

# Group 2 Innate Lymphoid Cells: New Players in Human Allergic Diseases

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## ■ Abstract

Allergic diseases are characterized by tissue eosinophilia, mucus secretion, IgE production, and activation of mast cells and TH2 cells. Production of TH2 cytokines including IL-4, IL-5, IL-9, and IL-13 has mainly been attributed to CD4+ T<sub>H</sub>2 cells. However, the recent discovery of group 2 innate lymphoid cells (ILC2s) in humans and findings from experimental disease models have challenged conventional concepts associated with the contribution of specific cells to type 2 inflammation in allergic diseases. ILC2s produce high levels of T<sub>H</sub>2 cytokines and have been detected in human lung tissue, peripheral blood, the gastrointestinal tract, skin, and sinonasal tissue, suggesting that ILC2s could contribute to chronic rhinosinusitis, asthma, atopic dermatitis, and gastrointestinal allergic disease. Moreover, depletion of ILC2s in animal models suggests a role for these cells in atopic dermatitis and asthma. This review will focus on the role of ILC2s in human allergy and asthma and provide a mechanistic insight from animal models.

**Key words:** ILC2. ILC2s. Group 2 innate lymphoid cells. Allergy. Asthma. Atopic dermatitis. Chronic rhinosinusitis. Allergic rhinitis.

## ■ Resumen

Las enfermedades alérgicas se caracterizan por una eosinofilia tisular, producción de moco, producción de IgE, y por activación de mastocitos y células Th2. A estas células se ha atribuido la producción predominante de citocinas IL-4, IL-5, IL-9 y IL-13. Sin embargo el descubrimiento reciente en humanos de las células linfoides innatas grupo 2 y los hallazgos en modelos experimentales de enfermedad han cuestionado nuestros conceptos convencionales sobre las células que contribuyen a la inflamación tipo 2 presente en las enfermedades alérgicas. Las células ILC2 producen grandes cantidades de citocinas Th2 y se han podido detectar en el pulmón humano, en sangre periférica, tracto gastrointestinal, piel y tejido rinosinusal. Este hecho sugiere que estas células ILC2 podrían contribuir a la fisiopatología de la rinosinusitis, asma, dermatitis atópica y enfermedad gastrointestinal. Además la deplección de estas células en modelos animales sugiere su papel en la dermatitis atópica y en el asma. Esta revisión trata sobre el papel de las células ILC2 en alergia y asma humana, y ofrece una visión mecanística basada en resultados obtenidos en modelos animales.

**Palabras clave:** ILC2. ILC2s. Células linfoides innatas grupo 2. Alergia. Asma. Dermatitis atópica. Rinosinusitis crónica. Rinitis alérgica.

## Introduction

Tissue eosinophilia, epithelial mucus metaplasia, and IgE production are hallmarks of allergic diseases such as asthma [1]. These features are largely due to the downstream effects of the  $T_H2$  cytokines IL-4, IL-5, IL-9, and IL-13, which are currently the targets of novel biologic therapy [2]. The primary source of  $T_H2$  cytokines has been attributed to  $CD4^+$   $T_H2$  cells that require antigen experience and differentiation prior to cytokine production [3]. Findings from early animal models and human samples supported the notion that  $CD4^+$   $T_H2$  cells primarily directed allergic responses, whereas  $T_H1$  cells protected against allergic disease [1,4,5]. Although the  $T_H1/T_H2$  cell paradigm has provided significant insight into the pathogenesis of allergic diseases, evidence has emerged over the past decade that non-T-cell populations might significantly contribute to  $T_H2$  cytokine-mediated disease. Importantly, a 2009 study demonstrated that a population of  $CD34^+$  non-B/non-T lymphocytes detected in human asthmatic sputum produced IL-5 and IL-13 in response to inhalational allergen challenge [6]. In 2010, the authors of 3 independent studies reported the presence of innate lymphocytes (non-B non-T cells) in mice that produced  $T_H2$  cytokines and were protective against helminth infections [7-9]. These cells were initially termed natural helper cells, nuocytes, and innate helper 2 cells, although they are now referred to as group 2 innate lymphoid cells or ILC2s.

## ILC2s: Identification of a Novel Cell Type in $T_H2$ Inflammation

ILC2s are lineage-negative lymphocytes, that is, they are negative for surface expression of known lineage markers. Studies vary somewhat in their lineage antibody staining approaches, although most have used a combination of CD3, CD4, CD8, TCR $\beta$ , TCR $\delta$ , CD5, CD19, B220, NK1.1, Ter-119, GR-1, Mac-1, CD11c, and Fc $\epsilon$ R1 in mice to exclude T, B, NK, and NKT cells, as well as mast cells basophils, and macrophage/monocyte populations [7-9]. In humans, combinations of CD1a, CD3, CD4, CD11b, CD11c, CD14, CD16, CD19, CD20, CD123, TCR $\beta$ , TCR $\delta$ , CD235a, and Fc $\epsilon$ R1 have been used to exclude analogous lineage-positive cells [10,11]. Positive markers for mouse ILC2s include CD45, Thy1.2, CD44, CD69, and c-kit, although some differences exist in levels of Sca-1, T1/ST2 (IL-33 receptor), and IL-7R $\alpha$  [7-9]. Human ILC2s express the prostaglandin D2 (PGD $_2$ ) receptor DP2 (also known as CRTH2), as well as IL-7R, IL-33R, c-kit, T1/ST2, and CD161 [10,12].

