

At the Bench: Understanding group 2 innate lymphoid cells in disease

Taylor A. Doherty¹

Department of Medicine, University of California San Diego, La Jolla, California, USA

RECEIVED AUGUST 5, 2014; REVISED OCTOBER 21, 2014; ACCEPTED OCTOBER 29, 2014. DOI: 10.1189/jlb.5BT0814-374R

► SEE CORRESPONDING ARTICLE ON PAGE 469

ABSTRACT

The conventional paradigm of type 2 inflammatory responses is characterized by activation of CD4⁺ Th2 cells that produce IL-4, IL-5, and IL-13, resulting in tissue eosinophil infiltration, mucus metaplasia, AHR, and IgE production. However, the recent discovery of ILC2s in mice and humans has brought forth a novel pathway in type 2 immunity that may work independent of, or in concert with, adaptive Th2 responses. ILC2s were described initially as lineage-negative lymphocytes that produce high levels of Th2 cytokines IL-5 and IL-13 in response to IL-25 and IL-33 and promote protection against helminth infections. More recent investigations have identified novel upstream regulators, as well as novel ILC2 products. ILC2s are found in mucosal surfaces, including respiratory tract and skin, and studies from experimental asthma and atopic dermatitis models support a role for ILC2s in promoting type 2 inflammatory responses. There are many unanswered questions about the role of ILC2s in chronic allergic diseases, including how ILC2s or upstream pathways can be targeted for therapy. As ILC2s are not antigen specific and may be activated after exposures to a variety of infectious agents and irritants thought to contribute to respiratory and skin diseases, future strategies to target ILC2 function in human disease may be promising. Our intent is to identify priority areas for ILC2 translational research based on basic research insights. *J. Leukoc. Biol.* 97: 455–467; 2015.

Introduction

Type 2 inflammation, including tissue eosinophilia, epithelial mucus metaplasia, and IgE production, is characteristic of allergic diseases, such as asthma [1]. Classically, conventional

CD4⁺ Th2 cells that secrete IL-4, IL-5, and IL-13 have been considered the primary orchestrators of the allergic response in tissues. Although the study of conventional Th2 cells has provided significant insight into the pathogenesis of asthma and allergy, many phenotypes of human disease are not adequately explained by the Th2 cell paradigm. For example, viruses are thought to be a primary trigger of asthma exacerbations, and evidence is accumulating that viruses may play a role in the development of asthma [2]. Furthermore, environmental insults, such as tobacco smoke and ozone exposure, stimulate innate-immune pathways and may contribute to asthma pathogenesis [3, 4]. This suggests that additional innate-immune pathways are active, and recent studies support ILC2s as important contributors in models of type 2 diseases. Here, we will review cellular and molecular mechanisms of ILC2s in several animal models and stress their potential contributions to diseases of the respiratory tract and skin. The accompanying review by Peebles [5] will focus on the roles of ILC2s in clinical disease and highlight the need for strategies to target ILC2s in humans.

DISCOVERY OF ILC2S: A NEW ERA IN TYPE 2 IMMUNITY

The presence of a non-B, non-T cell population that produces Th2 cytokines has been suggested for over 1 decade. Beginning in 2001, investigations of IL-25-induced responses in mice led to the discovery of novel non-B, non-T cells that produced Th2 cytokines [6–8]. Three pivotal studies reported in 2010 confirmed the presence of innate lymphocytes that robustly produce Th2 cytokines and are now termed ILC2s by consensus [9–12]. The first report showed that the IL-1 family cytokine IL-33 induced high levels of IL-5 and IL-13 from lineage-negative (lack of expression for CD3, CD4, CD8, TCR- β , TCR- δ , CD5, CD19, B220, NK1.1, Ter-119, GR-1, Mac-1, CD11c, and Fc ϵ R1) and c-kit⁺ Sca-1⁺ lymphocytes, present in the mesenteric fat of mice [11]. The cells were termed NHCs, and the authors showed that IL-33 stimulation of 5000 purified NHCs induced microgram levels of IL-5 and IL-13, demonstrating a robust Th2 cytokine production capacity. Furthermore, adoptive transfer of NHCs

Abbreviations: ^{-/-} = deficient, γ c = γ chain, AHR = airway hyper-responsiveness, BAL = bronchoalveolar lavage, CLP = common lymphoid progenitor, CRS = chronic rhinosinusitis, CRSwNP = chronic rhinosinusitis with nasal polyp, CRTH2 = chemoattractant receptor homologous molecule expressed on Th2 lymphocytes, CysLT = cysteinyl leukotriene, DC = dendritic cell, DP2 = PGD2R, DR3 = death receptor 3,

(continued on next page)

1. Correspondence: Dept. of Medicine, University of California San Diego, Biomedical Sciences Bldg., Room 5080, 9500 Gilman Dr., La Jolla, CA 92093-0635, USA. E-mail: tdoherty@ucsd.edu

into RAG2/ γ c knockout mice that lack NHCs resulted in intestinal goblet cell hyperplasia after *Nippostrongylus brasiliensis* infection. A subsequent report showed that IL-25 and IL-33 given to IL-13 reporter mice led to the accumulation of an IL-13⁺ non-T cell population in the MLNs and small intestines [9]. The cells were named “nuocytes,” and further work showed that nuocyte expansion after helminth infection required IL-25 and IL-33. Importantly, nuocyte IL-13 production was also required for helminth expulsion. The 3rd report described a similar population of lineage-negative IL-13⁺ cells, termed Ih2s, which accumulated in the MLNs, spleens, livers, and lungs of mice after *N. brasiliensis* infection. Ih2s were also sufficient to induce worm expulsion after stimulation with IL-25 in the absence of adaptive immunity [10]. Collectively, these landmark studies demonstrated that NHCs, nuocytes, and Ih2s (now all collectively referred to as ILC2s) were novel Th2 cytokine-producing cells critical for early helminth immunity (see Table 1).

ILC2s: Innate lymphocytes that do not express surface markers for common lineage markers (lineage negative) and secrete type 2 cytokines, including IL-4, IL-5, and IL-13. ILC2s are also not antigen specific and can be activated by a variety of mediators present in type 2 inflammatory diseases. ILC2s include nuocytes, NHC, and Ih2s.

ILC2 DEVELOPMENT

Similar to other ILCs, ILC2s originate from CLPs that require Id2, Notch, and IL-7R that signals through the common γ c for development [11, 13–15] (see Fig. 1). A complete absence of mesenteric fat-associated c-kit⁺ Sca-1⁺ NHCs was shown initially in Id2 knockout mice and is consistent with the requirement of Id2 for innate lymphoid development [11]. A follow-up report demonstrated that bone marrow NHCs are marked by a history of RAG expression and developed from common lymphoid, but not myeloid or erythroid, progenitors after studies were performed of progenitors transplanted into irradiated congenic mice [13]. IL-7 or IL-7R is required for development of ILC2s, and IL-7R^{-/-} mice lack innate type 2 lung inflammation after exposure to the fungal allergen *Alternaria alternata* compared with a robust response induced in RAG^{-/-} mice that have ILC2s but lack T or B cells [11, 16, 17]. Although several ILC2 studies have used IL-7R^{-/-} mice, interpretations of ILC2 function in these mice can be challenging, given the potential role of IL-7R in eosinophil, T cell, and macrophage function that could contribute to type 2 inflammation [18, 19].

The master Th2 cytokine transcription factor GATA3 has been shown to play a prominent role in ILC2 development and

function and represents shared transcriptional programming of ILC2s and Th2 cells [20–22]. Aside from a clear role in the development of ILC2s, GATA3 is also critical to the development of ILC3s and all IL-7R-expressing ILCs [23, 24]. This suggests that GATA3 has a broad, early role in ILC development, apart from a later role in ILC2 cytokine production. In addition to GATA-3 expression, our group has reported that human peripheral blood ILC2s and mouse lung and bone marrow ILC2s highly express the transcription factor Ets-1 [17], which has been shown to be critical for NK and NKT cell development (25, 26). Further, Ets-1^{-/-} T cells cultured in Th2-polarizing conditions are impaired in Th2 cytokine production, suggesting that Ets-1 may be required for ILC2 development and/or Th2 cytokine production similar to GATA-3 [27].

GATA-3: Master transcription factor for Th2 cytokine production and development of ILC2s. GATA-3 is also highly expressed in conventional Th2 cells after differentiation from naïve T cells.

Since the discovery of ILC2s, investigations have identified novel transcription factors that regulate ILC2 development. The nuclear receptor ROR α was shown to be required for ILC2 development from CLPs into nuocytes in vitro, and IL-25 administration to ROR α knockout mice resulted in severely impaired nuocyte expansion, as well as reduced goblet cell hyperplasia compared with WT mice [28]. A subsequent report confirmed that ROR α contributed to NHC development and importantly, showed that responses from ILCs distinct from ILC2s, as well as conventional Th2 cells, were not impaired [29]. In contrast to GATA-3 regulation of ILC2s and Th2 cells, ROR α may specifically regulate ILC2s without a significant role in CD4⁺ Th2 cell differentiation or cytokine production. Interestingly, GATA3^{-/-} CLPs do not express ROR α or Id2 mRNAs, suggesting that ILC2s undergo complex, sequential regulation by several molecules in their development (Fig. 1) [21]. From a translational aspect, ROR α has been identified previously as an asthma-susceptibility gene in genome-wide association studies and possibly links ILC2s to human asthma [30].

ROR α : A transcription factor required for ILC2 development that does not appear to be required for conventional CD4⁺ Th2 development or function.

In addition to ROR α , Notch signaling member TCF-1 has been shown to be required for the development of functional ILC2s, as mice that lack TCF-1 displayed impaired ILC2 cytokine responses [31]. Additionally, the transcription factor PLZF has been detected at high levels in all ILC precursors, although bone marrow chimera studies revealed that PLZF appears to be required more specifically for development of ILC2s, in addition to a known role for NK T cell development [32]. Overall, the development of ILC2s from lymphocyte progenitors depends on GATA3-induced Id2 and ROR α , in addition to Notch, TCF-1, PLZF, and IL-7R signaling.

