protein (PLZF, encoded by Btb16), T-cell factor-1 (TCF-1) [26, 27], and zinc finger protein growth factor independent-1 (Gfi1) [28] are essential for the sequential specification and commitment of the ILC2 lineage. Indeed, TCF-1 acts through both Gata3-dependent and -independent pathways to promote the generation of ILC2s [27] but precisely how these two factors orchestrate the ILC2 programming is incompletely understood.

In adults, mature ILC2s are thought to originate from bone marrow progenitors and IL-33 promotes their egress [29-31]. However, under certain circumstances, ILCs may also arise in the thymus where the levels of expression of transcription factors dictate the fate outcome of early T-cell progenitors to become either adaptive or innate immune cells [29, 32]. At least part of this program may be regulated by the transcriptional enhancer E-box proteins (E proteins) and Id proteins which modulate ILC2 levels. Indeed, overexpression of Id1 or the dual deletion of E2A and HEB results in hyper-inflammatory ILC2 responses following allergen challenge with papain and enhanced capacity to eliminate *N. brasiliensis* [29]. Id1 itself is not generally expressed by immune cells but overexpression of Id proteins, or removal of their E protein binding partner, enhances Id activity and drives the development of cells that depend on it. ILC2 production can be generated in the thymus by culture with IL-7 and IL-33 [22, 23] suggesting that modulating the balance of transcription factors may determine an ILC fate outcome *in vivo* [29, 32].

Single Cell ILC-omics Uncovers the Identity of the ILC2p

ILCs exhibit considerable heterogeneity in terms of cell surface antigen and gene expression. This reflects differential responses that occur to continuous encounters with fluctuating stimuli at mucosal surfaces such as the lung and specializations in individual tissues. The capacity to trace ILC fate has been limited by the relative rarity of these cells, however, significant

advances in genomics have enabled the detailed temporal mapping of the dynamic and quantitative contributions of transcriptional regulators in defining cellular trajectory.

Defining the earliest ILC progenitors has been elusive in both mouse models and humans. However, recent studies of small cell numbers and single cell analyses have uncovered new markers of early checkpoints in ILC development. Analysis of the common innate lymphoid progenitor (CILP) revealed that the dedicated ILC2 progenitor unexpectedly expressed the surface receptor programmed cell death protein 1 (PD-1), which in combination with IL-25R serves as a hallmark for this progenitor [33, 34]. Notably, the ablation of PD-1 in progenitors did not impact the development of ILC2p or ILC2s [33]. PD-1 is a major target in immunotherapy and is an important negative regulator of effector gut KLRG1$^+$ ILC2s (also known as iILC2) [17, 35]. The emergence of KLRG1$^+$ ILC2s in the lung also appears to rely on PD-1. We speculate that differential regulation in distinct tissues could account for the higher abundance of KLRG1$^+$ ILC2s in the lung that are associated with inflammation and asthma [35]. ILC2s also dynamically express the ligand PD-L1 during immune responses in the lung, and its ligation with PD-1 on Th2 cells acts as innate regulatory checkpoint for the adaptive response leading to Gata3 and IL-13 upregulation by T-cells [36]. Inhibiting PD-L1 or IL-13 during early-life bacterial infection of the respiratory system prevents more severe allergic airway inflammation in later-life [37, 38]. However, blockade of PD-L1 during the first two weeks postpartum in the absence of pathogenic infection maintained exaggerated responsiveness to HDM challenge of mice in early life [39]. The expression of these two key checkpoint factors on ILC2s warrants a deeper dissection of the consequences of therapy as although this may augment anti-tumor immunity, autoimmune sequelae are potential unexpected outcomes of treatment.

Genome-wide probing of ILCs using RNA-seq, ChIP-seq and ATAC-seq combined with mass cytometry enables multivariate mapping of transcriptional and epigenetic identity of

thousands of gene profiles and regulatory elements in single cells with functional outputs such as cytokine expression and metabolic regulation. This has allowed the detailed exploration of regulatory circuits in mammalian cells [40-46]. This type of detail challenged the simplified schema for ILCs previously proposed [3] and suggests that there may be as many 15 different subcategories reflecting differences in tissue localization and the influence of various stimuli, including commensal bacteria, in shaping the epigenetic landscape of ILCs. Some subsets, however, exhibit characteristics typically associated with other or multiple subtypes. ILC2s are typified by their expression of *Gata3, Hes1, Areg, Il5* and *Il13* transcripts, a subclass of ILC2, ILC1/2, also express *Gzma, Hopx* and *Epas1* normally associated with ILC1, while ILC2/3 produce *Cxcl2, Cxcl3* and *Arg1* characteristic of ILC3 [40]. In all, four different subclasses of ILC2s were reportedly delineated. While these subsets all exhibited the signature gene *Gata3*, segregation into subsets was based on their graded expression of *Gata3* and also *Klf4, Llrg1, Ly6a* and *Il2ra*. The ILC2d subclass exhibited the highest levels of these genes as well as *Il5* and *Csf2* and was distinguished from other subsets by their expression of *Areg*. These subclasses may result from heterogeneity introduced as a consequence of cues from their environment. This pattern was similar to that observed for T-bet suggesting that ILC subsets are not static, but their phenotypes reflect the dynamic equilibrium that occurs in implementing immune responses and maintaining homeostasis. The existence of these subclasses that do not neatly fit into the original proposed schema [3] and imply that the plasticity reported by other groups in *in vitro* analyses are insights into such changes that can be induced in ILCs. Whilst this structure should be maintained it is a simplification of the numbers of subclasses that are encountered at different locations and during homeostasis or challenge [47]. A detailed understanding of this plasticity *in vivo* is warranted.

A comprehensive model describing how human ILCs develop, similar to that already undertaken in mice, has until recently been lacking, mainly as a result of technical limitations

in probing rare immune populations. Recent advances in genomics and mass cytometry have, however, enabled the elucidation of these developmental steps in man [45, 48, 49]. A striking initial finding identified that the transcriptional regulator RORγt (encoded by *RORC2* in man) is not limited to ILC3s as found in mice but is broadly expressed in human ILC progenitors including those of ILC2 [50]. $Lin^{-}CD34^{+}CD45RA^{+}CD117^{+}IL\text{-}1R1^{+}ID2^{+}ROR\gamma t^{+}$ progenitors were found in secondary lymphoid tissues and gave rise to all ILC subsets, but not other cell lineages [50]. This raised the concept that differentiation could differ between the two species and that RORγt may play a potential role in humans distinct from that in mice. Aligned with this finding, although signature factors such as *GATA3* were strongly associated with and most highly expressed in human ILC2s, this transcription factor was also expressed by other subsets such as ILC1s and ILC3s [49]. Consequently, it appears that the G protein-coupled receptor CRTH2 (also known as DP_2) is the most reliable marker for separating human ILC2s from ILC3s. This is also the case in the mouse where ILC1s and 3s are intermediate for *Gata3* and ILC2s have high expression.