ILC2s develop from common lymphoid progenitors that require inhibition of DNA binding 2 (Id2), Notch, and the IL-7 receptor (IL-7R), which signals through the common  $\gamma$  chain ( $\gamma$ c) [9,13-15]. The master  $T_H2$  cytokine transcription factor GATA3 is critical for both ILC2 development and ILC2  $T_H2$  cytokine production and enables parallel transcriptional programming between ILC2s and  $T_H2$  cells [16-18]. Recently, the nuclear receptor RAR-related orphan receptor alpha (ROR $\alpha$ ) was shown to be necessary for development of ILC2s from common lymphoid progenitors into ILC2s in vitro.

Furthermore, ILC2 expansion and goblet cell hyperplasia were less evident in ROR- $\alpha$  knockout mice than in wild-type mice after administration of IL-25 [19]. A subsequent report also showed that ROR- $\alpha$  contributed to the development of ILC2s and demonstrated specificity of ROR- $\alpha$  in controlling ILC2s, as the development and function of other ILCs and  $T_H2$  cells did not require ROR- $\alpha$  [20]. Interestingly, genome-wide association studies in human asthma have revealed the ROR- $\alpha$  locus to be a potential asthma susceptibility gene [21]. Notch signaling member T-cell factor 1 (TCF-1) also contributes to development of functional ILC2s, as mice that lack TCF-1 displayed impaired ILC2 cytokine responses [22]. Thus, the development of ILC2s from lymphocyte progenitors depends on GATA3, Id2, ROR- $\alpha$  Notch, and IL-7R signaling.

## ILC2 Effector Functions

Early studies of ILC2s in mice showed that these cells produced high levels of IL-5 and IL-13 ( $\mu$ g/5000 cells), resulting in tissue eosinophilia, mucus production, and helminth expulsion [7-9]. Subsequent studies showed that ILC2s also produced IL-4 in the presence of thymic stromal lymphopoietin (TSLP) or leukotriene (LT) D4 [16,23]. Production of IL-13 by lung ILC2s is necessary for airway hyperresponsiveness (AHR) in mice infected with influenza virus or that have been administered IL-25 [24,25]. Additional investigations demonstrated that ILC2s also produce IL-6 and IL-9 [9,26,27]. A study of mice with a genetic tag for IL-9 expression that were challenged with the protease papain revealed that ILC2s were a dominant source of IL-9 [27]. Interestingly, IL-9 production by ILC2s was transient and dependent on IL-2 and intact adaptive immunity. Furthermore, IL-9 induced production of ILC2 IL-5, IL-6, and IL-13, suggesting that IL-9 could amplify ILC2 function.

Apart from the contribution of ILC2s to type 2 inflammation and AHR in mice, a role has emerged for ILC2s in tissue repair responses and normal homeostatic functions. For example, one study showed that ILC2-depleted RAG knockout mice (which lack T and B cells but have ILC2s) infected with influenza virus were profoundly hypoxemic and hypothermic and developed loss of lung epithelial barrier integrity [28]. Adoptive transfer of ILC2s or administration of amphiregulin restored airway integrity and lung function. We have also found that lung ILC2s rapidly produce amphiregulin after challenge with the fungal allergen *Alternaria*, suggesting that the ILC2-mediated repair response is not specific to influenza as a mucosal insult [26].

ILC2s also appear to contribute to normal tissue homeostasis and glucose metabolism. One report showed that under homeostatic conditions, ILC2s are the dominant source of IL-5 in many murine tissues including the brain, heart, lung, kidney, skin, intestine, and uterus [29]. Vasoactive intestinal peptide (a gut hormone activated by eating), as well as eating itself, induced production of  $T_H2$  cytokines by ILC2s, suggesting that stimulation of ILC2s by neuropeptides contributed to normal homeostasis. ILC2s have also been detected in visceral adipose tissue and contributed to eosinophil accumulation [30]. Moreover, IL-5 and eosinophil-deficient mice displayed impaired glucose control after a high-fat diet,

suggesting a role for ILC2s in glucose tolerance. Another study found that RAG-deficient mice that were fed a high-fat diet and depleted of ILC2s developed obesity and impaired glucose tolerance [31]. Thus, ILC2s contribute to  $T_H2$  cytokine production, which results in tissue disease under some conditions, although they also maintain homeostasis and tissue repair in other circumstances.