The varied surface-marker expression and tissue distribution initially reported with mouse Ih2s, nuocytes, and NHCs have led to some confusion regarding whether these populations are indeed distinct from each other. The common surface-marker

(continued from previous page)

EGFR = epidermal growth factor receptor, Ets-1 = E26 transformation-specific 1, GATA3 = transacting T cell-specific transcription factor 3, Id2 = inhibitor of DNA binding 2, Ih2 = innate helper 2 cell, ILC2 = group 2 innate lymphoid cell, LT = leukotriene, LXA4 = lipoxin A4, MLN = mesenteric lymph node, NHC = natural helper cell, OX40L = OX40 ligand, PLZF = promyelocytic leukemia zinc finger, RAG2 = recombination-activating gene 2, ROR α = retinoic acid receptor-related orphan receptor α , TCF-1 = T cell factor 1, TL1A = TNF-like ligand 1A, TSLP = thymic stromal lymphopoietin, WT = wild-type

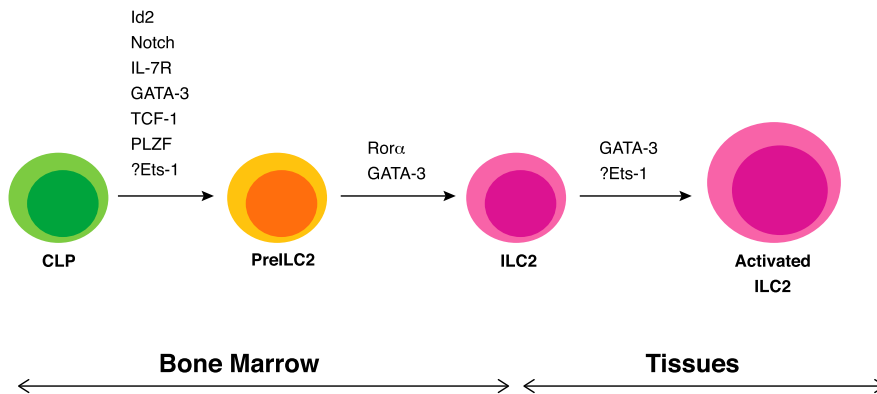


Figure 1. ILC2 development. ILC2s derive from a CLP through expression of Id2, Notch, IL-7R, GATA-3, TCF-1, PLZF, and possibly, Ets-1. Pre-ILC2s require GATA-3 and ROR α for maturation into ILC2s. GATA-3 and possibly Ets-1 are required for activated ILC2 function.

expression shared by these populations includes CD45, Thy1.2, CD44, CD69, and c-kit, but differences exist in surface expression of Sca-1, T1/ST2 (IL-33R), and IL-7R α (Table 1) [9–11]. However, the commonality that Ih2s, nuocytes, and NHCs are lineage-negative lymphocytes that produce Th2 cytokines has led to a consensus to include these populations as ILC2s [12]. Differences detected among these cell populations may relate to the level of activation, kinetics, and tissue distribution, as well as methods of detection or stimulation. Human ILC2s have also been detected in multiple tissues, including the skin, respiratory, and gastrointestinal tract, and are defined as lineage-negative lymphocytes that express the PGD2R (or DP2; also known as CTRH2) and CD161 [33]. Ongoing work will, no doubt, identify novel ILC2 surface markers, and the hope is that a more ILC2-specific marker is discovered to allow for improved detection and possibly targeting.

CTRH2: Also known as DP2, is one of the 2 receptors for PGD2, a lipid mediator that is released rapidly in allergic inflammation by mast cells, eosinophils, and macrophages. CTRH2 mediates activation and chemotaxis of immune cells, including ILC2s, Th2 cells, and eosinophils.

PRODUCTS OF ILC2S IN HEALTH AND DISEASE

Studies with animal models have shown that the products of ILC2s contribute to normal tissue homeostasis, pathologic changes in disease, and repair responses after mucosal insult. The initial studies of ILC2s demonstrated an early role in helminth immunity, as ILC2s were shown to produce high levels of IL-5 and IL-13, leading to tissue eosinophilia, mucus production, and worm expulsion [9–11]. Interestingly, the quintessential Th2 cytokine IL-4 was not detected after ILC2 stimulation with IL-33 in these early reports [9, 11]. Subsequent studies have shown that TSLP and LTD4 can induce IL-4 production by ILC2s, suggesting that under specific conditions, ILC2s are a source of IL-4 [20, 34]. Notably, IL-33 stimulation of conventional T cells is reported to induce a Th2 cell population that produces IL-5, but not IL-4, and thus, lack of IL-4 production by ILC2s in the early reports may be related to a specific effect of IL-33R signaling [35].

The first study to demonstrate a role for lung ILC2s in a mouse model of disease showed that ILC2 IL-13 expression was required for AHR in mice challenged with influenza virus [36]. A

TABLE 1. Initially discovered ILC2 subsets

ILC2 member	Tissue localization	Marker expression	Function	Ref.
NHC	Mesenteric fat associated Lymphoid clusters	CD45, CD44, CD69, c-kit, CD90.2, CD25, CD27, CD38, IL-7R α , Sca-1, T1/ST2, GITR	NHC IL-5 and IL-13 production after IL-33 stimulation; NHC required for helminth expulsion in RAG2/ γ c knockout mice	[11]
Nuocyte	Bone marrow, lung, MLNs, spleen	CD45, CD44, CD69, c-kit, CD90.2, CD43, CD49d, CD127, CD132, IL-7R α , Sca-1, T1/ST2, MHC-II, Itgb7, ICOS, ICAM-1, CCR9	Accumulation of non-T cell IL-13 ⁺ cells after IL-25/IL-33 administration; nuocyte IL-13 required for helminth expulsion in IL-17RB (IL-25R) knockout mice	[9]
Ih2	Bone marrow, liver, lung, MLNs, peritoneum, spleen	CD45, CD44, CD69, c-kit, CD90.2, CD25, CD122	Accumulation of IL-13 ⁺ lineage- negative cells after IL-25 stimulation; Ih2 required for helminth expulsion in RAG2/ γ c knockout mice	[10]

GITR, Glucocorticoid-induced TNFR; Itgb7, integrin- β 7.

subsequent report showed that adoptively transferred WT ILC2s were sufficient to restore AHR in IL-13 knockout mice after IL-25 exposure, thus further highlighting the importance of ILC2s as a source of IL-13 [37]. Lung ILC2s have since been shown to produce IL-5 constitutively, which controls eosinophil homeostasis, although IL-13 production requires stimulation with IL-25 or IL-33 [38].

Additional studies have demonstrated that ILC2s also produce IL-6 and IL-9 [11, 39]. IL-9 is also produced by Th2 or Th9 cells and contributes to airway eosinophilia, mast cell accumulation, and mucus production in animal asthma models [40]. The first report, showing that ILC2s produce IL-9, demonstrated that mouse lung ILC2s were a dominant source of IL-9 in WT mice challenged with the protease papain [39]. ILC2 IL-9 production was transient after papain challenge, peaking at 12 h and minimally present by 24 h, and promoted production of ILC2 IL-5, IL-6, and IL-13. Interestingly, ILC2 IL-9 production was dependent on IL-2 and intact adaptive immunity, thus suggesting adaptive immune cells can control ILC2 function.

Early studies provided important insight into the contribution of ILC2s to pathologic changes in type 2 immune disease, including tissue eosinophilia, mucus production, and AHR. However, the finding that ILC2s also promote tissue-repair responses through production of the EGFR ligand amphiregulin has changed our perspective of these novel cells [41]. Monticelli et al. [41] showed that depletion of ILC2s in RAG knockout mice infected with influenza virus led to hypoxemia, hypothermia, and loss of epithelial barrier integrity. ILC2s were found to produce high levels of amphiregulin and adoptive transfer of ILC2s, or administration of amphiregulin, into ILC2-depleted mice restored airway integrity after influenza infection. Our group has also found that lung ILC2s rapidly produce amphiregulin after innate challenge with the fungal allergen *Alternaria* [17]. Whether ILC2 amphiregulin can also contribute to pathologic remodeling, as is found in asthma, is unknown. Overall, the context of disease may dictate whether ILC2s contribute to pathogenic responses or play a role in normal repair processes.

Amphiregulin: *EGFR ligand produced by ILC2s and involved in lung repair responses after viral infection in mice. Although many ILC2 cytokines are pathogenic in lung-disease models, the production of amphiregulin suggests dual roles of ILC2 in promoting inflammatory as well as repair responses.*

Recently, the role of ILC2s in conditions of homeostasis has been investigated. With the use of IL-5 reporter and deleter mice, a study showed that ILC2s are the dominant source of IL-5 in many tissues at rest, including brain, heart, lung, kidney, skin, intestine, and uterus [38]. In the naïve mouse lung, IL-5 production by ILC2s was constitutive, but IL-13 required further stimulation. In contrast, ILC2s in the small intestine constitutively produced IL-13, which was increased further after food intake. Interestingly, vasoactive peptide, a hormone activated by eating, induced further ILC2 IL-5 production, suggesting that homeostatic control of ILC2s may occur through neuropeptide stimulation. Another report by the same group demonstrated that ILC2s were the major source of IL-5 and IL-13 in mouse

visceral adipose tissue and contributed to eosinophil and alternatively activated macrophage accumulation [42]. The study also showed that IL-5 and eosinophil-deficient mice displayed impaired glucose control after a high-fat diet. A similar investigation found that depletion of ILCs in RAG^{-/-} animals fed a high-fat diet led to increased obesity and impaired glucose tolerance [43]. Adoptive transfer of ILC2s or NKT cells into obese mice induced weight loss and improved glucose control. Together, these reports suggest that ILC2s are critical in maintaining tissue eosinophil homeostasis, as well as visceral fat abundance and glucose control. Overall, the functions of ILC2s under homeostatic conditions and in disease should be greatly explored before targeting these cells for therapy in human type 2 inflammatory diseases.

Clinical questions: *Can ILC2s be selectively targeted, given that no specific marker has been identified? If so, how can such a therapy preserve homeostatic or tissue-repair responses?*

REGULATION OF ILC2 FUNCTION

The list of mediators that can modulate ILC2 function is continually expanding and is summarized in **Fig. 2**. The cytokines IL-33 and IL-25 were first described to induce ILC2 cytokine production and proliferation [6–11]. IL-33 exists in a chromatin-bound, biologically active proform, present in epithelial cells, endothelial cells, and macrophages, and can be processed into a more potent cleaved protein by neutrophil elastase and cathepsin G [44, 45]. IL-33 binds to a heterodimer of ST2 and the IL-1R accessory protein (T1/ST2). IL-25 (IL-17E) is a member of the IL-17 family and binds specifically to the heterodimer of IL-17RB and IL-17RA. IL-25 is secreted by many cell types, including Th2 cells, eosinophils, as well as epithelial cells [46]. Ligation of T1/ST2 by IL-33 or IL-25R by IL-25 results in activation of MAPK and NF- κ B signaling [36, 46, 47]. IL-25 and IL-33 are induced in type 2 inflammatory responses in mice and are thus available for potent ILC2 Th2 cytokine production [17, 48, 51]. Notably, a report has shown that IL-33 is more potent than IL-25 in the induction of ILC2 IL-13 production responsible for AHR in mice [52]. Shortly after the characterization of mouse ILC2 responses, human ILC2s from fetal gut and peripheral blood were shown to produce IL-13 in response to IL-25 and IL-33 [33]. Thus, IL-33 and IL-25 induce activation of human and mouse ILC2s, and the availability of these cytokines from different cell types may dictate the dominant pathway of ILC2 activation. Aside from ILC2 activation, IL-25 and IL-33 activate many cells, including DCs, eosinophils, macrophages, basophils, T cells, and NKT cells that could contribute amplification of the inflammatory response and indirectly activate ILC2s [47, 53].

