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CME Review

Innate lymphoid cells and allergic disease

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At the conclusion of this activity, participants should be able to:

- Describe the variety if innate lymphoid cells (ILC), their major functions, their development, and their role in the pathogenesis of allergic disease
- Discuss the correlations between ILC and human allergic disease, especially in regard to ILC numbers, activation state, and cytokine production.

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Introduction

Allergic diseases, including asthma, atopic dermatitis, and allergic rhinitis, are substantial causes of morbidity and mortality in developing and developed countries. An expansive set of cellular mediators and mechanisms have been identified that contribute to the initiation or exacerbation of allergic disease. During the past decade, the discovery of a new subset of leukocytes known as innate lymphoid cells (ILCs) has great expanded our understanding of the pathogenesis of allergic disease.

ILCs are effector lymphocytes that are embedded at mucosal sites and are involved in tissue homeostasis and response to diverse environmental insults. Derived from common lymphoid progenitors, ILCs develop into 3 major cell subsets: groups 1, 2, and 3 ILCs.[1] Group 1 ILCs (ILC1s) are driven by the transcription factor T-bet and produce interferon (IFN) γ. Group 2 ILCs (ILC2s) are governed by the transcription factor GATA3 and express interleukin (IL) 4, IL-5, IL-9, IL-13, and amphiregulin. Group 3 ILCs (ILC3s) are controlled by the transcription factor RORγt and produce IL-17 and/or IL-22. These transcription factor signatures and effector functions largely mirror $CD4^+$ T_H1, T_H2, and T_H17 cells, respectively. However, ILCs exhibit several key differences that make them distinct from their $CD4^+$ T_H cell counterparts. In contrast to $CD4^+$ T cells, ILCs are primarily at mucosal sites even at baseline and are only found in low numbers in secondary lymphoid organs, such as lymph nodes and the spleen.[2] Moreover, even in ILC-rich tissues, ILCs are found in significantly fewer numbers than $CD4^+$ T cells. ILCs do not depend on antigen presentation and can be activated immediately from stimuli in the tissue milieu, such as cytokines, positioning them as innate counterparts to $CD4^+$ T cells.[3–5] We discuss the development and homeostasis of ILCs, mechanistic insights into the role of ILCs in the pathogenesis of allergy, and the contribution of ILCs to human allergic disease, with a particular focus on ILC2s and their contributions to allergic disease pathogenesis.

ILC Development

Our understanding of ILC development comes largely from preclinical models and is summarized in Figure 1. In mice, ILCs derive from common lymphoid progenitors (CLPs).[6] The expression of the transcriptional regulator Id2 halts T- and B-cell development and promotes the differentiation of CLPs to α-integrin–expressing lymphoid precursor (αLP) cells, early ILC progenitors (EILPs), and common helper innate lymphoid progenitor (CHILP) cells.[5,7–9] αLP/EILP cells require T-cell factor 1 for development and have multilineage potential, with the capability to form all ILC helper subsets and classic natural killer (NK) cells and lymphoid tissue inducer (LTi) cells, whereas CHILP cells can generate all these subsets except NK cells.[9,10] The expression of NFIL3 (nuclear factor, IL-3 regulated) and TOX (thymocyte selection-associated high-mobility box protein) are also critical for differentiation of lymphoid progenitors to αLP/EILP and CHILP cells.[11,12] CHILP cells depend on the transcription factor ETS1 for appropriate fitness.[13] Expression of PLZF (promyelocytic leukemia zinc finger) protein and the upregulation of the cell surface receptor programmed death 1 (PD-1) marks the differentiation of CHILP cells into ILC progenitor cells, which can repopulate all helper ILC lineages but not LTi cells.[14,15] ILC development requires IL-2, IL-7, and Notch signaling,[5,6,16–19] whereas *RAG* (recombination-activating gene) expression is dispensable consistent with a lack of antigen specificity in ILC.[3–5]

The lineage specification of ILC progenitor cells into committed precursors of ILC1s, ILC2s, and ILC3s remains an area of ongoing investigation. The expression of Bcl11b, Gfi1, and RORα are vital for the generation of ILC2 precursors (ILC2P), the terminally differentiated ILC2 lineage cell in the bone marrow and most comparable cell to mature peripheral ILC2s.[20,21] GATA3 is also a critical marker of ILC2-committed cells, although GATA3-deficient mice lack all ILC subsets.[22–25] It is thought that GATA3 expression in early progenitor cells is required for advancement toward all ILC development but within the mature ILC compartment that high GATA3 expression demarcates the ILC2 fraction.[22] It is unclear whether terminally differentiated ILC1 progenitor and ILC3 progenitor cells that are comparable to ILC2 progenitor cells exist within the bone marrow or other sites of ILC lymphopoiesis.

An ample population of ILC progenitors exists in the bone marrow that may serve as the source of early ILC tissue seeding and as a reservoir for repopulation of tissues throughout life.[26] Additional evidence suggests that ILC lymphopoiesis may happen outside primary lymphoid tissues, including in the fetal gut and liver in mice.[14,19] In addition, in humans, circulating ILC progenitor cells have been identified in the peripheral blood.[27] The establishment and maintenance of ILC populations in tissue are further detailed below.

