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Group 2 Innate Lymphoid Cells: Team Players in Regulating Asthma

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Abstract

Type 2 immunity helps protect the host from infection, but it also plays key roles in tissue homeostasis, metabolism, and repair. Unfortunately, inappropriate type 2 immune reactions may lead to allergy and asthma. Group 2 innate lymphoid cells (ILC2s) in the lungs respond rapidly to local environmental cues, such as the release of epithelium-derived type 2 initiator cytokines/alarmins, producing type 2 effector cytokines such as IL-4, IL-5, and IL-13 in response to tissue damage and infection. ILC2s are associated with the severity of allergic asthma, and experimental models of lung inflammation have shown how they act as playmakers, receiving signals variously from stromal and immune cells as well as the nervous system and then disseminating cytokine cues to elicit effector functions and potentiate CD4⁺ T helper cell activation, both of which characterize the pathology of allergic asthma. Recent breakthroughs identifying stromal- and neuronal-derived microenvironmental cues that regulate ILC2s, along with studies recognizing the potential plasticity of ILC2s, have improved our understanding of the immunoregulation of asthma and opened new avenues for drug discovery.



1. INTRODUCTION

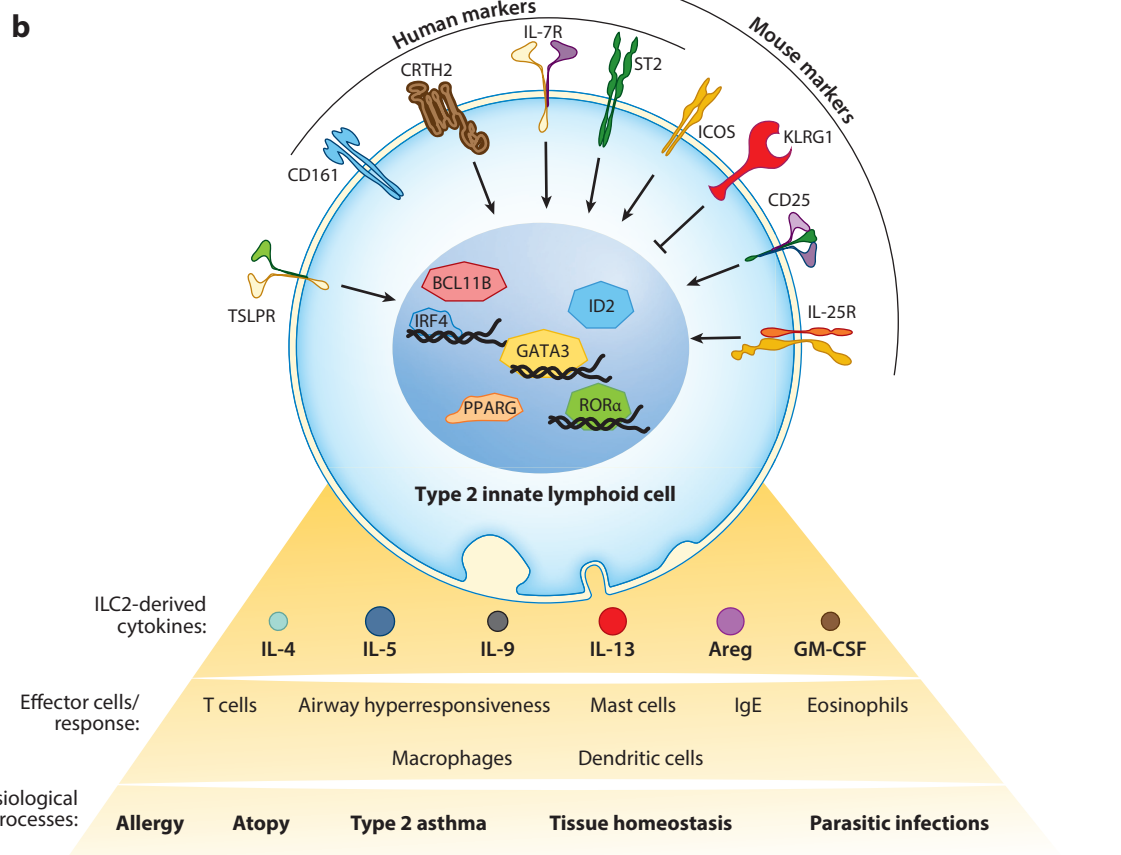
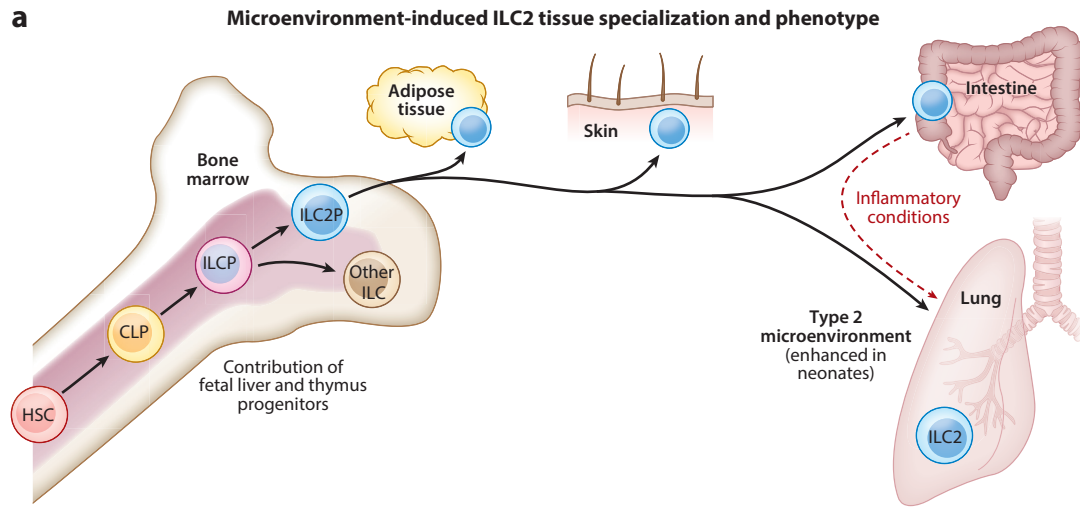
Asthma is a heterogeneous respiratory disease characterized by chronic lower airway inflammation, airway hyperresponsiveness (AHR), and airway remodeling. According to World Health Organization statistics, approximately 235 million people suffer from asthma, globally (1), resulting in a high health and economic cost. Asthma-associated immune phenotypes (endotypes) can vary widely. For example, allergen-induced type 2 immune reactions characterized by cytokines, including IL-4, IL-5, and IL-13, derived predominantly from group 2 innate lymphoid cells (ILC2s) and adaptive T helper 2 (Th2) cells, and eosinophilia are associated with the type 2–high asthma endotype (2). Indeed, blood eosinophil levels are a strong determinant of patient responsiveness to type 2 cytokine–targeting antibody treatments. By contrast, the type 2–low asthma endotype, associated with a marked increase in IL-17A and neutrophilic or paucigranulocytic airway inflammation, is frequently linked with exposure to irritants or obesity (2). However, these features are not always mutually exclusive and can be strongly influenced by comorbidities such as allergic rhinitis, respiratory viral infection, chronic rhinosinusitis, nasal polyps, atopic dermatitis, and obesity. Although asthma is a common human respiratory illness, many of our mechanistic insights into asthma are derived from animal models. This has included the discovery of lymphocyte specialization with the characterization of polarized Th cell subsets within the adaptive arm of the immune system (3) and, more recently, novel ILC populations, which have been shown to play central roles in asthma-associated immune reactions (4, 5).

Key among the roles of ILC2s is their ability to integrate signals from the epithelium, stromal cells, nervous system, and other immune cells and to subsequently orchestrate downstream effector functions. In this review, we discuss how the discovery of ILC2s in mice and humans has added greater complexity, but also a greater appreciation of the immune mechanisms that underlie asthma, and how breakthroughs in understanding ILC2 biology may offer new pathways for potential therapeutic intervention.

1.1. Type 2 Lymphocytes in Asthma

Traditionally, allergic asthma was considered a Th2 cell–driven pathology. These cells are characterized by their expression of the cytokines IL-4, IL-5, IL-9, and IL-13, which stimulate so many of the critical mediators of allergic asthma, including Th2 cell polarization, eosinophilia, mastocytosis, goblet cell hyperplasia, muscle contraction, IgE switching, and mucus hypersecretion (4, 6–10). However, research in mouse models led to the discovery of ILC2s as new protagonists in protective type 2 immunity and allergic asthma (4, 11). In contrast to T cells, ILCs lack somatically recombined antigen-specific receptors and respond to microenvironmental cues, such as cytokines, rather than directly to antigen stimulation. ILCs are divided into five categories: natural killer (NK) cells, ILC1s, ILC2s, ILC3s, and lymphoid tissue inducer (LTi) cells. NK cells are innate cytotoxic cells, comparable to cytotoxic T cells, whereas ILC1s, ILC2s, and ILC3s are analogous to Th1, Th2, and Th17 cells, respectively. LTi cells are essential for secondary lymphoid organ development (12).