Emerging Anti-inflammatory Role for ILC2s

ILC2s are well known inducers of type-2 inflammatory responses, but recent evidence indicates that they are also involved in the resolution of inflammation and prevention of cell death through IL-9 [51, 52]. ILC2s are the dominant source of IL-9 during the resolution of arthritis and $IL\text{-}9^{+}$ ILC2s were located in close proximity to regulatory T (Treg) cells in inflamed synovium [51]. In the absence of IL-9, ILC2-induced proliferation and activation of Tregs was impaired leading to chronic arthritis. In contrast, treatment with rIL-9 promoted ILC2-dependent Treg activation and effectively resolved inflammation [51]. A more recent study demonstrated that artificially increasing ILC2s significantly attenuated experimental arthritis

in an IL-4/13-dependent manner [53]. Clinical relevance was demonstrated in remitting rheumatoid arthritis patients who had high numbers of ILC2s and IL-9$^+$ ILC2s in their joints and circulation that inversely correlated with disease severity [51, 53]. In a mouse cecal and ligation puncture model, sepsis induced IL-33 release and subsequent expansion of IL-9-secreting ILC2 in the lung, which prevented lung endothelial cell pyroptosis by attenuating caspase-1 activation [52]. Collectively, these data indicate a pivotal role for ILC2s in resolving chronic inflammation, and the potential for their therapeutic manipulation [51]. Further exploration of their anti-inflammatory capacity in other tissues and diseases is required.

Bidirectional Relationships between ILC2s and Tregs

A complex bidirectional relationship exists between pulmonary ILC2s and Tregs. Transient depletion of Tregs induced IL-2– and CD25-dependent proliferation of ILC2s, suggesting that ILC2s can directly access IL-2 in the lung and that Tregs restrain the IL-2–dependent expansion of these tissue-resident ILCs [54]. Human and murine ILC2s express both ICOS and ICOS-ligand (ICOS-L) and ICOS:ICOS-L interactions on these cells promotes type-2 cytokine production and ILC2 survival through STAT5 signaling [55, 56]. A lack of ICOS on murine ILC2s reduced airway hyperresponsiveness (AHR) and lung inflammation, and blocking ICOS:ICOS-L interactions in human ILC2s suppressed their pro-inflammatory effects [55]. Induced Tregs (iTreg) but not natural Tregs, inhibited the production of ILC2-drived IL-5 and IL-13 *in vitro* and *in vivo* [57]. iTreg mediated suppression of ILC2s required ICOS-ICOS-L-dependent cell-to-cell contact in addition to the suppressive cytokines TGF-β and IL-10 [57]. Human iTreg can suppress human ILC2s through ICOSL to control airway inflammation in a humanized ILC2 mouse model [57]. Furthermore, human ILC2s express IL-10RA and TGFBR2 receptors and the addition of rIL-10 or TGF-β to *ex vivo* ILC2 cultures suppressed type-2 cytokine production

[58]. In contrast, TGF-β is also a chemoattrant for ILC2s that express TGFBR2 that contributes to pulmonary responses to allergen.

Other cytokines such as IFN-γ can suppress IL-33-induced ILC2 activation and control Treg numbers and type-2 responses [56]. ILC2s express the IFN-γ receptor [42] and IFN-γ directly represses ILC2 activation, cytokine production and proliferation *in vitro* [56]. *In vivo* IL-33-induced lung ILC2 proliferation and accumulation was blocked by co-administration of IFN-γ and mice that overexpress IFN-γ had fewer ILC2s. *Listeria monocytogenes* infection, which elicits potent IFN-γ responses, also suppressed ILC2 function in an IFN-γ-dependent fashion [56]. OX40L expression by ILC2s is also required for IL-33-driven Treg and Th2 cell expansion [59]. Collectively, these studies highlight a multifaceted interplay between ILC2s and Tregs and the factors that regulate their function.

New Influencers of ILC2 Function

The immune system is subject to both environmental and intrinsic influences. Recent advances highlight the importance of circadian rhythm, sex hormones, age and neurotransmitters in regulating ILC2 function, especially in the lung.

Circadian Rhythm

The circadian clock has emerged as an important factor influencing the efficiency of immune response generation [60]. Its daily oscillations are mediated principally *via* the suprachiasmatic nucleus which entrains peripheral body clocks [61, 62]. In mammals, this

clock depends on highly conserved transcriptional regulators such as CLOCK, BMAL1 and REV-ERBα nuclear proteins that bind to E-box sequences. The circadian clock involves many other genes such as Id2 and the repressor nuclear factor interleukin-3 (NFIL3, also known as E4BP4), which are also associated with the immune system and influence the core and secondary feedback loops involving Rev-ERBα. Rorα, a critical transcription factor for ILC2 development [22], is an activator of *Bmal1* transcription within the suprachiasmatic nucleus [63]. It is required for normal BMAL1 expression and consolidation of daily locomotor activity and is regulated by the core clock in the suprachiasmatic nucleus. This suggests that opposing activities of Rorα and REV-ERBα, are important in the maintenance of circadian clock function [63]. NFIL3 is required for the development of the ILC progenitor [24, 25], which may be independent of the cellular clock, however, it also regulates Th17 cells by linking their development to the circadian network though REV-ERBα [64]. REV-ERBα represses proinflammatory cytokine production and controls the amplitude of pulmonary inflammation to inhaled endotoxin [65, 66]. Similarly, BMAL1 regulates the diurnal rhythms of inflammatory monocytes facilitating their mobilization in immune defense [67]. This effect is at least partially mediated through inhibiting NF-κB activation and the induction of miR-155 [68]. miR-155 deletion ablates the circadian rhythmicity of cytokine production and leads to increased susceptibility to lipopolysaccharide-induced sepsis. miRNAs are key regulators of ILC2-induced allergic inflammation and miR-155 is increased in ILC2s in response to stimulation with IL-33. Moreover miR-155$^{-/-}$ mice have reduced IL-33-induced ILC2 proliferation and cytokine production [69, 70]. Tissue ILC2s regulate

eosinophilopoiesis and accumulation in tissues through constitutive cytokine production, particularly IL-5. They also express the vasoactive intestinal peptide receptor type-2 (VPAC2) which responds cyclically and potentially provides a mechanism by which ILC2 are directly linked to circadian and metabolic rhythms and maintain eosinophil homeostasis [71, 72]. Collectively, multiple facets of the immune system are influenced by the circadian clock and factors critical for the development and maintenance of ILCs are implicated in secondary regulation of circadian oscillations. Further experimental confirmation of these observations is warranted.