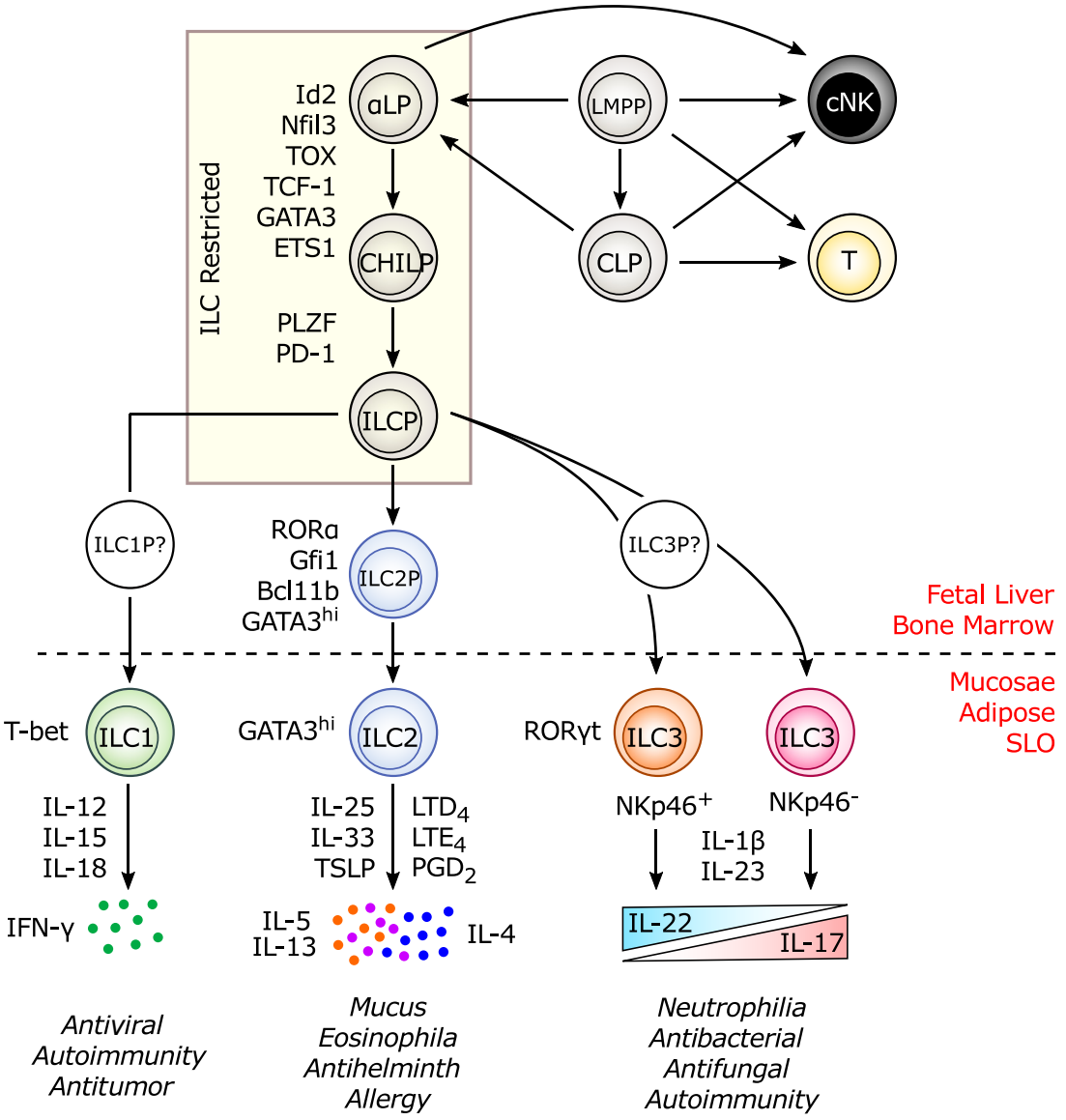

Figure 1. Innate lymphoid cell (ILC) development and effector functions. ILCs develop from lymphoid-primed multipotent progenitor (LMPP) or common lymphoid progenitor (CLP) cells similar to T and classic NK (cNK) cells in the bone marrow and fetal liver. ILC development proceeds through several stepwise, ILC-committed progenitors and requires a number of transcription factors that are expressed at unique points throughout development. Mature ILC1s, ILC2s, and ILC3s represent the major lineages of ILCs with unique transcriptional governance, stimuli, and cytokine production. These are primarily found at mucosal sites, in adipose tissue, and in limited quantities in secondary lymphoid organs (SLO). αLP, α-integrin–expressing lymphoid precursor; CHILP, common helper innate lymphoid progenitor; IFN, interferon; IL, interleukin; ILCP, innate lymphoid cell progenitor; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; PGD_2, prostaglandin D_2; TSLP, thymic stromal lymphopoietin.