## Upstream Modulation of ILC2s

### *Cytokines (IL-25, IL-33, and TSLP)*

IL-25 and IL-33 were the first cytokines demonstrated to activate ILC2s in mice [7-9,32-34]. Both are induced during type 2 inflammatory responses and are thus available for potent production of  $T_H2$  cytokines by ILC2s [11,26,35-37]. IL-25 is a member of the IL-17 family and is secreted by  $T_H2$  cells, eosinophils, and epithelial cells. It binds to IL-25R (IL-17E), a heterodimer of IL-17RB and IL-17RA [38]. IL-33 is present in epithelial cells, endothelial cells, and macrophages as a biologically active proform, binds to a heterodimer of ST2 and the IL-1 receptor accessory protein (T1/ST2) after release, and can be further activated by neutrophil elastase [39,40]. In humans, fetal gut and peripheral blood ILC2s were shown to produce IL-13 after stimulation with IL-25 and IL-33, as in the early mouse studies [10]. Although ILC2s are potently activated by IL-25 and IL-33, other immune cells (eg, basophils, T cells, NKT cells, DCs, eosinophils, and macrophages) are also activated by these cytokines and contribute to type 2 inflammatory responses [5,41].

Studies showing that ILC2s were activated by IL-25 and IL-33 were followed by reports that TSLP could activate ILC2s [16,42,43]. TSLP is elevated in the epithelium of asthmatic airways and in the skin of patients with atopic dermatitis and was initially reported to prime dendritic cells for adaptive  $T_H2$  responses [44,45]. Mjosberg et al [16] showed that aside from its role in adaptive responses, TSLP enhanced human peripheral blood and nasal polyp ILC2 production of IL-4, IL-5, and IL-13 beyond IL-33 alone and further induced GATA-3 expression in human ILC2s from peripheral blood and nasal polyps. The results of murine models have also demonstrated that TSLP activates ILC2s in the lung and independently of IL-33 in skin [42,43].

### *Lipid Mediators (Prostaglandin D<sub>2</sub>, Cysteinyl Leukotrienes)*

Lipid mediators have long been recognized as contributors to type 2 inflammatory diseases. Eicosanoids, including prostaglandins and leukotrienes, are derived from arachidonic acid and are rapidly produced by activated mast cells, eosinophils, dendritic cells, and macrophages [46]. Importantly, PGD<sub>2</sub> and cysteinyl leukotrienes have recently been shown to promote ILC2 responses and thus link a novel  $T_H2$  cytokine-producing cell type with lipid mediators that regulate allergic inflammation [23,47-49]. PGD<sub>2</sub> binds to CRTH2 (also known as DP2) expressed on human ILC2s and to other cell types, including eosinophils and  $T_H2$  cells. CRTH2 has been shown to mediate chemotaxis, cytokine production, and cell survival and is a therapeutic target in

allergic diseases [47,48,50-53]. Recently, PGD<sub>2</sub> was shown to potentiate production of IL-13 by ILC2s in peripheral blood more than the combination of IL-2, IL-25, and IL-33 [47]. The increase in production of IL-13 by ILC2s was also detected after stimulation with a DP2 (CRTH2) agonist, suggesting that CRTH2 is the active receptor. The authors further demonstrated that lipoxin A<sub>4</sub> (LXA<sub>4</sub>) reduced levels of IL-13 produced by ILC2s that were treated with IL-2, IL-25, IL-33, and PGD<sub>2</sub>. Our group and others have reported that PGD<sub>2</sub> also induces chemotaxis of human ILC2s through CRTH2 [48,49]. This finding suggests that PGD<sub>2</sub> may have multifaceted effects on ILC2s, including recruitment and activation that may be relevant to severe asthma, as PGD<sub>2</sub> has recently been detected at increased levels in samples from these patients [51].

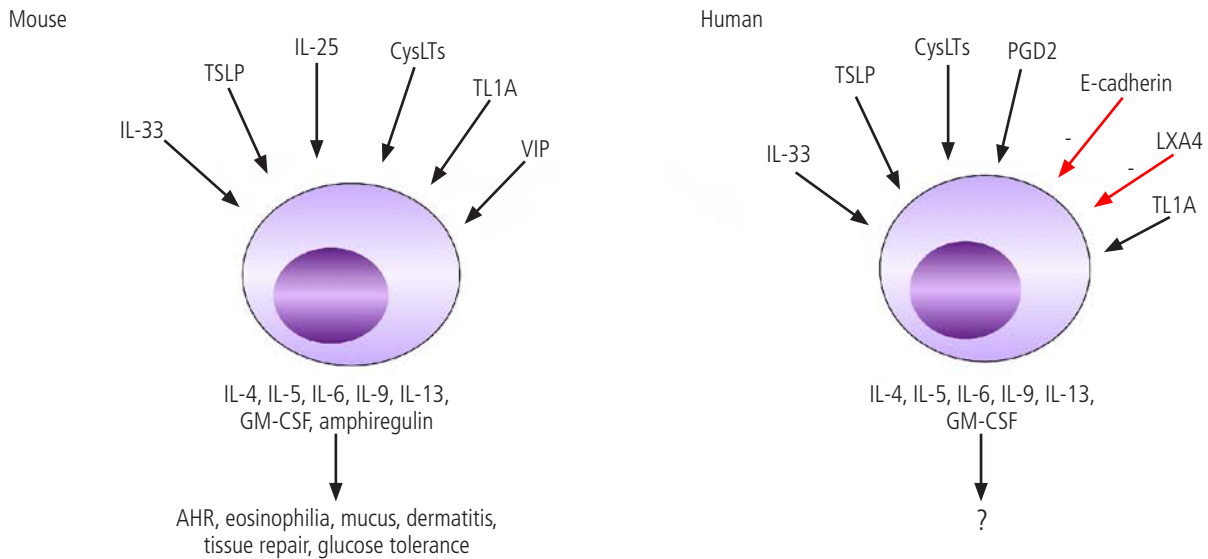
Cysteinyl leukotrienes include LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub> and are increased in asthma and chronic rhinosinusitis [46]. The cysteinyl leukotriene 1 receptor (CysLT1R) binds to LTD<sub>4</sub> with high affinity and is blocked by the antagonist montelukast. Our group has shown that mouse lung ILC2s express CysLT1R, which in turn mediates ILC2 calcium influx and  $T_H2$  cytokine production, including secretion of IL-4 after stimulation of ILC2s by LTD<sub>4</sub> [23]. Cytokine production and calcium influx were abrogated when ILC2s were cultured with montelukast, suggesting that CysLT1R was the active receptor. We further showed that LTD<sub>4</sub> enhanced ILC2 proliferation and lung eosinophilia in RAG2 knockout mice (which have ILC2s but lack T and B cells) receiving *Alternaria* airway challenges. The fact that leukotrienes induced IL-4 production from ILC2s, in contrast with IL-33, which does not induce production of IL-4 by ILC2s, could be an important mechanism by which ILC2s support  $T_H2$  cell differentiation [54]. A subsequent study with human ILC2s showed that montelukast blocked ILC2 cytokine production in the presence of mast cell supernatants, thus suggesting that our findings in mice translate to humans [48]. Therefore, the lipid mediators present in human disease appear to be an important source of molecules that dictate ILC2 responses.

