



REVIEW

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Group 2 innate lymphoid cells (ILC2s): The spotlight in asthma pathogenesis and lung tissue injury

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Abstract

Asthma is a heterogeneous disease with ranging etiology and severity. Asthma is a disease of chronic inflammation of the airways, with clinical symptoms of wheezing, breathlessness, cough, and chest tightness manifested as chronic fixed or variable airflow obstruction and airway hyperresponsiveness that predispose the airway epithelium to repeated injury, repair, and regeneration. In recent years, innate lymphoid cells (ILC1, ILC2, and ILC3) have been discovered. The predominant ILC type found in the lung tissue is group 2 innate lymphoid cells (ILC2s). Upon damage to the airway epithelium mediating the release of epithelial cytokines (TSLP, IL-33, and IL-25) ensued the activation of ILC2 in an antigen-independent manner. Activated ILC2 produces a significant amount of type 2 cytokines (IL-4, IL-5, IL-9, and IL-13), altogether contributing to type 2 inflammation in the airways. ILC2s are mediators of type 2 immunity for many type 2 inflammatory diseases such as asthma, since ILC2s were reported to play an important role in asthma pathogenesis. Here we discuss the role of ILC2 in the development of asthma and ILC2 effector cytokines (IL-4, IL-5, and IL-13) contributing to airway epithelial structural changes.

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Introduction

Asthma is a chronic clinical condition characterized by episodic symptoms of airflow obstruction, bronchial hyperresponsiveness, and airway inflammation. Asthma is the most prevalent respiratory airway disease worldwide, affecting >300 million people of all ethnicity,^{1,2} and is the most common chronic disease in children.³ Familial history of atopic disease, genetics, environmental factors (pollutants, microbes, and exposure to passive smoking),⁴ and allergens, such as house dustmite allergens,⁵ cockroaches,⁶⁻⁸ pets (especially cat dander),^{9,10} and fungi *Alternaria*,^{11,12} are significant inducers of an asthma attack. Asthma is a phenotypically heterogeneous disease with different phenotypes.¹³ Asthma symptoms result from a cascade of inflammatory phase and ILC2s that release type 2-derived cytokines, which play a vital role in asthma inflammation.¹⁴ ILC2s are a recently discovered subtype of innate lymphoid cells; they produce high amounts of type 2 cytokines, exhibit lymphoid morphology, and are non-T and non-B cells that lack antigen specificity.¹⁵ Asthma is typically regarded as a Th 2 cell-mediated disease. However, as in recent years, ILC2s have shown a critical role in the symptoms associated with asthma pathogenesis. Type 2 inflammation is the presiding mechanism underlying asthma;¹⁶ studies have shown a predominant increase of ILC2s in asthmatic patients.¹⁷ The ILC2 pathway is activated by epithelial cell-derived cytokines (IL-33, IL-25, and TSLP) upon stimulation and proceed by resulting in the production of a significant amount of its effector cytokines (IL-5, IL13, and IL-4).¹⁸ Here, in this review, we discuss our understanding of the development and activation of ILC2s and how these cells contribute to type 2 inflammation in the context of asthma.

Inflammation in asthma

Asthma is a disease of chronic inflammation that affects the lower airways, characterized by reversible airflow obstruction, airway inflammation, and airway hyperresponsiveness (AHR), with associated symptoms such as coughing, difficulty in breathing, wheezing, and chest-tightening.¹⁹ All these symptoms are mediated by immune cells such as T-lymphocytes,²⁰ mast cells,²¹ neutrophils,²² eosinophils,²³ epithelial cells,²⁴ macrophages,²⁵ NK cells,²⁶ and ILC2s.²⁷ Among all the pro-inflammatory mediators produced by the immune cells, the main elements are cytokines, histamines, and leukotrienes.²⁸ In the past, clinicians classified asthma as an allergic or non-allergic disease. Nevertheless, this concept has fallen out of favor, and now it is known that asthma is a much more heterogeneous disease and differs in management and clinical severity.²⁹ Prolonged inflammation in asthma results in swelling and inflammation of the lining of airways, thus eventually leading to structural changes in airways,³⁰ as asthma inflammation occurs in both large (trachea) and small airways (bronchi and bronchioles).³¹

