

Type 2 Innate Lymphocytes in Allergic Airway Inflammation

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Abstract CD4⁺ T helper-2 (Th2) cells, which produce a unique profile of IL-4, IL-5 and IL-13 pro-inflammatory cytokines, are thought to be central in the orchestration and amplification of allergic asthma. However, a novel non-T/non-B lymphoid cell population, named type 2 innate lymphocytes (ILC2s), that produces high amounts of IL-5 and IL-13 was recently discovered. Unlike Th2 cells, these ILC2s are not antigen-restricted and are activated by epithelial cell-derived cytokines IL-25 and IL-33. In this review, we will focus on recent studies, mainly involving allergen-based mouse models, that have provided evidence for a significant contribution of ILC2 to allergic airway inflammation.

Keywords Innate lymphoid cells (ILC) · Allergic airway inflammation · Mouse models · Cytokines · IL-5 · IL-13 · IL-25 · IL-33 · TSLP · Gata3 · HDM · House dust mite · Influenza · Type 2 innate lymphocytes

Introduction

Asthma is a disorder of the conducting airways affecting over 300 million people worldwide [1, 2]. The disease is characterized by chronic airway inflammation and hyperreactivity

(AHR) leading to shortness of breath, coughing and recurrent wheezing, and often presents at childhood. Multiple pathogenic environmental factors and more than 100 major and minor susceptibility genes have been identified [3–5]. Patients manifest symptoms of asthma in response to inhalation of airborne allergens, such as house dust mite (HDM), molds, or animal dander [1, 2]. In addition, asthma can be induced by virus infection, obesity, exercise, cold air or exposure to air pollution, ozone, cigarette smoke, and diesel exhaust particles.

Many cell types participate in allergic inflammation, including innate and adaptive immune cells, epithelial cells, smooth muscle cells, and fibroblasts. Although various T cell subsets, including Th17 cells, NKT cells, CD8⁺ T cells, and regulatory T cells, show involvement in asthma pathogenesis (recently reviewed in [6]), allergen-specific T helper type 2 (Th2) are thought to be central in the orchestration of allergic asthma. They regulate production of the key type 2 inflammatory cytokines IL-4, IL-5, and IL-13 that fulfill specific roles in asthma: IL-4 induces IgG1 and IgE class switching in allergen-specific B cells, IL-5 recruits eosinophils, and IL-13 triggers mucus production and AHR. In addition to this trio of classic Th2 cytokines, IL-9 and GM-CSF play important roles, as they regulate differentiation and maturation of mast cells (IL-9), maturation and survival of eosinophils, and recruitment of basophils (GM-CSF) [7].

Next to Th2 cells, innate cells such as mast cells, eosinophils and basophils also produce Th2 cytokines. Most importantly, a novel population of non-B/non-T innate lymphoid cells (ILC) that have the capacity to produce large amounts of IL-5 and IL-13 was recently identified in the intestine and in the lung. Because of their Th2 cytokine production, they have been named type 2 innate lymphoid cells or ILC2. These ILC2 belong to the family of developmentally related innate lymphocytes [8•]. For a long time, NK cells and lymphoid tissue inducer (LTi) cells were thought to be the only members of this family, but recent research has shown that there are more innate lymphocytes that play an important role mainly in mucosal homeostasis. It was recently proposed that ILC should be categorized into

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three groups based on their cytokine profile: group 1 ILC contains IFN γ -producing ILC1 and NK cells, group 2 contains the Th2 cytokine-producing ILC2, and group 3 ILC includes LT α i cells, which have been identified almost two decades ago [9, 10] and more recently identified innate lymphocytes that produce IL-17A and IL-22 [11]. The developmental relationships between the various ILC lineages, their function in protective immunity, and their role in immune pathology have very recently been reviewed [8•]. Here, we will focus on recent studies that have provided evidence for a significant contribution of ILC2 to allergic airway inflammation, in particular in mouse models.

Thymic Stromal Lymphopoietin (TSLP), IL-25 and IL-33

Allergens do not directly act on T cells or ILC2. Differentiation of Th2 effector cells from naïve T cells requires intermediate players that sample the airway lumen, process and present antigens, and produce cytokines and chemokines that instruct other immune cells. IL-4 has widely been recognized as the key instructing cytokine for Th2 differentiation. Likewise, ILC2 activation is dependent on a specific group of cytokines that are often produced by non-immune cells: TSLP, IL-25, and IL-33. These cytokines were shown to have crucial roles in allergic asthma, because they act as important instructing cytokines in type 2 immunity.