ILC Tissue Maintenance and Trafficking

Tissue seeding by ILC2s occurs in the perinatal period in mice. Before birth, ILC2s are virtually absent in the lungs.[28–30] Across the first 2 weeks, ILC2s rapidly accumulate in the lungs and reach levels comparable to adult mice.[28–31] This accumulation of ILC2s coincides with a wave of IL-33 expression in the lungs, primarily from nonhematopoietic ($CD45^-$) epithelial cell adhesion molecule expressing ($EpCam^+$) lung epithelial cells.[29] Conceptually, this IL-33 expression and ILC2 accumulation occurs during the alveolarization period of murine lung development with expansion and maturation of type 2 pneumocytes. Mice that lack IL-33 signaling had reduced numbers of ILC2s in the lungs at postnatal days 7, 10, and 14, consistent with a critical role for IL-33 in establishing the ILC2 compartment within the lungs.[28,29,31] In the lungs during the postnatal period, ILC2s are the major source of type 2 cytokines, and the recruitment of type 2–associated effector cells, such as eosinophils and anti-inflammatory M2 macrophages, critically depends on ILC2s.[29,31] Collectively, these changes promote type 2 immune skewing in the postnatal period that can be exacerbated with exposure to allergen and persist into adulthood. Although ILC2 accumulation has been well characterized in the lungs, substantially less is known about ILC2 accumulation in other tissues.

ILC lineages are generally considered tissue resident under steady-state conditions. Pulse-chase experiments, wherein newly divided cells are tracked via the incorporation of the nucleoside analog bromodeoxyuridine, demonstrate that ILC2s are long-lived cells that exhibit self-renewing capacity in situ within tissues.[30] Parabiosis studies of naive mice, wherein 2 mice are surgically connected and share a vascular supply, allowing for the tracking of cells migrating through the blood from one mouse to another, demonstrate that ILC1s, ILC2s, and ILC3s are minimally replenished from hematogenous sources across short-term (40 days) or long-term (130 days) experiments, with recovery of approximately 2%