### *TNF Member TL1A*

TNF-like ligand 1A (TL1A) is a member of the TNF family that was recently shown to activate ILC2s [55]. TL1A binds to death receptor 3 (DR3), which is expressed on mouse and human ILC2s. Furthermore, TL1A directly promotes ILC2 cytokine production in vitro and in vivo and leads to expansion of ILC2s in vivo. As additional novel mediators that modulate ILC2 function in mice and humans are discovered, the overall picture of ILC2 regulation in tissues becomes more complex. Dominant pathways that are present under certain conditions may be absent under others, as shown with IL-33-dependent and -independent ILC2 responses [43,56]. Additionally, targeting 1 upstream pathway of ILC2 activation may not be sufficient given the potential redundancy of multiple pathways. The key modulators of ILC2 function in humans and mice are summarized in the Figure.

## Human Asthma and ILC2s

Peribronchial inflammation, epithelial mucus production, AHR, and remodeling are the principal features of human



**Figure.** ILC2 responses in mice and humans. The epithelial cytokines TSLP, IL-33, and IL-25, as well as the lipid mediators PGD<sub>2</sub> and CysLTs produced by mast cells, activate ILC2s to produce T<sub>H</sub>2 cytokines, including IL-4, IL-5, IL-9, and IL-13, in addition to IL-6 and GM-CSF. TNF member TL1A produced by dendritic cells might further promote human and mouse ILC2 activation through binding of DR3. In mice, the hormone VIP regulates intestinal ILC2 function. The epidermal growth factor receptor ligand amphiregulin secreted by mouse ILC2s induces postviral repair responses. In mice, ILC2 products have been shown to promote AHR, eosinophilia, mucus production, and skin inflammation, as well as tissue repair and glucose tolerance. In humans, lipoxin A4 and E-cadherin may have inhibitory effects on ILC2 function. The relative contribution of human ILC2s to disease is not known, although targeting T<sub>H</sub>2 cytokines has benefited some patients. TSLP indicates thymic stromal lymphopoietin; CysLTs, cysteinyl leukotrienes; VIP, vasoactive intestinal peptide; GM-CSF, granulocyte-macrophage colony-stimulating factor; AHR, airway hyperresponsiveness; PGD, prostaglandin D; TL1A, TNF-like ligand 1A.

**Table.** Studies of ILC2s in Human Allergic Diseases and Asthma

Human Disease	Study	Outcome	Reference
Chronic rhinosinusitis	Peripheral blood ILC2s after nasal cat allergen challenge in allergic rhinitis	Increased CD84 <sup>+</sup> ILC2s after nasal cat allergen challenge	81
	Peripheral blood ILC2s in patients with seasonal allergic rhinitis undergoing SCIT	ILC2s increased during pollen season and reduced by SCIT	82
	Nasal polyp ILC2 cytokine responses	ILC2 T <sub>H</sub> 2 cytokine production dependent on GATA-3	16
	Ethmoid sinus mucosal ILCs in nasal polyp patients	Increased numbers of mucosal ILC2s in patients with polyps vs patients without polyps	77
	Correlation between nasal polyp ILC2 and allergic status and T <sub>H</sub> 2 cells	ILC2s correlated with allergic sensitization and T <sub>H</sub> 2 cells	78
	Correlation between nasal polyp ILC2 and polyp endotype and corticosteroid use	ILC2s correlated with eosinophilic endotype and response to corticosteroids	73
Asthma	Peripheral blood ILC2 levels	No difference in peripheral blood ILC2 levels between patients with severe disease, patients with mild disease, and healthy controls.	47
	Peripheral blood ILC2 cytokine responses	Increased numbers of peripheral blood ILC2s and T <sub>H</sub> 2 cytokine responses in allergic asthmatics vs allergic rhinitis and controls	57
Atopic dermatitis	Skin ILC2 levels	Increased skin ILC2s in patients vs healthy individuals.	43
	Skin ILC2 levels and function	Increased skin ILC2s in patients vs healthy individuals; increased IL-33-mediated ILC2 T <sub>H</sub> 2 cytokine production that was inhibited by E-cadherin	12

