



New Kids on the Block

Group 2 Innate Lymphoid Cells and Type 2 Inflammation in the Lung

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Inflammatory diseases of the lung are a major cause of morbidity and mortality. Allergic lung inflammation often stems from the overproduction of type 2 cytokines. The resulting type 2 inflammation is frequently caused by an inappropriate immune response to relatively harmless allergens and often associates with asthma. Until recently, the primary contributors of type 2 cytokines were believed to be T helper (Th) 2 cells. This concept was challenged by the discovery of group 2 innate lymphoid cells (ILC2s) in the lung, which represent a major source of type 2 cytokines during the acute inflammatory phase. Recent advances in our understanding of the regulation and development of ILC2 have redrawn the roadmap of type 2 inflammation. Indeed, ILC2s appear to be critical for the induction of adaptive immunity and, thus, play a central role for immune regulation. As one of the first responders in the entire Th2 cascade, ILC2 might serve as the early tile in a Th2 domino effect. As such, ILC2s present an attractive target for future drug development.

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Abbreviations: ILC2 = group 2 innate lymphoid cell; ROR α = retinoic-acid-receptor orphan receptor α ; Th = T helper; TSLP = thymic stromal lymphopoietin

Inflammation of the airways is a complex process and can be caused by a wide variety of stimuli.¹ The inflammatory response can be broadly divided into several types of immune reactions based on the cytokines produced and the profile of effector cells involved.² Each type of response is geared toward eliminating a distinct class of pathogen, although dysregulation of the immune system may instigate unwanted reactions to self-antigens or relatively harmless allergens. The allergic immune response often assumes a type 2 profile, perpetuated by the production of type 2 cytokines such as IL-4, IL-5, IL-9, and IL-13 (Table 1) and elevated antigen-specific IgE titers.³ Although the type 2 response is most frequently encountered in atopic

asthma and allergic airway inflammation, other immune responses that involve cytokines such as IL-17A, IL-17F, and interferon- γ also constitute major subclasses of lung disease.^{1,2} The main cellular source of type 2 cytokines was previously believed to be CD4⁺ T helper (Th) 2 cells, which are activated by allergen-derived antigens. This paradigm was challenged by the observation that T-cell-deficient RAG-deficient mice can still mount an innate type 2 response.^{4,5} Several laboratories independently confirmed this finding with the discovery of a new class of innate type 2 cytokine-producing lymphocyte, initially called nuocytes,⁶ natural helper cells,⁷ and Ih2.⁸ A consortium of experts later determined that these cells belong to the same lineage and proposed the unified name of group 2 innate lymphoid cell (ILC2).⁹ ILC2s were initially discovered in the gut, where they proved essential for mounting the innate type 2 response to cytokine stimulation and, more importantly, to helminth infection.^{6–8} Indeed, ILC2-deficient *Rag2*^{−/−}*Il2rg*^{−/−} or *Il17rb*^{−/−} mice, which have functionally impaired ILC2 responses, indicating the important role for ILC2 in clearing parasite infections.^{6,8} The presence of ILC2 in the lung was initially noted⁸ or hypothesized and subsequently

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Table 1—Type 2 Cytokines

Cytokine	Produced by	Function
IL-4	CD4 ⁺ T cells Basophils	Th2 differentiation B-cell class switching
IL-5	CD4 ⁺ T cells ILC2	Eosinophilia
IL-9	CD4 ⁺ T cells Innate lymphocytes	B-cell class switching Mast cell recruitment Mucus production
IL-13	CD4 ⁺ T cells ILC2	Mucus production Airway remodeling Smooth muscle contraction Airway hyperresponsiveness