TSLP. TSLP was originally discovered in media of a thymic stromal cell line that supported B development and thymocyte proliferation [12]. While its name suggests otherwise, TSLP is mainly expressed by epithelial cells lining the skin and mucosal surfaces. Its expression is regulated by pro-inflammatory cytokines, such as IL-1 α , IL-1 β , and tumor necrosis factor (TNF)- α , and involves NF- κ B signaling [13]. Many Toll-like receptor (TLR) agonists, allergen sources, and chemicals induce TSLP expression by epithelial cells (reviewed in [14]). The functional TSLP receptor consists of a dimer of the TSLPR chain and the IL-7R α chain (shared with the IL-7 receptor). The common gamma chain (γ c), which is required for the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, is thus not required for TSLP binding and function. In addition to JAK/STAT signaling, TSLP also induces activation of NF- κ B. TSLP, which is highly expressed in the epidermis in skin lesions of patients with atopic dermatitis [14], has often been implicated in allergic asthma. Genome-wide association studies (GWAS) have associated TSLP with an increased asthma risk [3–5]. In mouse models of allergic asthma, TSLP was found at increased levels and blockade or targeted deletion of the TSLPR reduced allergic airway inflammation [15–17].

IL-25. Interleukin-25 is also known as IL-17E and was originally discovered as a cytokine secreted by Th2 cells [18]. Epithelial cells, basophils, eosinophils, and mast cells are sources of IL-25 [19–22]. The receptor for IL-25 is constituted by IL-17RA (shared with the receptor of IL-17A) and IL-17RB (shared with the receptor of IL-17B). IL-25 activates NF- κ B signaling, MAPK, AP-1, and C/EBP via recruitment of Act1 and TRAF6 [23]. The initial description of IL-25 included a demonstration of its potency as a cytokine that induces type 2 immunity: intra-peritoneal injection of IL-25 leads to eosinophilia, increased mucus production, and epithelial cell hyperplasia, probably mediated by increased levels of IL-4, IL-5, and IL-13, and accompanied by increased serum IgE, IgG1, and IgA [18]. Several studies have linked IL-25 to airway inflammation, showing that overexpression of IL-25 in airway epithelium induces a type 2 response [19] and that intranasal administration of IL-25 induces AHR [24]. Likewise, administration of anti-IL25 antibodies or *Il25*-deficiency in mice reduced airway inflammation [24–26]. Furthermore, polymorphisms in the human *IL17RB* gene have been associated with asthma [27].

IL-33. In an attempt to discover distinct markers for Th1 and Th2 cells, two receptors for IL-1 family members were discovered: Th1 cells selectively express IL-18R and Th2 cells selectively express ST2 [28]. Later on, ST2 was recognized as the unique chain of the IL-33R [29]. The IL-1 receptor accessory protein (IL-1RAcP) is the other chain of the functional IL-33R [30, 31]. Like other IL-1 family members, the IL-33 signaling pathway signals via the MyD88 pathway that recruits IRAK1 and IRAK4, leading to downstream phosphorylation of several signaling molecules, including ERK1/2, p38, and NF κ B [29]. ST2 is not only expressed by TH2 cells, but also by many innate immune cells: mast cells, eosinophils, and basophils all increase cytokine and chemokine production upon IL-33 stimulation [32–34]. IL-33 also impacts directly on naïve Th cells and polarizes to a typical Th2 phenotype, characterized by IL-5 and IL-13 production, but not IL-4 [35, 36]. In polarized Th2 cells, IL-33 stimulation can induce cytokine expression without TCR triggering [37]. Numerous studies in humans and mice show that IL-33 is widely expressed in stromal cells of most organs including lung, and in immune cells (e.g., macrophages, DCs, and mast cells) and in epithelial cells, fibroblasts, and smooth muscle cells [38]. Importantly, IL-33 expression increases in inflammatory conditions: in macrophages, DCs, and fibroblasts, TLR stimulation increases *Il33* mRNA expression [39], and in epithelial cells, TLR4 signals induces the release of IL-1 α , which then acts in an autocrine manner to trigger IL-33 release [40]. There is increasing evidence that IL-33 is not secreted as a classical cytokine, but rather acts as an ‘alarmin’ that is released in necrotic cells [41]. Interestingly, IL-33 levels correlate with