The airway epithelium, a defense barrier lining the airway and a major key factor in asthma pathogenesis,³² after irritation by stimulants (injury, allergens, microbes, or pollutants) tend to release a significant amount of molecules

such as chemokines, cytokines, prostaglandin E2 (PGE2), endothelin, nitric oxide, and others.³³ These epithelial-derived molecules cause the activation of inflammatory cells, resulting in the airways inflammation.³⁴ Therefore, activated inflammatory cells release pro-inflammatory mediators such as cytokines, chemokines, histamines, and leukotrienes. This results in mucus hypersecretion that plugs the airways and makes it difficult to breathe, rendering vascular permeability, leading to migration of other inflammatory cells; aggravating inflammation and smooth muscle contraction; bronchial constriction; and increased bronchial permeability.³⁵

Development and survival of ILC2s

ILC2s are the subgroup of the ILC family developed from common lymphoid progenitor (CLP) and are affiliated to the lymphoid lineage negative cells.³⁶ ILC family (ILC1s, ILC2s, and ILC3s) is categorized based on surface markers (ILC1s: IL-7R α , NKp44, NKp46, sca-1, and Thy-1; ILC2s: IL-7R α , CCR2, GATA binding protein 3 [GATA3], ST2, KLRB1, IL-17RB, and SCA-1; and ILC3s: IL-7R α , c-kit, IL-23R, NKp44, and NKp46) (Figure 1),^{37,38} transcription factors, and production of cytokines. ILCs orchestrate immune response mediated by production of cytokine (ILC1s: TNF γ ; ILC2s: IL-5, IL-13, and IL-4; and ILC3s: IL-17, IL-22, and GM-CSF) (Figure 1). ILC subsets hold a close resemblance, concerning cytokine production, to the T helper subsets Th1, Th2, and Th17, respectively (Figure 1).³⁹ Activated ILC2s by its alarmins (IL-33, IL-25, and TSLP) are released by the epithelial cells upon epithelial injury and irritation.⁴⁰ ILC2s can be classified into natural ILC2 cells (nILC2) and inflammatory ILC2 cells (iILC2). Notably, nILC2 and iILC2 cells play different roles in the lung: iILC2 cells mobilize to the lung from the gut to participate in rapid and transient type 2 immunity, while nILC2 cells mainly reside in the tissue and respond to viral infection.⁴¹ Also, iILC2 cells develop significant amounts of IL-13 and small amounts of IL-5 and IL-4 *in vivo* and are capable of producing large amounts of IL-13, IL-5, and IL-4 following *in vitro* stimulation of phorbol myristate acetate (PMA)/ionomycin.⁴² Furthermore, nILC2 cells express methionine enkephalin (MetEnk),⁴¹ a peptide with an immunological role in increasing numbers of cytotoxic cells against viral infection and tumor cells. Treatment with MetEnk inhibits influenza A virus (H1N1) replication⁴³—whether iILC2 cells express MetEnk is to be determined.⁴¹ Similarly, nILC2 cells produce amphiregulin, which plays a vital role in maintaining the integrity of epithelial barrier during inflammation; however, whether iILC2 cells produce amphiregulin to mediate tissue repair is yet to be determined.⁴¹

The principal transcriptional factors implicated in the differentiation and development of ILC2s are GATA3 and retinoic acid-related orphan receptor alpha (ROR α).⁴⁴ Human ILC2s highly express GATA3 and are found to be enriched in nasal polyps of patients with chronic rhinosinusitis, a typical type 2-mediated disease. Nasal polyp epithelial cells express thymic stromal lymphopoietin (TSLP), which promotes activation of signal transducer and activator of transcription 5 (STAT5) and GATA3 expression.⁴⁵