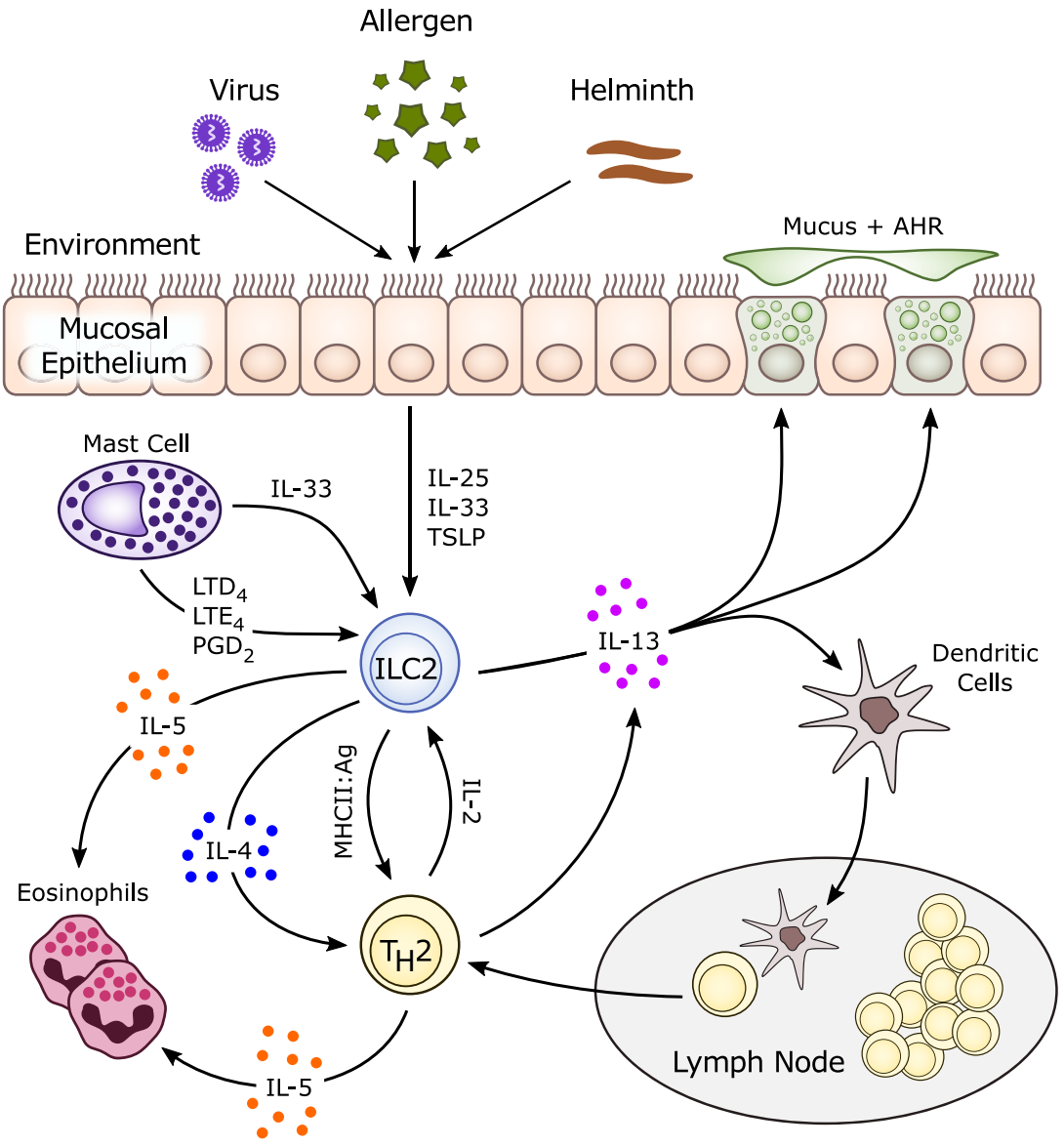

Figure 2. Mechanisms of innate lymphoid cell group 2 (ILC2)–mediated allergic disease. Environmental insults, including viruses, allergens, and helminths, damage the epithelial surfaces at mucosal sites, leading to the expression and release of danger signals interleukin (IL) 25, IL-33, and thymic stromal lymphopoietin (TSLP) that activate ILC2s. Activated ILC2s produce IL-5 to recruit eosinophils and IL-13 to mediate mucous metaplasia and release, induce airway hyperreponsiveness (AHR) in the case of the lungs, and mobilize dendritic cells to migrate to the lymph nodes and prime $CD4^+$ T cells into T_H2 cells. Infiltrating $CD4^+$ T_H2 cells dialog with resident ILC2s via an antigen presentation/IL-2 loop and further contribute IL-5 and IL-13, leading to augmented allergic inflammation. Mast cells produce IL-33 and proinflammatory lipid mediators, the latter of which are associated with ILC2 production of IL-4. ILC2-derived IL-4 promotes $CD4^+$ T_H2 cell differentiation. LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; MHCII:Ag, major histocompatibility complex class II antigen; PGD_2, prostaglandin D_2.

to 5% of donor ILCs in the lung, small intestine, salivary gland, and adipose tissue of the opposing parabiont.[32,33] However, in the context of infection with the helminth *Nippostrongylus brasiliensis*, which induces a strong type 2 immune response, ILC2s but not other ILC lineages are recruited to the lung in higher quantities than in mice receiving a mock inoculum.[33] The signals that support and maintain ILCs in tissue at steady state are largely unknown. T-cell–derived IL-2 is posited to support ILC2, in tissues via major histocompatibility complex (MHC) class II–mediated dialogue,[34] although a separate study suggests IL-2 may be dispensable for ILC2 maintenance at baseline.[33] ILC2s express inducible T-cell costimulator (ICOS), and ICOS-deficient mice have reduced numbers of ILC2s in the lungs and small intestine at baseline, suggesting a homeostatic role for ICOS signaling in supporting ILC2 populations at mucosal sites.[35,36] Although metabolic processes tied to arginase-1, a key enzyme in the metabolism of arginine to ornithine and urea as part of the urea cycle, and fatty acid processing support ILC2 responses to environmental challenge, disruptions of these systems have no effect on ILC2s at baseline.[37,38] These data collectively demonstrate that ILC2s are primarily tissue-resident cells in adult mice and that recruitment of cells from the bloodstream may occur in response to disrupted homeostasis and inflammation.

A limited number of signaling pathways have been identified that influence ILC2 trafficking. Germline C-C chemokine receptor type 9 (CCR9) deficiency reduced the trafficking of ILC2s to the small intestine, suggesting a possible defect in early ILC2 seeding of the gastrointestinal tract dependent on CCR9.[39] Using Kaede transgenic mice that express a photoconvertible fluorescent protein, allowing for cell tracking, ILC2s were found to traffic from the small intestinal lamina propria to the mesenteric lymph node rapidly within a 24-hour period.[40] In addition, prostaglandin D_2

(PGD_2) signaling via one of its receptors, CRTH2, and the integrin-β2 (CD18) both promote accumulation of ILC2s in the inflamed lung.[41,42] Collectively, these data suggest that ILC2 may transit dynamically within local regions or systemically during postnatal development of the organism or under conditions of inflammatory stress.

ILC2 Activators and Inhibitors

ILC2s are the subset of ILCs most extensively studied in the context of allergic inflammation because of their production of proallergic mediators, including IL-4, IL-5, IL-9, and IL-13. A variety of environmental stimuli are known to activate ILC2s. The 3 canonical activators of ILC2s are IL-33, IL-25, and thymic stromal lymphopoietin (TSLP).[4,5,43] ILC2s express the receptors for all 3 of these cytokines in both mice and humans,[4,44] though ILC2s from different tissues may depend more critically on one cytokine over the others.[44–46] IL-33 is a proinflammatory IL-1 family member that mediates its effects by binding to its cognate receptor ST2. Epithelial cells, including type 2 pneumocytes within the lung and keratinocytes within the skin, and endothelial cells are major sources of IL-33.[47,48] Macrophages and mast cells can also express IL-33, as do other cell types in smaller frequencies.[47,49,50] IL-33 is stored preformed within the nucleus of these cells, serving as a readily available inflammatory mediator.[51] In vitro, IL-33 robustly activates ILC2s, increasing proliferation and expression of IL-5 and IL-13.[4,5] IL-33 signaling deficiency or blockade of the IL-33 receptor attenuates ILC2 activation and associated physiologic responses in murine models, such as influenza infection,[52] *N brasiliensis* infection,[4] and atopic dermatitis.[46] Exogenous delivery of recombinant IL-33 elicits strong ILC2 responses in tissues, such as the lungs and gastrointestinal tract.[4,5,53] Maximal ILC2 responses to IL-33 require a second signal that potentiates NFAT signaling, such as leukotrienes.[54]