Recent work has identified mediators, apart from IL-25 and IL-33, that regulate ILC2 responses including the epithelial cytokine TSLP, which has been detected at increased levels in the airway epithelium of asthmatics and in the skin of atopic dermatitis patients and was initially was found to induce expression of the OX40L on DCs leading to Th2 cell differentiation [54, 55]. More recently, a role for TSLP in ILC2

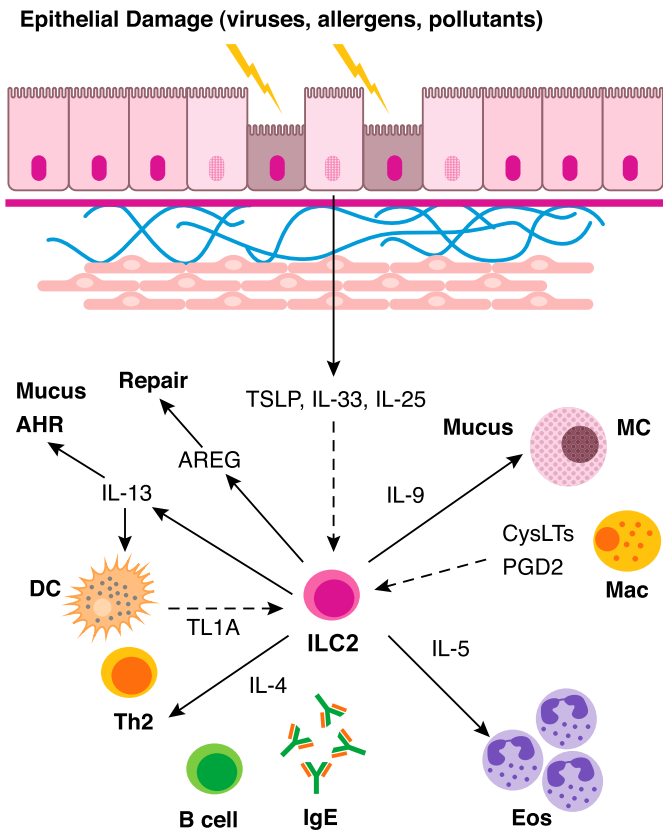


Figure 2. Tissue ILC2 responses. Epithelial cytokines TSLP, IL-33, and IL-25 can activate ILC2s to produce Th2 cytokines, including IL-4, IL-5, IL-9, and IL-13. PGD2 and LTD4, produced by mast cells, eosinophils (Eos), and macrophages (Mac), can also activate ILC2s, whereas LXA4 may have inhibitory effects on ILC2 function. TNF member TL1A, produced by DCs and other cells, might further promote ILC2 activation through binding of DR3. ILC2s may also respond to basophil IL-4 production to promote tissue eosinophilia (not shown). ILC2 and Th2 cells work in parallel to promote tissue type 2 responses. IL-5 production stimulates recruitment, activation, and survival of eosinophils. IL-13 induces AHR, DC migration, and along with IL-9, promotes mucus production. ILC2 IL-4 secretion may contribute to Th2 cell differentiation and IgE production by B cells. Amphiregulin (AREG) secreted by ILC2s binds to EGFR in the airway epithelium to induce repair responses and possibly pathologic remodeling. MC, Mast cell.

responses was demonstrated as human peripheral blood and nasal polyp ILC2s cultured with TSLP and IL-33 displayed enhanced Th2 cytokine production (including IL-4) above IL-33 alone [20]. TSLP has also been shown to activate mouse ILC2s and independent of IL-33 in the skin [56, 57].

GATA3 is considered a master Th2 cytokine transcription factor that controls ILC2 development, as well as function [17, 20–22]. Interestingly, TSLP stimulation of human ILC2s was shown to increase the level of GATA3 expression [20]. Forced GATA3 expression in DP2-negative (CRHT2-negative) human ILCs also up-regulated DP2 (CRHT2), T1/ST2, and TSLPR levels and led to Th2 cytokine production compared with control vector-transduced ILCs. Furthermore, knockdown of GATA3 in human ILC2s led to reduced IL-13 levels. Overall, studies with human and mouse ILC2s have convincingly shown that GATA3 regulates much of the ILC2 differentiation and

activation program. Although ILC2s and Th2 cells share high GATA3 expression, ILC2s express GATA3 in the bone marrow, suggesting that they are primed for Th2 cytokine production, in contrast to conventional Th2 cells that differentiate in the periphery [17, 22].

Cytokines, including IL-25, IL-33, and TSLP, were the first mediators discovered to activate ILC2s, but more recent reports have demonstrated that lipid mediators present in type 2 inflammatory diseases also modulate ILC2 activity. Eicosanoids, including PGs and LTs, are arachidonic acid-derived lipid mediators produced by many cells, including eosinophils, mast cells, DCs, and macrophages that have been shown to modulate ILC2 function [34, 58, 59]. PGD2 binds to DP2 (CRTH2), expressed on human ILC2s, as well as Th2 cells and eosinophils, and promotes chemotaxis, cytokine production, and survival [58, 60–64]. In human peripheral blood ILC2 cultures, PGD2 potentiated IL-13 production above IL-2, IL-25, and IL-33 stimulation that was also mimicked by a DP2 (CRTH2) agonist [58]. The same study also demonstrated that human ILC2s express LXA4R/N-formyl peptide receptor 2, the receptor for LXA4, and LXA4 inhibited ILC2 IL-13 production in the presence of IL-2, IL-25, IL-33, and PGD2. In addition to a role in cytokine production, DP2 (CRTH2) ligation by PGD2 functions in recruitment of many inflammatory cells into tissues, including basophils, eosinophils, and Th2 cells [60]. Our group [65] and others [61] have recently shown that PGD2 induces chemotaxis of human ILC2s via DP2 (CRTH2). PGD2 has also been tied to severe asthma, and PGD2 binding to DP2 (CRTH2) on ILC2s may have multiple functional consequences, including chemotaxis and cytokine production in asthma [62].

CysLTs are another group of eicosanoids that are elevated in asthma and CRS [59]. LTD4 binds with high affinity to the CysLT1R that is highly expressed on the surface of mouse lung ILC2s, as we have reported [34]. In vitro, LTD4 potently induced ILC2 calcium influx and cytokine production, including release of IL-4. Furthermore, intranasal LTD4 potentiated allergen-induced ILC2 proliferation and lung eosinophilia in RAG2 knockout mice (that have ILC2s but lack T and B cells). As eicosanoid molecules, including PGs, LTs, and lipoxins are largely produced by nonepithelial cells, including macrophages, mast cells, eosinophils, and DCs, these reports suggest novel ways in which ILC2s can be modulated (Fig. 2) [59].

CysLTs: Lipid mediators derived from arachidonic acid that induce bronchial constriction, as well as chemotaxis and activation of immune cells in allergic disease. CysLTs are produced by eosinophils, mast cells, macrophages, and DCs.

The TNF/TNFR family members control a wide array of innate and adaptive immune responses [66, 67]. The TNF member TL1A is produced by activated DCs and T cells and binds to DR3, resulting in signaling through TNFR-associated death domain protein and intracytoplasmic death domain [68, 69]. A recent report demonstrated that DR3 is expressed on human and mouse ILC2s, and TL1A directly induces ILC2 cytokine production in vitro and promotes ILC2 cytokine production and expansion in vivo [70]. Importantly, DR3^{-/-} mice were impaired

in clearance of *N. brasiliensis* infection and had reduced lung inflammation and numbers of ILC2s compared with controls. A role for TL1A/DR3 was shown previously to be critical for the development of allergic lung inflammation in an OVA model of asthma, and DR3⁺ ILC2s may have contributed to these findings [68]. Overall, the expanding numbers of mediators, including cytokines and lipid mediators that modulate ILC2 function, support the idea that the inflammatory milieu created by varying neighboring cell types likely dictates ILC2 activity. Thus, the potential for redundancy and context specificity in ILC2 activation will require further exploration if therapy to target upstream mediators of ILC2 activation in humans is undertaken.

Clinical question: Will targeting a single upstream mediator of ILC2 activation modulate ILC2 function, given the presence of several potentially redundant pathways of ILC2 activation?

ILC2 CROSS-TALK WITH OTHER IMMUNE CELLS

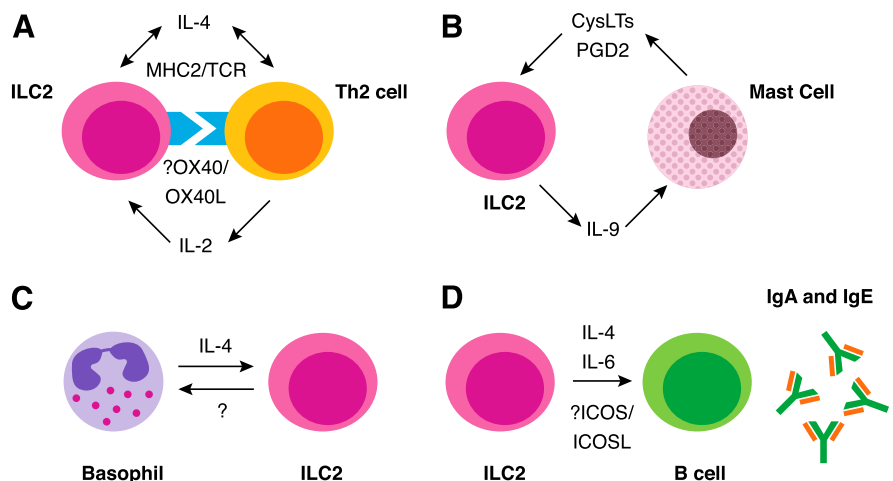
The relationship between ILC2s and other immune cells is summarized in **Fig. 3**. ILC2s have been shown recently to participate in cross-talk with DCs, CD4⁺ T cells, B cells, mast cells, and basophils. Early studies revealed that γ c cytokines IL-2 and IL-7 support ILC2 survival and cytokine production in vitro [11, 56]. As conventional T cells are a source of IL-2, this suggested that adaptive immune cells could be involved in the maintenance of ILC2 responses. A recent study investigated such interactions between mouse ILC2s and naïve CD4 cells and found that anti-CD3/CD28-stimulated naïve CD4⁺ cells induced ILC2 proliferation and IL-4 and IL-5 production that was dependent on IL-2 [71]. Furthermore, ILC2s induced contact-dependent CD4⁺ T cell-Th2 cytokine production, and ILC2s could act as MHCII-dependent APCs in vitro to stimulate T cell responses. In an attempt to translate these findings in vivo, the authors adoptively transferred OVA-specific CD4⁺ cells and ILC2s into ST2^{-/-} mice challenged with IL-33 plus OVA. Only cotransfer of ILC2s with CD4 cells resulted in MLN OVA-specific IL-13 production. A subsequent report showed that ILC2s promoted CD4 T cell responses in vitro

that were partially dependent on OX40/OX40L interactions and IL-4 [72]. Although these reports suggest that ILC2s induce CD4 cell proliferation and Th2 cytokine production in a contact-dependent manner in vitro, whether ILC2 and CD4 cells require direct contact in vivo for promoting Th2 responses is unclear, especially given the low numbers of ILC2s in tissues and lymph nodes. Additionally, these studies have used naïve and primary activated T cells and not memory T cells that likely propagate chronic type 2 inflammatory diseases and trigger exacerbations.

The production of IL-4 from ILC2s could provide an important signal leading to Th2 cell polarization in vivo [20, 34, 71, 72]. However, a recent study showed that ILC2 IL-13, but not IL-4, was required for papain-induced Th2 cell responses in ROR α ^{-/-} bone marrow transplant mice that lack ILC2s but have normal Th2 cell responses [73]. In another report, the same group showed that ROR α ^{-/-} bone marrow transplant mice were impaired in generating Th2 cell responses in a house dust-mite model, but not an OVA/alum model, suggesting that the type of antigen and/or route of sensitization (mucosal vs. systemic) may dictate whether ILC2s contribute to Th2 differentiation [74]. Although the precise role of ILC2s in initiating adaptive Th2 responses may depend on the context or model, the idea that ILC2s could be critical for development of adaptive type 2 inflammatory conditions has broad implications.