Abbreviations: ILC2, group 2 innate lymphoid cell; SCIT, subcutaneous immunotherapy.



asthma. In many asthmatics, eosinophilic inflammation and increased levels of the  $T_H2$  cytokines IL-5 and IL-13 are present, and triggers include respiratory viruses and aeroallergens [2]. A 2009 report identified the presence of a non-B/non-T lymphocyte population that produced IL-5 and IL-13 in asthmatic sputum after airway challenge with allergen, suggesting a non-T-cell source of  $T_H2$  cytokines [6]. It is not known whether these cells are the same population as the ILC2s subsequently found in human lung and bronchoalveolar lavage fluid [28,47]. The first study to examine ILC2s in asthmatics found that peripheral blood ILC2 cells (lineage-negative CRTH2<sup>+</sup> IL-7R<sup>+</sup>) were similar in number in patients with severe asthma compared with mild asthmatics and healthy controls [47]. Interestingly, peripheral blood ILC2 levels varied significantly between patients (1.78% to 27.9% in healthy patients, 1.08% to 24.2% in mild asthmatics, and 1.08% to 17.8% in severe asthmatics), thus supporting the heterogeneity of peripheral ILC2 pools. The same study demonstrated that IL-13 production by peripheral blood ILC2s stimulated with IL-2, IL-25, and IL-33 was enhanced by PGD<sub>2</sub> and partially inhibited by LXA<sub>4</sub>. Additionally, c-kit<sup>+</sup> CD161<sup>+</sup> tryptase-negative cells (reported to be ILCs) in human lung were colocalized with mast cells and near small and medium size airways.

In contrast, a subsequent report showed that levels of peripheral blood ILC2s, defined as lineage-negative IL-7R<sup>+</sup> CRTH2<sup>+</sup> cells, were higher in patients with allergic asthma than in patients with allergic rhinitis and healthy individuals [57]. Of note, IL-25 and IL-33 induced greater peripheral blood cell production of IL-5 and IL-13 in allergic asthmatics than in other groups, suggesting functional consequences of having greater numbers and/or enhanced function of ILC2s. Heterogeneous patient populations and possibly differences in the allergic status of individuals may account for differences between the 2 studies. Although the role of lung ILC2s in asthmatic patients is not known, upstream cytokines and mediators (eg, IL-25, IL-33, TSLP, leukotrienes, and PGD<sub>2</sub>) are elevated in human asthma, suggesting an environment that favors activation of ILC2s that could promote disease [51,58-61]. The main studies of ILC2s in human allergic diseases and asthma are summarized in the Table.

## ILC2s in Experimental Asthma

Airway challenges with viruses or allergens in mice induce features of human asthma, including AHR, peribronchial inflammation, mucus production, and lung remodeling. Most asthma models to study ILC2s have utilized RAG knockout mice (which lack B and T cells but have ILC2s) or have evaluated ILC2 responses only during early innate responses. Although the contribution of ILC2s compared with other cell types during an ongoing adaptive response remains unknown, significant insight into lung ILC2 responses has been gained from mouse models.

### Viral Models of Lung Inflammation

Lung ILC2s from mice were first shown to contribute to AHR after influenza virus exposure [24]. The authors treated RAG2 knockout mice with anti-Thy1.2 antibody to

deplete ILC2s and showed that ILC2-depleted mice had reduced AHR after infection. It is noteworthy that transfer of wild-type ILC2s, but not IL-13-knockout ILC2s, into IL-13 knockout mice restored AHR, suggesting that production of IL-13 by ILC2s was required for influenza-induced AHR. In addition to promoting AHR, ILC2s have been shown to induce tissue repair responses after influenza infection [28]. The authors showed that depletion of ILC2s in RAG knockout mice infected with influenza virus resulted in hypoxemia, hypothermia, and loss of epithelial barrier integrity. ILC2 microarray analysis revealed that ILC2s produced large amounts of the epidermal growth factor receptor ligand amphiregulin. Adoptive transfer of ILC2s or administration of amphiregulin in ILC2-depleted mice restored airway integrity and lung function after influenza infection. Thus, ILC2s could play a role in promoting pathogenesis and repair responses after influenza infection.

Rhinovirus exposure is a primary cause of asthma exacerbation in humans and therefore a major cause of morbidity in asthmatics [62]. Aside from a role in exacerbations, viruses have also been implicated in the development of asthma [62,63]. A recent study investigated the effect of rhinovirus infection on ILC2 responses in neonatal mice and found that rhinovirus expanded IL-13-producing lung ILC2s [64]. ILC2 expansion, mucus metaplasia, and AHR were dependent on IL-25 and were not observed in infected adult mice. The increased ILC2s persisted for 3 weeks and were thus available for a more potent response to further stimulation that might lead to asthma features.