ILC2s are potent cytokine producers, expressing predominantly IL-5 and IL-13 but also IL-4 and IL-9, among others (11–14) (**Figure 1**). Like T cells and B cells, ILC2s are derived from common lymphoid progenitor (CLP) cells in the bone marrow via an ILC progenitor intermediate that can further differentiate into ILC1s, ILC2s, ILC3s, and NK cells (15). ILC2 commitment requires the transcription factors GATA3, retinoic-acid-receptor-related orphan receptor α (ROR α), and B cell lymphoma leukemia 11b (BCL11b) protein (15–17). Both fetal-liver-derived and bone marrow progenitors can differentiate into ILC2s, and during the first couple of weeks after birth, ILC2 numbers expand greatly in the lung. This has been attributed to their stimulation by IL-33



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Figure 1 (Figure appears on preceding page)

ILC2 development and identification. (a) Like other hematopoietic cells, ILC2s derive from HSCs that differentiate into CLPs and further commit toward the innate lymphocyte lineage as ILCPs, and ILC2Ps. Though ILC2s first develop in fetal tissues, a significant fraction of them arise during the neonatal period, whereupon they colonize most peripheral tissues (skin, intestine, lung, and visceral adipose tissue). Here they become resident lymphocytes and adapt to the tissue microenvironment by acquiring a specialized genetic program tailored to each tissue. In particular, at least half of the ILC2s residing in the lung originated in the neonatal period, when lungs display a strong type 2–biased microenvironment and allergic sensitization likely occurs. However, under inflammatory conditions such as parasite infection and allergic reactions, the lung receives a transient ingress of ILC2s from the small intestine, which are characterized by increased sensitivity to IL-25 and higher expression of KLRG1 and contribute to lung inflammation. (b) ILC2s can be identified by their lack of lineage-specific markers (CD3, CD4, CD8a, CD11b, CD11c, CD19, FcεRI, Ly6C, Ly6G, NK1.1/CD56, TCRαβ, TCRγδ, and Ter119) and expression of IL-7Rα (CD127) and high levels of the transcription factor GATA3. In addition, other molecules can serve to identify them in humans (i.e., CD161, CRTH2, and ST2) and mice (ST2, CD25, KLRG1, ICOS). Despite also providing contact-dependent signals, the foremost contribution of ILC2s to allergy and asthma is their overwhelming capacity to rapidly produce type 2 cytokines upon stimulation by cytokines/alarmins (IL-25, IL-33, TSLP). In turn, ILC2-derived cytokines orchestrate the spectrum of immune effector cells that mediate the immunopathology of asthma, such as eosinophils and mast cells. Abbreviations: Areg, amphiregulin; CLP, common lymphoid progenitor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HSC, hematopoietic stem cell; ILCP, ILC progenitor; RORα, retinoic-acid-receptor-related orphan receptor α; TSLP, thymic stromal lymphopoietin; TSLPR, TSLP receptor.

released by airway epithelial cells due to mechanical stress induced by the onset of breathing (18–20). Indeed, in adult mice, the majority of tissue ILC2s are of early postnatal source rather than fetal or adult origin (21).

During homeostasis, ILC2s are primarily located in mucosal barrier sites such as the lung and intestine, but they are also found in many other tissues (12), where they take on tissue-specific characteristics dictated by microenvironmental cues (22, 23). Gene expression profiles have helped to differentiate ILC subsets (24, 25) and even subdivide the major subsets based on differential gene expression (26–30). One such subdivision defined natural ILC2s [characterized by IL-5, arginase 1, and IL-33R (ST2) expression] and inflammatory ILC2s [expressing IL-25R (IL-17BR) and elevated IL-13] (26–28, 31). However, ever-increasing resolution of single-cell omics, such as mass cytometry and single-cell RNA sequencing, has indicated that ILC2s represent a continuum of cells that adapt to microenvironmental cues and become specialized to the tissues where they reside (22, 23, 29, 32). In this way, skin-resident ILC2s respond to IL-18 by expressing IL-18Rα, while gut-resident ILC2s express IL-25R, making them particularly sensitive to IL-25, and stomach and lung ILC2s display higher levels of ST2 (23, 33). However, despite being primarily tissue-resident cells (34), ILC2s can migrate to distant tissues upon immune challenges and acquire the phenotype characteristic of the target tissue (22, 26, 27).

Although ILC2s play a pivotal role in helminth parasite clearance and postclearance tissue repair (11, 35), ILC2 dysregulation is implicated in various allergic diseases such as asthma, rhinitis, and atopic dermatitis. Strikingly, numerous ILC2-related genes have been identified by genome-wide association studies of asthma, with single-nucleotide polymorphisms located in the genes encoding IL-13, RORα, ST2, and thymic stromal lymphopoietin (TSLP) (36–39). Early mouse studies also implicated innate mechanisms in lung inflammation, with Rag2-deficient mice, which lack T and B cells, continuing to display airway inflammation and asthma-like symptoms during allergen challenge (40). This non-B cell, non-T cell compartment has been defined as ILC2s (4, 41), and its roles in human asthma are the subject of extensive investigation.

1.2. Human Asthma

Nasal polyps associated with chronic rhinosinusitis were one of the first human tissues found to harbor ILC2s (42). Since then, ILC2s have been shown to be consistently expanded in

atopy/asthma patients within the peripheral blood and lung (43, 44), and their frequencies rise during allergy season, significantly correlating with symptoms (45). In humans, upon allergen challenge, the numbers of CD161⁺CRTH2⁺ ILC2s decrease among peripheral blood mononuclear cells and accumulate in the lung [as measured in bronchoalveolar lavage (BAL)] (46). In addition, BAL ILC2s increase their expression of genes encoding the transcription factors aryl hydrocarbon receptor, GATA3, BATF, PPARG, and IRF4, which promote ILC2 activity, and the cytokines and cytokine receptors that drive ILC2 function [including IL-2RA, IL-4R α , ST2 (IL1RL1, IL-33R), IFNGR1, IL-25R (IL-17RB), IL-13, and AREG] (46).

Asthmatic patients have increased frequencies of ILC2s in blood, sputum, and BAL, which are associated with elevated IL-5 and IL-13 expression (47–54). However, the correlation between asthma severity and ILC2 levels has varied between studies. Smith et al. (49) reported higher blood and sputum ILC2 numbers in patients with severe asthma compared to those with mild asthma, while Yu et al. (50) reported that patients with mild asthma display higher frequencies of ILC2s than those with moderate or severe asthma. Such differences may be due to multiple factors, e.g., the changing frequencies of eosinophils and CD4⁺ T cells, differences in disease classification criteria, and varied treatment histories in the two studies. Notably, successful asthma treatment significantly reduces blood ILC2 levels (51), while allergen rechallenge increases type 2 cytokine-expressing ILC2s in sputum (52). Pediatric severe therapy-resistant asthma (STRA) patients show significantly higher levels of ILC2s in blood and sputum as compared to patients with difficult asthma and controls (55, 56). STRA patients also showed increased frequencies of IL-13-positive ILC2s. Notably, systemic steroid treatment of STRA patients reduced ILC2 levels, which were accompanied by improvements in the disease. Asthmatic patients also show elevated levels of ILC2 initiator cytokines like IL-33 (53) and TSLP (54) in the BAL as compared to controls. Collectively, these studies demonstrate associations between ILC2 levels and human asthma.

Human ILC2s have been commonly defined as CD45⁺Lin[−]IL-7R α ⁺CRTH2⁺ cells in human blood. However, a recent report has suggested that human ILC2 populations are more heterogeneous than previously appreciated (44). Indeed, IL-7R α ⁺CRTH2⁺, IL-7R α ⁺CRTH2[−], and IL-7R α [−]CRTH2[−] ILC2s expressing IL-5 were identified in blood and BAL. These ILC2 subpopulations show differential expression of ILC2 markers such as GATA3, IL-25R, KLRG1, ICOS, and CD161, but all three subpopulations caused AHR when transferred to *Rag2*^{−/−}*Il2rgc*^{−/−} mice. Further studies are required to determine whether these three populations are bona fide subpopulations of ILC2s or occur at different stages of differentiation/activation/plasticity and how they may impact disease.