Sex Hormones

The prevalence of certain diseases may differ in incidence and severity between males and females. Men are more susceptible than women to developing severe asthma in childhood, however, following puberty, this trend reverses with females having the highest incidence of allergies in adulthood [73, 74], which correlate with higher circulating ILC2 numbers [75]. The mechanisms underlying these differing susceptibilities has been elusive but the patterns of pathogenesis implicate sex hormones. Several immune cell lineages, including myeloid cells and lymphocytes express receptors for estrogen, progesterone and androgens (testosterone, dihydrotestosterone and androstenedione) and are hormone regulated and influence both innate and adaptive immune responses [76]. In macrophages, estrogen receptor signaling inhibits the production of NF-κB-regulated proinflammatory genes such as IL-6 while the activation of ERα46 impairs leukocyte migration by inhibiting CCL2 expression [77, 78].

Similarly, in dendritic cells (DCs), estrogen-dependent activation of ERα regulates the development and/or functional responses of particular subsets [79, 80]. Progesterone also has both stimulatory and suppressive roles in immunity. Progesterone receptors are expressed principally by T-cells, including Tregs and NK cells, in addition to DCs and mesenchymal stem cells [81-83]. In NK cells, progesterone downregulates the secretion of IFN-γ, dampening down uterine NK cell function [84]. The active metabolite of testosterone is dihydrotestosterone which irreversibly binds to the androgen receptor (AR) [85]. The AR is expressed at various levels by a variety of leukocytes such as neutrophils and macrophages [86]. Most recently, the AR was identified on ILC2s and signaling through this receptor reduced the susceptibility to IL-33- and alternia extract-driven lung inflammation in part by reducing the expansion and reactivity of ILC2 [75, 87]. Signalling through the AR pathway provides support for the protective role of androgens in allergic asthma and the dimorphic switch that occurs after puberty. The lungs of female mice harbor significantly greater numbers of ILC2s during homeostasis, mostly due to the presence of KLRG1$^-$ ILC2s that are largely absent in male lungs [88]. These KLRG1$^-$ ILC2s are capable of producing type-2 cytokines and increased with age and sexual maturity, suggesting the existence of a unique functional ILC2 subset in females. The frequency of PLZF$^+$ ILC precursors were higher in males and further increased by androgens, suggesting that male sex hormones inhibit the conversion of ILC precursors to ILC2s. However, these sex dependent effects appear to be specific for tissue location and disease context. In contrast to the lung, adult male mice have increased ILC2 numbers driven by mast cell-derived IL-33 in the central nervous system (CNS), which is an immune-protective mechanism and may contribute to the sexual dimorphism

observed in the demyelinating disease multiple sclerosis to which females are more susceptible [89, 90]. IL-33 responsive ILC2 are also present in the uterus and regulated by estrogen, however, lung ILC2s were not altered by estrogen administration or in ovariectomized mice [91].

Age

The first breath and resulting inflation of the lung triggers IL-33 production by type II alveolar epithelial cells and induces the expansion of ST2$^+$ ILC2s in newborn mice [92, 93]. Consequently, few ST2$^+$ ILC2s are present in the lungs embryonically and in newborn mice, but numbers are markedly increased by postnatal week 1, peak during postnatal week 2 to levels three times that of adult mice and then decrease and stabilize by week 6 [72, 93-95]. However, the mechanisms responsible for ILC2 contraction are incompletely understood [94]. Interestingly, neonatal mice had fewer ILC2s in their liver and small intestine compared to adults, suggesting that the functional relevance of increased ILC2s in early-life is likely limited to the lung [93]. Importantly, a significant proportion of neonatal lung and draining mediastinal lymph node ILC2s co-expressed intracellular IL-5 and IL-13 and had increased proliferative capacity compared to adult ILC2s *in vitro* [93]. This is in contrast to adult lung ILC2s that appear to be only constitutive IL-5 producers, with these IL-5$^+$ ILC2s found embedded in collagen-rich regions near the confluence of medium-sized blood vessels and airways but absent from the alveolar structures [2, 72]. However, the precise location of ILC2s in the developing neonatal lung is unknown, and they may reside in the alveolar compartment. Furthermore, neonatal lung ILC2s have different cell surface antigen expression with less CD90.2 and CD25 than adult lung ILC2s, but more ST2 and comparable intracellular GATA3 levels [95]. Collectively, these data indicate phenotypic and functional differences between neonatal and adult ILC2s in the lung, suggesting that their impact in lung development is not just related to ILC2s numbers.

Early-life Th2-associated immune skewing and susceptibility to allergy are often considered remnants of feto-maternal symbiosis [94]. Indeed, neonates are prone to sensitization by allergens and Th2 cell-driven allergic disease as their immune system is Th2-biased [96, 97]. The IL-33-dependent expansion of ILC2s during the neonatal period may be important in the reportedly exaggerated HDM-induced AHR in newborns [39]. During the alveolarization phase of postnatal lung development, HDM exposure further increased IL-33, which increased cytokine production by ILC2s and activation of $CD11b^+$ DCs driving greater Th2 skewing [94]. These data indicate that the alveolarization period has type-2 dominant immunity with exaggerated innate immune responses to allergens. This may promote Th2-skewed immune responses that may explain increased asthma prevalence in childhood.