asthma severity [42, 43] and genetic variants of IL-33 or IL-33R have been associated with susceptibility to asthma in humans [3–5]. Various groups have assessed the potential of IL-33 as a trigger for allergic disease, and demonstrated activation of eosinophils [44], macrophages [35], DCs [40], and Th2 cells [35]. Studies using IL-33-deficient animals showed that the development of AHR was abolished compared to control animals [45]. Blocking of the IL-33 receptor using T1/ST2 neutralizing antibodies or fusion proteins decreased allergic airway inflammation [46].

ILC2 and Cytokine-Mediated Airway Inflammation

The major source of type 2 cytokines in most models of murine asthma is the population of allergen-specific Th2 cells. However, Rag1/Rag2-deficient mice that lack mature B and T cells can still produce substantial amounts of type 2 cytokines and induce an asthma phenotype. In 2001, the first observation of a non-B/non-T cell population that responded to IL-25 treatment by production of IL-5 and IL-13 was made by Fort et al. [18]. Subsequent reports have shown a similar population that responded to IL-33 treatment [35, 47]. These publications preluded the discovery of a novel family of cytokine-producing lymphocytes that lack classic lineage markers, but that, among other surface markers, express the lymphocyte-associated receptors for IL-2 and IL-7. Multiple groups identified lineage-negative lymphocytes that produce large amounts of IL-5 and IL-13, but little IL-4. These lineage-negative lymphocytes were studied in the context of helminth immunity in the intestine, and were termed natural helper cells [48•], nuocytes [49•], or type 2 multipotent progenitors [50•]. Phenotypically, the different populations show major similarities, and are now referred to as group 2 ILC [11]. Interestingly, studies that quickly followed the initial descriptions of ILC2 in the intestine described a similar population of type 2 cytokine-producing innate lymphoid cells in the pulmonary tract of man and mice [51•, 52•, 53•]. ILC2 were studied in many models of respiratory inflammation, including cytokine-based lung inflammation, various allergic airway inflammation models, pulmonary influenza or helminth infection, as well as in patients with chronic allergic rhinitis (summarized in Table 1).

ILC2 are identified as lineage-negative cells that, among other markers, express the receptors for the cytokines IL-7 (IL-7R α and c γ), IL-25 (IL-17RA and IL-17RB), and IL-33 (ST2 and IL1RAcP) [11, 54]. ILC2 are present in Rag1/Rag2-deficient mice, but absent in mice that additionally lack c γ expression. While the absence of ILC2 from *Il7ra*^{-/-} and the strong reduction of ILC2 in *Il7*^{-/-} mice indicates that IL-7 probably functions in development and homeostasis of ILC2

[48•, 51•, 55•], IL-25 and IL-33 were shown to induce proliferation and production of the effector cytokines IL-5 and IL13 [55•, 56•, 57•, 58•, 59•, 60•] (Fig. 1).

Local or systemic administration of IL-25 or IL-33 to mice induced the expansion of ILC2 in lungs, BAL fluid, and mediastinal lymph nodes [55•, 56•, 57•, 58•, 59•, 60•]. While both IL-25 and IL-33 can induce proliferation of and cytokine expression by ILC2, IL-33 induces ~5-fold more ILC2 than a similar dose of IL-25 [60•]. This response is independent of B and T lymphocytes, as similar levels of airway inflammation, eosinophil recruitment, and cytokine production were observed in Rag1/Rag2-deficient mice compared with wild-type mice [55•, 56•, 58•, 59•]. In addition, experiments in which wild-type ILC2 were transferred to *Rag2*^{-/-}*Il2rg*^{-/-} double-deficient animals show that ILC2 are sufficient to induce airway inflammation in response to IL-33 [61]. Furthermore, we found that IL-25 and IL-33 substantially increased the fraction of cytokine-producing ILC2 [58•]. Transfer experiments illustrated that ILC2-derived IL-13 is required for AHR induction in cytokine-induced airway inflammation models, as transfer of wild-type but not *Il13*^{-/-} ILC2 to *Il13*-deficient mice could restore IL-25-induced AHR [59•, 60•].