Type 2 cytokines are produced mainly by CD4⁺ Th cells, which can be further subdivided into functionally distinct populations.² ILC2s comprise another major source of type 2 cytokines. Various studies also implicate other myeloid or lymphoid lineages as potential type 2 cytokine-producing cells (eg, basophil-derived IL-4), although no clear consensus has been established about their role in type 2 inflammation of the airways. Production of type 2 cytokines in the airways has pleiotropic effects but, in most cases, result in the influx of inflammatory cells, mucus hyperproduction, and airway remodeling. ILC2 = group 2 innate lymphoid cell; Th = T helper.

confirmed by studies that characterized lung ILC2 in inflamed and naive wild-type mice¹⁰⁻¹³ and in human lung tissue.¹⁴ Lung ILC2s respond to viral infection^{10,11} or allergen-induced tissue damage mediated by the ILC2-activating cytokines IL-33 plus IL-2, IL-7, IL-25, and thymic stromal lymphopoietin (TSLP).^{13,14} Lung ILC2s are a potent source of IL-5 and IL-13 but do not produce IL-4.¹³ Additionally, lung ILC2s play a role in tissue repair through the production of amphiregulin.¹¹ IL-9-producing ILC have also been identified in the lung.¹⁵ Activation of ILC2 occurs within hours after exposure to allergens, followed by eosinophilia and IL-13-mediated mucus hyperproduction, independent of the adaptive immune system.¹³ Thus, our understanding of the type 2 response has now expanded to include ILC2s as critical mediators of acute type 2 lung inflammation. In this article, we discuss the current understanding of ILC2 regulation and interaction with the immune system as a whole, their role in perpetuating airway inflammation and disease, and their potential as a future target for drug development.

ILC2 PHENOTYPE

Characterization of ILC2 in mice and man has yet to yield a single lineage-restricted marker for identification by flow cytometry or in tissue sections. Instead, a panel of markers must be used to exclude other known CD45⁺ leukocytes. Among these lineage-negative cells, ILC2s are known to express a number of cell surface molecules by which they may be identified (Table 2). Different groups have established comparable strategies to detect ILC2 in mouse tissues.¹⁶ It should be

noted that the expression of cell surface markers on ILC2 may differ depending on which tissue is analyzed and possibly on the mouse strain being investigated. The functional capabilities of ILC2 (proliferic production of IL-5 and IL-13) also are often used to detect and confirm the ILC2 population. Reporter mice, intracellular cytokine detection, and *in vitro* culture have been used successfully to study ILC2.

Recently, the transcription factors retinoic-acid-receptor orphan receptor α (ROR α)^{17,18} and GATA3^{19,20} were discovered to be critical for ILC2 development and function. GATA3 is essential for the transcription of type 2 genes²¹ and is required for the function and development of Th2 cells. GATA3 is also essential for earlier stages of T-cell development.²² Because ROR α is ILC2 lineage restricted among other innate and most adaptive leukocytes, it may serve as a future target for generating ILC2-reporter animals, although expression has been reported in some Th17 cells.²³

In humans, ILC2s have been identified in fetal lung and nasal polyps.¹⁴ Phenotypically, human ILC2s express similar markers (Table 2) and produce IL-5 and IL-13 in response to IL-2 plus IL-25 or IL-33 stimulation. Identification of ILC2 in healthy or inflamed adult human lung tissue by flow cytometry is challenging because they represent a relatively small population, but analysis of human patients with atopic dermatitis suggested that ILC2s are associated with disease.²⁴ Tissue explant cultures or humanized immune system mice may eventually assist us in understanding the exact phenotype and biology of human lung ILC2.