The structural features of postnatal lung development which consists mainly of the formation and remodeling of alveoli are similar between humans and mice, despite obvious timeframe differences. The most active phase of alveolar development occurs during the second postnatal week in mice and between years 2-3 in humans. The discovery of substantially increased IL-33 production and accumulation of ILC2s during the alveolarization phase of lung development raises the question of why a spontaneous IL-33-dependent type-2 immune cell microenvironment evolved in mammalian lungs. It is tempting to speculate that type-2 immunity controls lung development or remodeling of the lung postnatally. Indeed, IL-33 is known to activate alternatively activated M2 macrophages that control tissue remodeling and postnatal branching morphogenesis of the lung [92, 98, 99]. However, despite this no gross abnormalities in lung alveolarization were observed in IL-33-deficient mice [92, 94]. It has been suggested that ILC2s may also be involved in lung regeneration and may be a potential immunomodulatory target to stimulate alveologenesis in adult mice [100]. ILC2s and lung macrophages modulate the regenerative microenvironment to support alveolar epithelial stem cell proliferation and differentiation [100]. $IL\text{-}5^+$ ILC2s were found near small airways and in

alveolar spaces post pneumonectomy, however, no direct studies were performed indicating the requirement for ILC2s in lung regeneration or alveolarization [100]. T- and B-cell deficient mice had normal lung mass indicating that adaptive immunity is unlikely to play a role in lung regeneration, highlighting the importance of innate immunity and possibly ILC2s [100]. Indeed, ILC2s have also been shown to promote lung tissue homeostasis after infection as well as disrupting bronchial epithelial barrier integrity in asthmatic patients [5, 101, 102]. Thus, the role of ILC2s in lung development, repair and homeostasis is complex and remains to be fully elucidated.

Neurotransmitters

Initial evidence that neurotransmitters may modulate immune responses was that their release from nervous tissue could lead to signaling through lymphocyte cell surface receptors [103]. Leukocytes express receptors for the main brain neurotransmitters such as glutamate, dopamine and serotonin [103], and release neurotransmitters that act as autocrine or paracrine modulators [104]. Neuromedin U (NMU) is a neuropeptide expressed by the CNS, but also various peripheral organs including the lung and gastrointestinal tract, where ILC2s are abundant. Very recently, ILC2s have been identified in the mouse CNS [89, 105]. NMU interacts with two G protein-coupled receptors, NMU-R1 and NMU-R2. NMU-R2 is expressed in a specific region of the brain and NMU-R1 is expressed in various peripheral tissues, including immune and hematopoietic cells [106]. Early work demonstrated that NMU is involved in type-2 immune responses including mast cell-mediated inflammation, activation of murine and human eosinophils and allergen-induced lung eosinophilia [107, 108]. However, the underlying mechanisms remain elusive. A recent triad of work demonstrate a clear role for NMU in activating mucosal ILC2 and type-2 inflammation [109-111]. Profiling of lung resident ILCs from mice at baseline and after stimulation with IL-25 or IL-33 using parallel droplet-based ScRNA-seq, identified the neuropeptide receptor *Nmur1*

as a novel ILC2-specfic gene that was selectively expressed by mature ILC2 and ILC2p, but not other hematopoietic cells at baseline or after IL-25 stimulation [109-111]. ILC2s also co-localized with cholinergic neurons that express NMU [109]. NMU activated ILC2s *in vitro*, and administration of NMU alone induced lung inflammation [109] and when co-administered with IL-25, substantially increased allergic airway inflammation by expanding iILC2s [109, 110]. Furthermore, administration of NMU *in vivo* induced potent type-2 cytokine responses that resulted in accelerated expulsion of *N. brasiliensis* [109]. This was supported by autonomous ablation of Nmur1 in ILC2 that resulted in poor worm control [111]. Loss of NMU-NMUR1 signaling reduced ILC2 frequency and effector function and altered transcriptional programs after HDM challenge *in vivo* [110]. Interestingly, NMU expression in asthmatic bronchial brushings correlates with disease severity [112], which could be related to NMU-mediated ILC2 activation. These studies raise the question of whether NMU potentiates airway inflammation when high levels of innate type-2 cytokines like IL-25 are present after virus-induced asthma exacerbations [113]. These data indicate that NMU-NMRU1 signaling provides a selective mechanism that integrates the enteric nervous system and innate immune system to induce rapid type-2 immune responses at mucosal sites [109].

Another mechanism of neuronal-associated ILC2 activation involving a rare airway epithelial cell population known as pulmonary neuroendocrine cells (PNECs) has been recently shown to act through calcitonin gene-related peptide to stimulate ILC2s [114]. PNECs also act through the neurotransmitter gamma-aminobutyric acid (GABA) to induce mucus secreting-cell hyperplasia in the airways, and the lungs of human asthmatics had increased PNECs. PNECs reside in close proximity to ILC2s and can stimulate ILC2 cytokine production and likely form neuro-immune modules at airway branches to amplify allergic asthma [114]. Moreover, certain neuronal cues suppress ILC2 function. Murine ILC2s express the β_2 adrenergic receptor (β_2AR) and colocalize with adrenergic neurons in the intestine [115]. β_2AR

deficiency resulted in exaggerated ILC2 responses and type-2 inflammation in the intestine and lung. Conversely, β_2AR agonist treatment impaired ILC2 responses and reduced inflammation *in vivo*. Mechanistically, the β_2AR pathway is a cell-intrinsic negative regulator of ILC2 responses through inhibition of cell proliferation and effector function. This study provides the first evidence of a neuronal derived regulatory circuit that limits ILC2-dependent type-2 inflammation [115]. Given the importance of ILC2s in driving type-2 immune responses it appears that β_2AR may function as a molecular rheostat to fine-tune ILC2 responses and prevent pathologic type-2 inflammatory responses. These studies also highlight the contrasting functions of adrenergic and cholinergic neurons in regulating ILC2 function. These opposing functions appear to have evolved in the mammalian nervous system as a dual mechanism to rapidly repress or activate ILC2s to protect the host against diverse inflammatory stimuli [115].

ILC2s – Innate Gatekeepers of Respiratory Immunity and Homeostasis in Chronic Lung Diseases and Infection

Emerging evidence strongly implicate ILCs in the pathogenesis of chronic respiratory inflammation and diseases including asthma, COPD, pulmonary fibrosis and cystic fibrosis. ILCs also regulate immune responses and restoration of lung homeostasis following respiratory infections, and are involved in the infectious induction, exacerbation and onset of severe phenotypes of chronic respiratory diseases. Defining the roles of ILC2s in respiratory disease will clarify their use as diagnostic markers and their manipulation will highlight their potential for therapeutic targeting in disease prevention (**Table** 2). There have been few studies of human ILCs and additional studies are clearly warranted.

ILC2s are Critical Mediators of Allergic Airway Inflammation and Asthma

The airway epithelium is a crucial barrier enabling transport of gases and molecules whilst protecting from environmental challenges, inhaled particles and pathogens. In the healthy state ILCs are present in low numbers in the submucosa and maintain tissue homeostasis, but they are early immune responders and mediate tissue repair in response to challenge, and can also drive airway inflammation and chronic respiratory diseases [116]. ILC stimulation depends on their microenvironment and activation by cytokines, lipids, microbes and their metabolites and contact with other cells in the respiratory tract [117].