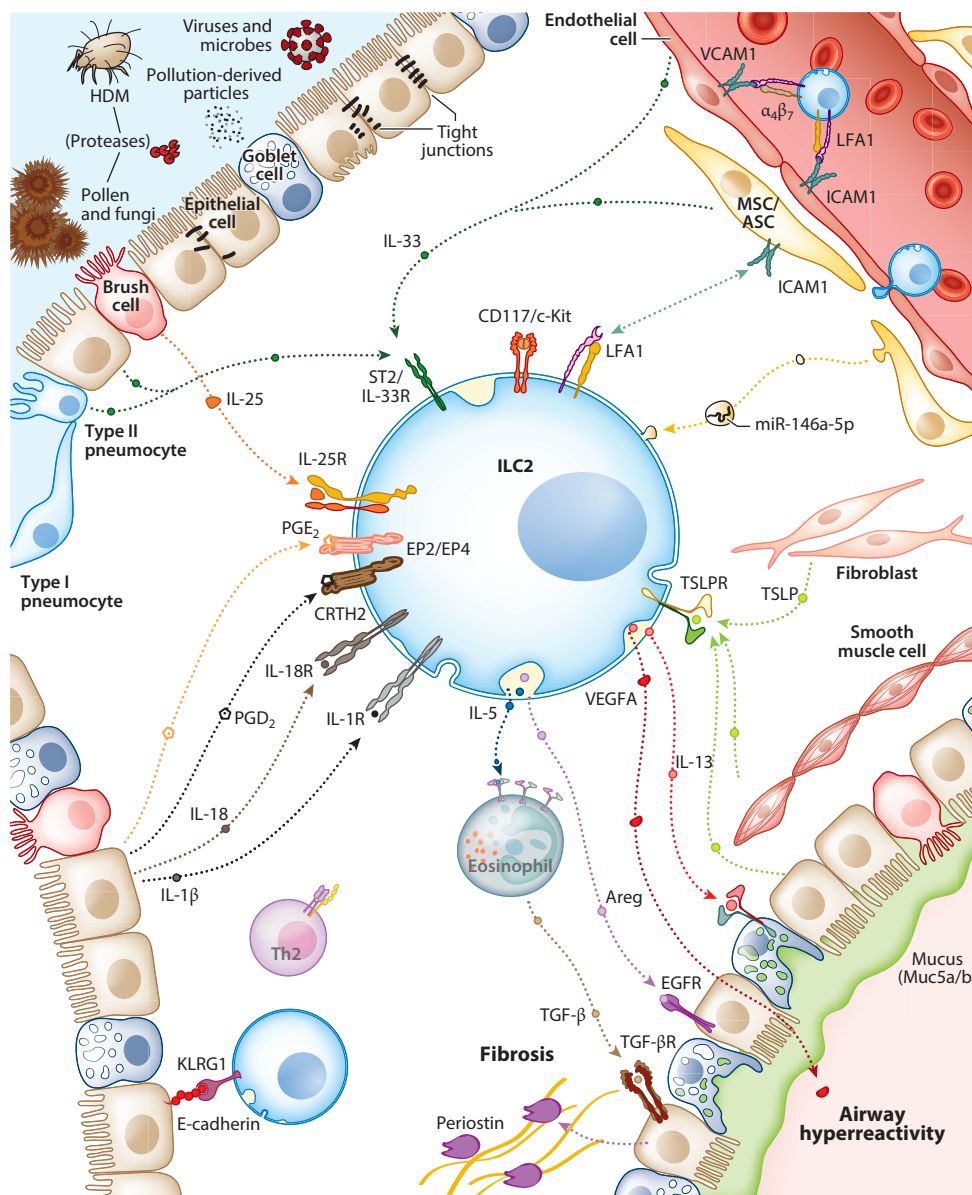
Viral infections generally provoke a type 1 immunity profile. However, infections of the respiratory viruses rhinovirus (57) and influenza virus (58) promote ILC2 lung infiltration and exacerbate lung inflammation and airway hyperreactivity to secondary infections and allergen challenges in an ILC2-dependent manner (59). Asthma patients also present increased IL-25 production upon rhinovirus infection, and mouse models indicate that IL-25R blockade is sufficient to suppress rhinovirus-induced IL-33 and TSLP expression and subsequent ILC2 and eosinophilic lung infiltration.

2. STIMULATION OF LUNG ILC2s BY EPITHELIUM- AND STROMAL CELL-DERIVED FACTORS

An intact airway epithelium is critical for lung homeostasis, and dysregulation of this physical barrier leads to the release of cytokines or cytokine-like alarmin molecules that activate juxtaposed immune cell sentinels. ILC2s express and display a spectrum of cytokine receptors that allow them



to sense initiator alarmins/cytokines that stimulate their proliferation and promote the secretion of type 2 effector cytokines (**Figure 2**). Inhaled allergens, infectious agents, or airborne pollutants can all induce epithelial cell stress or cell death. For example, various plant-derived, insect-derived, or fungal allergens contain proteases that can directly damage epithelial cell tight junctions or activate airway epithelial cells via protease-activated receptors (60–62). Three major activators of ILC2s are IL-33, TSLP, and IL-25. These may be produced at homeostatic levels or released and/or upregulated upon infection or tissue damage.



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Figure 2 (Figure appears on preceding page)

ILC2s sense the tissue microenvironment and initiate the type 2 immune response. As tissue-resident lymphocytes, ILC2s are strategically positioned in the mucosal barrier sites, poised to monitor the release of alarmins by epithelial, endothelial, and stromal cells. Epithelial cells, brush cells, MSCs, ASCs, and pneumocytes (together with immune cells such as alveolar macrophages and dendritic cells) can detect allergens and irritants and, in turn, release IL-33, IL-25, and TSLP. Together with IL-1 β and IL-18 liberated by dying cells, these alarmins stimulate ILC2s to secrete IL-5, IL-13, amphiregulin, and VEGFA, which engage their respective receptors in epithelial cells, goblet cells, and smooth muscle cells, among others. The activation of these cells leads to airway hyperresponsiveness, mucus production, fibrosis, and cardinal pathophysiological features of asthma and allergy. Endothelial cells also respond to the local inflammation and support further recruitment of ILC2s and other immune effector cells from the blood and distal tissues (e.g., in the intestine), boosting the inflammatory process. Abbreviations: Areg, amphiregulin; ASC, adventitial stromal cell; EGFR, epidermal growth factor receptor; EP2/EP4, prostaglandin E₂ receptor 2/4; HDM, house dust mite; ILC2, group 2 innate lymphoid cell; MSC, multipotent stromal cell; PGD₂, prostaglandin D₂; PGE₂, prostaglandin E₂; Th2, type 2 T helper cell; TSLP, thymic stromal lymphopoietin; TSLPR, TSLP receptor; VEGFA, vascular endothelial factor A.

2.1. IL-33

IL-33 is a nuclear protein that is released as an alarmin. In humans, airway bronchial epithelial cells and endothelial cells are major sources of IL-33 (63–66). In mice, type II alveolar pneumocytes (67) and adventitial stromal cells (ASCs) (68) constitutively produce IL-33, while endothelial cells produce IL-33 only under chronic inflammation (69–71). Transgenic expression of IL-33 causes severe inflammation in multiple organs (72). The mechanism of IL-33 release into the extracellular space remains elusive, though epithelial necrosis or active secretion has been suggested (70, 73–75). Once released, mast cell- and neutrophil-derived extracellular proteases are proposed to process IL-33 into its more active form (73, 76), which binds to ST2 (IL-1RL1, IL-33R) (77), expressed on ILC2s in the lungs. In the absence of processing, IL-33 is quickly oxidized and its activity neutralized (78).

The importance of IL-33 in allergic lung inflammation has been shown in multiple models (53, 79–82). Subsequent studies demonstrated that asthmatic patients display higher levels of IL-33 in their BAL, sputum, serum, and human bronchial epithelial cells (HBECs) as compared to healthy controls (53, 64, 83). IL-33 potently activates ILC2s, and ST2- or IL-33-deficient animals display reduced AHR, ILC2, and IL-5/IL-13 levels in the BAL and lung as compared to controls (41, 84). ILC2-derived type 2 cytokines can subsequently recruit eosinophils and other immune cells to the lung. ILC2 expression of IL-4 and IL-13 may further compromise epithelial tight junctions by altering their structure (85, 86). Indeed, ILC2s cocultured with HBECs in the presence of IL-33 produced IL-13 that disrupted epithelial cell tight junctions, an effect prevented by inclusion of anti-IL-13 neutralizing antibody (87). In vivo, intranasal administration of IL-33 increased airway epithelial permeability in *Rag2*^{-/-} mice but not in *Rag2*^{-/-}*Il2rgc*^{-/-} or ILC2-deficient *Rora*^{tg/tg} mice (87). Allergen-induced uric acid production in the airways of mice has been shown to enhance IL-33 release by airway epithelium and thereby promote a type 2 response (75), and a similar mechanism involving IL-33 and TSLP has been reported to induce lung ILC2s following respiratory syncytial virus infection (88).

2.2. TSLP

TSLP is produced by various nonimmune and immune cells, but the major sources of TSLP in the lung are epithelium, fibroblasts, and smooth muscle cells (89, 90), which produce TSLP in response to allergen, infection, or trauma (5, 89, 91, 92). TSLP signaling occurs via a heteromeric receptor composed of TSLP receptor (TSLPR) and IL-7R α (93) expressed on ILC2s.



Significantly, TSLP levels are increased in the serum, BAL, and sputum of asthmatic patients as compared to healthy controls (94–96). Overexpression of TSLP in mice caused spontaneous development of airway inflammation, while TSLPR-deficient mice displayed decreased airway inflammation, lymphocyte infiltration, and AHR (97). TSLPR-deficient mice also have fewer lung ILC2s and reduced IL-5 and IL-13 secretion in response to *Alternaria alternata*-induced acute lung inflammation and respiratory syncytial virus infection (98, 99). However, in vitro, TSLP alone does not induce ILC2 proliferation and type 2 cytokine secretion but instead acts synergistically with IL-33 (5). Indeed, these cytokines also appear to reciprocally regulate the expression of the others receptor on ILC2s (98). Notably, TSLP in the BAL fluids of patients with severe refractory asthma correlated with steroid resistance (54, 100), and airway ILC2s (but not blood ILC2s) from these patients showed dexamethasone resistance. Dexamethasone treatment increased IL-7R α expression on ILC2s and thereby increased TSLP-mediated pSTAT5 and MEK signaling. Inhibition of MEK and STAT5 reversed ILC2 steroid resistance. Therefore, combining anti-TSLP neutralizing antibody with conventional therapy could potentially help treat severe refractory asthma (54, 100).

2.3. IL-25

In mice, IL-25 overexpression or exogenous IL-25 administration induces type 2 immune responses including an increase in type 2 cytokine secretion, airway inflammation, lymphocyte infiltration, AHR, and fibrosis (4, 26, 41). The IL-25 receptor, which consists of IL-17RB and IL-17RA subunits, is differentially expressed on ILC2s, dependent on tissue location (23). Asthmatic patients display higher serum IL-25 levels than healthy controls (101), and IL-25 neutralization during allergen-induced experimental asthma reduces all hallmarks of asthma (102). In mice, though, IL-33 is a more potent activator of lung ILC2s and AHR (41). The cellular source of IL-25 remained elusive for a long time. However, in 2016 multiple groups independently discovered intestinal tuft cells as the source of IL-25 (103–105), which, in a feed-forward mechanism, stimulates IL-13 expression by ILC2s that can lead to further tuft cell differentiation from progenitor cells. The source of IL-25 in the lungs is somewhat more ambiguous. von Moltke et al. (103) reported that mouse DCLK1⁺ lung tuft cells (known as brush cells in the trachea) also express IL-25, and this was supported by further studies in which *A. alternata* and house dust mite (HDM)-induced allergic lung inflammation promoted the proliferation of IL-25-positive brush cells (106). However, it has been proposed that DCLK1⁺ solitary chemosensory cells in the human upper airways are the source of human IL-25 in patients with nasal polyposis and that the presence of these IL-25-producing cells is correlated with ILC2 levels (107, 108).