Basophils have also been implicated in promoting Th2 responses, including those induced by papain [75, 76]. Recently, a potential link between basophil IL-4 production and ILC2 activation in papain-induced lung inflammation was reported [77]. The authors generated IL-4 enhancer mutant mice (IL-4 3'UTR^{-/-} mice) that selectively lacked basophil IL-4 production, whereas mast cells and CD4⁺ T cells were capable of IL-4 production. IL-4 3'UTR^{-/-} mice were impaired in developing eosinophilic lung inflammation, mucus production, and AHR after 3 papain challenges compared with WT mice. In addition, lung ILC2 activation and numbers were reduced in the IL-4 3'UTR^{-/-} mice. Lung ILC2s express IL-4R α , as we have previously reported [17], and this study showed that IL-4 stimulation of ILC2s resulted in production of CCL11, CCL3, CCL5, and IL-9, as well as potentiation of IL-33-induced IL-5 and IL-13 secretion. Importantly, adoptive transfer of IL-4^{-/-} basophils in basophil-depleted mice led to reduced numbers of

Figure 3. ILC2 cross-talk. (A) ILC2s and CD4⁺ T cells may functionally interact directly through MHC2/TCR signaling and possibly OX40/OX40L. IL-2, secreted by T cells, supports ILC2 survival and proliferation, and IL-4, secreted by ILC2s or Th2 cells, may lead to mutual stimulation. (B) Mast cell release of lipid mediators, including CysLTs and PGD2, can lead to chemotaxis (PGD2) and activation of ILC2s. In turn, activated ILC2s produce IL-9 that leads to mast cell accumulation in tissues. (C) Basophils are an innate source of IL-4 required for ILC2 activation after airway exposure to protease. (D) ILC2s produce IL-4 and IL-6 that can induce B cell production of IgE and IgA, respectively. ILC2s express ICOS, and B cells express the ICOS ligand (ICOSL), although an interaction between these cells via ICOS/ICOSL is unknown.



ILC2s, eosinophilic lung inflammation, and mucus production compared with mice receiving WT basophils. Thus, basophil/ILC2 cross-talk may initiate early type 2 responses to protease allergens. Whether these findings might translate to ongoing allergic inflammation is not yet clear.

In addition to basophil/ILC2 cross-talk, mast cells may communicate with ILC2s. Mast cells and ILC2s were found to be colocalized in the dermis of mice, and interactions between these cell types were visualized for up to 30 min [78]. Furthermore, mast cell release of IL-6 and TNF- α was reduced by IL-13, suggesting that ILC2 IL-13 production may have a suppressive role in mast cell function. Mast cells and ILC2s have also been detected in close proximity in human lung [58]. One possible reason for being such close neighbors could be a result of mast cell production of PGD₂ that is chemotactic for ILC2s, as our group [65] and others [61] have shown. In turn, ILC2 IL-9 production could induce mast cell accumulation in tissues [78].

Finally, B cells and ILC2s may have meaningful interactions through direct contact, as well as cytokine production. ILC2s express the costimulatory molecule ICOS and could be stimulated by ICOS ligand-expressing B cells, but any functional significance of this possible interaction has yet to be shown [9]. The initial description of ILC2s in mesenteric fat demonstrated that production of IL-5 and IL-6 led to IgA antibody secretion by B1 B cells [11]. Furthermore, an ILC2-like population that is Sca-1⁺ Thy1⁺ IL-18R⁺ but does not express IL-7R or T1/ST2 was shown to promote B cell IgE production in vitro in the presence of ILC2 stimulated with IL-18 [79].

In addition to interactions with several immune cell types, ILC2s may have an important influence on epithelium, smooth muscle cells, and fibroblasts, as is suggested by the report that ILC2 amphiregulin production promotes lung epithelial tissue repair [41]. Overall, ILC2s have been shown to interact with many immune cell types known to contribute to type 2 diseases, and the uncovering of the functional significance of such interactions will likely improve our understanding of the role of tissue ILC2s.

Clinical question: *If ILC2s are targeted, what will be the impact on the function of other immune and structural cell types?*

ILC2S IN EXPERIMENTAL ASTHMA AND LUNG DISEASE MODELS

Human asthma is characterized by peribronchial inflammation, AHR, epithelial mucus production, and airway remodeling with principle triggers that include respiratory viruses and allergens. Mouse model protocols use airway challenges with viruses or allergens to induce airway inflammation and have been able to replicate features of human asthma to varying extents. Lung ILC2s from mice were first shown to contribute to AHR after influenza virus exposure [36]. Adoptive transfer of WT ILC2, but not IL-13 knockout ILC2, restored AHR in IL-13 knockout mice, suggesting that ILC2 IL-13 was critical for influenza-induced AHR. Since this initial report, several groups have shown that ILC2s contribute to type 2 lung inflammatory responses and AHR in mice exposed to

multiple allergens, including *Alternaria*, papain, house dust mite, and OVA, and findings are summarized in **Table 2** [16, 17, 36, 37, 56, 80].

Most of the lung disease models have used RAG knockout mice (that lack adaptive immunity) or have evaluated ILC2 responses before generation of adaptive immunity. Thus, the contribution of ILC2s compared with other cell types during an ongoing adaptive response is unclear. However, a few studies have investigated the relative levels of Th2 cytokine-producing cell types during early and later antigen challenges [37, 80]. One study demonstrated that the numbers of lung IL-5⁺ ILC2s were about 1/2 of IL-5⁺ T cells after 1 and 3 challenges but similar after 10 challenges with house dust mite [80]. Lung IL-13⁺ ILC2s remained less than 1/2 of IL-13⁺ T cells after 1, 3, and 10 house dust-mite challenges. However, ILC2 and T cell IL-5⁺ and IL-13⁺ cells were similar in BAL regardless of the number of challenges. With the use of an OVA model, the same study showed that ILC2s were a major source of IL-5, but neither T cells nor ILC2s were the dominant source of IL-4 and IL-13. Another study used IL-4 and IL-13 reporter mice and compared numbers of IL-4⁺ and IL-13⁺ cells within the CD4⁺ and CD4⁻ compartments in the BAL after a 12 or 25 day OVA model [37]. The IL-13⁺ CD4⁺ cell levels were 3 \times that of CD4⁻ (which included ILC2 and other CD4⁻ cells) in the 12 day model and nearly 10 \times greater in the 25 day model. IL-4⁺ cells in the BAL appeared to be neither CD4⁺ cells nor ILC2s. Together, these reports suggest that the numbers of cytokine-positive ILC2s may be less than CD4⁺ and other cell types during later antigen challenges, but ILC2s continue to produce Th2 cytokines at later stages of inflammatory responses.

One limitation of these studies is that the methods do not account for the level of cytokine production/cell, and previous reports suggest that ILC2s may have a larger capacity for Th2 cytokine production in vitro compared with other cell types. On a per-cell basis, ILC2 have been reported to produce significantly more IL-5 and IL-13 (microgram amounts/5000 cells) compared with mast cells, basophils, NKT cells, and Th2 cells when stimulated with IL-33 [11, 84–87]. Strategies to deplete ILC2s, beginning at later stages of allergic inflammation, as has been done with CD4 cells [88], will be required to address their role in chronic antigen-driven responses.

Clinical questions: *As the role of ILC2s during an ongoing adaptive immune response is unknown, what effect will targeting ILC2s have in chronic human diseases? Furthermore, what is the relative contribution of ILC2s during different stages of type 2 inflammation compared with T cells, mast cells, eosinophils, basophils, and NK T cells?*

Mice exposed to the fungal allergen *Alternaria* develop rapid increases in IL-33, activation of lung ILC2s, and airway eosinophilia, dependent on IL-33 [16, 48]. *Alternaria* is a protease allergen, and activation of ILC2s through IL-33 may be a common pathway shared with other proteases, including papain [56]. We have reported further that single intranasal administration of *Alternaria* induces rapid CysLT production that could activate ILC2s through CysLT1R, suggesting that IL-33-independent pathways of ILC2 activation are present early

TABLE 2. ILC2s in selected lung and skin disease models

Allergen/virus/compound	ILC2 involvement in model	Ref.
Lung inflammation models		
Influenza	AHR dependent on IL-33 and ILC2 IL-13 (RAG2 ^{-/-} mice) Epithelial tissue repair dependent on ILC2 amphiregulin (RAG1 ^{-/-} mice) ILC2 depletion by CD90.1 antibody in RAG ^{-/-} mice	[36, 41]
Fungal allergen (<i>Alternaria</i>)	Initiation of airway eosinophilia (RAG2 ^{-/-} mice) dependent on IL-33 ILC2 transfer to IL-7R ^{-/-} sufficient for early eosinophilic airway inflammation	[16, 17]
OVA	Th2 cytokine producing ILC2s comparable with number of CD4 ⁺ Th2 cells ILC IL-13-restored AHR restored in IL-13 ^{-/-} mice Partial ILC2 steroid resistance in OVA + IL-33 model	[37, 80, 81]
House dust mite	ILC2 promotion of Th2 cell priming in RORα ^{-/-} BMT mice	[74, 80]
Papain (cysteine protease)	Initiation of lung eosinophilia (RAG1 ^{-/-} mice) ILC2 IL-9 production ILC2 promotion of Th2 cell priming in RORα ^{-/-} BMT mice ILC2 activation by basophil IL-4	[39, 56, 77]
Dermatitis models		
MC903 (vitamin D analog)	ILC2 responses and skin inflammation dependent on TSLP, not IL-33 signaling Ear swelling reduced in RORα ^{sg/sg} and CD90.1-depleted RAG1 ^{-/-} mice Ear swelling and skin ILC2 number dependent on IL-25, IL-33, and TSLP ILC2 activation by basophil IL-4	[57, 82, 83]

BMT, Bone marrow transplantation; ^{sg/sg}, staggerer.

after allergen challenge [34]. Our work has also shown that *Alternaria*-induced ILC2 activation in mice occurs during a conventional adaptive response to a completely different allergen, suggesting that high *Alternaria* spore exposure could worsen lung inflammation caused by another allergen [89]. From a translational aspect, *Alternaria* exposure is associated with severe human asthma, including fatal attacks, although whether rapid IL-33/ILC2 activation occurs in humans is unknown [90].

RAG1 and -2: *RAG1 and -2 are required for T and B cell development. RAG1 and RAG2 knockout mice have ILC2s, despite lacking B and T cells, and much of the work in mouse models of disease has excluded contributions of adaptive immunity.*

In addition to promoting pathogenic type 2 lung responses, there is evidence that ILC2s induce tissue repair responses after viral exposure. Depletion of ILC2s in RAG knockout mice infected with influenza virus led to hypoxemia, hypothermia, and loss of epithelial barrier integrity [41]. ILC2s were found to produce high levels of amphiregulin and adoptive transfer of ILC2s, or administration of amphiregulin to ILC2-depleted mice restored airway integrity after influenza infection. We have also reported that ILC2 production of amphiregulin and epithelial expression of the amphiregulin receptor EGFR are rapidly up-

regulated after one *Alternaria* challenge, thus supporting a role for ILC2s in tissue repair postallergen challenge as well [17].