ILC2 in Murine Allergen-Based Airway Inflammation Models

Our understanding of the pathogenesis of allergic asthma has greatly advanced thanks to the development of various animal models for asthma. The initially studied models included mice that were sensitized to protein antigens, such as ovalbumin (OVA). Subsequently, models based on more physiological antigens such as house dust mite (HDM) have been developed. Various allergen-based mouse models contributed to our understanding of the role of ILC2 in allergic airway inflammation.

OVA-Induced Asthma. OVA-induced airway inflammation is a long-used model for studying allergic inflammation and is thought to mimic important aspects of human allergic asthma. Barlow et al. [60•] observed that the numbers of ILC2 in BAL and lung increased in an OVA-asthma model. OVA-asthma models in IL-4- and IL-13-reporter mice showed that a substantial proportion of IL-13-producing cells were ILC2, while most IL-4-producing cells were lineage-positive cells. Also, our analysis of the IL-5⁺ and IL-13⁺ populations in OVA-induced airway inflammation showed a similar number-wise contribution of ILC2 and Th2 in the lung, while ILC2 produced very little IL-4 [58•].

In an OVA-asthma model, Taube and colleagues [62] found that IL-22 increased upon allergen exposure and was mainly produced by a population resembling intestinal

Table 1 ILC2 in pulmonary inflammation models

Mouse		
Allergen-based models		
Allergen (type)	Mechanism	References
Papain (protease)	ILC2-dependent eosinophilia and AHR. IL-2-dependent IL-9 production drives IL-5 and IL-13 production.	[56•, 57•]
<i>Alternaria alternata</i> (fungus)	ILC2 required in initial, innate phase of inflammation.	[55•, 65]
House dust mite	ILC2 required for inflammation in acute response. During challenges, similar numbers of IL-5 ⁺ /IL-13 ⁺ ILC2 and Th2	[56•, 57•, 58•]
<i>Sphingomonas</i> spp. (Gram-negative bacteria)	NKT cells activated by Cd1d-ligands from <i>Sphingomonas</i> spp. directly induce AHR via IL-13 and ILC2/MΦ activation via IL-33.	[59•]
Ovalbumin	Similar contribution of ILC2 and Th2 to IL-5 and IL-13 production. Limited IL-4 production by ILC.	[58•, 59•, 60•]
Influenza virus infection models		
Influenza strain	Mechanism	References
Influenza PR/8 (H1N1)	ILC2 crucial for tissue repair by production of EGF-family member amphiregulin	[52•]
Influenza H3N1	IL-13 from ILC2 induces AHR, irrespective of adaptive immunity	[51•]
Other pulmonary inflammation models		
Model	Mechanism	References
Cytokine-based IL-25/33	Strong expansion of ILC2 and induction of cytokine production, mainly IL-5 and IL-13, but also IL-4.	[55•, 56•, 57•, 58•, 59•, 60•]
<i>Nippostrongylus brasiliensis</i> (helminth)	After <i>N. brasiliensis</i> infection, pulmonary ILC2 (and CD4 ⁺ T cells) produce IL-13 in a Gata3-dependent fashion	[92]
<i>Strongyloides venezuelensis</i> (helminth)	Pulmonary <i>S. venezuelensis</i> stimulates alveolar epithelial cells to produce IL-33. ILC2 accumulate and produce IL-5 and IL-13 in response to IL-33.	[97]
Human		
Model	Mechanism	References
n/a	Mechanism unknown; ILC2 are found in lung, intestine and peripheral blood. ILC2 are increased locally in chronic rhinitis patients.	[53•, 65]
n/a	Mechanism unknown; ILC2 are found in lung and BAL fluid of lung transplantation patients.	[52•]