### Allergen-Induced Lung Inflammation

Since the discovery of ILC2s, several reports have shown that they contribute to type 2 lung inflammation and AHR in mice exposed to multiple allergens including ovalbumin (OVA), *Alternaria*, papain, and house dust mite [11,24,25,42,65,66]. The fungal allergen *Alternaria alternata* is associated with severe human asthma, and unsensitized mice challenged with even 1 dose of *Alternaria* develop rapid increases in IL-33, activation of lung ILC2s, and IL-33 dependent airway eosinophilia [11,26,65,67]. Our group also demonstrated that *Alternaria* enhances ILC2 activation during a conventional adaptive response to a completely different allergen (ryegrass), suggesting that high *Alternaria* exposure could worsen lung inflammation caused by a separate allergen [68]. *Alternaria* exposure in some asthmatics is associated with severe asthma symptoms, including fatal attacks, although the presence of rapid IL-33/ILC2 activation in humans exposed to *Alternaria* has not been demonstrated [67].

It is not clear whether ILC2s play a significant role during ongoing exposure to allergens in the presence of intact adaptive immunity. However, some studies have compared levels of different  $T_H2$  cytokine-producing cell types during early and late allergen challenges in mice [25,66]. One report showed that the number of lung IL-5<sup>+</sup> ILC2s was about half that of the IL-5-producing T cells after 1 and 3 challenges, but similar after 10 challenges with house dust mite [66]. One explanation could be that ILC2s require time for robust expansion (given their low numbers [10 000 to 20 000] in naïve mouse lung) to compete with the numbers of cytokine-producing T cells [11].

A separate study determined the numbers of CD4<sup>+</sup> and CD4<sup>-</sup> cells (ILC2s and other CD4<sup>-</sup> cells) that produced IL-4<sup>+</sup> and IL-13<sup>+</sup> cells in the bronchoalveolar lavage fluid of IL-4 and IL-13 reporter mice after a 12- and 25-day OVA model [25]. The IL-13<sup>+</sup> CD4<sup>-</sup> cell levels were 3 times that of CD4<sup>-</sup> in the 12-day model and 10 times greater in the 25-day model. These reports suggest that ILC2s continue to produce T<sub>H</sub>2 cytokines at later stages of type 2 inflammatory responses, although the absolute number of cytokine-producing ILC2s may be less than that of CD4<sup>+</sup> T<sub>H</sub>2 cells. The major limitation of these studies is that the methods did not enable detection of cytokine levels per cell, as ILC2s had previously been reported to have a larger capacity for T<sub>H</sub>2 cytokine production ( $\mu\text{g}/5000$  cells) than other cell types [9,69-72]. Therefore, a comparison of numbers of T<sub>H</sub>2 cytokine-producing cells may not be adequate to address the relative contribution of ILC2s during ongoing adaptive responses.

Severe asthma is associated with resistance to corticosteroids. The issue of whether ILC2s are corticosteroid-resistant has been investigated [2,73,74]. One report showed that ILC2s were not reduced in wild-type mice given dexamethasone after intraperitoneal sensitization with OVA/alum followed by IL-33 and OVA challenges [74]. However, ILC2s from TSLP receptor knockout mice showed partial sensitivity to dexamethasone, and stimulation of naïve lung ILC2s with TSLP in vitro also led to partial dexamethasone resistance. This suggests that TSLP confers partial ILC2 resistance to corticosteroids. Based on a model where IL-33, and not TSLP, is primarily active, we recently reported that systemic corticosteroids induce apoptosis of mouse lung ILC2s during repetitive intranasal *Alternaria* challenges [73]. Future studies will need to determine whether human ILC2s are refractory to corticosteroids under specific circumstances, as this could have implications for the pathogenesis of severe asthma.

## ILC2s in Human Chronic Rhinosinusitis

### Nasal Polyposis

Chronic rhinosinusitis (CRS) is characterized by inflammation of the nasal and paranasal mucosal surfaces and is often associated with allergic disease, asthma, aspirin sensitivity, and cystic fibrosis [75,76]. Nasal polyps occur in some patients with CRS and can be classified as either eosinophilic or noneosinophilic “endotypes” [76]. ILC2s are enriched in nasal polyps and are detected in higher numbers in the ethmoid mucosa of patients with nasal polyps than in those without polyps [10,77]. Additionally, a recent study demonstrated a positive association between nasal polyp ILC2s, allergic sensitization, and CD4<sup>+</sup> T<sub>H</sub>2 cells [78]. We recently showed that ILC2 counts were higher in eosinophilic polyps than in noneosinophilic polyps and that they were reduced in patients who had received systemic corticosteroids [73]. Together, these studies implicate ILC2s in CRS with eosinophilic nasal polyposis. Importantly, the cytokines TSLP and IL-33, as well as cysteinyl leukotrienes, have been found at higher levels in patients with CRS and are thus available for ILC2 activation [77,79,80].