Rhinovirus exposure is a major cause of morbidity for human asthmatics and is the primary cause of asthma exacerbation [2]. In addition to asthma flares, viruses are implicated in the development of asthma [2, 91]. A very recent study reported that rhinovirus infection in neonatal mice, but not adult mice, expanded IL-13⁺ lung ILC2s [92]. ILC2 expansion as well as mucus metaplasia and AHR were dependent on IL-25. Interestingly, the expanded ILC2s persisted for 3 weeks after infection and could provide a robust type 2 response to additional stimuli, resulting in the development of asthma features.

Corticosteroid resistance can be associated with severe asthma and whether ILC2s are steroid resistant has important therapeutic implications [93]. A recent study suggested that mouse lung ILC2s may be corticosteroid resistant in the presence of TSLP [81]. The authors performed OVA/alum i.p. sensitizations, followed by IL-33 and OVA challenges, in mice and determined that dexamethasone administration did not reduce lung ILC2s. Furthermore, an absence or blockade of TSLP led to a partial increase in steroid sensitivity of lung ILC2s in vivo, and TSLP stimulation of naïve lung ILC2s in vitro led to partial dexamethasone resistance. Our recent work suggests that systemic corticosteroids induce apoptosis of mouse lung ILC2s during

repetitive intranasal *Alternaria* challenges [94]. This suggests that ILC2 corticosteroid resistance may occur under specific conditions that need to be explored further.

In addition to a role for ILC2s in asthma models, ILC2s have been implicated in pulmonary fibrosis. A recent report showed that lung ILC2s expanded 10-fold by Day 7 after bleomycin treatment, and ST2^{-/-} (IL-33R) mice were resistant to lung fibrosis and lacked ILC2 expansion [95]. Furthermore, adoptive transfer of ILC2s into WT mice receiving bleomycin increased lung inflammation and collagen production as well as lung IL-13 and TGF- β 1 mRNA levels. IL-33 also induced M2 polarization of lung macrophages that were required for lung fibrosis. Although the precise role of ILC2s during bleomycin-induced fibrosis is not clear, the suggestion is that IL-33 induces ILC2 IL-13 production, which may have multiple inflammatory and profibrotic effects on several cell types, including macrophages, alveolar epithelium, and fibroblasts.

Clinical question: Do ILC2s contribute to pathologic airway remodeling (fibrosis, smooth muscle increases) in asthma that is thought to be responsible for a gradual decline of lung function in asthmatics?

ILC2S IN HUMAN ASTHMA

A report in 2009 demonstrated the presence of a CD34⁺ non-B/non-T lymphocyte population that produced IL-5 and IL-13 in asthmatic sputum after airway challenge with allergen [96]. Whether these cells are the later-described CRTH2-expressing ILC2s is not known, but the work does suggest that a non-T cell Th2 cytokine-producing cell type may be active in human asthma. Subsequently, ILC2s were shown to be present in human lung and BAL [41, 58]. Barnig et al. [58] reported that peripheral blood ILC2s (lineage-negative CRTH2⁺ IL-7R⁺) cells were not different in numbers among severe asthmatics, mild asthmatics, and controls. The group went on to show that IL-13 production by peripheral blood ILC2s was enhanced by PGD2 and inhibited by LXA4. A more recent report demonstrated that peripheral blood ILC2s (lineage-negative CRTH2⁺ IL-7R⁺) were increased in patients with allergic asthma compared with those with allergic rhinitis and healthy controls [97]. Furthermore, PBMC production of IL-5 and IL-13, in response to IL-25 and IL-33, was increased in those with allergic asthma. Although both studies differ in correlation of peripheral blood ILC2 numbers with asthma, the patient populations are likely heterogeneous, and the allergic status of individuals in the former study is not clear. Although the function of ILC2s in the lungs of asthmatic patients is not known, upstream cytokines and mediators of ILC2 activation, including IL-25, IL-33, TSLP, LTs, and PGD2, are elevated in human asthma [62, 98–101].

Clinical question: Do rapid ILC2 activation and elaboration of type 2 cytokines participate in human asthma exacerbation where viruses (rhinovirus, influenza) are largely the trigger?

ILC2S IN HUMAN CRS

CRS affects up to 12–13% of the adult general population and is characterized by inflammation of the nasal and paranasal mucosal

surfaces, often associated with asthma, allergic disease, aspirin sensitivity, and cystic fibrosis [102, 103]. A subset of patients with CRSwNPs that cause significant morbidity and has led to classification of “endotypes” within CRS [103]. ILC2s were first reported as enriched in nasal polyps in 2011 [33], and a subsequent study identified an increased percentage of ILC2 in inflamed sinonasal mucosa from patients with CRSwNP compared with CRS without nasal polyps [104]. Another report showed a correlation with increased nasal polyp ILC2s and allergic sensitization, as well as numbers of polyp Th2 cells [105]. Our group recently reported that ILC2s were increased in eosinophilic compared with a noneosinophilic polyp endotype and were reduced in patients that had received systemic corticosteroids [94].

Further investigation into mechanisms of ILC2s in nasal polyps revealed that nasal polyp ILC2s produced IL-13 in a GATA3-dependent manner after stimulation with IL-2, IL-33, and TSLP [20]. Furthermore, TSLP enhanced the level of ILC2 GATA3 expression, possibly accounting for increased ILC2 cytokine production by TSLP. Similar to asthma, mediators that activate ILC2s, including epithelial cytokines TSLP and IL-33, as well as LTs, have been detected at higher levels in patients with CRS and are thus available for potent ILC2 stimulation [104, 106, 107].

CRS also includes patients with chronic allergic rhinitis. Our group investigated changes in peripheral blood ILC2s immediately after cat-allergen challenge in allergic rhinitis subjects with positive cat challenges [108]. We found that the percent of CRTH2⁺ ILC2s approximately doubled after cat-allergen challenge compared with diluent control challenges in the same patients performed at a separate visit. In support of this, a subsequent report showed that peripheral blood ILC2s are increased in grass pollen allergic rhinitis subjects during the pollen season and are reduced by s.c. immunotherapy [109]. Taken together, the available literature suggests that ILC2s may have a role in CRS, including nasal polyposis and allergic rhinitis, and further studies are needed to characterize the relative functional role of ILC2s compared with mast cells, basophils, T cells, and eosinophils.

ILC2 IN SKIN-DISEASE MODELS

Atopic dermatitis is a common chronic skin condition characterized by itchy, scaly rashes, along with skin barrier disruption, eosinophilic infiltration, and high serum IgE. Mouse models of atopic dermatitis are limited, although transgenic expression or instillation of cytokines as well as use of a vitamin D analog have recapitulated some of these clinical features. ILC2s are found in the skin of mice, and the majority of IL-13-positive cells under homeostatic conditions in the skin of IL-13 reporter mice are CD3 negative [78]. This is consistent with other reports showing that ILC2s are the primary Th2 cytokine-producing cell type in different organs in the absence of disease [38, 42].

In a mouse model of atopic dermatitis induced by the vitamin D analog calcipotriol, ILC2s contributed to dermal thickening and inflammation that was dependent on TSLPR signaling but independent of IL-33 [57]. This model has been shown previously to be TSLP dependent, and whether IL-33 is available and active is unclear [110]. Moreover, another recent report showed that skin-specific overexpression of IL-33

TABLE 3. Summary of questions for future clinical-translational ILC2 research

- 1) What is the role of ILC2s in the presence of ongoing adaptive-immune responses that occur in chronic diseases, including asthma and atopic dermatitis?
- 2) To what extent do ILC2s contribute to human allergic diseases relative to other cell types known to be involved including T cells, mast cells, and eosinophils?
- 3) Are there novel and more selective ILC2 markers that may be used both in research and clinically for ILC2 detection?
- 4) Could in-depth screening and molecular analysis of tissue ILC2s in human disease lead to novel as well as specific targets that will also improve our understanding of ILC2 function?
- 5) What are the unintended consequences of ILC2 targeting that may disrupt metabolic homeostasis or tissue repair?
- 6) How will targeting ILC2s affect other immune and structural cell functions?

induced atopic dermatitis-like pathology with increased dermal eosinophils, skin Th2 cytokines, and expansion of ILC2s [111]. Thus, the availability of 1 or more upstream mediators of ILC2 activation, such as TSLP and/or IL-33, likely drives ILC2 cytokine responses and accumulation that could lead to dermatitis. Interestingly, IL-2 administration to RAG1^{-/-} mice also resulted in skin ILC2 expansion, Th2 cytokine production, and pathologic features of dermatitis, including epidermal hyperplasia, dermal thickening, and eosinophilic infiltration, suggesting that multiple cytokines under specific conditions can promote ILC2-mediated dermatitis [78]. Similar to lung-disease models, basophils have also been shown recently to support ILC2 accumulation in the skin through IL-4 production [82]. Overall, ILC2s appear to contribute to dermatitis in these studies, but models of atopic dermatitis are limited, and relevance to human disease is largely unclear.

ILC2S IN HUMAN ATOPIC DERMATITIS

Elevated levels of TSLP and IL-33 have been detected in the skin of atopic dermatitis patients, suggesting that ILC2s may also be active in the disease [112, 113]. ILC2s have also been found at higher levels in the skin of atopic dermatitis patients compared with healthy controls [57, 83]. A recent study of ILC2s in human atopic dermatitis demonstrated that human skin ILC2s from diseased patients produce Th2 cytokines in response to IL-33 but not TSLP or IL-25 [83]. Human skin ILC2s express killer cell lectin-like receptor subfamily G member 1, which binds to the epithelial adhesion molecule E-cadherin that is down-regulated in atopic dermatitis. The authors found that E-cadherin inhibited ILC2 proliferation and IL-5 and IL-13 production [83]. Furthermore, ILC2s were recruited to skin of house dust mite-allergic subjects administered intradermal house dust mite. Thus, there may be a role for ILC2s in atopic dermatitis, in part, as a result of reduced inhibition by keratinocyte E-cadherin and increased recruitment upon allergen exposure.

E-Cadherin: Cell-cell adhesion glycoprotein highly expressed in epithelial cells with a critical role in tissue formation and suppression of cancer. In the skin, E-cadherin appears to inhibit ILC2 activation and suppress undesirable inflammatory responses.

Clinical question: Can ILC2s be targeted specifically in the skin where a topical therapy might be used?

TARGETING ILC2S IN HUMAN DISEASE

Many challenges remain in specifically targeting ILC2s in human disease. First, there is no specific marker or target currently known to be present on ILC2s. Second, the targeting of upstream cytokines, including IL-33, TSLP, and PGD2, will likely have effects on multiple inflammatory and structural cell types known to be regulated by these mediators, and the targeting of 1 mediator may not be sufficient. Finally, some caution going forward is prudent in the targeting of tissue ILC2s that may have a role in metabolic homeostasis and tissue repair. As information regarding the role of ILC2s in human disease accumulates, there is reason to be hopeful that such therapeutic targeting in some patients may be promising.