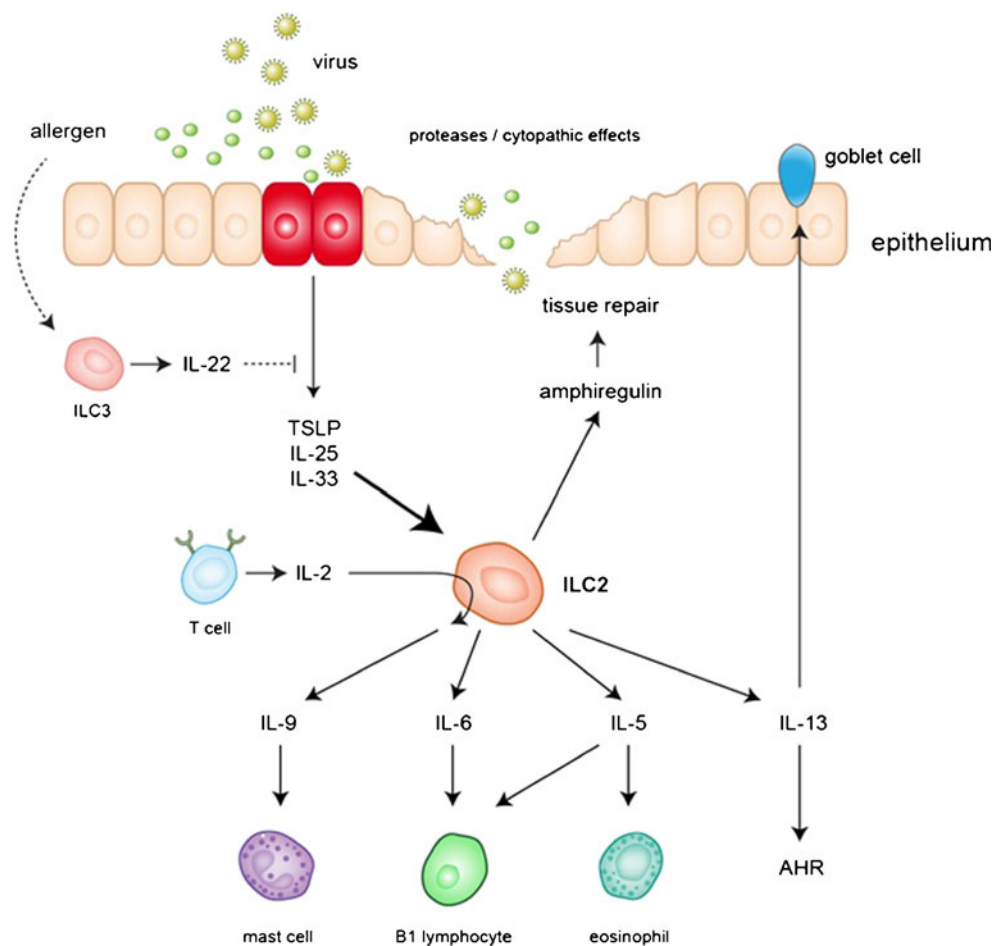
IL-22⁺RORγt⁺ ILC3. Administration of recombinant IL-22 to ovalbumin-exposed mice reduces AHR, eosinophilia, and IL-13 levels, while *Il22*^{-/-} mice show an aggravated asthma phenotype, including higher levels of IL-33. ILC3 may thus dampen airway inflammation via the effects of IL-22 on epithelium, which reduces IL-33 secretion and subsequent activation of Th2 cells and ILC2 (Fig. 1). The mechanisms by which ILC3 are activated in allergic airway inflammation remain to be elucidated.

HDM-Induced Asthma. Although HDM-based airway inflammation models classically involve a sensitization and a challenge phase, HDM also induces an acute inflammatory reaction. Halim et al. [56•] administrated 3 daily doses of 100 μg HDM to WT, *Rag1*^{-/-}, and *Rag2*^{-/-}*Il2rg*^{-/-} mice. Analysis the next day showed increased mucus production and a mild increase in BAL eosinophils, only when ILC2

were present (in WT and *Rag1*^{-/-} mice). This effect could be abolished by depletion of ILC2 using an anti-CD25 antibody in *Rag1*^{-/-} mice. This data suggests that ILC2 may play a role in the early, innate response to HDM, providing an early source of Th2 cytokines, although it remains unclear what cytokines were produced in this model.