Allergic asthma is an archetypal type-2 immune-mediated airway disease that typically develops in childhood, often consequent to bronchiolitis or wheezing induced by respiratory viral (respiratory syncytial virus [RSV], rhinovirus [RV]) or bacterial (*Chlamydia*, *Mycoplasma*) infections [118-120]. It is also exacerbated by these and influenza A virus (IAV) infections, which may drive more severe type-1/-17 associated disease [121-123]. Polymorphisms in IL-33, ST2, RORα and IL-13 are associated with type-2 high asthma and are critical for ILC2 development and activation [124]. IL-33 and ILC2s are elevated in the airways and blood of asthma patients [125, 126], and along with the levels of IL-5 and IL-13, increase with asthma severity [126-128].

The mechanisms of induction of ILC2 responses that drive asthma are beginning to be unraveled and involve innate type-2 cytokines and metabolic changes **(Figure 1A)**. ILC2s respond to eicosanoids, prostaglandin D2 and leukotrienes produced by mast cells, macrophages and eosinophils. Important to asthma pathogenesis, with or without co-stimulation with IL-25 or IL-33, ILC2s produce IL-4 and IL-13, activate and mobilize DCs and their release of Th2-cell recruiting CCL17, present antigen through MHC-II, and drive Th2 responses including IL-4, IL-5 and IL-13 production that is independent of antigen [129, 130]. Conversely the pro-resolving mediator lipoxin A4 and E-cadherin (ligand for KLRG1) from neutrophils, epithelial cells or M2 macrophages reduce cytokine production by ILC2s [129, 130].

Recent studies showed that the expression of the intracellular metabolic factor arginase-1 (Arg-1) increased in mouse and human ILC2s during acute and chronic lung inflammation [131]. This occurred through the inhibition of Arg-1 enzyme activity that disrupted numerous ILC2 metabolic processes including altered arginine catabolism and reduced polyamine biosynthesis and aerobic glycolysis [131]. Collectively these data show that type-2 immune factors drive ILC2 development and metabolic factors regulate ILC2 activity that may promote asthma development. Furthermore, the roles in other phenotypes of asthma, including severe steroid resistant forms, which are often associated with respiratory infections, remain to be resolved and could be elucidated using mouse models that recapitulate the hallmark features of the human disease as well as human tissues and cells [122, 123, 132]. These murine studies are being translated into human therapies. To date, treatment with antibodies against IL-33 and TSLP suppressed airway inflammation and AHR after allergen challenge, and anti-prostaglandin D2 (PGD2) receptor (anti-CRTH2 antagonist) improved asthma control and lung function [133, 134] (**Table 2**).

ILC2s in Other Chronic Respiratory Diseases

There is a paucity of information on the roles of ILCs in the pathogenesis of other chronic respiratory diseases. ILC2 numbers are lower in the lung tissue of severe COPD patients compared to mild COPD or healthy controls but the numbers of total $CD45^+$ lymphocytes were not altered [13]. An important study by Kearley *et al*., in COPD patients indicated that increased IL-33 levels occur that correlated with reduced lung function (FEV1), and its production was induced by viral but not *Alternaria* infection of human bronchoepthelial cells (BECs) [135] **(Figure 1B)**. In mice, acute or chronic cigarette smoke exposure increased pulmonary IL-33 expression, viral infection with IAV or RSV but not fungal infection with *Alternaria* induced its release, and administration of rIL-33 exacerbated virus-induced inflammation. These

studies also showed that acute smoke exposure reduced IL-5 and -13 responses to rIL-33 as well as the numbers of IL-13-expressing ILCs and their production of IL-5 and -13 in response to IL-33. Smoke also suppressed ST2 expression on ILC2s whilst increasing expression on macrophages and NK cells, and reduced cytokine production by ILC2 and NK cells. The absence of IL-33, in IL-33 or ST2-deficient mice or treatment with an ST2 inhibitor, suppressed IAV-induced exacerbation of acute cigarette smoke-driven inflammation. Mechanistically they showed that IL-33 enhances macrophage and NK responses to IAV following acute smoke exposure in wild-type but not ST2-deficient mice, which were supported by *ex vivo* mouse studies. Thus, smoke exposure increases IL-33 expression and infection induces its release amplifying type-1 inflammatory responses by promoting macrophage and NK cell function. More generally, smoke alters lung immunity to facilitate IL-33 exaggeration of pro-inflammatory responses to infection that exacerbates the underlying disease [14]. The roles of ILC2s in the pathogenesis of COPD and infection rather than in acute smoke exposure remain to be defined. These roles could be elucidated using mouse models that recapitulate the hallmark features of the human disease as well as human tissues and cells [136-138].

In support of the generalizability of these observations to chronic respiratory diseases, IPF patients have increased IL-25 expression and ILC2s in their BALF, and IL-33 is constitutively or inducibly expressed in lung BECs and macrophages, respectively, in bleomycin-induced experimental pulmonary fibrosis [139]. Experimentally, *Schistosoma mansonii*-induced granulomas and fibrosis were dependent on IL-25 and -17, and IL-25-induced fibrosis required ILC2s in wild-type compared to ILC2-deficient (*Rora*$^{sg/sg}$) mice [140]. They extended these findings by showing that ILC2s regulate fibrosis in anti-CD90.2 treated T- and B-cell-deficient mice, and that IL-13^{+} ILC2s regulate collagen deposition in adoptive transfer studies in *Il13*$^{-/-}$ mice. Others showed that combined targeting of TSLP, IL-25 and -33 suppressed type-2-driven inflammatory and IL-13 producing ILC2 responses, airway

remodeling and fibrosis during *S. mansonii* infection and HDM-induced allergic lung inflammation [141]. Inhibition of IL-33 activity or depletion of alveolar macrophages decreased, whereas treatment with rIL-33 or adoptive transfer of ILC2s increased, inflammation and fibrosis. Indeed, IL-33 induced M2 macrophage polarization and expansion of ILC2s with pro-inflammatory and -fibrotic responses *in vitro* and *in vivo*. Thus, IL-33 is pro-inflammatory and -fibrotic and initiates and progresses pulmonary fibrosis involving macrophages and ILC2s.