The cytokine TGF- β is another epithelial cell-derived activator of ILC2s. Lung ILC2s express TGF- β RII, and in vitro treatment with TGF- β enhances ILC2 chemokinesis (109). Furthermore, following HDM allergen immunization, the deficiency of airway epithelium-derived TGF- β leads to reduced BAL lymphocyte infiltration, reduced eosinophil recruitment, and diminished AHR, as compared to control animals. TGF- β -deficiency specifically impaired ILC2 accumulation without affecting T lymphocytes (109). Therefore, the beneficial effects of TGF- β neutralization (110, 111) in asthma might, at least partially, be mediated by ILC2s.

3. ILC2 EFFECTOR FUNCTIONS

The cardinal type 2 cytokines IL-4, IL-5, IL-9, and IL-13 are all produced by ILC2s (representing up to 50–80% of all IL-5 and IL-13 producers) and have been shown to contribute to the induction of goblet cell hyperplasia, mucus hypersecretion, eosinophilia, AHR, and fibrosis in the lung (4, 6, 9, 12, 112, 113).

Mucus hypersecretion is a major cause of airway obstruction and is accompanied by goblet cell hyperplasia. Epidemiological studies have demonstrated a strong association between early life rhinovirus infection with asthma in later life (114). In neonatal mice, rhinovirus infection led to an increase in lung IL-25, IL-33, and TSLP secretion, with airway epithelium-derived IL-25 inducing ILC2-mediated IL-13 production, which subsequently promoted mucus hyperproduction and goblet cell hyperplasia (115–117). Adoptive transfer of ILC2s from infected neonatal mice to healthy neonatal recipients was sufficient to cause mucosal cell metaplasia (117). In vivo allergen challenge also promotes murine ILC2s to produce amphiregulin, which further contributes to mucus overproduction by epithelial cells. In an HDM mouse model, ILC2-derived amphiregulin can activate the epidermal growth factor receptor (EGFR) signaling pathway, which ultimately causes mucus hypersecretion by epithelial cells.

Although IL-4 and IL-13 can enhance histamine-induced AHR in human bronchi (118), any role for ILC2s in this process remains to be shown. However, in contrast to wild-type mice, mice deficient in either IL-25 or IL-33 receptor and double knockout mice, which cannot elicit ILC2 responses, have impaired AHR in allergen-induced experimental asthma models (41). Shen et al. (119) have recently demonstrated that ILC2-derived vascular endothelial factor A (VEGFA) is crucial for AHR. In IL-33- or *A. alternata*-induced airway allergy models, adoptive transfer of ILC2s to *Rag2^{-/-}Il2rgc^{-/-}* mice was sufficient to induce AHR. In this model, inhibition of VEGFA signaling by SU1498 abolished AHR, while neutralization of IL-13 was only partially effective. Notably, VEGFA also regulated IL-13 secretion by ILC2s in an autocrine manner (119).

Recently ILC2s have been implicated in lung fibrosis. In an HDM model with overexpression of smad5, IL-25 neutralization reduced bronchiolar collagen deposition and airway remodeling (120), while ILC2-deficient mice displayed diminished collagen deposition and pulmonary fibrosis following IL-25-induced acute lung inflammation (112). Adoptive transfer of ILC2s also exacerbated bleomycin-induced pulmonary fibrosis (121). Experimental lung fibrosis is also reduced in ST2-deficient mice (121). The relative importance of specific ILC2-derived cytokines in fibrosis is unclear. In an allergen-induced experimental asthma model, ILC2-derived IL-5 recruited eosinophils to the lung and eosinophil-derived TGF- β contributed to lung fibrosis, with IL-5-deficient mice exhibiting reduced airway remodeling (122, 123). Furthermore, both IL-4-deficient and IL-13-deficient mice display reduced subepithelial fibrosis and periostin deposition. However, this reduction is also accompanied by a reduction in TGF- β 1 level, making it difficult to delineate the direct and indirect effects of IL-4 and IL-13 (124, 125). Indeed, the differential contribution of IL-4 and IL-13 derived from ILC2s, Th2 cells, mast cells, or basophils to subepithelial fibrosis is yet to be determined.

4. ILC2 MIGRATION/HOMING/INTEGRINS

Although ILC2s are often referred to as tissue resident, they are also recruited from the blood circulation and other tissues during inflammation (27, 34). Indeed, many studies using human ILC2s harvest them from peripheral blood. During asthma, lung endothelial cells upregulate the expression of the cellular adhesion molecules ICAM1 and VCAM1 (126), and recent studies have implicated both of these molecules in ILC2 recruitment to the lung (127, 128). Karta et al. (127) demonstrated, using bone marrow chimeras, that the increase in lung ILC2 levels following *A. alternata* challenge is due to both local proliferation and migration from bone marrow and circulation. This migration from the blood circulation to the airways required $\alpha_L\beta_2$ (LFA1) integrin expression (common to both human and mouse ILC2s), and blocking either β_2 or α_L inhibited allergen-induced ILC2 recruitment to the lung (127). The authors went on to propose



ICAM1, expressed by lung endothelial cells and a known ligand of $\alpha_L\beta_2$, as a potential partner for ILC2-expressed $\alpha_L\beta_2$, supporting a role for endothelial cells in ILC2 recruitment to the airways (127). The contribution of endothelial cell-mediated ILC2 recruitment was further supported by the findings that allergen-induced IL-33 secretion promotes RAGE (receptor for advanced glycation end products)-mediated VCAM1 expression by lung endothelial cells and that antibodies that inhibit VCAM1 binding to $\alpha_4\beta_7$ on ILC2s repress lung ILC2 recruitment in response to HDM- or IL-33-induced airway inflammation (128).

Recruitment of ILC2s into the lungs requires CCR4 and/or CCR8 in mice (129, 130), with IL-33 inducing CCR8 (and CCR1) but not CCR4 (129). CCR8 inhibition reduced ILC2 recruitment and type 2 inflammation (129, 130). Importantly, human peripheral blood ILC2s also express CCR8, raising the potential for therapeutic benefit in asthma.

Although ILC2s display tissue residence and adaptation in humans and mice (21, 22, 32, 34), they can also participate in and/or coordinate immunity in distal organs (22, 27, 131, 132). For example, during murine allergen challenges, gut IL-25R⁺KLRG1^{hi} ILC2s migrate from the lamina propria to the lung in an S1P-dependent manner, substantially contributing to allergic inflammation (22, 27, 31). Reciprocally, lung-resident ILC2s also migrate toward the intestine to contribute to the antiparasitic response (22). Additionally, intestinal ILC2s are able to detect IL-33 released from keratinocytes upon mechanical skin injury. In turn, these ILC2s activate intestinal mast cells via IL-4 and IL-13, which mediate allergen-induced anaphylaxis (132). This raises the question of whether a similar mechanism could potentiate asthma in atopic patients.

5. ILC2 IMMUNE NETWORKING

Complex diseases, like asthma, involve an interplay of numerous immune and nonimmune cells. ILC2s are a pivotal hub in the cross talk between innate immune cells (e.g., macrophages, dendritic cells (DCs), and granulocytes) and adaptive lymphocytes (B and T cells) (**Figure 3**). ILC2s modulate the immune response by sending and receiving multiple ligands and soluble factors that induce immune cell activation as well as stromal cell reactions.