### Allergic Rhinitis

Two studies have reported changes in ILC2s in the peripheral blood of patients with allergic rhinitis after allergen exposure [81,82]. The first study assessed changes in peripheral blood ILC2s 4 hours after cat allergen challenge in patients with allergic rhinitis who had positive cat challenge results [81]. The percentage of CRTH2<sup>+</sup> ILC2s was increased 2-fold after cat allergen challenge compared with a diluent control challenge given to the same patients at a separate visit. A subsequent report showed that peripheral blood ILC2s were increased during the pollen season in patients with allergic rhinitis induced by grass pollen and were reduced by subcutaneous immunotherapy [82]. The role of increased ILC2s in peripheral blood after allergen exposure is not clear, but may represent bone marrow egress of ILC2s bound for tissues that could then contribute to inflammatory responses. CRTH2 is present on human ILC2s and binds to PGD<sub>2</sub>, a lipid mediator that has a known role in the chemotaxis of immune cells. High serum levels of the major PGD<sub>2</sub> metabolite 9 $\alpha$ ,11 $\beta$ -PGF<sub>2</sub> are induced within 5 minutes of airway allergen challenge, suggesting that PGD<sub>2</sub> is rapidly available for systemic recruitment of CRTH2<sup>+</sup> cells after allergen exposure and could thus promote ILC2 chemotaxis in CRS [49,83].

## ILC2s in Human Atopic Dermatitis

Atopic dermatitis is a common chronic skin condition characterized by itchy scaly rashes together with skin barrier disruption, eosinophilic infiltration, and high serum IgE. TSLP and IL-33 as well as ILC2s have been detected at higher levels in the skin of atopic dermatitis patients compared with healthy controls, suggesting that ILC2s may also be involved in pathogenesis [12,43,84,85]. A recent study demonstrated that human skin ILC2s from diseased patients produce T<sub>H</sub>2 cytokines after stimulation with IL-33, but not TSLP or IL-25 [12]. Skin ILC2s have also been shown to express killer cell lectin-like receptor G1 (KLRG1), which binds to E-cadherin, an epithelial adhesion molecule that is downregulated in atopic dermatitis. Interestingly, E-cadherin directly inhibited ILC2 proliferation and IL-5 and IL-13 production, suggesting that healthy skin may inhibit ILC2 function and that the lack of E-cadherin–ILC2 interaction leads to uncontrolled inflammation in atopic dermatitis [12]. Finally, ILC2s accumulated in the skin of house dust mite–allergic patients who were challenged with intradermal house dust mite. Thus, ILC2s may play a role in atopic dermatitis, in part owing to reduced E-cadherin–mediated inhibition and increased allergen-induced recruitment.

## ILC2s in Skin Disease Models

Skin ILC2s are present in mice, and most of the IL-13–positive cells in the skin of IL-13 reporter mice are CD3<sup>-</sup> under homeostatic conditions [86]. Mouse models of atopic dermatitis are limited, but features of the disease that can be induced include eosinophilic infiltration, epidermal hyperplasia, dermal thickening, and systemic IgE production. ILC2s have been shown to contribute to dermal thickening and inflammation,

which are dependent on the TSLP receptor and independent of IL-33 signaling [43]. In this model, mice were administered the vitamin D analog MC903, which has previously been shown to induce high levels of TSLP, although it is unclear whether IL-33 is available and active [87]. In fact, skin-specific overexpression of IL-33 was also shown to induce atopic dermatitis-like disease with expansion of ILC2s and increased dermal eosinophilia and tissue  $T_{H2}$  cytokines [88]. Furthermore, administering IL-2 to RAG1<sup>-/-</sup> mice led to skin ILC2 expansion and  $T_{H2}$  cytokine production, as well as epidermal hyperplasia, dermal thickening, and eosinophilic infiltration [86]. These reports suggest that the presence of 1 or more upstream mediators including TSLP and/or IL-33 likely dictates the resulting ILC2 response, which could promote features of dermatitis.

## ILC2 Networks With Other Immune Cells

ILC2s have recently been shown to communicate significantly with several types of immune cells, including CD4<sup>+</sup> T cells, mast cells, basophils, and B cells. Apart from interactions with the several immune cell types detailed below, ILC2s may also have effects on structural cells, including epithelial cells, smooth muscle cells, and fibroblasts, as supported by secretion of amphiregulin by ILC2s, which was shown to promote lung epithelial tissue repair [28]. A better understanding of interactions between ILC2s and other cell types is critical to uncovering their role in allergic inflammation and may help us to bridge the gap between innate and adaptive type 2 immune responses.

### *ILC2s and CD4 T Cells*

The  $\gamma$  chain cytokines IL-2 and IL-7 support ILC2 proliferation and survival, and as conventional T cells are a source of IL-2, adaptive immune cells were initially thought to be involved in promoting ILC2 responses [9,42]. In support of this hypothesis, a recent study by Mirchandani et al [89] showed that activated naïve CD4<sup>+</sup> cells induced ILC2 proliferation and IL-2-dependent IL-4 and IL-5 production. ILC2s also express MHCII and the authors showed that ILC2s could act as MHCII-dependent antigen-presenting cells to stimulate T-cell responses in vitro. Another study showed that OX40/OX40L interactions and production of IL-4 by ILC2s promoted CD4 T-cell responses in vitro [90]. Although it is not clear whether contact-dependent interaction between ILC2s and CD4 T cells contributes to ongoing inflammatory responses in vivo, a very recent report has shown that IL-13 knockout mice receiving MHCII-deficient ILC2s during helminth infection are impaired in worm clearance and in generating an adaptive  $T_{H2}$  response compared with mice receiving wild-type ILC2s [91]. Further work in the same report showed that ILC2s can process and present antigen, as well as promote antigen-specific T-cell responses.