SUMMARY

The recent discovery of ILC2s has generated significant excitement and has led to an improved understanding of allergic diseases and asthma. ILC2s produce large amounts of Th2 cytokines and are found in human tissues, including respiratory tract and skin. Mouse models have shown that ILC2s can contribute to eosinophilic infiltration, mucus production, AHR, and skin disease. Furthermore, ILC2s are implicated in Th2 cell sensitization, thus linking ILC2s with conventional adaptive Th2 cell paradigms of allergic disease that include specific IgE production. Importantly, ILC2s appear to have roles in metabolic homeostasis and tissue repair, suggesting multifaceted effector functions. Studies correlating ILC2 levels or function with human diseases are emerging, and the targeting of ILC2s may represent a potential therapeutic strategy. However, several important questions regarding the role of ILC2s in allergic diseases remain, including how ILC2s can be specifically targeted and to what extent ILC2s contribute during chronic allergic inflammation (**Table 3**). The hope is that the discovery of ILC2s will eventually translate into therapy for human disease.

ACKNOWLEDGMENTS

T.A.D. is supported by U.S. National Institutes of Health Grants 1R01AI114585 and 1K08AI080938, American Lung Association/American Academy of Allergy, Asthma & Immunology Allergic Respiratory Diseases Award, University of California San Diego Clinical and Translational Research Institute Pilot Award, and ALA Biomedical Research Award.

DISCLOSURES

The authors report no conflict of interest in the preparation of this manuscript.

REFERENCES

- Busse, W. W., Lemanske, Jr., R. F. (2001) Asthma. *N. Engl. J. Med.* **344**, 350–362.
- Dulek, D. E., Peebles, R. S., Jr. (2011) Viruses and asthma. *Biochim. Biophys. Acta* **1810**, 1080–1090.
- Guarnieri, M., Balmes, J. R. (2014) Outdoor air pollution and asthma. *Lancet* **383**, 1581–1592.
- Silvestri, M., Franchi, S., Pistorio, A., Petecchia, L., Rusconi, F. (2014) Smoke exposure, wheezing, and asthma development: a systematic review and meta-analysis in unselected birth cohorts. *Pediatr. Pulmonol.* doi: 10.1002/ppul.23037. [Epub ahead of print].
- Peebles, Jr., S. (2014) At the Bedside: The emergence of group 2 innate lymphoid cells in human disease. *J. Leuko. Bio.* **97**, 469–475.
- Fort, M. M., Cheung, J., Yen, D., Li, J., Zurawski, S. M., Lo, S., Menon, S., Clifford, T., Hunte, B., Lesley, R., Muchamuel, T., Hurst, S. D., Zurawski, G., Leach, M. W., Gorman, D. M., Rennick, P. G. (2001) IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity* **15**, 985–995.
- Hurst, S. D., Muchamuel, T., Gorman, D. M., Gilbert, J. M., Clifford, T., Kwan, S., Menon, S., Seymour, B., Jackson, C., Kung, T. T., Brieland, J. K., Zurawski, S. M., Chapman, R. W., Zurawski, G., Coffman, R. L. (2002) New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J. Immunol.* **169**, 443–453.
- Fallon, P. G., Ballantyne, S. J., Mangan, N. E., Barlow, J. L., Dasvarma, A., Hewett, D. R., McIlgorm, A., Jolin, H. E., McKenzie, A. N. (2006) Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J. Exp. Med.* **203**, 1105–1116.
- Neill, D. R., Wong, S. H., Bellosi, A., Flynn, R. J., Daly, M., Langford, T. K., Bucks, C., Kane, C. M., Fallon, P. G., Pannell, R., Jolin, H. E., McKenzie, A. N. (2010) Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* **464**, 1367–1370.
- Price, A. E., Liang, H. E., Sullivan, B. M., Reinhardt, R. L., Eisle, C. J., Erle, D. J., Locksley, R. M. (2010) Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc. Natl. Acad. Sci. USA* **107**, 11489–11494.
- Moro, K., Yamada, T., Tanabe, M., Takeuchi, T., Ikawa, T., Kawamoto, H., Furusawa, J., Ohtani, M., Fujii, H., Koyasu, S. (2010) Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature* **463**, 540–544.
- Spits, H., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J. P., Eberl, G., Koyasu, S., Locksley, R. M., McKenzie, A. N., Mebius, R. E., Powrie, F., Vivier, E. (2013) Innate lymphoid cells—a proposal for uniform nomenclature. *Nat. Rev. Immunol.* **13**, 145–149.
- Yang, Q., Saenz, S. A., Zlotoff, D. A., Artis, D., Bhandoola, A. (2011) Cutting edge: natural helper cells derive from lymphoid progenitors. *J. Immunol.* **187**, 5505–5509.
- Yokota, Y., Mansouri, A., Mori, S., Sugawara, S., Adachi, S., Nishikawa, S., Gruss, P. (1999) Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2. *Nature* **397**, 702–706.
- Satoh-Takayama, N., Lesjean-Pottier, S., Vieira, P., Sawa, S., Eberl, G., Vosschenrich, C. A., Di Santo, J. P. (2010) IL-7 and IL-15 independently program the differentiation of intestinal CD3–NKP46+ cell subsets from Id2-dependent precursors. *J. Exp. Med.* **207**, 273–280.
- Bartemes, K. R., Iijima, K., Kobayashi, T., Kephart, G. M., McKenzie, A. N., Kita, H. (2012) IL-33-responsive lineage- CD25+ CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs. *J. Immunol.* **188**, 1503–1513.
- Doherty, T. A., Khorram, N., Chang, J. E., Kim, H. K., Rosenthal, P., Croft, M., Broide, D. H. (2012) STAT6 regulates natural helper cell proliferation during lung inflammation initiated by *Alternaria*. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **303**, L577–L588.
- Siddiqui, S., Secor, Jr., E. R., Silbart, L. K. (2013) Broncho-alveolar macrophages express chemokines associated with leukocyte migration in a mouse model of asthma. *Cell. Immunol.* **281**, 159–169.
- Kelly, E. A., Koziol-White, C. J., Clay, K. J., Liu, L. Y., Bates, M. E., Bertics, P. J., Jarjour, N. N. (2009) Potential contribution of IL-7 to allergen-induced eosinophilic airway inflammation in asthma. *J. Immunol.* **182**, 1404–1410.
- Mjösberg, J., Bernink, J., Golebski, K., Karrich, J. J., Peters, C. P., Blom, B., te Velde, A. A., Fokkens, W. J., van Drunen, C. M., Spits, H. (2012) The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. *Immunity* **37**, 649–659.
- Klein Wolterink, R. G., Serafini, N., van Nimwegen, M., Vosschenrich, C. A., de Bruijn, M. J., Fonseca Pereira, D., Veiga Fernandes, H., Hendriks, R. W., Di Santo, J. P. (2013) Essential, dose-dependent role for the transcription factor Gata3 in the development of IL-5+ and IL-13+ type 2 innate lymphoid cells. *Proc. Natl. Acad. Sci. USA* **110**, 10240–10245.
- Hoyler, T., Klose, C. S., Souabni, A., Turqueti-Neves, A., Pfeifer, D., Rawlins, E. L., Voehringer, D., Busslinger, M., Diefenbach, A. (2012) The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity* **37**, 634–648.
- Yagi, R., Zhong, C., Northrup, D. L., Yu, F., Bouladoux, N., Spencer, S., Hu, G., Barron, L., Sharma, S., Nakayama, T., Belkaid, Y., Zhao, K., Zhu, J. (2014) The transcription factor GATA3 is critical for the development of all IL-7Rα-expressing innate lymphoid cells. *Immunity* **40**, 378–388.
- Serafini, N., Klein Wolterink, R. G., Satoh-Takayama, N., Xu, W., Vosschenrich, C. A., Hendriks, R. W., Di Santo, J. P. (2014) Gata3 drives development of RORγt+ group 3 innate lymphoid cells. *J. Exp. Med.* **211**, 199–208.
- Walunas, T. L., Wang, B., Wang, C. R., Leiden, J. M. (2000) Cutting edge: the Ets1 transcription factor is required for the development of NK T cells in mice. *J. Immunol.* **164**, 2857–2860.
- Barton, K., Muthusamy, N., Fischer, C., Ting, C. N., Walunas, T. L., Lanier, L. L., Leiden, J. M. (1998) The Ets-1 transcription factor is required for the development of natural killer cells in mice. *Immunity* **9**, 555–563.
- Stempel, J. M., Grenningloh, R., Ho, I. C., Vercelli, D. (2010) Phylogenetic and functional analysis identifies Ets-1 as a novel regulator of the Th2 cytokine gene locus. *J. Immunol.* **184**, 1309–1316.
- Wong, S. H., Walker, J. A., Jolin, H. E., Drynan, L. F., Hams, E., Camelo, A., Barlow, J. L., Neill, D. R., Panova, V., Koch, U., Radtke, F., Hardman, C. S., Hwang, Y. Y., Fallon, P. G., McKenzie, A. N. (2012) Transcription factor RORα is critical for nuocyte development. *Nat. Immunol.* **13**, 229–236.
- Halim, T. Y., MacLaren, A., Romanish, M. T., Gold, M. J., McNagny, K. M., Takei, F. (2012) Retinoic-acid-receptor-related orphan nuclear receptor alpha is required for natural helper cell development and allergic inflammation. *Immunity* **37**, 463–474.
- Moffatt, M. F., Gut, I. G., Demenais, F., Strachan, D. P., Bouzigon, E., Heath, S., von Mutius, E., Farrall, M., Lathrop, M., Cookson, W. O.; GABRIEL Consortium. (2010) A large-scale, consortium-based genome-wide association study of asthma. *N. Engl. J. Med.* **363**, 1211–1221.
- Mielke, L. A., Groom, J. R., Rankin, L. C., Seillet, C., Masson, F., Putoczki, T., Belz, G. T. (2013) TCF-1 controls ILC2 and NKP46+ RORγt+ innate lymphocyte differentiation and protection in intestinal inflammation. *J. Immunol.* **191**, 4383–4391.
- Constantinides, M. G., McDonald, B. D., Verhoeve, P. A., Bendelac, A. (2014) A committed precursor to innate lymphoid cells. *Nature* **508**, 397–401.
- Mjösberg, J. M., Trifari, S., Crellin, N. K., Peters, C. P., van Drunen, C. M., Piet, B., Fokkens, W. J., Cupedo, T., Spits, H. (2011) Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat. Immunol.* **12**, 1055–1062.
- Doherty, T. A., Khorram, N., Lund, S., Mehta, A. K., Croft, M., Broide, D. H. (2013) Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production. *J. Allergy Clin. Immunol.* **132**, 205–213.
- Kurowska-Stolarska, M., Kewin, P., Murphy, G., Russo, R. C., Stolarski, B., Garcia, C. C., Komai-Koma, M., Pitman, N., Li, Y., Niedbala, W., McKenzie, A. N., Teixeira, M. M., Liew, F. Y., Xu, D. (2008) IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. *J. Immunol.* **181**, 4780–4790.
- Chang, Y. J., Kim, H. Y., Albacker, L. A., Baumgarth, N., McKenzie, A. N., Smith, D. E., Dekruyff, R. H., Umetsu, D. T. (2011) Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity. *Nat. Immunol.* **12**, 631–638.
- Barlow, J. L., Bellosi, A., Hardman, C. S., Drynan, L. F., Wong, S. H., Cruickshank, J. P., McKenzie, A. N. (2012) Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. *J. Allergy Clin. Immunol.* **129**, 1918 e1–4.
- Nussbaum, J. C., Van Dyken, S. J., von Moltke, J., Cheng, L. E., Mohapatra, A., Molofsky, A. B., Thornton, E. E., Krummel, M. F., Chawla, A., Liang, H. E., Locksley, R. M. (2013) Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* **502**, 245–248.
- Wilhelm, C., Hirota, K., Stieglitz, B., Van Snick, J., Tolaini, M., Lahl, K., Sparwasser, T., Helmby, H., Stockinger, B. (2011) An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. *Nat. Immunol.* **12**, 1071–1077.
- Soroosh, P., Doherty, T. A. (2009) Th9 and allergic disease. *Immunology* **127**, 450–458.