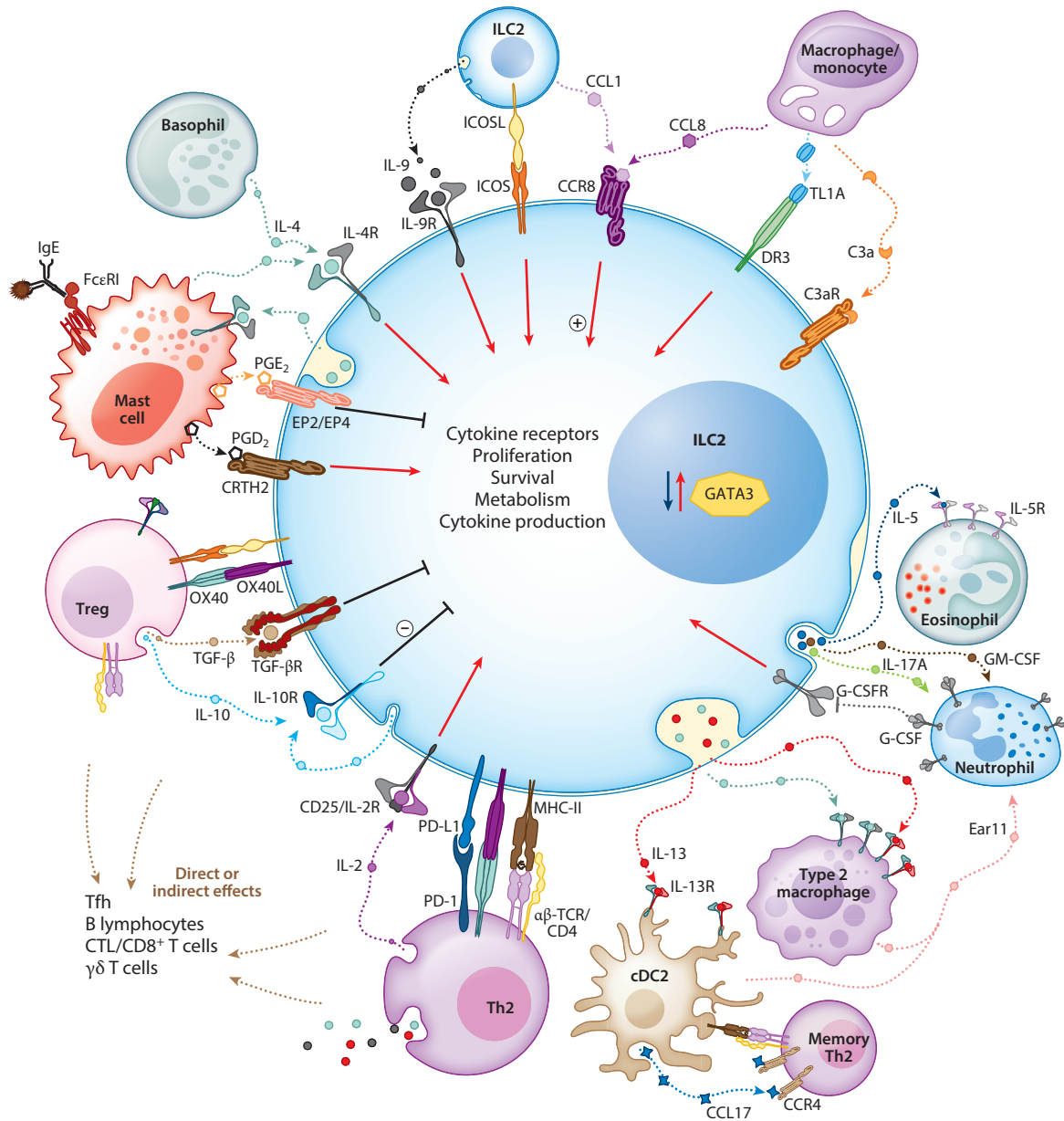
5.1. ILC2 Interactions with Stromal Cells

Lung ASCs have been reported to support ILC2 proliferation and cytokine production (68). In vitro, ASCs activate ILC2s principally via TSLP production. In vivo, ASC depletion reduces lung ILC2 numbers at least partly through the loss of ASC-derived IL-33. Reciprocally, depletion of ILC2s during helminth infection also reduces IL-33-expressing ASC expansion, suggesting a feedback circuit (68). However, further studies are required to uncover the signals involved in ILC2 recruitment to the adventitial cuffs and to understand the effect of ASC and ILC2 interactions in asthma. Of note, a recent study in adipose tissue has suggested that multipotent stromal cells (MSCs) in the adipose tissue can potentiate ILC2 proliferation and type 2 cytokine production that was attributable to integrin-mediated signaling via ICAM1 on stromal cells binding to LFA-1 on ILC2s (133). Consequently, ILC2-derived IL-4 and IL-13 feedback induces eotaxin secretion from MSCs, supporting eosinophil recruitment. With similar stromal cells present in the lungs, it is possible that a parallel circuit occurs in the airways alongside the ASC:TSLP pathway.

Interestingly, small extracellular vesicles (SEVs) derived from human mesenchymal stromal cells have been reported to ameliorate ILC2-dependent AHR following IL-33 administration. This inhibitory effect was microRNA (miR)-146-5p mediated, with SEVs isolated from mesenchymal stromal cells able to inhibit IL-5 and IL-13 secretion by ILC2s isolated from allergic rhinitis patients (134). However, the in vivo consequences of this interaction and the molecular mechanisms involved remain to be determined.

5.2. ILC2 Interactions with T Cells

Significantly, in addition to providing a potent innate source of type 2 cytokines, ILC2s can help potentiate adaptive Th2 cell responses, either directly or indirectly through third-party cells (6, 135–137). A proportion of mouse ILC2s express MHC class II molecules together with variable



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Figure 3 (Figure appears on preceding page)

ILC2s as central playmakers within the immune cell network in asthma. Since their discovery, ILC2s have been found to directly interact with most of the immune cells participating in asthma and allergy. ILC2s integrate the information provided by alarmins with the signals derived from other innate leukocytes such as basophils, neutrophils, macrophages, and mast cells. Cytokines (IL-2, IL-4, IL-9, IL-10, etc.), chemokines (CCL1, CCL8), ligand-receptor interactions (ICOS-ICOSL), lipid mediators (PGD₂, PGE₂, PGI₂, LTD₄, etc.), and other soluble factors will stimulate or suppress ILC2 expression of alarmin receptors, proliferation, survival, transcription factor levels (e.g., GATA3), and cytokine production. The net effect is the capacity of ILC2s to activate and positively modulate T cells, macrophages, and dendritic cells into becoming type 2 immune cells (Th2 cells, M2 cells, or cDC2s, respectively). In parallel, ILC2s also provide support for mast cells, eosinophils, and neutrophils, effectors that contribute extensively to the pathology associated with asthma. Abbreviations: cDC2, type 2 classical dendritic cell; CTL, cytotoxic T lymphocyte; EP2/EP4, prostaglandin E₂ receptor 2/4; G-CSF, granulocyte colony-stimulating factor; G-CSFR, G-CSF receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; ILC2, group 2 innate lymphoid cell; PGD₂, prostaglandin D₂; PGE₂, prostaglandin E₂; Tfh, T follicular helper; Th2, type 2 T helper; Treg, regulatory T cell.

levels of CD80 and CD86, giving them the capacity to present antigens to T cells (11, 136) to induce T cell proliferation (though not with the same efficiency as professional antigen-presenting cells). In turn, Th2 cells produce IL-2, which stimulates ILC2 expansion and cytokine production (136). The antigen-presenting function of ILC2s has also been reported to be at least partially dependent on ILC2-intrinsic C3ar1 expression (138). In humans, ILC2s are also able to take up, process, and present antigens on MHC class II molecules, and this requires stimulation with IL-1 β and IL-18 (136, 139). Furthermore, by rapidly producing IL-13, ILC2s promote the migration of DCs to the lymph nodes to prime T cells at the initiation of the adaptive immune response (6).

ILC2s can provide additional contact-dependent pro-type 2 signals to T cells. Besides expressing PD-1, ILC2s also display PD-L1 on their surface. Surprisingly, and in contrast to its role in promoting immune exhaustion, PD-L1 on ILC2s has been reported to promote Th2 differentiation and expansion in a Th2-intrinsic, PD-1-dependent manner (140). Lymphocyte-specific PD-L1-deficiency resulted in reduced numbers of ILC2s and DCs and diminished type 2 immunity (140). The costimulatory molecule OX40L, expressed by ILC2s in the lungs, also contributes to allergic airway disease by inducing the expansion of OX40-expressing Th2 cells and regulatory T cells (Tregs) upon IL-33 and allergen stimulation (137). Of note, engagement of OX40 on T lymphocytes has been shown to heighten expression of IL-9 by initiating chromatin reorganization characteristic of Th2/Th9 cells (141), and it would be interesting to know if such Th-derived IL-9 feeds back to stimulate ILC2s to promote a Th9 immune bias. Though ILC2s also express ICOSL, this molecule seems not to have a role in directly activating Th2 cells (142). In contrast, interaction in *trans* between ICOS and ICOSL on neighboring ILC2s supports the survival and expansion of ILC2s.

ILC2s were found to mediate their effects beyond the initiation of primary T cell responses by enhancing memory T cell migration to sites of secondary allergen challenge (135). ILC2-derived IL-13 was critical for eliciting the secretion of the Th2 cell-attracting chemokine CCL17 by IRF4⁺CD11b⁺CD103⁻ DCs. Thus, ILC2s can promote initial Th2 differentiation and cooperate with DCs to support memory Th2 cell responses to allergens.

5.3. ILC2 Interactions with Tregs

Tregs are a subset of T lymphocytes that mediate immune tolerance, e.g., by competing with other lymphocytes for IL-2 and by producing the immunosuppressive cytokines IL-10 and TGF- β (143, 144). Consequently, it is important to understand how Tregs interact with ILC2s (137, 145, 146). Normally ILC2s promote Treg fitness and function. Both ICOSL (145) and OX40L (137) expressed by ILC2s promote proliferation of Tregs expressing their cognate ligands (137, 145).

In addition, autocrine IL-9 expression by ILC2s induces GITRL, which promotes Treg function to resolve inflammation in arthritis models (146). Presumably, such pathways are ineffective in the lungs of people with asthma. Indeed, in food allergies, ILC2s bypass the immunoregulation induced by Tregs (143). Notably, in inflammatory airway disease, 5–10% of ILC2s express IL-10 (11, 147, 148). Interestingly, an increase in IL-10-producing ILC2s was associated with a reduction in eosinophilic infiltration (149), suggesting that skewing ILC2s toward IL-10 production may reduce type 2 inflammation.

5.4. Innate Th2 Cells and Memory ILC2s

The discovery of Th2 cells that appear to behave as innate lymphocytes, particularly after being primed by antigens from either allergens or parasites (150, 151), suggests that these cells may complement the innate functions of ILC2s in type 2 immunity. These innately licensed Th2 cells produce predominantly IL-13, and antigen-dependent stimulation is still required to induce their expression of IL-4. Although they could compensate to some extent in the absence of ILC2s, they were unable to protect from parasite infection (150). Conversely, it has been proposed that innate leukocytes can acquire a long-lasting memory-like state termed trained immunity that can epigenetically resemble that of their adaptive counterparts (152, 153). Memory-like ILC2s have been reported to arise following allergen sensitization and last for several months, making individuals more susceptible to subsequent challenges and more sensitive to unrelated allergens (154, 155).