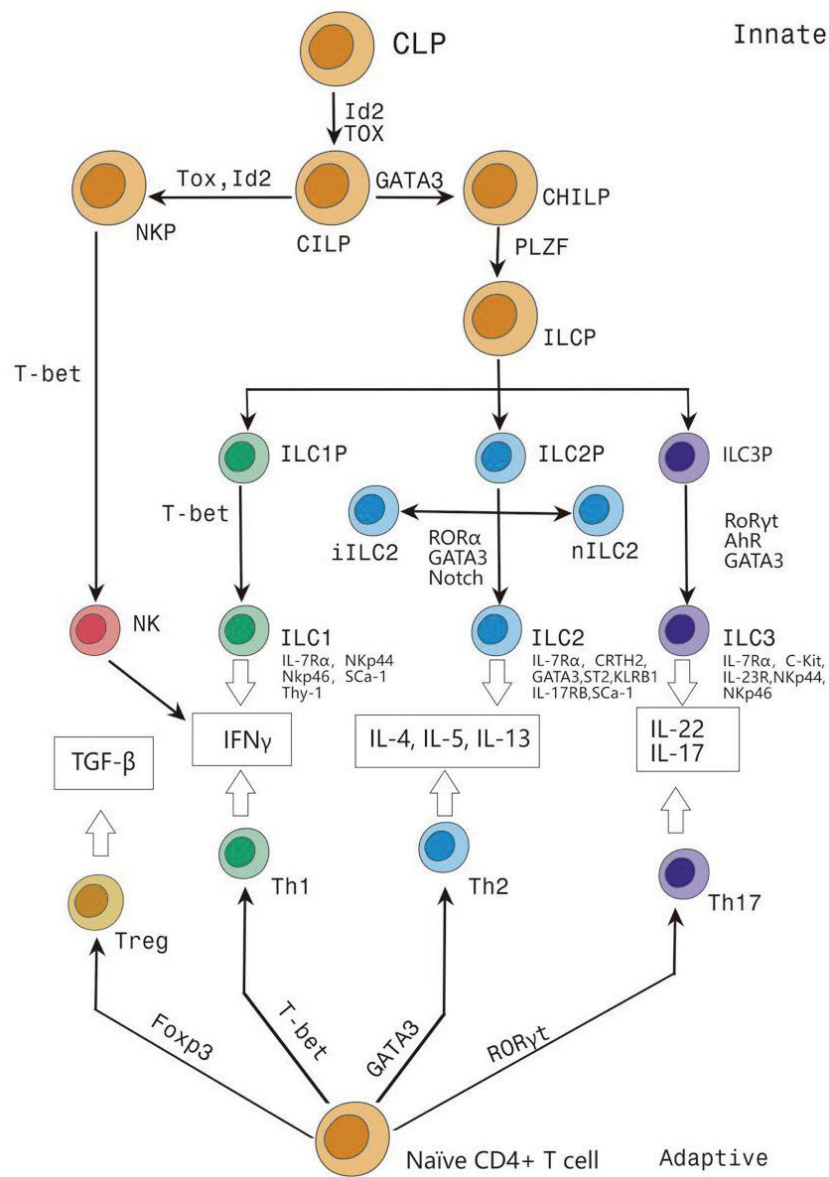


Figure 1 ILC family exhibit resemblance to TH2 cells in cytokine production and transcription factors. Shows ILC2 development and transcription factors. CLP: common lymphoid progenitor; CILP: common IL-R α + innate lymphoid progenitors; NKP: natural killer cell progenitor; CHILP: common helper innate lymphoid progenitors; ILCP: innate lymphoid cell progenitor; GATA3: GATA binding protein 3; ROR α : retinoic acid receptor-related orphan receptor alpha.

Deletion of GATA3 in all hematopoietic lineages at a very early stage of development abolish all helper-like ILCs. ILC2s depend on GATA3 for their development, and low levels of GATA3 are also required for the development of ILC3 subset (NKp46+); however, deletion of GATA3 later in the development results in the impaired differentiation of ILC2 cells, but not ILC3 cells, which shows that GATA3 is critical for the development and maintenance of ILC2s but not other ILCs.⁴⁶ The intracellular adhesion molecule-1 (ICAM-1) is also required for the development of ILC2s, where ICAM-1^{-/-} and ILC2s show impaired extracellular signaling-regulated kinase (ERK) pathway, which leads to diminished expression of GATA3 and production of type 2 cytokines.⁴⁷ ROR α

is identified as a regulator of ILC2 differentiation and function and this transcription factor is essential for the development of ILC2.⁴⁸ ROR α is highly expressed in the growth of ILC2 cells, but not other ILC subsets.⁴¹ Another role of ROR α is in directing ILC2 proliferation and effector function.