ILC2 REGULATION AND ROLE IN LUNG DISEASE

Unlike Th2 cells of the adaptive immune system, ILC2s do not express antigen-specific receptors. Instead, ILC2 function is regulated by the cytokine environment and possibly through cell-cell interactions.¹⁶ To date, we know that IL-33 plays a central role for ILC2 activation. IL-33 is a damage-induced cytokine released in active form from necrotic epithelial cells.²⁵ Constitutively produced IL-33 is sequestered in the nuclei of mouse lung epithelial cells²⁶ and type 2 pneumocytes²⁷ where, besides its role as an alarmin, it might have a regulatory function.²⁸ *Il33* expression is induced in type 2 pneumocytes upon exposure to a variety of allergens.²⁷ *In vitro* stimulation of lung ILC2 requires IL-33 signaling plus costimulation in the form of IL-2, IL-7, or TSLP.¹³ Skin ILC2s, on the other hand, have been reported to be activated mainly by TSLP and not IL-33 or IL-25 in mouse models,²⁴ although these results require confirmation.

TSLP is structurally related to IL-7 and signals through the TSLPR and IL-7R α heterodimer.²⁹ Produced by airway epithelial cells in response to PAR2 (protease-activated receptor 2), TSLP has been shown to act on

Table 2—ILC2 Identification in Man and Mouse

ILC2	Cell Surface Markers	Intracellular Protein	Gene Expression
Mouse	Lineage-, CD25, CD69, CD90, CD127, T1/ST2 (IL-33R), ICOS, KLRG1, Sca-1, IL-17BR (IL-25R), CD117 (c-Kit)	IL-5 IL-13	<i>Rora</i> , <i>Gata3</i> <i>Il4 reporter</i> <i>Il5 reporter</i> <i>Il9 reporter</i> <i>Il13 reporter</i>
Human	Lineage-, CD127, T1/ST2, CD25, CD161, CTRH2	IL-5 IL-13	<i>GATA3</i>

ILC2 detection is currently contingent on the use of fluorescent protein reporter mice or flow cytometry-assisted phenotypic analysis. Different laboratories have used combinations of cell surface markers to identify a highly pure population of ILC2 in mouse and man. Intracellular staining for hallmark ILC2-produced proteins (eg, type 2 cytokines) can also be used to identify ILC2. Finally, gene reporter mice have been useful in identifying and tracing ILC2. It should be noted that not all markers are similarly expressed on ILC2 in different tissues or in different mouse strains. See Table 1 legend for expansion of abbreviation.

multiple cell types, including dendritic cells and ILC2.³⁰ The protease allergen papain induces ILC2 activation in vivo and in lung explant cultures through TSLP and IL-33 signaling.¹³ Furthermore, TSLP is a potent coactivator of purified lung ILC2 in vitro¹³ and induces asthma-like disease in mice when administered intranasally as an adjuvant with ovalbumin antigen.³¹

IL-25 (IL-17E) is also known to induce lung ILC2 activation, although its exact mode of action is not well defined. IL-25 is produced by airway epithelial cells and binds to the IL-17RA and IL-17RB heterodimer, which is expressed on a variety of nonimmune and immune cells, including ILC2.^{6,32} Intranasal administration of IL-25 into RAG-deficient mice promotes the innate type 2 inflammatory response mediated by ILC2.⁵ Interestingly, IL-25 plus IL-33 does not directly activate lung ILC2 in vitro; instead, IL-25 seems to amplify the response of activated ILC2.¹³ Nevertheless, ILC2s express cell surface IL-25 receptors.⁶ Furthermore, IL-25 has an effect on the survival of lung ILC2 but not on bone marrow ILC2 precursors.¹⁷ The redundant roles of IL-33 and IL-25 were illustrated by a model of *Alternaria alternata* extract challenge.²⁷ A *alternata* administration induced ILC2 expansion in wild-type mice, and this was delayed but not prevented in the absence of IL-33. Only in the absence of both IL-33 and IL-25 signaling pathways was A *alternata*-induced ILC2 expansion ablated. Thus, IL-25 also plays a role in the expansion of ILC2 upon induction of lung inflammation.