5.5. ILC2s Regulate, and Are in Turn Regulated by, Mast Cells, Eosinophils, Neutrophils, and Basophils

Eosinophils are the most prominent population of effector cells in type 2 immunity-induced asthma, despite non-type 2 asthma indicating that they are not completely required for all cases of asthma. Indeed, mice lacking eosinophils either do not develop many of the features of allergic asthma or develop milder forms of them (156). Asthma preclinical models indicate that ILC2s contribute to eosinophil infiltration in lungs during allergen-driven type 2 immune responses (4–6). In patients with allergic rhinitis, allergen challenge induces a significant increase in ILC2 numbers in nasal tissue, strongly correlating with eosinophil infiltration (157).

Together with eosinophils, mast cells are pivotal in atopy/asthma (158). Intriguingly, both ILC2s and mast cells are activated by stem cell factor, which is elevated in sera of asthmatic patients (88). While ILC2s promote mast cell activation (159), the feedback provided by mast cells to ILC2s may have inflammatory or anti-inflammatory consequences. When activated by IgE binding to the FcεRI, mast cells produce IL-4, which can induce ILC2 proliferation and IL-13 production, thereby contributing to histamine/serotonin (5-HT)-induced anaphylaxis (160). Mast cells are also major producers of eicosanoids including leukotrienes and prostaglandins (161–164). Both mouse and human lung ILC2s express leukotriene receptor CysLT1R, which binds leukotriene D₄ (LTD₄) to induce ILC2 proliferation and type 2 cytokine production (165). Similarly, ILC2s respond to autocrine/paracrine (166) and mast cell-derived prostaglandin D₂ (PGD₂) by inducing IL-13 production and upregulating ST2 and IL-17RA expression, making these more responsive to IL-33 and IL-25 activation (167–169). On the other hand, the lipid mediators prostaglandin E₂ (170), prostaglandin I₂ (171), and lipoxin A₄ (172) exhibit inhibitory effects on ILC2s. Similarly, IL-33-activated mast cells produce IL-2, which has been shown in murine papain-induced asthma to promote Tregs that curb ILC2 inflammatory effects through IL-10 (173).

The association between ILC2s and basophils in the context of asthma has not been extensively studied. However, in atopic dermatitis models, IL-33 directly stimulates basophils, which in



turn produce IL-4 that synergizes with IL-33 to directly enhance ILC2 activation (174). By contrast, basophil inhibition reduces ILC2 accumulation in inflamed skin (175). Likewise, in papain-induced lung inflammation, basophil-derived IL-4 was required for ILC2 activation, IL-5 and IL-13 production, and induction of AHR (176). Given the increased numbers of basophils in the sputum of asthmatic patients (177–179) and that, in humans, basophils respond to allergen by up-regulating IL-4 (180), it is certainly possible that an equivalent mechanism for promoting ILC2 proliferation exists in humans.

Neutrophilic infiltration is commonly associated with chronic obstructive pulmonary disease (COPD) or non-type 2 asthma (181, 182), but even in asthmatic patients with a type 2 asthma, endotype allergen challenge promotes lung neutrophilic infiltration (46). Usually they are a minor population, and they were often considered to be the escapees from the suppressive effects of IL-4 and the type 2 environment (183). However, recent studies have identified signals that can drive neutrophil recruitment during type 2 immunity, including the M2 macrophage-derived molecule Ym1 (encoded by *Chil3*) (184, 185). ILC2s also contribute to neutrophil recruitment in the type 2 microenvironment (186). ILC2s promote the induction of myeloid-derived Ear11, an RNase-like protein that recruits neutrophils upon *A. alternata* and ragweed challenges.

The modulation between ILC2s and neutrophils seems to be mutual, as it has been recently reported that neutrophils cap or curtail ILC2 responses (187). Neutrophils consume granulocyte colony-stimulating factor (G-CSF), and an excess of G-CSF upon neutrophil depletion has a dual effect: There is an increase in ILC2s with higher production of IL-4 and IL-13 (both in lungs and BAL). Both human and murine ILC2s upregulate the expression of the G-CSF receptor (G-CSFR) upon IL-33 stimulation, and in turn, the neutrophil-depletion-related excess of G-CSF enhances the expression of GATA3, IL-5, and IL-13. Interestingly, this did not intensify AHR, suggesting that exacerbation of type 2 immunity without further abnormalities is not always sufficient to induce asthma.

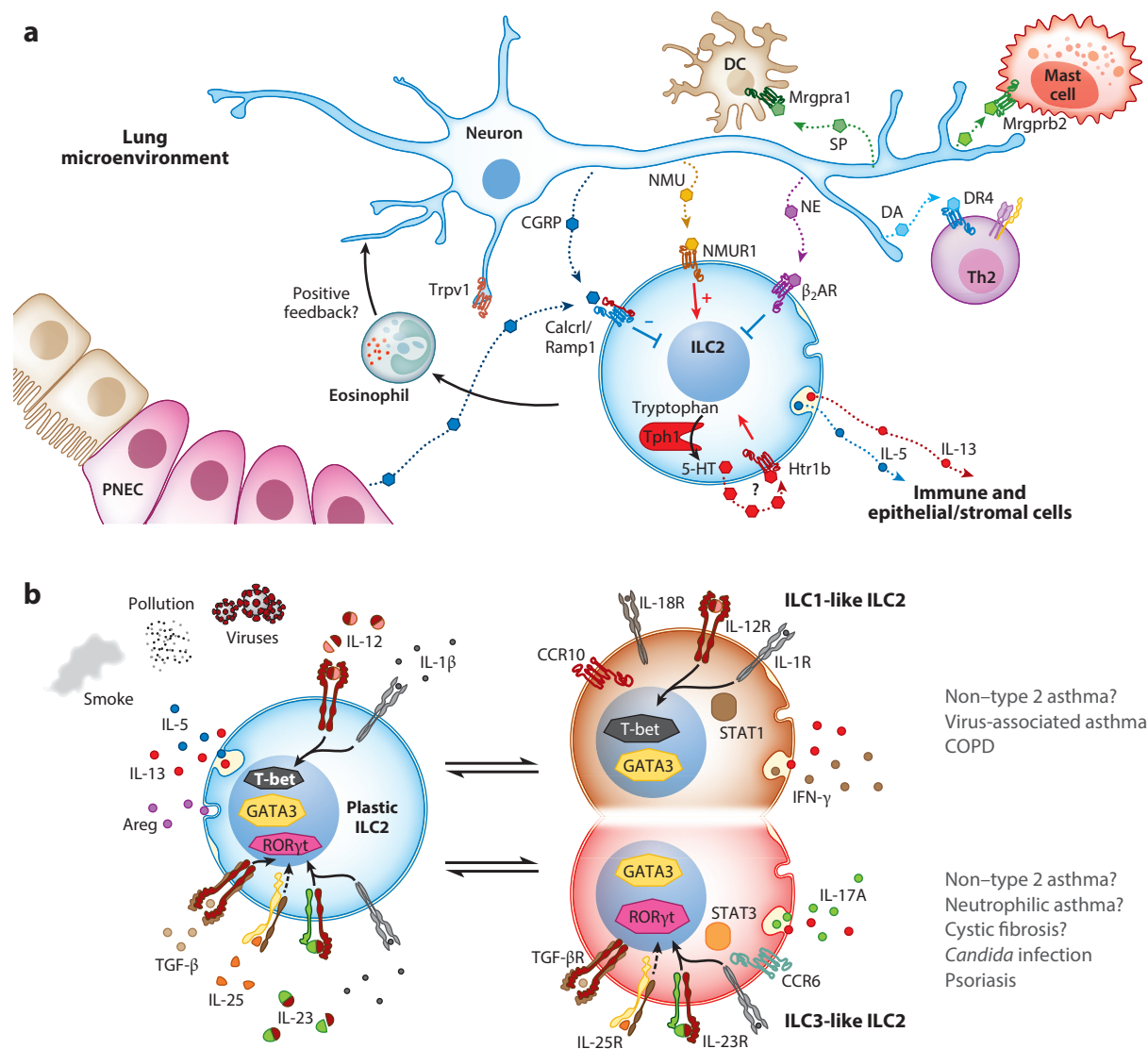
In the mucosal barrier sites, activated myeloid cells and endothelial cells are also sources of the cytokine TL1A (Tnfsf15), which can activate both mouse and human ILC2s via death receptor 3 (DR3) (188–190). In response to ovalbumin or intranasal papain challenge, *DR3*^{−/−} mice showed reduced accumulation of ILC2s in BAL and lung compared to controls (188, 189). However, TL1A signaling is not necessary for ILC2 differentiation, and IL-33 and IL-25 signaling remain functional in *DR3*^{−/−} ILC2s (188). Interestingly, TL1A neutralizing antibody ameliorates lymphocyte and eosinophil accumulation in the BAL of rats undergoing ovalbumin-induced experimental lung inflammation (191). Thus, TL1A signaling might be a potential therapeutic target in asthma, though the contribution of ILC2s remains to be identified.