Production of IL-4 by ILC2s can initiate  $T_{H2}$  cell polarization, and production of IL-2 by T cells could reciprocally maintain ILC2 levels and activation [16,23,89,90]. Apart from IL-4 and direct contact, a recent report suggested that IL-13 produced by ILC2s was required for papain-induced

lung  $T_{H2}$  cell responses, possibly through control of dendritic cell migration [92]. The study utilized ROR- $\alpha$ -deficient bone marrow transplant mice that lack ILC2s but have normal  $T_{H2}$  cell responses, thus making it possible to test the intact T-cell response in an ILC2-deficient mouse. In a separate report, the same ROR- $\alpha$ -deficient bone marrow transplant mice were challenged with house dust mite and found to have reduced  $T_{H2}$  cell responses, suggesting that the ILC2- $T_{H2}$  cell axis may be more broadly important in several  $T_{H2}$  models [93].

### *ILC2s and Basophils*

Two recent reports suggest a link between production of IL-4 by basophils and activation of ILC2 in lung and skin inflammation models [94,95]. In the first report, the authors generated basophil-specific IL-4-deficient mice that had impaired eosinophilic lung inflammation, mucus production, and AHR after 3 papain challenges compared with wild-type mice. Furthermore, lung ILC2 activation and numbers were reduced in the mutant mice, suggesting that the ILC2s responded to basophil-produced IL-4. Lung ILC2s were previously known to express IL-4R $\alpha$  [11] and stimulation of ILC2s by IL-4 resulted in production of CCL11, CCL3, CCL5, and IL-9, as well as enhanced IL-5 and IL-13. A subsequent report showed that basophils were a dominant source of IL-4 in the inflamed skin of mice treated with the vitamin D analog MC903 [95]. Importantly, basophil-produced IL-4 was required for ILC2 accumulation and proliferation in skin. Therefore, induction of ILC2 responses by basophil-produced IL-4 promotes early type 2 responses in lung and skin disease models, although it is not known whether this pathway is active during ongoing inflammation.

### *ILC2s and Mast Cells*

Mast cells and ILC2s have been reported to be colocalized in mouse dermis, and close interactions between both cell types were visualized for up to 30 minutes using intravital microscopy [86]. The report went on to show that production of IL-6 and TNF- $\alpha$  by mast cells was reduced by IL-13, an ILC2 product that could dampen mast cell proinflammatory responses. ILC2s and mast cells have also been detected in close proximity in human lung, suggesting that this interaction is neither tissue- nor species-specific [47]. The close proximity of ILC2s and mast cells could be due to production of PGD<sub>2</sub> by mast cells, which induces the chemotaxis of ILC2s observed in humans [48,49]. ILC2s also produce IL-9, which could lead to accumulation of mast cells in tissues [27].

### *ILC2s and B Cells*

ILC2s in the mesenteric fat of mice were initially reported to produce IL-5 and IL-6 that led to IgA antibody secretion by B1 B cells [9]. A subsequent study reported an IL-18 receptor-expressing ILC2-like population (not expressing IL-7R or IL-33R) in mouse spleen that promoted B-cell IgE production after coculture studies and stimulation with IL-18 [96]. Additionally, ILC2s express the inducible T-cell costimulator molecule (ICOS) and could interact with ICOS ligand-expressing B cells, although the significance of this possible interaction is unknown [7]. Finally, as ILC2s can be a source of IL-4 in the



presence of leukotrienes and TSLP, B cells could be induced to class switch to IgE through IL-4R signaling [16,23].

## Summary

The wealth of recent information about ILC2s has improved our understanding of the mechanisms that contribute to allergic diseases, including rhinosinusitis, asthma, and atopic dermatitis. ILC2s rapidly and robustly produce  $T_H2$  cytokines in tissues and are found on the skin and many mucosal surfaces including those of the respiratory tract. Animal models have shown that ILC2s promote features of asthma and atopic dermatitis. ILC2s have also been reported to initiate  $T_H2$  cell sensitization and may play a broader role in the development of allergic diseases. Targeting ILC2s as a therapeutic strategy in human allergic diseases may be appealing, but many challenges exist, including the lack of ILC2-specific markers or targets. Furthermore, targeting upstream cytokines, including IL-33, TSLP, and PGD<sub>2</sub>, will likely have effects on multiple cell types or be insufficient to affect ILC2 responses owing to redundancy. Importantly, unintended consequences of targeting ILC2 repair or homeostatic functions may result in untoward side effects. The hope is that further exploration into the role of ILC2s in human health and disease will lead to novel pathways, targets, and drug discovery that will eventually produce treatments for patients who desperately need them.

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## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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