Other recent studies found elevated levels of IL-9 in airways of cystic fibrosis patients, and in mice that IL-9 induces IL-2 production by mast cells that promotes IL-25$^+$ ILC2 and Th9 cell proliferation [142]. Blocking IL-9 or c-Kit (CD117) prevented these effects. Thus, targeting IL-9 may reduce Th9 and ILC2-associated lung inflammation in fibrotic lung diseases.

ILC2s in Respiratory Infections

ILC2s have been implicated in both protection against and pathogenesis of parasitic, viral and bacterial infections and their exacerbations of chronic respiratory diseases **(Figure 1)**. Their involvement in parasitic infections has been extensively reviewed elsewhere [143]. Therefore, we focus on role of ILC2s in respiratory viral infections.

Rhinovirus

Recent studies have made substantial advances in understanding the role of ILC2s in RV infections in early life, which induce mucus hypersecretion and AHR [144]. Profiling of responses of neonatal (6 day-old) or adult (8 week-old) mice to RV infection over 28 days showed that neonatal mice had increased IL-13 production from ILC2s, but reduced IFN-γ, IL-12 and TNF-α expression compared to adults [144]. IL-25 attenuated increases in ILC2s, mucus hypersecretion and AHR. They then showed that intranasal administration of recombinant

(r)IFN-γ protein or Rorα inhibitors reduced these RV-induced IL-13 and ILC2 responses and mucus hypersecretion [145, 146]. Treatment of lung ILC2s *ex vivo* with rIFN-γ reduced IL-5, IL-13, IL-17RB, ST2 and GATA3 expression, or with Rorα inhibitor blocked the expansion of IL-25- or IL-33-induced ILC2s and IL-13 release [146, 147]. Infected *Rora*$^{sg/sg}$ mice had reduced expansion of ILC2s, and sorted ILC2s induced an asthma-like phenotype in naïve young or adult mice [146]. Most recently IL-33 and TSLP were shown to be induced in airway epithelial cells by neonatal infection and were required for maximal IL-25 expression, ILC2 development, mucus hypersecretion and AHR [147]. Thus, early-life RV infection induces an interplay of IL-25, IL-33 and TSLP-driven type-2 and ILC2 responses that require Rorα and contribute to mucus hypersecretion, AHR and potentially asthma. In contrast, IFN-γ inhibits ILC2 expansion, IL-13 expression and mucus hypersecretion.

Translational studies have been performed in experimental RV-induced asthma exacerbations in human adults [148]. Infection increased IL-4, IL-5, IL-13 and IL-33 in asthmatic airways that correlated with disease severity compared to healthy controls. IL-33 levels also correlated with IL-5 and IL-13 levels. *In vitro* RV infection of BECs induced IL-33 and culture of pBECs, peripheral T-cells or ILC2s with supernatants from RV-infected BECs induced IL-33-dependent Th2 cytokine release without affecting IL-25, IL-33 or TSLP. This shows that RV-induced asthma exacerbations in adults involve the induction of type-2 cytokines including IL-33, and that T-cells and ILC2s are mechanistic links. Thus, IL-33 may be a therapeutic target in asthma exacerbations.

Respiratory Syncytial Virus

Similar observations have been made with RSV strengthening generalizability. IL-33-induced increases in ILC2s play critical roles in the pathogenesis of RSV infection only in early life. Increased levels of IL-33 and -13 were detected in nasal secretions of hospitalized infants with

RSV that subsided during recovery [95]. In mice RSV infection in neonates but not adults induced rapid increases in IL-33 expression and ILC2s in the lungs. Suppression of IL-33 responses inhibited RSV-induced Th2 inflammation airway eosinophilia, mucus hypersecretion and AHR with opposite effects induced by rIL-33 administration. Others investigated the mechanisms involved and showed that RSV infection in wild-type mice increased IL-13, IL-33 and TSLP levels and the numbers of IL-13 producing ILC2s [149]. Deletion of TSLP reduced IL-13 levels, IL-13-producing ILC2s, mucus hypersecretion, AHR and weight loss without affecting viral load. They also showed that RSV-induced STAT1 responses were required for the control of immunopathologic IL-5$^+$ and -13$^+$ ILC2s. Both intrinsic and extrinsic factors caused this dysfunction with extrinsic IL-33 promoting ILC2s. These studies show that IL-33 and TSLP are required to induce IL-13$^+$ ILC2s and Th2-associated disease during neonatal RSV infection, and that STAT1 opposes these effects. Thus, again IL-33 and TSLP may be therapeutic targets.

Influenza A Virus

IAV infection of mice leads to the accumulation of ILC2s in the lungs, and their depletion during infection caused the loss of epithelial integrity and impaired lung function and airway remodeling [5]. These events could be reversed by ILC2-derived amphiregulin. Respiratory viral infections typically induce type-1 pro-inflammatory responses though type-2 responses also arise in a tissue protective role and may lead to asthma exacerbations. Infection with pandemic strains of IAV induces IFN-γ responses that restricts protective ILC2 function. A recent study showed that genetic IFN-γ deficiency or anti-IFN-γ treatment during IAV infection did not increase ILC2s but enhanced their activity and release of IL-5 and amphiregulin and improved tissue protection without affecting viral load or clearance [150]. These effects were dependent on IL-5 and were not observed in ILC2-deficient mice.

Asthma and COPD patients are more susceptible to IAV infections that exacerbate the underlying disease and ILC2s may be involved. However, the mechanisms involved are poorly understood. IAV infection induced AHR independently of T- and B-cells in *Rag2*$^{-/-}$ mice but did require ST2 [151]. Infection lead to IL-33 production in alveolar macrophages and increased the numbers of ILCs, which did not occur in *Il13*$^{-/-}$ or *Rag2*$^{-/-}$ mice or with anti-CD90.2 treatment. Others showed that IAV infections in mice also induced robust but transient IL-5 production from infiltrating c-kit^{+} ST2^{+} ILC2s and concomitant eosinophil influx into the airways, particularly during recovery [152]. The effects were abrogated with anti-CD90.2 treatment. In these studies, NKT cells and alveolar macrophages were the sources of IL-33. A follow up study found increased ILC1s in COPD patients that were associated with disease severity and exacerbation susceptibility [14]. Experimentally they showed that ILC2s can also be plastic and during IAV infection. They developed ILC1 characteristics with reduced GATA-3 expression and produced IFN-γ, IL-12 and -18. These ILC1s reinforced T-bet dependent virus-induced inflammation. Translating their findings, they showed that IL-12 converted human ILC2s into ILC1s.