Although neither activates ILC2s to any substantial effect in isolation in vitro, IL-25 and TSLP significantly enhance ILC2 proliferation and cytokine expression in combination with other cytokines, namely, IL-33 (IL-25 and TSLP) or IL-2 (IL-25).[4,5] Mice deficient in the IL-25 receptor (also known as IL-17RB) have impaired ILC2-mediated immunity to intestinal helminth infection, whereas exogenous delivery of IL-25 to wild-type mice enhances ILC2 activity.[5] Furthermore, deficiency in TSLP signaling attenuates ILC2 responses in the skin, whereas overexpression of TSLP activates ILC2.[44] TSLP and IL-25 are expressed by epithelial cells, with a major source of IL-25 being tuft cells in the small intestine.[55,56]

Several other ILC2 activators have been characterized. The lipid mediators leukotriene D_4, leukotriene E_4, and PGD_2 induce cytokine expression from ILC2s.[57–59] Interestingly, leukotriene D_4 and PGD_2 extend ILC2 cytokine-producing capacity to include IL-4, and PGD_2 is a potent chemoattractant of ILC2s. Additional activators of ILC2 include IL-1β and tumor necrosis factor–like ligand 1A.[60,61]

Conversely, there is intense interest in understanding inhibitors of ILC2s that may serve as therapies in allergic disease. Endogenous inhibitors of ILC2s include Spred1, a suppressor for the Ras-ERK pathway, and the cell surface receptor PD-1.[62,63] Lipoxin A_4 and the cyclooxygenase product prostaglandin I_2 also negatively regulate ILC2 proliferation and cytokine production,[64,65] and type 1 and 2 IFNs and their convergent downstream target signal transducer and activator of transcription 1 negatively regulate ILC2 viability and function, including in a model of allergic airway disease and respiratory viral infection.[53,66–69] Signaling through the α_7-nicotinic acetylcholine receptor similarly attenuates ILC2 function.[70] ILC2s are also attenuated by T-regulatory cells and their products IL-10 and transforming growth factor β.[71–73]

Therapeutics that target IL-25, IL-33, and TSLP are of significant interest to the field, and several candidates have been advanced to clinical trial.[74] In one phase 1 study, patients were randomized to receive placebo or AMG-157, an anti-TSLP neutralizing antibody, and received doses on days 1, 29, and 57 of the study. Experimental allergen challenges were completed on days 41 to 43 and 83 to 85 of the protocol and demonstrated that patients receiving AMG-157 had a smaller reduction in forced expiratory volume in 1 second compared with placebo treatment during the early- and late-phase allergen response.[74] Several other investigational new drugs are in the pipeline or in preclinical development targeting IL-33 binding to the IL-33 receptor ST2. One candidate therapeutic, AMG-282, is an anti-ST2 antibody and is currently in phase 1 clinical trials for mild allergic asthma and chronic rhinosinusitis (NCT01928368 and NCT02170337). A second candidate therapeutic, ANB020, is being evaluated in phase 2 trials for the treatment of peanut allergy (NCT02920021).

ILC2 Mechanisms of Pathogenesis in Allergic Disease

ILC2s are important mediators of allergic disease, and a significant amount of literature sheds light on the mechanistic importance of ILC2s in response to allergens (summarized briefly in Fig 2). The initial reports describing ILC2s all demonstrated their physiologic importance in type 2 immune responses using a mouse model of infection with the helminth *N brasiliensis*.[3–5] These data suggested an important role for ILC2s in type 2 immune responses and by extension posited a potential role in allergic reactions. Subsequent work implicated ILC2s as important type 2 immune effector cells in mouse models of aeroallergen exposure to *Alternaria alternata* and papain, and adoptive transfer of ILC2s into lymphocyte-deficient mice demonstrated ILC2s to be sufficient to induce airway mucous accumulation and eosinophilia independent of the adaptive immune system.[43,75] Compared with $CD4^+$ T_H2 cells, ILC2s were found to be considerable independent expressers of IL-5 and IL-13 in house dust mite–induced allergic inflammation, and ILC2s have greater than 10-fold per-cell cytokine-producing capacity compared with $CD4^+$ T_H2 cells.[76]

Related work using murine models of atopic dermatitis has shed additional light on the role of ILC2s in allergic disease. Depletion of ILC2s in the skin of $Rag^{-/-}$ mice ablated skin inflammation and thickening in a vitamin D analogue–induced model of atopic dermatitis.[44] The activation of ILC2s within the skin may involve multiple circuits. Although much attention has been focused on IL-33–induced activation of ILC2s in the lungs, all 3 of the cytokines, IL-25, IL-33, and TSLP, have been implicated in ILC2-driven allergic reactions within the skin.[44,46,77]