We compared the contribution of ILC2 and Th2 cells in a classic HDM model in which mice were sensitized and then challenged 1, 3, or 10 times with HDM [58•]. We found that the number of ILC2 increased with increasing numbers of challenges in both lung and BAL. ILC2 substantially contributed to IL-5 and IL-13 but not to IL-4 production in lung and BAL. Together with the finding that IL-5 transcription levels per cell were very high in ILC2, compared with Th2 cells, we concluded that ILC2, next to Th2 cells, are important cytokine producers in HDM-induced asthma [58•].

Fig. 1 The multifaceted role of ILC2 in airway inflammation. Upon allergen exposure or influenza virus infection, the epithelium and immune cells that guard the epithelial wall rapidly produce substantial amounts of IL-25, IL-33, and TSLP. These cytokines induce activation and proliferation of ILC2. The ILC2 contribute to allergic airway inflammation by production of IL-5 and IL-13 that induce eosinophil recruitment and AHR, respectively. If ILC2 are co-stimulated with IL-2 derived from T cells, ILC2 can transiently produce IL-9 that will activate mast cells. ILC2 may also serve protective roles in influenza infection by stimulating tissue repair via amphiregulin and through stimulation of B1 lymphocytes that produce virus-neutralizing antibodies. Upon allergen exposure, lung ILC3 may be stimulated to produce IL-22, which limits IL-33 production



Fungal Allergen-Induced Asthma. Fungal allergens, especially *Alternaria alternata* are clinically and epidemiologically associated with asthma [63–65]. When exposed to *A. alternata*, BALB/c and *Rag1*^{−/−} or *Rag2*^{−/−} mice responded with classical allergic lung inflammation to a similar level in the early phase, resulting in detectable eosinophilia within 12 h of inhalation [55•, 65]. In BALB/c mice only, airway eosinophilia further increased on day 5. This indicates that the adaptive immune system is involved at later time points to further amplify the initial innate response. Interestingly, using *Alternaria* infection experiments in *Il7r*^{−/−} mice or adoptive transfers of ILC2 to *Il7r*^{−/−} mice that lack ILC2, it was shown that *A. alternata*-mediated airway inflammation is probably mediated mainly by ILC2, although a contribution of other *Il7r*-dependent populations cannot be excluded [55•, 65].

Glycolipid-Mediated Airway Inflammation. Glycolipid antigens that can stimulate NKT cells have been found in pollen, house dust and bacteria [66–69]. Administration of the NKT ligand α -GalCer to wild-type mice rapidly induces AHR and airway inflammation. This response relies on IL-33 produced by alveolar macrophages, DCs and type II pneumocytes [45, 46]. Interestingly, in vitro experiments show that α -GalCer-activated NKT cells, but not Th2 cells, stimulate macrophages

to produce IL-33 that induce activation and expansion of ILC2 [59•]. *Sphingomonas* bacteria are often found in poorly controlled asthma and have been associated with AHR [70, 71]. Glycolipid antigens from *Sphingomonas* are known to activate NKT cells that produce both IL-13 and IL-33. Kim et al. [59•] showed that most IL-13 produced in *Sphingomonas*-challenged mice derived from ILC2 that relied on NKT-derived IL-33 for their activation and expansion. Innate-like NKT cells thus serve a double role in asthma pathogenesis: they directly contribute to AHR by producing IL-13 and via IL-33 they activate other cells including ILC2 (Fig. 1).

Papain-Induced Airway Inflammation. The protease papain is a known cause of occupational asthma [72] and was reported to cause asthma symptoms independent of B or T lymphocytes [45]. Halim et al. [56•] demonstrated that papain-induced airway inflammation (characterized by eosinophilia, increased levels of IL-5 and IL-13 in BAL fluid, and mucus production) can be mediated by ILC2. Depletion of ILC2 from *Rag1*^{−/−} mice using an anti-CD25 antibody abolished the papain-induced inflammatory response, while transfer of ILC2 to *Rag2*^{−/−}*Il2rg*^{−/−} mice restored this response.