ILC2-like cells were detected in peripheral tissues of ROR α -deficient mice; these cells fail to expand in response to stimulating cytokines.⁴⁸ Hence, GATA3 and ROR α are essential for the development, maintenance, and functioning of ILC2s.⁴⁹ Similarly, transcription factor 1 (TCF-1) and notch signaling also play a role in the development of ILC2, as notch signaling induces TCF-1. TCF-1 is a critical transcription factor required for the expression of GATA3

and ROR α (two important transcription factors known to be vital for the development of ILC2); it also appears to be an important factor for the expression of IL-33R by ILC2. A loss of TCF-1 expression impairs the ability of ILC2 to produce type 2 effector cytokines (IL-5 and IL-13).⁵⁰ TGF- β (TGF β) exhibits a cell-intrinsic role in programming the development of ILC2 by upregulation of ST2 expression in their progenitors (CHILP and ILC2P) mediated partially via MEK1/2-dependent but Smad3- and TAK1-independent pathways. TGF β simultaneously plays an important role in the functioning, homeostasis, and maintenance of ILC2. It is reported that deficiency in TGF β signaling results in the unsuccessful development of ILC2 and a significant decline in total numbers of ILC2s.⁵¹ IL-33 and IL-25 signaling might also play a role in the development of ILC2.⁴¹ In addition, E3 ubiquitin ligase Von Hippel-Lindau VAL, a new molecule identified recently, plays a significant role in promoting maturation and development of ILC2s. It was reported that a deletion of E3 ubiquitin ligase VHL in innate lymphoid progenitors minimally affected early-stage bone ILC2s but caused a selective and intrinsic decrease in mature ILC2 numbers in peripheral non-lymphoid tissues, resulting in reduced type 2 immune responses.⁵² Proliferation and survival of ILC2 cells, the cytokine-independent function of ILC2 cells, could directly regulate T cell activation, and ILC2 expression of MHC class II could present antigen to T cells, triggering T cell-derived IL-2 production. As a result, such IL-2 would promote ILC2 proliferation.⁵³ IL-9 signaling is crucial for the survival of activated ILC2s, as shown in a study, where IL-9 receptor (IL-9R)-deficient mice displayed reduced numbers of ILC2s in the lung after infection, resulting in impaired IL-5, IL-13, and amphiregulin levels.⁵⁴ Another cytokine, IL-7, promotes ILC2 proliferation and survival.⁵⁵

ILC2 in Inflammation

ILC2s belong to one of the three subsets of ILC populations arising from common lymphoid progenitors.⁵⁶ Among ILC subsets, ILC2s are the prominent cells in the lung tissue, and upon ILC2 activation, they produce large amounts of type 2 cytokines (IL-5, IL-13, IL-9, and IL-4).⁵⁷ ILC2s influence both adaptive and innate immunity and are regarded as the counterpart of the Th2 immune pathway.⁵⁸ ILC2 cells are essential drivers of inflammation;⁵⁹ they are the spotlight of type 2 immune response⁶⁰ and have been a source of inflammation in many clinical diseases,⁶¹ such as in inflammatory bowel disease (IBD),⁶² intestinal helminths infection,⁶³ and allergic rhinitis.⁶⁴