6. NEUROIMMUNE MODULATION OF ILC2s AND ASTHMA

Many neuropeptides, biogenic amines, and neurotransmitters promote communication between the nervous system and immune cells. The lungs are innervated by a complex network of sensory, sympathetic, and parasympathetic neurons (192, 193), with ILC2s residing in close proximity (28, 194, 195) (**Figure 4**). Notably, allergen-induced airway hyperreactivity in mice is abrogated when the vagal lung-innervating neurons responsible for the detection of noxious stimuli (nociceptive Trpv1-expressing neurons) are inhibited or deleted (196). It has become apparent that either depleting or blocking Trpv1⁺ lung afferents modulates lung ILC2s, prompting the concept that ILC2s are regulated by neuronal cues (197). Recently, Drake and colleagues (198) found that patients with persistent asthma have denser innervation and higher substance P expression in lung biopsies than patients with intermittent asthma or healthy individuals, and this was associated with a failure to respond to bronchodilation treatment. In mice, they found that IL-5-induced

eosinophil infiltration promoted airway remodeling, expansion of lung innervation, and airway hyperreactivity (198). Although they did not examine the source of IL-5, it is tempting to speculate whether ILC2-derived IL-5 may underlie this phenotype, with ILC2s and afferent neurons creating a positive-feedback loop (via eosinophils) that may contribute to the development of asthma.

ILC2s are enriched in receptors for neuronal cues (194, 199, 200), including the neuropeptides neuromedin U (NMU) and calcitonin gene-related peptide (CGRP) and β_2 adrenergic receptor (β_2 AR) agonists. In the lungs of mice, NMU receptor 1 (NMUR1)-positive ILC2s respond to NMU and IL-25 to promote eosinophilia and AHR (28). Furthermore, NMUR1-deficient mice fail to mount productive type 2 immune responses against helminths or HDM challenge



(Caption appears on following page)

Figure 4 (Figure appears on preceding page)

Environmental and microenvironmental cues leading to typical and atypical ILC2s in asthma. (a) ILC2s express many receptors for neurotransmitters and neuropeptides, as well as exceptionally high levels of certain enzymes implicated in the synthesis of neurotransmitters. Both sympathetic and vagal sensory neurons project their afferent fibers within lung parenchyma. In particular, Trpv1-expressing nociceptive neurons, which specialize in the detection of noxious stimuli from the environment (e.g., irritants, extreme temperatures, tissue damage), appear to play key roles in promoting airway hyperresponsiveness. Besides activating cDC2s and mast cells through the production of substance P and promoting Th2 cell differentiation through the release of dopamine, lung afferent neurons release many peptides and biogenic amines that directly target ILC2s that reside in close proximity to neural fibers. Both NMU and VIP promote ILC2 expansion and Th2 cytokine production, while norepinephrine and CGRP curb the activation of ILC2s. Furthermore, unusually for immune cells, ILC2s can secrete CGRP and express the enzymatic machinery necessary to produce 5-HT. Murine models have shown that inhibition or deletion of nociceptive neurons prevents or decreases the severity of allergic immune responses and asthma, suggesting that modulation of neuroimmune units can represent a targetable treatment approach. (b) ILC2s are characterized by the expression of cardinal type 2 cytokines (i.e., IL-4, IL-5, IL-9, IL-13, and Areg) and the transcription factor GATA3. Nonetheless, ILC2s exhibit a remarkable ability to adapt their biology to match their environment. This reversible plasticity allows ILC2s to acquire ILC1- and ILC3-like features such as specific expression of T-bet and ROR γ t and IFN- γ and IL-17A, respectively. Indeed, the changes induced by IL-1 β and IL-12, in the case of ILC1-like ILC2s, or by IL-1 β , IL-23, and TGF- β , for ILC3-like ILC2s, suggest a profound modulation in the transcriptional signature. Both human and murine ILC2s present this capacity in vitro, but we are only starting to understand the prevalence of this process in vivo. Interestingly, the plasticity of ILC2s provides a plausible hypothesis to understand how ILC2s may participate and promote COPD and non-type 2 asthma, which are characterized by type 1 and type 3 immunity, respectively. Abbreviations: Areg, amphiregulin; β_2 AR, β_2 adrenergic receptor; cDC2, type 2 classical dendritic cells; CGRP, calcitonin gene-related peptide; COPD, chronic obstructive pulmonary disease; DA, dopamine; DC, dendritic cell; 5-HT, serotonin; ILC2, group 2 innate lymphoid cell; NE, norepinephrine; NMU, neuromedin U; PNEC, pulmonary neuroendocrine cell; ROR γ t, retinoic-acid-receptor-related orphan receptor γ t; SP, substance P; Th2, type 2 T helper; Trpv1, transient receptor potential cation channel subfamily V member 1; VIP, vasoactive intestinal peptide.

(195, 200). However, NMU-deficient mice do not present gross alterations in response to HDM (28), and the extent of NMU involvement in allergen-induced airway inflammation and lung eosinophilia has not been reported in *Nmur1*-deficient mice (28). The consequence of deleting NMU in specific neurons, and restricting *NMUR1*-deficiency to ILC2s, will help further delineate the specific neural regulation of ILC2s by the NMU pathway. While NMU boosts ILC2 activity, the effects of CGRP on ILC2s are more heterogeneous. Clusters of rare epithelial pulmonary neuroendocrine cells (PNECs) secrete CGRP, promoting ILC2 expansion, IL-5 and IL-13 production, and infiltration of Th2 lymphocytes and eosinophils in an OVA-challenge model of lung inflammation (201). Furthermore, asthmatic patients show increased PNEC levels as compared to healthy controls, suggesting a role in human asthma (201), though the mechanism by which allergens stimulate PNECs still requires further investigation. Indeed, by contrast, recent publications have reported that CGRP curbs ILC2-induced inflammation in response to either IL-25 or IL-33 (199, 202, 203). By affecting chromatin regulatory elements, CGRP reduced expression of proinflammatory genes such as *Il4*, *Il5*, *Il9*, *Il13*, *Il17a*, and *Il17f* and promoted an immunoregulatory gene signature in ILC2s (e.g., *Areg* and *Il10ra* expression), even in response to IL-33 and NMU (199, 202, 203). Mice challenged with intranasal IL-33 experienced a reduction in ILC2-dependent eosinophilia, type 2 cytokines, and AHR when CGRP was coadministered, and CGRP-deficient mice displayed an increase in ILC2 proliferation upon papain challenge (202). In both mice and humans, the β_2 AR is highly expressed by ILC2s from the lungs, and β_2 AR agonists impair ILC2 responses, while β_2 AR deficiency enhances ILC2 activation (194).

ILC2s also express tryptophan hydroxylase 1 (Tph1), one of the enzymes required to produce 5-HT, and an intrinsic lack of Tph1 expression precludes activation of ILC2s during parasitic infection (204). Though this study did not evaluate the role of ILC2-specific 5-HT or Tph1 expression in murine allergy models, 5-HT has been implicated in asthma (205), and Tph1 is positively

regulated by GATA3 in ILC2s (206), making it a potential modulator during allergic/asthmatic type 2 responses.

Although ILC2s appear to be a hub for signals from the nervous system, other immune cells may also complement the neuroimmune modulatory axis during type 2 inflammation. Indeed, lung T cells express the dopamine receptor DR4, which synergizes with STAT5 signaling to induce T cell proliferation and Th2 programming upon allergen challenge (207). Interestingly, dopaminergic fibers are more abundant in the lungs of both human and mouse infants and children/pups and have the potential to contribute to the polarization of the type 2 lymphocytes that prevail in lungs early after birth (18, 207). In addition, substance P–producing nociceptive neurons are able to detect cysteine protease activity derived from HDM and activate MRGPRB2⁺ mast cells and MRGPRA1⁺ type 2 classical DCs to initiate the inflammatory response (208, 209).

Thus, ILC2s sit within a complex neuroimmune network in which their production of, or response to, neurotransmitters can help maintain tissue homeostasis or contribute to the pathophysiology of lung disease. The development and analysis of more refined experimental models will allow a greater understanding of neuro-ILC2 interactions.

6.1. Sex Hormone Modulation of ILC2s

Male and female sex hormones differentially impact ILC2s, with females having higher numbers of ILC2s as compared to males. This mirrors the sex bias in asthma: Women display greater susceptibility and severity of asthma than men (210). In mice, *A. alternata*– or HDM-induced models of airway inflammation resulted in fewer ILC2s and less inflammation in males as compared to females. Moreover, ILC2s isolated from females produced higher levels of IL-5 and IL-13 (211). Orchiectomy in male mice exacerbated airway inflammation in response to *A. alternata*, and adoptive bone marrow transfer from the androgen receptor (AR)-deficient mice into wild-type mice demonstrated that androgen influences ILC2 function in a cell-intrinsic manner (211). In female mice, the KLRG1[−] ILC2 subset predominates but can be diminished by an increase in androgen levels (212). In addition, AR signaling in ILC2 progenitors suppresses their differentiation into ILC2s (212). AR is also expressed by lung epithelium, and testosterone suppresses IL-33 and TSLP secretion, further suppressing ILC2 proliferation in a cell-extrinsic manner (211).

7. ENVIRONMENTAL FACTORS

Circadian rhythms have been associated with asthma (213), and it is notable that ILC2s (at least in the gut) show marked oscillations in circadian regulator genes (214). This is also in line with ILC2-mediated circadian regulation of eosinophil homeostasis (215). Such changes are probably linked to feeding behavior, which has been shown to promote IL-5 and IL-13 production by ILC2s (215). Upon activation, ILC2s upregulate the glycolytic pathway, a process that requires arginase 1 (216, 217). Supporting this, both fasting and a ketogenic diet, which fail to provide fuel for glycolysis, reduce IL-13 production (215, 217) and result in less severe allergic responses (217). Furthermore, fiber-rich diets also downmodulate airway inflammatory disease in mice, due to intestinal-microbiota-derived short-chain fatty acid metabolites acting to curtail ILC2 metabolism and inhibit histone deacetylases, thereby suppressing GATA3 expression, ILC2 proliferation, and cytokine production (218, 219).