Wilhelm et al. [57•] employed an IL-9-reporter mouse in a papain-induced model of airway inflammation to show that

IL-9 is strongly induced upon administration of the protease. Interestingly, most IL-9 derived from ILC2 cells that appeared to lack IL-7R α expression. Kinetic experiments showed that IL-9 production by ILC2 was rapidly lost while cells continued to express IL-5 and IL-13. The authors went on to show that the observed IL-9 production depended on IL-2 derived from adaptive immune cells. Whether IL-9 production is a common trait of ILC2, or whether expansion of this population is confined to papain-based models of allergic airway inflammation, remains to be shown. Next to its function as a growth factor for mast cells, IL-9 was also shown to induce the production of IL-22 [73].

ILC2 in the Human Respiratory System

Studies of human lung tissue or BAL fluid from lung transplantation patients identified a population of lineage-negative CD25⁺CD127⁺ST2⁺ lymphocytes, thus sharing important cytokine receptors with murine ILC2 [52•, 53•]. Human ILC2 also express the NK cell marker CD161 and CCR2 [53•, 65], a chemoattractant that is also expressed by Th2 cells [74]. Human ILC2 were also detected in fetal tissues, including lung and intestine, and at low frequencies in peripheral blood, suggesting that they recirculate. Similarly, Monticelli et al. [52•] identified Lin⁻ populations in healthy lung parenchyma and BAL fluid from patients that underwent lung transplantation. Mjösberg et al. [53•] reported that ILC2 were increased locally in nasal polyps of chronic rhinitis patients compared with healthy controls. *IL5* and *IL13* levels are increased in patients compared to healthy controls, suggesting that ILC2 are an important source of type 2 cytokines. Nasal polyp epithelial cells expressed TSLP, which enhanced type 2 cytokine production in ILC2s [75]. Although chronic rhinitis belongs to the ‘Th2 diseases’, it remains to be shown whether ILC2 play a role in human asthma.

ILC2 in Influenza Virus Infection Models

Influenza virus infection induces IL-33 production in the lung [52•, 76]. Chang et al. [51•] used influenza A virus subtype H3N1 to show that influenza induces IL-13-mediated AHR and airway inflammation, irrespective of the presence of Th2 cells. Instead, the ILC2 population expanded ~5-fold in response to IL-33 production by alveolar macrophages and were responsible for most of the IL-13 produced. Whereas *Il13*^{-/-} animals or ILC2-depleted animals were protected from AHR, adoptive transfer of IL13-competent ILC2 to *Il13*^{-/-} hosts restored the AHR phenotype. These findings indicate that ILC2 are responsible for IL-13 production and AHR in response to H3N1 influenza virus infection.

The ILC2 population was also substantially increased in H1N1 PR/8 influenza virus infection, but was shown to fulfill a protective role [52•]. ILC2 depletion impaired lung function and tissue repair, leading to decreased integrity of lung epithelium. This protective function of ILC2 is independent of IL-13 and IL-22, but instead relies on the production of the epidermal growth factor family member amphiregulin, which was enhanced by IL-33 stimulation. Amphiregulin has been linked to tissue remodeling and repair in various diseases of acute epithelial damage and asthma [77–80]. In particular, levels of amphiregulin in BAL were found increased as early as 3 h after a single allergen challenge with the fungal allergen *Alternaria*, while surface amphiregulin expression on ILC2 was increased after 6 h [65]. The seeming contradiction in the detrimental and beneficial roles for ILC2 [51•] may be caused by the properties of the virus proper. H1N1 virus infection has not been reported to cause AHR [52•, 81], while this was demonstrated for H3N1 infection [51•]. Interestingly, experiments in mice show that B1 lymphocytes are the most important producers of IgM in influenza virus infection and are crucial for control of infection [82]. While most ILC2 reports focus on the production of IL-5, ILC2 also produce IL-6 [48•]. IL-5 is an essential growth factor for B1 cells [83] and IL-6 functions as a general B cell growth factor [84, 85]. ILC2 may thus serve another, indirect protective role in influenza virus infection by the stimulation of B1 cells via production of IL-5 and IL-6 (Fig. 1).

An interesting hypothesis to test is whether ILC2 play a role in asthma exacerbation, which is often triggered by exposure to respiratory pathogens and an important cause of morbidity often requiring hospitalization [86].