In the context of asthma inflammation, ILC2 cells line the mucosal surface of the airway to protect host from adversity of pathogenic microbes, allergens, and pollutants.⁶⁵ Pathogenic recognition, allergen exposure, or damage to the barrier function of the airway epithelium enhances mucosal permeability of foreign substances into the airway epithelium of the patients with asthma;⁶⁶ these initiate the release of epithelial cell-derived cytokines, such as TSLP, IL-25, and IL-33, hence ILC2s alarms.⁶⁷ They activate ILC2s in an antigen-independent manner via their respective receptors TSLPR, IL-17RB, and ST2.⁶⁸ Overexpression of TSLP in the lungs leads to

the development of AHR.⁶⁹ Likewise, TSLPR-deficient mice reported having impaired type 2 responses.⁷⁰ IL-25 is expressed in the epithelial lung cells following allergen exposure. In airway epithelial cells, transgenic overexpression of IL-25, and intraperitoneal or intranasal administration of IL-25, promotes type 2 responses. Correspondingly, IL-25-deficient mice reported having decreased AHR in a model of asthma.⁷¹

IL-33, or IL-2/33, is a potent activator of ILC2, resulting in enhanced production of critical effector cytokines IL-5 and IL-13, which are responsible for the development of allergic airway inflammation.^{72,73} On the other hand, IL-33-deficient mice have impaired AHR, and mice deficient in IL-33 receptor, ST2 mice have declined parasitic expulsion.⁶⁹ On the contrary, other cytokines have shown to inhibit mouse and human ILC2 responses, such as IFN- β , IL-10, TGF- β , IFN γ , and IL-27.⁷⁴ In response to epithelial cell-derived cytokines, ILC2s produce a significant amount of type 2 cytokines (IL-5, IL-13, and IL-4) (Figure 2).^{44,75,76} IL-5 functions in eosinophil proliferation, differentiation, maturation, migration to tissue sites and survival, as well as in the prevention of eosinophil apoptosis; eosinophils are vital players in the pathogenesis and severity of asthma.⁷⁷ IL-13 acts on the airway smooth muscle cells, inducing AHR, promoting differentiation of goblet cells, and contributing to hypersecretion of the mucus.⁷⁸ ILC2s have been found to be an important source of IL-9 after the administration of papain.⁵⁸ IL-9 promotes mast cell hyperplasia,⁷⁹ AHR, and mucus overproduction.⁸⁰ Moreover, IL-9+ILC2s have been shown to promote resolution of lung inflammation by impairing caspase-1 activation, which prevents lung endothelial cells from pyroptosis, and particularly treatment with rIL-9 recombinant protein promotes ILC2-dependent Treg activation and successfully resolves inflammation.⁸¹⁻⁸³ Subsequently, IL-4 induces IgE isotype switching in B cells and promotes eosinophil transmigration across endothelium.⁸⁴

ILC2 provokes inflammation in asthma

ILC2-associated asthma is frequently associated with higher eosinophil counts. Eosinophilia is a predictor of the severity of asthma and possible exacerbations; it also predicts the outcome of asthma care with ICS.⁸⁵ Late-onset asthma that develops in adulthood is often associated with chronic rhinosinusitis and nasal polyps; thus, increased numbers of ILC2s have been detected in these nasal polyps, resulting in severe eosinophilia and persists despite inhaled corticosteroid treatment.⁸⁶ Besides, Nagakumar et al.⁸⁷ surveyed children with severe therapy-resistant asthma (STRA), children with difficult asthma (DA), and a control group where sputum ILC2s were significantly higher in STRA patients compared to DA and disease controls; but ILC2s decreased *in vivo* post-intramuscular triamcinolone. Liu et al.⁸⁸ reported persistent steroid resistance in ILC2-associated asthma in asthmatic patients with increased TSLP levels in an MEK- and STAT5-dependent manner. However, this effect was reversed by MEK and STAT5 inhibitors. Likewise, Verma et al.⁸⁹ demonstrated, using ST2 knockout (KO) mice, deletion of ST2, subsequently leading to increased production of TSLP, hence the increased number of ILC2s; this resulted in increased airway inflammation and persistence