Activation of ILC2s in tissues profoundly changes their surface phenotype, a finding consistent with several feed-forward loops that may promote the allergic response. Notably, the high-affinity subunit of the IL-2 receptor complex, CD25, is significantly upregulated in the setting of inflammation in the lungs and skin.[75,78] Increased CD25 expression positions ILC2s to better respond to IL-2, a potent IL-33 synergistic signal for ILC2s in mice and humans. Inflammation in the lungs and skin also upregulates the IL-33 receptor ST2.[78–80] In vitro stimulation of human ILC2s increases the expression of the receptors for IL-25, IL-33, and TSLP, suggesting similar feed-forward loops may boost ILC2 responses in humans.[81] In addition, ILC2s but not $CD4^+$ T_H2 cells exhibit corticosteroid resistance in mice and humans, particularly when they are stimulated by TSLP, suggesting ILC2s as pathophysiologic mediators in corticosteroid-resistant asthma.[82–85]

Several transcriptional regulators maintain ILC2 identity and enhance ILC2 activity during allergic inflammation. GATA3 is a hallmark transcriptional regulator of ILC2s that defines the ILC2 lineage and when phosphorylated binds to the IL-5 and IL-13 promoters to drive cytokine production.[8,24] Transgenic mice overexpressing GATA3 had significantly increased IL-5 and IL-13 production and ST2 and IL-25 receptor expression in lung ILC2s compared with WT ILC2s or GATA3 hemizygous ILC2s.[25] GATA3

overexpressing ILC2s also had upregulated ST2 expression and cytokine production in a murine model of house dust mite–induced allergic airway inflammation.[86] Whole transcriptome analysis of mature ILC2s with or without a conditional deletion of GATA3 demonstrates substantial changes in the overall ILC2 program, reinforcing the importance of GATA3 in ILC2 function.[22] Similarly, overexpression of GATA3 increased ST2 and TSLP receptor expression and IL-5 and IL-13 cytokine production from human ILC2s stimulated in vitro.[23] In addition, Gfi1 promotes ILC2 activity by increasing the expression of the IL-33 receptor, stabilizing GATA3, and blocking IL-17A production.[80] Mice with a conditional deletion of Gfi1 fail to respond effectively to aeroallergen challenge and exhibit a hybrid ILC2-ILC3 effector state. Bcl11b critically stabilizes GATA3 and Gfi1 to promote the ILC2 program and functionality. Bcl11b-deficient mice fail to respond appropriately to IL-33 stimulation or *N brasiliensis* infection, shifting toward an ILC3-like phenotype.[20,87] Moreover, PLZF enhances IL-5 and IL-13 expression during allergen challenge from ILC2s but not $CD4^+$ T_H2 cells.[88]

Additional ILC2-intrinsic signals enhance the contribution of ILC2s to allergic disease. In addition to playing a role in ILC2 homeostasis, signaling via ICOS on ILC2s enhances cytokine production during IL-33–induced airway inflammation.[36] MicroRNAs may also play a role in shaping ILC2 responses, with miR-155 upregulated after IL-33 stimulation of ILC2s and contributing to ILC2 proliferation, IL-13 expression, and stabilization of GATA3.[89]

Although ILC2s have sufficient capacity to induce pathophysiologically significant responses in isolation, ILC2 responses to allergens in intact organisms and humans occur in the context of a broader type 2 immune response. Several key interactions have been described between ILC2s and other cells of the immune system that have potential relevance in allergic disease. ILC2–T-cell crosstalk appears to be a vital circuit for promoting robust innate and adaptive type 2 immune responses. ILC2s express MHC class II and are capable of antigen uptake, processing, and presentation to $CD4^+$ T cells via peptide display in MHC class II. ILC2s interact with antigen-specific T cells via antigen-loaded MHC class II, triggering T-cell expression of IL-2 that signals back onto the ILC2 to promote ILC2 proliferation and IL-13 production.[34] Conversely, mice that lack ILC2s have severe impairments in the number of $CD4^+$ T_H2 cells in lymph nodes of *N brasiliensis*–infected mice.[34,90] Effective $CD4^+$ T_H2 cell differentiation and responses require ILC2-derived IL-13 to mobilize dendritic cells to migrate to the lymph nodes for T_H2 cell priming during primary and memory responses in adult and early postnatal mice.[28,91] Transfer of ILC2s and $CD4^+$ T cells, but not either individually, into *Il7ra*$^{-/-}$ mice was required for a robust antigen-specific allergic response.[92] Furthermore, ILC2-derived IL-4 promotes the differentiation of $CD4^+$ T_H2 cells in mice infected with the helminth *Heligmosomoides polygyrus*.[93] ILC2-derived IL-4 also promotes mast cell sensitivity to degranulation and inhibits T-regulatory cell induction, contributing to a shift away from tolerance that may predispose individuals to food allergy. Similarly, ILC2s synergize with $CD4^+$ T_H2 cells to promote food allergy via IL-13 production.[94] These data suggest a key collaboration between ILC2s and adaptive $CD4^+$ T cells in promoting allergic type 2 immune responses.[73]