Thus, ILC2s are crucial in restoring the airway epithelium after IAV infection, but IFN-γ restricts their function promoting pathogenesis. Consequently, increasing ILC2 activity is a potential therapeutic strategy. In exacerbations of chronic respiratory diseases IAV-induces AHR through an IL-13/-33/ILC2 axis, and interactions between ILC2s and IL-33 producing NKT cells and alveolar macrophages leads to high levels of IL-5 production by ILC2s and eosinophilopoiesis during recovery from IAV infection that may exacerbate asthma. In contrast, ILC2 plasticity toward ILC1s exacerbates virus-induced inflammation that may have adverse consequences in COPD. Thus, early IL-13 or -33 and/or later anti-IL-5 treatment may be beneficial in asthma but maintaining ILC2 function may be protective in COPD.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Despite the recent explosion of studies, particularly using 'omics technologies, investigating the biology of ILC2s in multiple contexts, namely in the respiratory tract, there are still many unanswered questions. These include, a consensus for consistency in identifying and reporting of ILC2s to limit issues with reproducibility in the identification and isolation of ILC2s in both mice and humans, clarifying their roles in different disease states, and defining the potential strategies for therapeutic manipulation. There are emerging anti-inflammatory roles for ILC2s that interact with Tregs that need to be defined. Numerous influencers of ILC2 function are being identified including novel roles for circadian rhythm, sex hormones, age, and neurotransmitters. ILC2s have important roles in chronic lung diseases such as asthma, COPD and infection that are only just beginning to be unraveled. They may promote the development of asthma and be involved in pulmonary fibrosis, but their numbers and function are reduced in COPD. ILC2s may also have pathogenic roles in RV and RSV infections, particularly in early life, but may be critical for tissue restoration after IAV infection. Their profiles and roles in disease states may be further defined using single cell sequencing. ILC2s may both modulate and be modulated by microbiota in the lung and gut and have local and systemic effects that differentially affect chronic respiratory diseases and infections. This may be dependent on host genetics and consequently susceptibility to infection. Fully elucidating the roles of ILC2s in development and disease and the generation of new ways to specifically modulate them has the potential to substantially impact the ways that these issues are prevented and treated.

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Conflict of Interest

MRS has no conflict of interest.

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Figure 1. ILCs in respiratory diseases

A. In asthma ILC2 development and activity is driven by eicosanoids, prostaglandin D2 (PGD2) and leukotrienes from mast cells, macrophages and eosinophils, as well as innate type-2 cytokines IL-33, -25, TSLP and neuromedin U (NMU). This is accompanied by increases in ILC2 markers such as Arginagse-1 and the neuropeptide receptor Nmur1. Activated ILC2s release IL-5 and -13 that activate DCs and Th2 cells to reinforce type-2 immunity and asthma pathogenesis by inducing eosinophilic airway inflammation, mucus hypersecretion, airway remodeling and airway hyperresponsiveness (AHR, wheezing). These responses are opposed by lipoxin A4 and E-cadherin actions released from neutrophils, epithelial cells and macrophages, and by increases in type I IFNs, IFN-γ and IL-27 that are induced by typical viral infections. **B.** In COPD cigarette smoke and likely air pollution induce the increased expression of IL-33 that is released upon viral infection and exacerbates the underlying disease. However, acute smoke exposure reduces IL-5 and -13 responses and ST2 expression on ILC2s. ST2 inhibition suppresses virus-induced exacerbation of acute cigarette smoke-driven inflammation, IL-33 enhances macrophage and NK cell killing of virus, and ILC2s and IL-33 promote airway fibrosis. **C.** Helminth infections induce granulomas and fibrosis that are dependent on IL-33, -25, TSLP, IL-17 and IL-13^{+} ILC2s. Combined targeting of IL-33, -25 and TSLP suppressed type-2 driven inflammation, IL-13+ ILC2s, airway remodelling and fibrosis. IL-5^{+} and IL13^{+} ILC2s induce macrophage, eosinophil and mucus activity that destroy and clear helmniths but eosinophils promote fibrosis and allergy. **D.** In early life, rhinovirus (RV) and respiratory syncytial virus (RSV) induce IL-33, -25, TSLP and IL-13^{+} ILC2s, airway eosinophilia, mucus hypersecretion and AHR and predispose to the development of asthma. IFN-γ suppresses ILC2s, but infection reduces IFN-γ, IL-12 and TNF expression. **E.** Asthma and COPD patients are more susceptible to bacterial and viral infections such as with influenza A virus (IAV), which induce the accumulation of ILC2s in the lung that

are tissue protective. They also typically induce type-1 responses that restrict ILC2s but also type-2 responses that are responsible for tissue protection but IL-13 and -33 contribute to asthma exacerbations. IAV infection induces AHR independently of Th2 cells in an IL-13/-33/ST2/ILC2-mediated axis. IAV infection induces IL-33 production from alveolar macrophages and NKT cells that increase ILC2s, and causes transient IL-5 production, and the influx of c-kit^{+} ST2^{+} ILC2s and associated eosinophils that exacerbate asthma. In COPD ILC2s are plastic and upon IAV infection acquire an ILC1 phenotype, with reduced GATA-3 expression and produce IFN-γ, IL-12 and -18. These cells reinforce virus-induced inflammation and exacerbations.