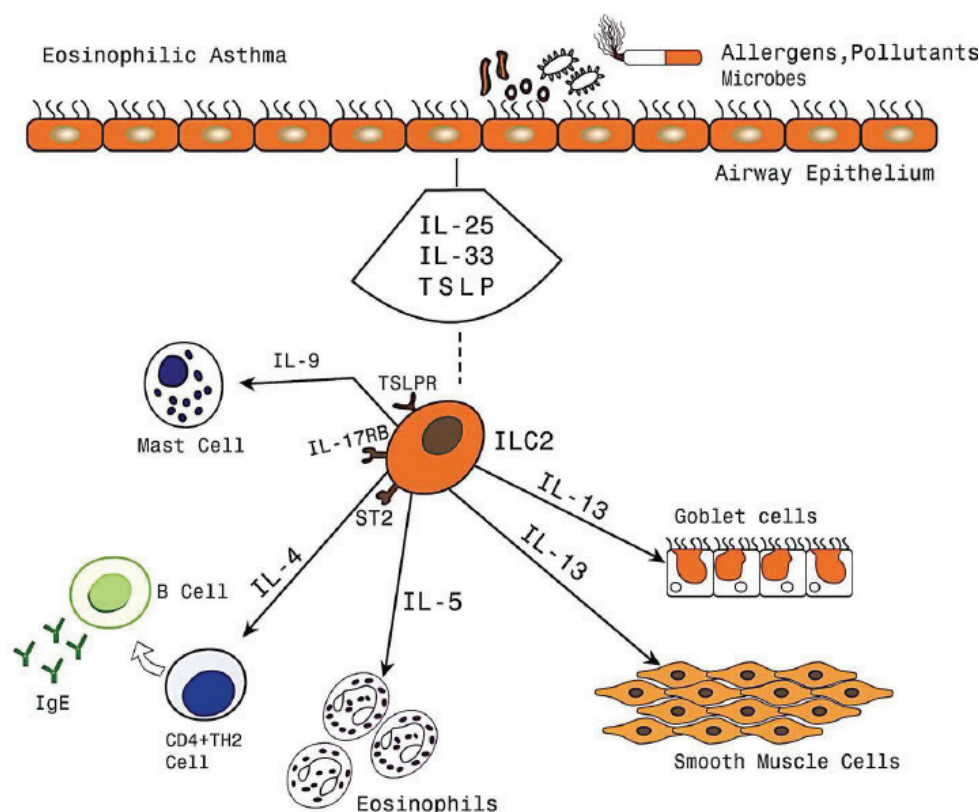


Figure 2 Damage to the epithelium induced the release of epithelial-derived cytokines (IL-25, IL-33, and TSLP) which activate ILC2s via their receptors (IL-17RB, ST2, and TSLPR, respectively). Activated ILC2s produce significant amount of type 2 cytokines (IL-4, IL-5, and IL-13), resulting in airway eosinophilia, mucus hypersecretion, and airway hyperreactivity. TSLP: thymic stromal lymphopoietin.

of asthma symptoms. Human ILC2s co-culture with bronchial epithelial cells, ILC2s (via IL13) impaired the epithelial barrier by disrupting epithelial tight junction (TJ), rendering barrier leakiness, hence the asthma pathogenesis.⁹⁰ Smith et al.,¹⁷ in their research, analyzed blood and sputum ILC2s and intracellular IL-5 and IL-13 in patients with severe asthma, since ILC2s are the predominant source of IL-5 and IL-13. Sputum IL-5(+) IL-13(+) ILC2s are significantly higher in patients with severe asthma compared to mild asthma and the control group, resulting in persistent airway eosinophilia, with sputum eosinophils >3%, despite high-dose eosinophilia. Similarly, in children with severe asthma, the number of ILC2s was more abundant in bronchial mucosa and broncho alveolar lavage (BAL) compared to non-asthmatic children, and ILC2s were correlated with bronchial mucosa eosinophilia and persistent symptoms.⁹¹ Moreover, Jia et al.⁹² reported a significant increase in ILC2s in asthma subjects relative to healthy groups and a significantly higher percentage of IL-13+ILC2s in circulating blood of patients in the uncontrolled group compared to a well-controlled group and stable control subjects. They further noted that IL-13+ILC2s isolated from peripheral blood mononuclear cells (PBMCs) were more resistant to glucocorticoid than Th2 cells in human asthma. Additionally, Lee et al.⁹³ investigated 28 asthmatics (12 non-severe and 16 severe) with chronic rhinosinusitis. They found that severe asthmatics had a higher expression of ILC2 cell counts,

TSLP, IL-25, IL-33, and Th2-driven cytokines (IL-4, IL-5, IL-9, and IL-13) in their nasal tissue.