ILC2 Development

All ILC lineages arise from common lymphoid progenitors (CLP) and, because ILC are absent in mice deficient for the helix-loop-helix transcription factor Id2, their development is dependent on Id2 (reviewed in [8•, 87]). In addition, ILC2 appear to require Notch signals for their generation: Wong et al. [88] described that ILC2 can be cultured in vitro from CLP in the presence of IL-7 and IL-33 using OP9 cells expressing Notch ligands (OP9 Δ 1). Signaling via IL-25 and IL-33 is important for ILC2 development, although not absolutely required. Importantly, ROR α and Gata3 have been implicated as essential transcription factors for ILC2 development.

ROR α . ILC2 express high levels of the transcription factor ROR α but not of the structurally related factor ROR γ t [88, 89]. ROR α -deficient ‘staggerer’ mice suffer from severe neurological defects and succumb soon after weaning [90]. Using BM chimeras, it was shown that ROR α was required in a cell-intrinsic manner for IL-25-induced expansion of ILC2 and mediation of type-2 immunity [88]. In in vitro cultures, ILC2

development from *Rora*^{-/-} CLP was very inefficient, although not completely abolished. Takei and co-workers [89] reported >10-fold reduction of ILC2s in lung and intestine of *Rora*^{-/-} mice. After adoptive transfer of BM into *Rag2*^{-/-}*Il2rg*^{-/-} mice, most lymphocytes, including Th2 cells, developed normally, but ILC2 numbers were significantly reduced. Challenge of ROR α -deficient mice with the allergen papain failed to induce eosinophilia and provoked less mucus production. By contrast, ROR γ t-deficient mice have normal numbers of ILC2 [48•, 49•]. These studies clearly demonstrate an important role for ROR α but not ROR γ t in ILC2 development and function. Interestingly, ILC2 are not completely absent in *Rora*-deficient mice; a possible explanation is that the function of ROR α is partly compensated by low-level ROR γ t expression.

Gata3. Gata3 acts as a key regulator for differentiation of Th2 effector cells: it orchestrates chromatin remodeling in the Th2 cytokine locus, thereby inducing high-level expression of IL-4, IL-5, and IL-13, and concomitantly suppresses Th1 development [91]. In the thymus, Gata3 is also essential for early stages of T cell development [91]. One report shows that Gata3 is required for the expression of IL-13 following infection with the helminth *Nippostrongylus brasiliensis* [92]. In a second report, Diefenbach and colleagues use an inducible knockout for *Gata3* to show that ILC2 development requires Gata3 in a cell-intrinsic fashion [93••]. A third report investigated the function of GATA-3 in human ILC2 and found that GATA3 controls expression of the cytokine IL-13 and the receptors for IL-33 and TSLP [75].

Conclusions

Studies in various mouse models have demonstrated that ILC2 constitute a significant and early source of type 2 cytokines in allergic airway inflammation. Nevertheless, the pathogenic role of ILC2 in conjunction with Th2 cells in asthma sensitization, challenge, and exacerbation remains unclear. Also, spatial distribution of ILC2 and the chemokines that attract them to the lungs during allergic inflammation remain to be elucidated. Because ILC2 accumulate in the lung after influenza infection in mice (thereby inducing hyperreactivity and promoting tissue repair), it is crucial to investigate whether ILC2 play a crucial role in virus infection-induced asthma exacerbations. Apart from studies in allergic rhinitis, no information exists on the contribution of ILC2 to type 2 conditions in humans. The central role of the Th2 pathway in allergic asthma prompted the development of biological targeting of the key type 2 cytokines in asthma, but the overall efficacy of humanized antibodies directed against IL-4, IL-5, and IL-13 appeared low [94]. As targeting the key activators of ILC2 (i.e. IL-25 and IL-33) is beneficial in mice, it is attractive to develop therapies that inhibit these cytokines in asthma patients.

Furthermore, it has already been shown that the glucocorticoid fluticasone suppresses translocation of GATA3 from the cytosol to the nucleus [95] and that inhibiting Gata3 expression by knock-down strategies reduces allergic inflammation in mice [96]. Therefore, the identification of ROR α and GATA-3 as key factors for ILC2 could open a road for the development of small molecule inhibitors of these transcription regulators as for therapeutic application in allergic asthma.

Conflict of Interest Roel G.J. Klein Wolterink declares that he has no conflict of interest.

Rudi W. Hendriks declares that he has no conflict of interest.

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