Table 1: Tools to explore ILC2 function

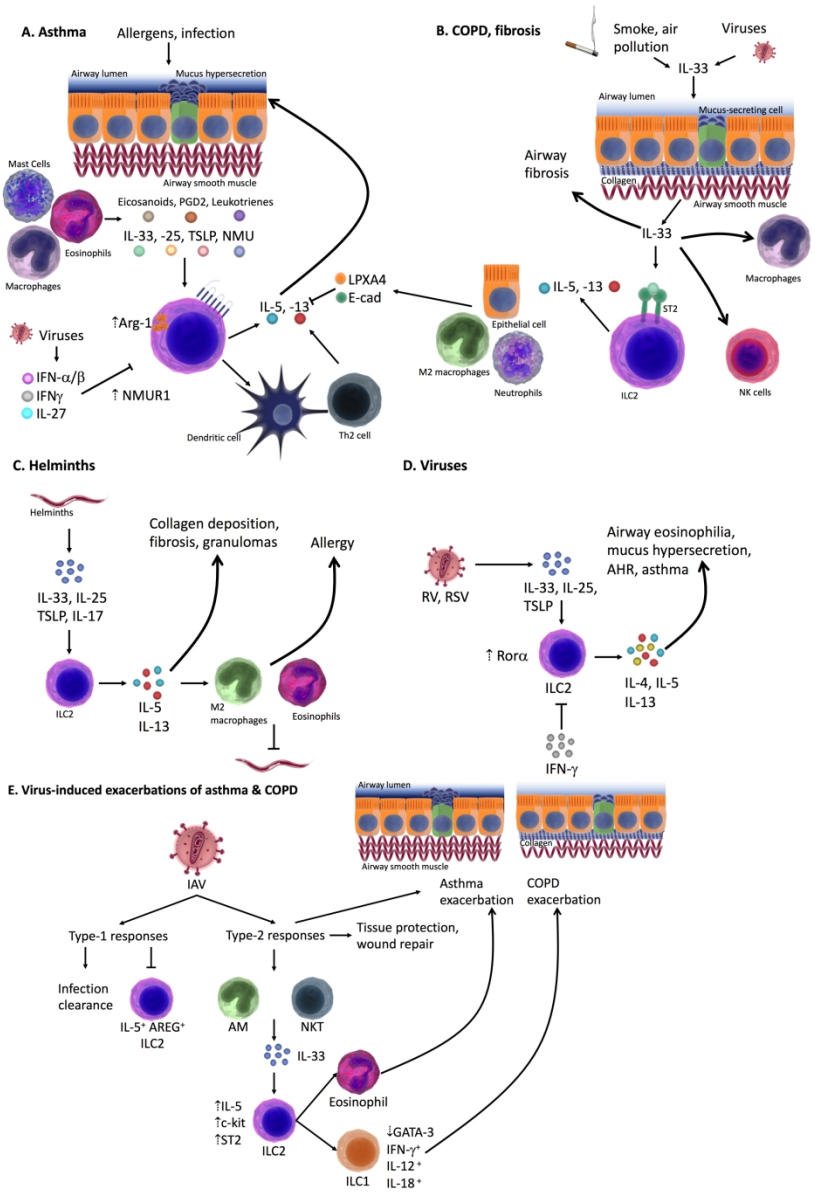
Location	*Cell surface markers*	*Stimuli*	*Gene expression or reporter*	
Lung and gut, skin, lymph nodes, bone marrow	*Lin-, CD25, CD69, CD90 (Thy1), CD127, T1/ST2 (IL-33R), ICOS, KLRG1, Sca-1, IL-17BR (IL-25R) CD117 (c-Kit)*	*IL-2 IL-7 IL-25 IL-33 TSLP*	*Rora, Gata3, Il-4 reporter Il-5 reporter Il-9 reporter Il-13 reporter*	
Engineered mice targeting ILC2				
Mouse line	*Deleted cells*			*References*
$Rora^{sg/fl}$ $Il7R^{Cre}$	*ILC2*			153
$Rora^{sg/sg}$	*Stagger mouse, deficient in Rora*	*Rora required for ILC2 development*		2
inducible ICOS-diphtheria toxin	*Temporal deletion of ILC2 with diphtheria toxoid*			153

receptor (iCOS-T)				
RAG-deficient R5/R5 deleter	*IL-5 reporter allele (tdTomato) for identification of ILC2s in adult mice*			72 154
RAG-deficient	*Deletion of T and B lymphocytes*	*CD90.2 antibody mediate deletion of ILCs*		5
Tcf7$^{-/-}$	*Deletion of Tcf7 in entire hemaotopoietic compartment*	*Tcf7 required for ILC2 generation*		27 26
Bcl11b$^{-/-}$ and *Bcl11b*$^{fl/fl}$ ERT2^{Cre}	*Global Bcl11b deficiency or temporal deletion with tamoxifen*	*Bcl11b required for ILC2 development*		155

Table 2: Potential ILC2 targets as novel therapies for chronic lung diseases

Class of target molecule	Drug	Key results	References
Molecules that activate ILC2s			
IL-25/IL-25R	Anti-IL-25 and anti-IL-25R	Pre-clinical. Inhibition of inflammation and airway hyperresponsiveness in experimental models of lung inflammation	156-158
IL-33/IL-33R (ST2)	Anti-ST2 antibody GSK3772847 RG6149/AMG282) Anti-IL-33 ANB020	Antibodies that inhibit IL-33 signaling are in clinical development	159
TSLP/TSLPR	anti-TSLP Tezepelumab	Administration reduced lung inflammation and airways bronchoconstriction in mild asthma, and lowered rates of asthma exacerbations in patients with uncontrolled asthma	133, 160
IL-9/IL-9R	Anti-IL-9 and anti-IL-9R MEDI-528	Humanized IL-9 antagonist that inhibits features of asthma in pre-clinical experimental models. No available data in humans	161
Prostaglandin pathway	CRTH2 antagonists OC000459 (Timapiprant) BI 671800 AZD1981 Fevipiprant	Compounds have entered clinical trials. Notably, Fevipiprant treatment of patients on inhaled corticosteroids improved Forced Expiratory Volume and reduced sputum eosinophilia	162-165
	Anti-huCRTH2 antibody	Pre-clinical humanized anti-huCRTH2 antibody causes depletion of CRTH2-expressing cells	166
Leukotriene pathway	Montelukast and Zafirlukast are cysteinyl leukotriene receptor antagonists	ILC2 expression of type-2 cytokines is partially inhibited by Montelukast *in vitro*	167, 168
Arginase	2(S)-amino-6-boronohexonic acid (ABH) or S-(2-boronoethyl)-*L*-cysteine (BEC)	Efficacy in pre-clinical experimental asthma models. Arginase inhibitors are in clinical trials as a cancer therapeutics.	169-172
Transcription factors			
GATA3	Antisense DNAzyme molecule (SB010) that cleaves GATA3 mRNA	Administration of SB010 in a small clinical trial resulted in improved lung function and reduced eosinophilia following allergen provocation	173-175
ILC2-derived cytokines			
IL-4	Anti-IL-4 receptor alpha (IL-4R) antibody	The anti-IL-4 receptor alpha (IL-4R) antibody (Dupilumab) that inhibits signaling	176, 177

	Dupilumab	by both IL-4 and IL-13 has proven efficacious in patients with asthma and atopic dermatitis	
IL-5	Anti-IL-5 antibody Mepolizumab	Has proven beneficial in the treatment of people with uncontrolled severe eosinophilic asthma	178-181
IL-13	Anti-IL-13 antibody Lebrikizumab Tralokinumab	Limited improvements in lung function in periostin-high and eosinophilic patients, but failed to reduce asthma exacerbations	182, 183



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