ILC2 mediates airway remodeling in asthma

Airway remodeling is an alteration in the size, mass, or number of tissue structural components that occurs in response to injury and inflammation, resulting from repeated tissue injury.⁹⁴ ILC2 is vital for maintaining tissue repair and contributes to chronic pathology such as airway fibrosis resulting in scars of lung tissue and airway narrowing.^{95,96} ILC2 activation promotes tissue remodeling induced through the expansion of ILC2 effector cytokine influence as airways vascularization, airways fibrosis, and airways smooth muscle hypertrophy.⁹⁷ Remarkably, ILC2-derived cytokines (IL-5, IL-9, and IL13) in a murine model have shown to contribute to airway remodeling in asthma. ILC2-derived IL-13 along with IL-9 promotes subepithelial fibrosis, AHR, smooth muscle increase, and epithelial mucus production. ILC2-derived IL-5 brings about the proliferation of eosinophils that express the pro-fibrotic growth factor TGF β .⁹⁸ TGF β (+) eosinophils activate the Smad-2/3 signaling pathway which contributes to airway remodeling observed in increased levels of collagen deposition and increased numbers of angiogenic blood vessels. A study done in human asthma reported that a single dose of antiIL-5 significantly reduced

the levels of TGF β (+) eosinophils, resulting in low levels of remodeling observed in reduced deposition of extracellular matrix-associated remodeling proteins procollagen and tenascin.⁹⁹ Additionally, the nILC2 cell population produces significant amounts of amphiregulin,⁴¹ a molecule of epidermal growth factor family linked to regulating tissue remodeling and repair during acute epithelial injury and asthma.¹⁰⁰

In the airway remodeling setting, ILC2 produced amphiregulin upon IL-33 stimulation. Amphiregulin expression was elevated in the lung following exposure to the H1N1 subtype of influenza A virus, which resulted in significant damage to the respiratory epithelium. In addition to tissue remodeling, ILC2 promotes a favorable role in tissue repair response in the lung following acute epithelial damage. Treatment with recombinant amphiregulin, but not IL-13, effectively restored lung function and epithelial repair in ILC-depleted influenza virus-infected mice, proposing that ILC2-derived amphiregulin is crucial in promoting airway epithelial repair.¹⁰¹ Also, ILC2-derived IL-9 promotes tissue repair, whereby IL-9 improved emphysema-like tissue damage after chronic helminth-induced lung injury.⁵⁴ Furthermore, CD25+CD90+ST2+ILC2 cells accumulated in the lung after H1N1 infection and depletion of ILC2s in influenza virus-infected Rag1KO hosts using an anti-CD90 monoclonal antibody treatment resulted in critically declined lung function, compromised lung epithelial barrier integrity, and increased host mortality, indicating that lung ILC2s are key modulators of lung tissue remodeling and epithelial repair.¹⁰¹ Meanwhile, just as the role of conventional CD4+T cells in the airway remodeling has remained unclear,¹⁰² so does the role of ILC2.⁹⁸ This requires further investigation to provide us with a clear understanding of the immune and inflammatory cell pathways involved in the progression of AHR, and probably provide key insight into biomarkers or genotypes to indicate the severity of AHR, as well as to serve as a therapeutic target to prevent or reverse airways' pathological changes in asthma.⁹⁹

Conclusion

ILC2s are in the spotlight in asthma pathogenesis, as ILC2s have been on the radar of many scientific studies in recent years. ILC2s studies have shown more evidence on the potential therapeutic approach in the management of type 2 airway diseases such as asthma. Moreover, full and vivid understanding that surrounds the ILC2 inflammation process will aid in therapeutic advancement, as some have already been developed and are quite useful in the treatment of asthma, while others are still working and progress is far from proven. Additionally, ILC2s in asthma could aid asthma phenotyping to establish an accurate diagnosis and provide appropriate treatment strategy. Lastly, the relationship between ILC2 and its counterpart (Th2 cells) in asthma is an intriguing area that requires further investigations.

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