Although ILC2s require glucose during their initial activation (217), they then rely on autophagy (219) and the acquisition of microenvironmental lipids (stored as lipid droplets) for anabolic processes such as proliferation and cytokine production (217, 220). As a consequence, ILC2s are particularly fit to thrive in environments characterized by inflammation-associated



tissue damage where Pla2g5-expressing macrophages engulf dead cells and provide ILC2s with free fatty acids (221). Taken together, these findings indicate it is possible that dietary intervention may prove beneficial in modifying type 2 responses generally, and ILC2 responses specifically, but large, carefully controlled trials are required (222).

Diet also affects the microbiome, which has also been linked to asthma susceptibility, but little is known about the possible role of ILC2s in this context (for an extensive discussion see References 223–225). Several lines of evidence indicate that allergy and asthma sensitization occur early in life (224, 226). Coincidentally, ILC2s accumulate in the lung during this period (postnatal days 10–14 in mice, 2–3 years in humans) and promote a type 2 immune microenvironment (18, 215) responsible for enhanced responses against HDM (20). This suggests that tuning of ILC2s during this period may prevent or promote asthma later in life. Indeed, averting microbial colonization during this time exacerbated IL-4, IL-5, and IL-13 production in subsequent allergic responses (227).

A recent study found that in mice, prepregnancy and prenatal maternal exposure to diesel exhaust particles (DEPs) enhanced allergic responses in the offspring (228). This process was dependent on IL-25R⁺ ILC2s and type 2 NK cells, which upon allergen-induced activation secreted granzyme B and IL-13, promoting further IL-25 production from bronchoalveolar epithelia. Furthermore, traffic-related particulate matter, such as DEPs, has been found to boost IL-4, IL-5, IL-13, and IL-17A expression in murine allergy models, with an active contribution of ILC2s to the related immunopathology (229, 230).

8. ILC2 PLASTICITY

Although typical type 2 cytokine-secreting ILC2s are associated with allergic asthma, changes in the local airway cytokine environment can instruct ILC2s to acquire type 1 (Th1) or type 3 (Th17) phenotypes (23) (**Figure 4**). Consequently, these reeducated ILC2s may contribute to nonallergic asthma and even COPD. IL-1 β is a key player in ILC2 plasticity and is produced by airway epithelial cells (231), resulting in higher levels of IL-1 β in the sputum of asthmatic patients as compared to healthy controls (232). IL-1 α/β induces IL-5 and IL-13 production from ILC2s, and when IL-1 β synergizes with IL-33, this potentiates the expression of ST2, IL17RB, and TSLPR on ILC2s, making them more responsive to these cytokines. Paradoxically, IL-1 β also induces T-bet and IL-12 receptor expression by ILC2s. This fosters mixed ILC1/ILC2 gene signatures in ILC2s (233) and conversion to an ILC1-like cell in the presence of IL-12 (234, 235). Indeed, several preclinical studies have also confirmed that triggers of COPD, such as cigarette smoke and respiratory infection with bacteria or viruses, promote an IL-12-dependent switch in ILC2s from GATA3⁺ST2⁺ cells to a GATA3^{lo}TBET⁺IL18R α ⁺ phenotype (70, 235). The plasticity of ILC2s has recently been linked to asthma (236). Beuraud et al. (236) report that severe asthma patients have increased frequencies of a subset of CCR10⁺ ILC2s that express IFN- γ and very little type 2 cytokine. This is mirrored by an increase in T-bet expression and a reduction in GATA3 expression. Strikingly, in mice, inhibition of CCR10⁺ ILC2 recruitment to the lungs exacerbated allergen-induced asthma, suggesting that repolarization to IFN- γ suppresses the type 2 response.

Murine ILC2s have also been reported to be able to switch to an ILC2-ILC3 hybrid phenotype upon IL-23- (237) or IL-25-driven (26, 27) inflammation. In these settings ILC2s acquired *Ror γ t* and IL-17A expression, while downregulating IL-5 and GATA3. These cells still coexpressed IL-13 and were associated with the development of psoriasis-like skin pathology (237). Likewise, human ILC2s develop a ROR γ t⁺CCR6⁺ ILC3-like reversible phenotype in skin lesions of patients with psoriasis (238). Additionally, in cystic fibrosis, a proportion of ILC2s appear to

reversibly transdifferentiate into ILC3-like cells, as a consequence of IL-1 β , TGF- β , and IL-23 (239). When ILC2s from nasal polyps of patients with cystic fibrosis were compared to ILC2s infiltrating nasal polyps of allergic patients, both groups were still strong IL-13 producers, but in the case of cystic fibrosis they were associated with neutrophilic infiltration. Thus, type 3 ex-ILC2s may contribute to neutrophilia observed in non-type 2 asthma.

9. CLINICAL RELEVANCE AND THERAPEUTIC POTENTIAL

Asthma treatment strategies are based on asthma endotype and biomarkers. Here, we discuss therapeutics only in the context of ILC2 function. The most common treatment involves inhaled β_2 agonists, which may be combined with corticosteroids, to induce airway smooth muscle relaxation. However, many immune cells, including mouse and human lung ILC2s, also express β_2 AR. Notably, deletion of β_2 AR from ILC2s increased their proliferation and cytokine expression, while β_2 agonist treatment attenuated ILC2 functions (194). Thus, the therapeutic effects of β_2 agonist might be due to cumulative effects on both smooth muscle and ILC2s.

In most patients, inhaled corticosteroids reduce inflammation in the lung. Although the majority of asthma patients respond to treatment, 3–10% of patients are corticosteroid resistant and account for the majority of asthma-related hospitalizations. Although corticosteroids can attenuate both human and mouse ILC2 viability and type 2 cytokine secretion (54, 100), TSLP-mediated corticosteroid resistance is observed in human and mouse lung ILC2s (54, 100). Notably, the anti-TSLP monoclonal antibody tezepelumab (AMG-157) has shown compelling benefit in asthmatic patients, decreasing annual exacerbation rates by up to 70% in a glucocorticoid nonresponsive patient cohort (240). Of the other ILC2-stimulating cytokines, IL-33 signaling inhibitors are also in phase 2 clinical trials, with anti-ST2 monoclonal antibody (GSK3772847) assessed for asthma treatment and anti-IL-33 antibodies (RG6149/AMG282 and ANB020) trialed in atopic dermatitis, where ANB020 has shown promising results. However, in spite of promising preclinical results, there has been very little progress in targeting IL-25 signaling in human asthma (57, 102, 241).

IL-5-neutralizing antibodies (mepolizumab and reslizumab) and the IL-5R-blocking antibody (benralizumab, which depletes IL-5R α -expressing cells by antibody-dependent cellular cytotoxicity) (242) effectively deplete eosinophil numbers and are approved for treating severe eosinophilic asthma (243–245). By contrast, anti-IL-13 antibody (lebrikizumab and tralokinumab) clinical trials for asthma have disappointed in phase 3 trials for asthma, despite early promise (246–248). The failure of anti-IL-13 antibodies may be attributable to the functional redundancy of IL-4 and IL-13, which both signal via IL-4R α . Indeed, dupilumab, which binds to IL-4R α and abrogates IL-4 and IL-13 signaling, reduces asthma exacerbations and is approved for clinical use in moderate to severe uncontrolled asthma (249). Dupilumab has also shown effectiveness in allergic-rhinitis-induced comorbid asthma, nasal polyposis, and atopic eczema (250–252). Interestingly, unlike the case of IL-5 inhibition, the patient blood eosinophil counts did not play any determinant role in dupilumab efficacy (253). Therefore, targeting multiple facets of ILC2 function might be more effective in controlling asthma than targeting any single cytokine.

Beyond antibody treatment options, prostaglandin and leukotriene signaling are additional targets for inhibiting ILC2 function. Human ILC2s express prostaglandin D₂ receptor (CRTH2) and its ligand PGD₂ activates ILC2s (166). Although fevipiprant, a CRTH2 antagonist, exhibited promising results in a phase 2 trial, it recently failed to meet primary end points in phase 3 LUSTER trials undertaken by Novartis. Although other CRTH2 antagonists remain in development, e.g., timapiprant, and positive effects continue to be reported for small studies, the LUSTER results have raised doubts as to whether such inhibitors will be effective in severe uncontrolled asthma (254).



10. CONCLUSIONS

While studies in experimental mouse models have highlighted the potential contribution of ILC2s in allergic lung diseases, their roles in human asthma are still being unveiled. In this regard, ILC2s appear among a field of players whose complex interplay builds momentum but ultimately leads to an immunological own goal. By responding to epithelium-derived cytokines (IL-33, IL-25, TSLP), ILC2s react rapidly to these first signals and help to kick off the type 2 immune response. Furthermore, due to their expression of receptors for neuropeptides (NMU) and neurotransmitters, they also receive signals from the nervous system allowing ILC2s to sense the wider field. These stimuli allow ILC2s to become the playmakers sending out cytokine passes (IL-4, IL-5, IL-9, IL-13) to bring eosinophils, mast cells, and goblet cells and push their adaptive Th2 cell relatives forward in the type 2 inflammation game. However, ILC2s are not content with a one-dimensional attack and can react flexibly (with plasticity) if they receive fresh team orders to play in a different formation by switching to a new cytokine pattern that can elicit type 1 or type 3 (IL-17) immunity. We are continuing to peel back the pages of the asthma playbook, and in so doing we are identifying new ways to defend from the resulting pathology and develop novel therapeutics.

DISCLOSURE STATEMENT

A.N.J.M. works for the MRC, which has licensed anti-IL-25R antibodies. A.N.J.M. is a scientific advisory board member of SinoMab.

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