With regard to other arms of the adaptive immune system, murine studies found that ILC2s interact with B lineage cells or possess potential to do so. ILC2s and B cells express ICOS and ICOS ligand, respectively, suggesting potential dialogue between these cells.[4] Coculture of ILC2s with B1 or B2-type B cells enhanced B-cell proliferation and antibody production, and adoptive transfer of ILC2s into *Il7r*$^{-/-}$ mice enhanced B1-cell antigen-specific IgM production.[95] Consistent with these findings, ILC2s promote IgA expression and self-renewal in B1 cells, likely via IL-5 and/or IL-6 expression.[5] ILC2s also have the capacity to produce IL-4, which may shape B-cell class switching.[58] In humans, ILC2s promote B-cell extrafollicular responses as evidenced by increased B-cell expression of EBI2 in human ILC2–B-cell coculture.[96] These data suggest that ILC2s may influence the B-cell and/or IgE compartments in allergic disease.[97] ILC2s also coordinate with cells of the innate immune system. Specifically, basophil-derived IL-4 is required for ILC2 responses in a mouse model of atopic dermatitis.[98]

A significant proportion of asthma exacerbations are triggered by viral infections, most notably with rhinovirus (RV), respiratory syncytial virus (RSV), and metapneumovirus. Emerging evidence suggests that ILC2s may play an important role in the pathophysiology of respiratory viral infection with consequence for allergic airway disease. The general paradigm is that viral infections, particularly of the respiratory tract, infect epithelial cells, leading to the expression of ILC2-activating cytokines, such as IL-33 (later in these infections, macrophages become the predominant source of IL-33) that stimulate ILC2s. Influenza infection triggers IL-5 and IL-13 production from ILC2s with concomitant eosinophilia and airway hyperreponsiveness in the presence and absence of an adaptive immune response.[99,100] RV infection in neonatal mice activates ILC2s via IL-25 to expand and express IL-13, contributing to airway mucous accumulation and airway hyperactivity.[101] In a murine model of RV-exacerbated asthma, RV infection increased the expression of IL-25 in the lungs, leading to an increase in ILC2s within the lungs that correlated with increased IL-5 and IL-13 cytokines in the bronchoalveolar lavage fluid.[102] ILC2s have also been implicated in the pathogenesis of severe RSV infection. In a mouse model, TSLP activates ILC2s during infections with several different pathologic clinical isolates of RSV, which correlates with increased airway mucous accumulation and airway hyperresponsiveness.[103] Additional work demonstrates that IL-33 activates ILC2s during RSV infection, particularly in the early postnatal period in mice.[79] Of note, ILC2 responses in the late stages of influenza infection are characterized by amphiregulin production from ILC2s that promotes tissue repair, suggesting that ILC2s may play pathologic and beneficial roles at different points during viral infection.[52]

ILC2s in Human Allergic Disease

Enhancements in ILC2 count and/or function have been observed in humans in the setting of allergic disease, including asthma, allergic rhinitis, and atopic dermatitis. ILC2s are twice as frequent in the blood of patients with allergic asthma compared with healthy controls. These ILC2s were also significantly more responsive to IL-33 and IL-25 ex vivo, expressing higher levels of IL-5 and IL-13 compared with healthy controls.[104] Increases in the number of ILC2s in the peripheral blood were substantially greater in patients with severe eosinophilic asthma compared with individuals with milder asthma or noneosinophilic asthma, with a strong positive correlation between the severity of eosinophilia in the sputum and ILC2 frequency of the peripheral blood.[105,106] Similar observations were made within the airways, with the total number of ILC2s and the number of IL-13–producing ILC2s increased in the bronchoalveolar lavage fluid of patients with asthma compared with controls without asthma.[107,108] Induction of airway inflammation in individuals with asthma by respiratory allergen challenge similarly increased ILC2 counts and cytokine production in the sputum,[109] and ILC2 activation was found to inversely correlate with effectiveness of corticosteroid treatment.[109,110] Of note, one study found no difference in the frequency of ILC2s in the peripheral blood of healthy controls, patients with mild asthma, and patients with severe asthma.[65]

Dynamic changes in ILC2 frequencies are also observed in patients with allergic rhinitis. In cat-allergic individuals, nasal challenge with cat antigen significantly increased the frequency of ILC2s in the peripheral blood compared with diluent challenge.[111] ILC2s are hypothesized to play a key role as proinflammatory cells in allergic

rhinitis because patients challenged with intranasal exposure to allergen have increases in the number of ILC2s in nasal secretions that strongly and positively correlated with airway eosinophilia.[112] Interestingly, the magnitude of ILC2 induction and cytokine production in patients with allergic rhinitis varies by antigen, with house dust mite monosensitized patients having significantly increased frequencies of circulating ILC2s compared with mugwort monosensitized patients.[113] However, in one study, no significant increase in ILC2s or their cytokine production was observed in patients with allergic rhinitis compared with healthy controls.[104] Pollen grass subcutaneous immunotherapy was effective in preventing ILC2 accumulation in the blood of patients with seasonal allergic rhinitis, indicating the potential for therapeutic intervention in attenuating ILC2 responses.[114] These data broadly support a role of ILC2s in the pathogenesis of allergic rhinitis, although factors such as specificity of the antigen may influence ILC2 reactivity.