Although not typically associated with the type 2 response, viral lung infections also involve ILC2. Mouse models of influenza infection show activation of ILC2 mainly through the release of IL-33 by epithelial cells and macrophages.^{10,11} These results suggest that ILC2s play a role in viral exacerbation of lung disease, a phenomenon commonly observed in patients with asthma.¹⁰ Interestingly, ILC2s also play a role in tissue repair through their expression of factors such as amphireg-

ulin. Dysregulation of this function may lead to lung fibrosis.¹¹

Following the discovery of ILC2s and their implication in murine models of type 2 lung inflammation, a possible role for ILC2 in human patients with rhinosinusitis has been suggested by their observed expansion.²⁰ These early results may substantiate previous asthma studies that identified genes directly associated with ILC2, including those expressed by ILC2 and responsible for driving their growth and development, such as *RORA*, *ILIRL1* (IL-33R), and those that act directly on ILC2 (*IL33* and *TSLP*).^{33,34}

In addition to susceptibility genes, elevated levels of type 2 cytokines are consistently detected in the sputum and serum of patients with atopic asthma,^{35,36} rhinosinusitis,³⁷ and allergic inflammation.³⁸ We now understand that besides Th2 cells, ILC2s may be important cellular mediators of inflammation. The division of labor between Th2 and ILC2 in different disease states remains to be determined, although mounting evidence in mouse models suggests a significant role of ILC2 even after induction of an allergen-specific adaptive type 2 response. In addition to type 2 cytokines, numerous studies have identified increased expression of alarmins in asthma. TSLP, IL-33, and IL-25 transcript and protein levels are elevated in patients with asthma.³⁹ Recent studies have established a clear link between these epithelial-derived cytokines and induction of ILC2 and type 2 inflammation.¹⁶ Importantly, the coinvolvement of ILC2 in type 2 inflammatory disease may explain the treatment failure of drugs that specifically target T cells.

ILC2 DEVELOPMENT

ILC2s are known to depend on ROR α for development and to heavily depend on GATA3 for development and function.¹⁷⁻²⁰ ILC2-committed precursors have been identified in the bone marrow of mice.^{17,19}

The preceding developmental stages for ILC2 are currently still undefined, although it is known that both the common lymphoid progenitor and lymphoid-primed multipotent progenitor have *in vivo* ILC2 potential^{17,18,40} and that Id2-regulated lineage restriction inhibits the differentiation of ILC precursors toward the B- and T-cell lineages.^{7,19,41} Rapid expansion of ILC2 after *in vivo* stimulation may be supported by infiltration of bone marrow-derived ILC2 or *in situ* expansion of resident cells. Chemokine and cytokine receptor analysis of ILC2 precursors and lung ILC2 show distinct differences,¹⁷ although further work is required to understand their development and migration in homeostasis or disease.

ILC2 INTERACTIONS

As the first source of type 2 cytokines, ILC2s might be instrumental in initiating a Th2 adaptive response by creating a type 2 milieu. Lung ILC2s are important for mediating the effector phase of acute type 2 inflammation. IL-5 promotes eosinophilia, and IL-13 has pleiotropic effects, including goblet cell hyperplasia, mucus secretion, and muscle contraction.^{42,43} In addition to secreting effector type 2 cytokines, which cause acute inflammation, ILC2s are hypothesized to have an effect on the adaptive immune response.^{3,16,44} A possible role in mesenteric B-1-cell regulation is supported by ILC2 and B-1-cell coculture experiments.⁷

Lung ILC2s are present in naive mice, where they appear to be poised to respond rapidly to activating signals. Expansion of ILC2 after *in vivo* challenges may depend on IL-33, IL-25, and other unknown factors. Indeed, the expression of ICOS and other cell surface molecules likely influence the function of ILC2 in their microenvironment through cell-cell interactions. Activated ILC2s in Rag2-deficient mice do not expand and are not maintained throughout helminth infection, thus providing evidence of cross-talk with the adaptive immune system.⁶ This may be due to IL-2, possibly derived from T cells, which is required to activate lung ILC2 through CD25 (IL-2R α) signaling.⁷

It is clear that ILC2s constitute a potent source of innate cell-derived cytokines that can, independently of the adaptive response, induce many of the pathologic features of the type 2 response. However, ILC2s may also provide important signals for the initiation of Th2-cell activation.⁶ The precise nature of these signals is not clear, but it is intriguing that ILC2s express major histocompatibility complex II and may, therefore, have the capacity for antigen presentation. Similarly, ILC2s produce factors that can modulate dendritic cell function, and these may then skew the response to a Th2 phenotype through antigen presentation to T cells.