41. Monticelli, L. A., Sonnenberg, G. F., Abt, M. C., Alenghat, T., Ziegler, C. G., Doering, T. A., Angelosanto, J. M., Laidlaw, B. J., Yang, C. Y., Sathaliyawala, T., Kubota, M., Turner, D., Diamond, J. M., Goldrath, A. W., Farber, D. L., Collman, R. G., Wherry, E. J., Artis, D. (2011) Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat. Immunol.* **12**, 1045–1054.
42. Molofsky, A. B., Nussbaum, J. C., Liang, H. E., Van Dyken, S. J., Cheng, L. E., Mohapatra, A., Chawla, A., Locksley, R. M. (2013) Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J. Exp. Med.* **210**, 535–549.
43. Hams, E., Locksley, R. M., McKenzie, A. N., Fallon, P. G. (2013) Cutting edge: IL-25 elicits innate lymphoid type 2 and type II NKT cells that regulate obesity in mice. *J. Immunol.* **191**, 5349–5353.
44. Carriere, V., Roussel, L., Ortega, N., Lacorre, D. A., Americh, L., Aguilar, L., Bouche, G., Girard, J. P. (2007) IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proc. Natl. Acad. Sci. USA* **104**, 282–287.
45. Lefrançois, E., Roga, S., Gautier, V., Gonzalez-de-Peredo, A., Monsarrat, B., Girard, J. P., Cayrol, C. (2012) IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. *Proc. Natl. Acad. Sci. USA* **109**, 1673–1678.
46. Gu, C., Wu, L., Li, X. (2013) IL-17 family: cytokines, receptors and signaling. *Cytokine* **64**, 477–485.
47. Lloyd, C. M. (2010) IL-33 family members and asthma - bridging innate and adaptive immune responses. *Curr. Opin. Immunol.* **22**, 800–806.
48. Doherty, T. A., Khorram, N., Sugimoto, K., Sheppard, D., Rosenthal, P., Cho, J. Y., Pham, A., Miller, M., Croft, M., Broide, D. H. (2012) *Alternaria* induces STAT6-dependent acute airway eosinophilia and epithelial FIZZ1 expression that promotes airway fibrosis and epithelial thickness. *J. Immunol.* **188**, 2622–2629.
49. Kouzaki, H., Iijima, K., Kobayashi, T., O'Grady, S. M., Kita, H. (2011) The danger signal, extracellular ATP, is a sensor for an airborne allergen and triggers IL-33 release and innate Th2-type responses. *J. Immunol.* **186**, 4375–4387.
50. Tamachi, T., Maezawa, Y., Ikeda, K., Kagami, S., Hatano, M., Seto, Y., Suto, A., Suzuki, K., Watanabe, N., Saito, Y., Tokuhisa, T., Iwamoto, I., Nakajima, H. (2006) IL-25 enhances allergic airway inflammation by amplifying a TH2 cell-dependent pathway in mice. *J. Allergy Clin. Immunol.* **118**, 606–614.
51. Angkasekwinai, P., Park, H., Wang, Y. H., Wang, Y. H., Chang, S. H., Corry, D. B., Liu, Y. J., Zhu, Z., Dong, C. (2007) Interleukin 25 promotes the initiation of proallergic type 2 responses. *J. Exp. Med.* **204**, 1509–1517.
52. Barlow, J. L., Peel, S., Fox, J., Panova, V., Hardman, C. S., Camelo, A., Bucks, C., Wu, X., Kane, C. M., Neill, D. R., Flynn, R. J., Sayers, I., Hall, I. P., McKenzie, A. N. (2013) IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J. Allergy Clin. Immunol.* **132**, 933–941.
53. Paul, W. E., Zhu, J. (2010) How are T(H)2-type immune responses initiated and amplified? *Nat. Rev. Immunol.* **10**, 225–235.
54. Ito, T., Wang, Y. H., Duramad, O., Hori, T., Delespesse, G. J., Watanabe, N., Qin, F. X., Yao, Z., Cao, W., Liu, Y. J. (2005) TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J. Exp. Med.* **202**, 1213–1223.
55. Al-Shami, A., Spolski, R., Kelly, J., Keane-Myers, A., Leonard, W. J. (2005) A role for TSLP in the development of inflammation in an asthma model. *J. Exp. Med.* **202**, 829–839.
56. Halim, T. Y., Krauss, R. H., Sun, A. C., Takei, F. (2012) Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* **36**, 451–463.
57. Kim, B. S., Siracusa, M. C., Saenz, S. A., Noti, M., Monticelli, L. A., Sonnenberg, G. F., Hepworth, M. R., Van Voorhees, A. S., Comeau, M. R., Artis, D. (2013) TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci. Transl. Med.* **5**, 170ra16.
58. Barnig, C., Cernadas, M., Dutile, S., Liu, X., Perrella, M. A., Kazani, S., Wechsler, M. E., Israel, E., Levy, B. D. (2013) Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma. *Sci. Transl. Med.* **5**, 174ra26.
59. Boyce, J. A. (2007) Mast cells and eicosanoid mediators: a system of reciprocal paracrine and autocrine regulation. *Immunol. Rev.* **217**, 168–185.
60. Hirai, H., Tanaka, K., Yoshie, O., Ogawa, K., Kenmotsu, K., Takamori, Y., Ichimasa, M., Sugamura, K., Nakamura, M., Takano, S., Nagata, K. (2001) Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J. Exp. Med.* **193**, 255–261.
61. Xue, L., Salimi, M., Panse, I., Mjösberg, J. M., McKenzie, A. N., Spits, H., Kleinerman, P., Ogg, G. (2014) Prostaglandin D2 activates group 2 innate lymphoid cells through chemoattractant receptor-homologous molecule expressed on TH2 cells. *J. Allergy Clin. Immunol.* **133**, 1184–1194.
62. Fajt, M. L., Gelhaus, S. L., Freeman, B., Uvalle, C. E., Trudeau, J. B., Holguin, F., Wenzel, S. E. (2013) Prostaglandin D2 pathway upregulation: relation to asthma severity, control, and TH2 inflammation. *J. Allergy Clin. Immunol.* **131**, 1504–1512.
63. Xue, L., Gyles, S. L., Wettestad, F. R., Gazi, L., Townsend, E., Hunter, M. G., Pettipher, R. (2005) Prostaglandin D2 causes preferential induction of proinflammatory Th2 cytokine production through an action on chemoattractant receptor-like molecule expressed on Th2 cells. *J. Immunol.* **175**, 6531–6536.
64. Xue, L., Barrow, A., Pettipher, R. (2009) Novel function of CRTH2 in preventing apoptosis of human Th2 cells through activation of the phosphatidylinositol 3-kinase pathway. *J. Immunol.* **182**, 7580–7586.
65. Chang, J. E., Doherty, T. A., Baum, R., Broide, D. (2013) Prostaglandin D2 regulates human type 2 innate lymphoid cell chemotaxis. *J. Allergy Clin. Immunol.* **133**, 899–901.e3.
66. Croft, M. (2009) The role of TNF superfamily members in T-cell function and diseases. *Nat. Rev. Immunol.* **9**, 271–285.
67. Ware, C. F. (2003) The TNF superfamily. *Cytokine Growth Factor Rev.* **14**, 181–184.
68. Meylan, F., Davidson, T. S., Kahle, E., Kinder, M., Acharya, K., Jankovic, D., Bundoc, V., Hodges, M., Shevach, E. M., Keane-Myers, A., Wang, E. C., Siegel, R. M. (2008) The TNF-family receptor DR3 is essential for diverse T cell-mediated inflammatory diseases. *Immunity* **29**, 79–89.
69. Meylan, F., Richard, A. C., Siegel, R. M. (2011) TL1A and DR3, a TNF family ligand-receptor pair that promotes lymphocyte costimulation, mucosal hyperplasia, and autoimmune inflammation. *Immunol. Rev.* **244**, 188–196.
70. Yu, X., Pappu, R., Ramirez-Carrozzi, V., Ota, N., Caplazi, P., Zhang, J., Yan, D., Xu, M., Lee, W. P., Grogan, J. L. (2014) TNF superfamily member TL1A elicits type 2 innate lymphoid cells at mucosal barriers. *Mucosal Immunol.* **7**, 730–740.
71. Mirchandani, A. S., Besnard, A. G., Yip, E., Scott, C., Bain, C. C., Cerovic, V., Salmond, R. J., Liew, F. Y. (2014) Type 2 innate lymphoid cells drive CD4+ Th2 cell responses. *J. Immunol.* **192**, 2442–2448.
72. Drake, L. Y., Iijima, K., Kita, H. (2014) Group 2 innate lymphoid cells and CD4+ T cells cooperate to mediate type 2 immune response in mice. *Allergy* **69**, 1300–1307.
73. Halim, T. Y., Steer, C. A., Mathã, L., Gold, M. J., Martinez-Gonzalez, I., McNagny, K. M., McKenzie, A. N., Takei, F. (2014) Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity* **40**, 425–435.
74. Gold, M. J., Antignano, F., Halim, T. Y., Hirota, J. A., Blanchet, M. R., Zaph, C., Takei, F., McNagny, K. M. (2014) Group 2 innate lymphoid cells facilitate sensitization to local, but not systemic, TH2-inducing allergen exposures. *J. Allergy Clin. Immunol.* **133**, 1142–1148.
75. Sokol, C. L., Barton, G. M., Farr, A. G., Medzhitov, R. (2008) A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat. Immunol.* **9**, 310–318.
76. Siracusa, M. C., Kim, B. S., Spergel, J. M., Artis, D. (2013) Basophils and allergic inflammation. *J. Allergy Clin. Immunol.* **132**, 789–801, quiz 788.
77. Motomura, Y., Morita, H., Moro, K., Nakae, S., Artis, D., Endo, T. A., Kuroki, Y., Ohara, O., Koyasu, S., Kubo, M. (2014) Basophil-derived interleukin-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation. *Immunity* **40**, 758–771.
78. Roediger, B., Kyle, R., Yip, K. H., Sumaria, N., Guy, T. V., Kim, B. S., Mitchell, A. J., Tay, S. S., Jain, R., Forbes-Blom, E., Chen, X., Tong, P. L., Bolton, H. A., Artis, D., Paul, W. E., Fazekas de St Groth, B., Grimbaldston, M. A., Le Gros, G., Weninger, W. (2013) Cutaneous immunosurveillance and regulation of inflammation by group 2 innate lymphoid cells. *Nat. Immunol.* **14**, 564–573.
79. Fukuoka, A., Futatsugi-Yumikura, S., Takahashi, S., Kazama, H., Iyoda, T., Yoshimoto, T., Inaba, K., Nakanishi, K., Yonehara, S. (2013) Identification of a novel type 2 innate immunocyte with the ability to enhance IgE production. *Int. Immunol.* **25**, 373–382.
80. Klein Wolterink, R. G., Kleinjan, A., van Nimwegen, M., Bergen, I., de Bruijn, M., Levani, Y., Hendriks, R. W. (2012) Pulmonary innate lymphoid cells are major producers of IL-5 and IL-13 in murine models of allergic asthma. *Eur. J. Immunol.* **42**, 1106–1116.
81. Kabata, H., Moro, K., Fukunaga, K., Suzuki, Y., Miyata, J., Masaki, K., Betsuyaku, T., Koyasu, S., Asano, K. (2013) Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation. *Nat. Commun.* **4**, 2675.
82. Kim, B. S., Wang, K., Siracusa, M. C., Saenz, S. A., Brestoff, J. R., Monticelli, L. A., Noti, M., Tait Wojno, E. D., Fung, T. C., Kubo, M., Artis, D. (2014) Basophils promote innate lymphoid cell responses in inflamed skin. *J. Immunol.* **193**, 3717–3725.
83. Salimi, M., Barlow, J. L., Saunders, S. P., Xue, L., Gutowska-Owsiak, D., Wang, X., Huang, L. C., Johnson, D., Scanlon, S. T., McKenzie, A. N., Fallon, P. G., Ogg, G. S. (2013) A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J. Exp. Med.* **210**, 2939–2950.
84. Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T. K., Zurawski, G., Moshrefi, M., Qin, J., Li, X., Gorman, D. M., Bazan, J. F., Kastelein, R. A. (2005) IL-33, an interleukin-1-like cytokine that

- signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* **23**, 479–490.
85. Ho, L. H., Ohno, T., Oboki, K., Kajiwara, N., Suto, H., Iikura, M., Okayama, Y., Akira, S., Saito, H., Galli, S. J., Nakae, S. (2007) IL-33 induces IL-13 production by mouse mast cells independently of IgE-FcεpsilonRI signals. *J. Leukoc. Biol.* **82**, 1481–1490.
 86. Smithgall, M. D., Comeau, M. R., Yoon, B. R., Kaufman, D., Armitage, R., Smith, D. E. (2008) IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells. *Int. Immunol.* **20**, 1019–1030.
 87. Suzukawa, M., Iikura, M., Koketsu, R., Nagase, H., Tamura, C., Komiya, A., Nakae, S., Matsushima, K., Ohta, K., Yamamoto, K., Yamaguchi, M. (2008) An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *J. Immunol.* **181**, 5981–5989.
 88. Doherty, T. A., Soroosh, P., Broide, D. H., Croft, M. (2009) CD4+ cells are required for chronic eosinophilic lung inflammation but not airway remodeling. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **296**, L229–L235.
 89. Kim, H. K., Lund, S., Baum, R., Rosenthal, P., Khorram, N., Doherty, T. A. (2014) Innate type 2 response to *Alternaria* extract enhances ryegrass-induced lung inflammation. *Int. Arch. Allergy Immunol.* **163**, 92–105.
 90. Bush, R. K., Prochnau, J. J. (2004) *Alternaria*-induced asthma. *J. Allergy Clin. Immunol.* **113**, 227–234.
 91. Holt, P. G., Strickland, D. H., Sly, P. D. (2012) Virus infection and allergy in the development of asthma: what is the connection? *Curr. Opin. Allergy Clin. Immunol.* **12**, 151–157.
 92. Hong, J. Y., Bentley, J. K., Chung, Y., Lei, J., Steenrod, J. M., Chen, Q., Sajjan, U. S., Hershenson, M. B. (2014) Neonatal rhinovirus induces mucous metaplasia and airways hyperresponsiveness through IL-25 and type 2 innate lymphoid cells. *J. Allergy Clin. Immunol.* **134**, 429–439.
 93. Walford, H. H., Doherty, T. A. (2014) Diagnosis and management of eosinophilic asthma: a US perspective. *J. Asthma Allergy* **7**, 53–65.
 94. Walford, H. H., Lund, S. J., Baum, R. E., White, A. A., Bergeron, C. M., Husseman, J., Bethel, K. J., Scott, D. R., Khorram, N., Miller, M., Broide, D. H., Doherty, T. A. (2014) Increased ILC2s in the eosinophilic nasal polyp endotype are associated with corticosteroid responsiveness. *Clin. Immunol.* **155**, 126–135.
 95. Li, D., Guabiraba, R., Besnard, A. G., Komai-Koma, M., Jabir, M. S., Zhang, L., Graham, G. J., Kurowska-Stolarska, M., Liew, F. Y., McSharry, C., Xu, D. (2014) IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. *J. Allergy Clin. Immunol.* pii: S0091-6749(14)00670-8 [Epub ahead of print].
 96. Allakhverdi, Z., Comeau, M. R., Smith, D. E., Toy, D., Endam, L. M., Desrosiers, M., Liu, Y. J., Howie, K. J., Denburg, J. A., Gauvreau, G. M., Delespesse, G. (2009) CD34+ hemopoietic progenitor cells are potent effectors of allergic inflammation. *J. Allergy Clin. Immunol.* **123**, 472–478.
 97. Bartemes, K. R., Kephart, G. M., Fox, S. J., Kita, H. (2014) Enhanced innate type 2 immune response in peripheral blood from patients with asthma. *J. Allergy Clin. Immunol.* **134**, 671–678 e4.
 98. Préfontaine, D., Lajoie-Kadoch, S., Foley, S., Audusseau, S., Olivenstein, R., Halayko, A. J., Lemièrre, C., Martin, J. G., Hamid, Q. (2009) Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J. Immunol.* **183**, 5094–5103.
 99. Oshikawa, K., Kuroiwa, K., Tago, K., Iwahana, H., Yanagisawa, K., Ohno, S., Tominaga, S. I., Sugiyama, Y. (2001) Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. *Am. J. Respir. Crit. Care Med.* **164**, 277–281.
 100. Corrigan, C. J., Wang, W., Meng, Q., Fang, C., Eid, G., Caballero, M. R., Lv, Z., An, Y., Wang, Y. H., Liu, Y. J., Kay, A. B., Lee, T. H., Ying, S. (2011) Allergen-induced expression of IL-25 and IL-25 receptor in atopic asthmatic airways and late-phase cutaneous responses. *J. Allergy Clin. Immunol.* **128**, 116–124.
 101. Ying, S., O'Connor, B., Ratoff, J., Meng, Q., Mallett, K., Cousins, D., Robinson, D., Zhang, G., Zhao, J., Lee, T. H., Corrigan, C. (2005) Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J. Immunol.* **174**, 8183–8190.
 102. Hamilos, D. L. (2011) Chronic rhinosinusitis: epidemiology and medical management. *J. Allergy Clin. Immunol.* **128**, 693–707, quiz 708–709.
 103. Akdis, C. A., Bachert, C., Cingi, C., Dykewicz, M. S., Hellings, P. W., Naclerio, R. M., Schleimer, R. P., Ledford, D. (2013) Endotypes and phenotypes of chronic rhinosinusitis: a PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. *J. Allergy Clin. Immunol.* **131**, 1479–1490.
 104. Shaw, J. L., Fakhri, S., Citardi, M. J., Porter, P. C., Corry, D. B., Kheradmand, F., Liu, Y. J., Luong, A. (2013) IL-33-responsive innate lymphoid cells are an important source of IL-13 in chronic rhinosinusitis with nasal polyps. *Am. J. Respir. Crit. Care Med.* **188**, 432–439.
 105. Miljkovic, D., Bassiouni, A., Cooksley, C., Ou, J., Hauben, E., Wormald, P. J., Vreugde, S. (2014) Association between group 2 innate lymphoid cells enrichment, nasal polyps and allergy in chronic rhinosinusitis. *Allergy* **69**, 1154–1161.
 106. Nagarkar, D. R., Poposki, J. A., Tan, B. K., Comeau, M. R., Peters, A. T., Hulse, K. E., Suh, L. A., Norton, J., Harris, K. E., Grammer, L. C., Chandra, R. K., Conley, D. B., Kern, R. C., Schleimer, R. P., Kato, A. (2013) Thymic stromal lymphopoietin activity is increased in nasal polyps of patients with chronic rhinosinusitis. *J. Allergy Clin. Immunol.* **132**, 593–600 e12.
 107. Steinke, J. W., Bradley, D., Arango, P., Crouse, C. D., Frierson, H., Kountakis, S. E., Kraft, M., Borish, L. (2003) Cysteinyl leukotriene expression in chronic hyperplastic sinusitis-nasal polyposis: importance to eosinophilia and asthma. *J. Allergy Clin. Immunol.* **111**, 342–349.
 108. Doherty, T. A., Scott, D., Walford, H. H., Khorram, N., Lund, S., Baum, R., Chang, J., Rosenthal, P., Beppu, A., Miller, M., Broide, D. H. (2014) Allergen challenge in allergic rhinitis rapidly induces increased peripheral blood type 2 innate lymphoid cells that express CD84. *J. Allergy Clin. Immunol.* **133**, 1203–1205.
 109. Lao-Araya, M., Steveling, E., Scadding, G. W., Durham, S. R., Shamji, M. H. (2014) Seasonal increases in peripheral innate lymphoid type 2 cells are inhibited by subcutaneous grass pollen immunotherapy. *J. Allergy Clin. Immunol.* pii: S0091-6749(14)01028-8 [Epub ahead of print].
 110. Li, M., Hener, P., Zhang, Z., Ganti, K. P., Metzger, D., Chambon, P. (2009) Induction of thymic stromal lymphopoietin expression in keratinocytes is necessary for generating an atopic dermatitis upon application of the active vitamin D3 analogue MC903 on mouse skin. *J. Invest. Dermatol.* **129**, 498–502.
 111. Imai, Y., Yasuda, K., Sakaguchi, Y., Haneda, T., Mizutani, H., Yoshimoto, T., Nakanishi, K., Yamanishi, K. (2013) Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. *Proc. Natl. Acad. Sci. USA* **110**, 13921–13926.
 112. Soumelis, V., Reche, P. A., Kanzler, H., Yuan, W., Edward, G., Homey, B., Gilliet, M., Ho, S., Antonenko, S., Lauerman, A., Smith, K., Gorman, D., Zurawski, S., Abrams, J., Menon, S., McClanahan, T., de Waal-Malefyt Rd, R., Bazan, F., Kastelein, R. A., Liu, Y. J. (2002) Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat. Immunol.* **3**, 673–680.
 113. Savinko, T., Matikainen, S., Saarialho-Kere, U., Lehto, M., Wang, G., Lehtimäki, S., Karisola, P., Reunala, T., Wolff, H., Lauerma, A., Alenius, H. (2012) IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. *J. Invest. Dermatol.* **132**, 1392–1400.

KEY WORDS:

ILC2 · allergy · asthma · atopic dermatitis