Atopic dermatitis in humans is also linked to dysregulation of ILC2s. ILC2s are markedly increased in the skin of patients with atopic dermatitis compared with skin samples collected from healthy controls.[44,46,98,115] Moreover, ILC2s in the setting of atopic dermatitis had features consistent with an activated phenotype, including the upregulation of CRTH2, CD161 (also known as killer cell lectin-like receptor subfamily B, member 1), ST2, TSLP receptor, IL-25 receptor, and KLRG1 (killer cell lectin-like receptor subfamily G, member 1).[44,46] Within the dermis of inflamed atopic skin, ILC2s were found close to basophils or T cells, suggesting possible coordination between these proinflammatory cell types.[98] Induction of an inflammatory response in allergic patients via exogenous, intraepidermal injection of house dust mite antigen significantly increased the number of ILC2s and their cytokine products IL-4, IL-5, and IL-13 at the site of injection compared with control saline instillation. Collectively, these data strongly suggest a pathologic role for ILC2s in the setting of atopic dermatitis.

Emerging evidence supports a potential role for ILC2s in the context of several other allergic diseases. Patients with aspirin-exacerbated respiratory disease challenged intranasally with nonsteroidal anti-inflammatory drugs had increased ILC2 burdens in nasal scrapings collected during challenge compared with ILC2 levels in nasal scrapings at baseline. This increase in ILC2 burden in the nasal scrapings was associated with a simultaneous decrease in ILC2 frequency in the blood, suggesting potential tissue infiltration of ILC2s from the blood to the nasal mucosa. Furthermore, this increase in ILC2 burden in the nasal scrapings positively correlated with severity of symptoms.[116] In addition, several eosinophilic disorders have been associated with ILC2 activation. Active eosinophilic esophagitis promotes an increase in the percentage of ILC2s in the esophagus compared with patients with quiescent disease or healthy controls.[117] Eosinophilic pleural effusion induces a marked increase in the number of ILC2s in the pleural fluid.[118]

ILC Plasticity and the Contributions of ILC1s and ILC3s to Allergic Disease

Given their production of well-known allergic mediators, such as IL-4, IL-5, and IL-13, ILC2s have been the primary focus of studies into the role of ILCs in allergic disease. However, non–type 2 cytokines, such as IFN-γ and IL-17, may also play a role in enhancing severe asthma.[119,120] In a mouse model of obesity-induced airway inflammatory disease, IL-17 from ILC3s was responsible for airway inflammation and airway hyperresponsiveness.[121] In this same study, IL-17–expressing ILC3s were recovered in increased frequencies from the bronchoalveolar lavage of patients with severe asthma compared with patients with mild asthma and healthy controls.

Moreover, conversion of ILCs between subsets may increase the available pool of pathogenic ILCs during allergic responses. ILC plasticity was initially observed within the ILC3 subset of mice, wherein signaling via IL-12 and IL-15 was inversely correlated with RORγt expression.[122] Additional in vitro studies with human ILCs found that IL-12 induces transdifferentiation of ILC3s into ILC1s (referred to as ex-ILC3) as indicated by loss of RORγt expression and increases in T-bet and IFN-γ expression.[123,124] Similarly, IL-23 induced ILC1s to convert to ILC3s in vitro as evidenced by the loss of IFN-γ production and the increased expression of RORγt and IL-22.[124] In vivo, adoptive transfer of purified human ILC1s into humanized mice led to the detection ILC3s in classically ILC3-rich tissues, such as the intestine.[124]

Analogous plasticity has been observed with ILC2s. Purified murine inflammatory ILC2s cultured under T_H17-skewing conditions (transforming growth factor β and IL-6) and exposed to the activators phorbol myristate acetate and ionomycin significantly expanded the number of IL-13 and IL-17 dual-producing cells.[125] Moreover, human ILC2s exposed to IL-12 or IL-18 develop the capacity to coproduce IL-13 and IFN-γ and to stably express T-bet, suggesting that these cells are acquiring an ILC1-like phenotype.[61,126,127] Conversely, IL-4 drives the transdifferentiation of human ILC1s to ILC2s.[128] Further studies will be necessary to understand more broadly the contributions of ILC1s and ILC3s to allergic disease and whether plasticity of the ILC subsets contributes to allergic pathophysiology.

Conclusion

The discovery and characterization of ILC populations, especially ILC2, have greatly expanded our understanding of allergic disease pathogenesis. ILCs are on guard at mucosal sites, equipped with substantial capacity to produce effector cytokines and ready to respond rapidly to environmental insults. They have been implicated across the spectrum of allergic diseases in humans, and elegant mouse models have found an essential role for ILCs in the initiation and exacerbation of allergic disease. The presence of ILCs, particularly ILC2s, at sites of allergic inflammation strongly suggests their role in the pathogenesis of allergen-driven disease. However, until we are able to effectively and specifically block ILC2 development or function in patients, the exact role for ILC2s in humans will remain inexact.

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