Type 2 inflammation is the underlying phenomenon in a variety of airway diseases such as atopic inflammation, rhinosinusitis, and allergic asthma. Standard therapy for inflammation is largely restricted to antiinflammatory corticosteroid therapy and β_2 -adrenoceptor agonists.⁴⁵ Although effective for most patients, this method treats the symptoms but fails to cure the disease. Asthma in particular remains a challenge to manage for many patients largely because of its heterogeneous and genetically complex nature.¹ Targeted therapies aimed at inhibiting the type 2 response by inhibiting type 2 cytokines and their receptors initially failed to achieve their promise.^{46,47} However, recent progress has demonstrated that when used to treat carefully stratified patient cohorts, both anti-IL-5 and anti-IL-13 have beneficial therapeutic applications.^{48,49} In the early 2000s, studies that identified epithelial cytokines important for type 2 inflammation (IL-25, TSLP, and IL-33) renewed the drive for novel therapies, which focused on what were believed to be direct upstream targets of Th2 activation. We now understand that ILC2s are intermediaries in the type 2 cascade and that many of these proposed therapeutics will directly modulate the biology of these cells. Thus, as our understanding of the initiation and progression of type 2 inflammation grows more refined, we become better equipped to strategically target specific aspects of type 2 lung disease.

Along these lines, novel strategies that target epithelial-derived alarmins are currently being evaluated. Neutralization of IL-25 has beneficial effects on type 2 lung inflammation in mice.^{50,51} Targeting IL-33 in mouse studies also shows promise as a possible new therapeutic strategy because blocking IL-33 signaling by antibody targeting results in attenuation of type 2 lung inflammation.⁵² Likewise, the targeting of TSLP may yield beneficial effects in type 2 inflammation through the attenuation of T-cell activation.⁵³

As we learn more about the development of ILC2s, we may be able to modulate their function. Already we know that the lineage-restricted nuclear receptor ROR α is critical for the development of ILC2 from a bone marrow precursor. Small molecule inhibitors may prove beneficial in halting their development or expansion.

The benefit of modulating ILC2 function as a therapeutic for type 2 lung inflammation and asthma is of great interest. However, one important question is whether ILC2s are required throughout the type 2 inflammatory response or simply pave the way for the adaptive immune system. If the latter is true, then we will likely fail to treat many type 2 inflammatory diseases by targeting ILC2, which would be akin to closing the stable door after the horse has bolted. Hence,

understanding their role in all aspects of disease progression is essential.

CONCLUSION

Type 2 inflammation of the airways is a major cause of morbidity and remains without effective cure despite decades of focused research. Reasons for this are the multifactorial origin and mechanism of the disease and, as we have described in this review, an incomplete understanding of the key players and pathways involved in mediating inflammation from the first encounter of immunogen to the hallmark physiologic features of type 2 inflammation. The discovery of ILC2s in the lung marks an important milestone given their critical role in the innate type 2 response and acute inflammation and likely role in regulating the adaptive immune system. ILC2s appear to be an important missing piece of the type 2 puzzle. We are now able to link the lung epithelial barrier, which first encounters the pathogen (or allergen), to the draining lymph node where naïve T cells are sensitized and primed to become Th2 cells. It is imperative that ILC2s are entered into the lexicon of both clinicians and scientists and that all past and future studies are evaluated with their function in mind.

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