

The role of type 2 innate lymphoid cells in asthma

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ABSTRACT

Asthma is a complex and heterogeneous disease with several phenotypes, including an allergic asthma phenotype, characterized by Th2 cytokine production and associated with allergen sensitization and adaptive immunity. Asthma also includes nonallergic asthma phenotypes that require innate rather than adaptive immunity. These innate pathways to asthma involve macrophages, neutrophils, as well as ILCs, newly described cell types that produce a variety of cytokines, including IL-5 and IL-13. We review the recent data regarding ILCs and their role in asthma. *J. Leukoc. Biol.* **94**: 933–940; 2013.

HETEROGENEITY OF ASTHMA

Asthma has been considered for several decades to be an immunological disease mediated by Th2 cells and adaptive immunity [1]. Many investigators have documented the important role of allergen-specific Th2 cells, eosinophils, and mast cells in asthma, particularly in mediating the inflammation in allergic asthma, the most common form of asthma. Th2 cells have been shown to have an important role in regulating type 2 immune responses, as they produce IL-4, IL-5, and IL-13. IL-4 is a switch factor for IgE, IL-5 is a growth and differentiation factor for eosinophils, and IL-13 can directly cause AHR, a cardinal feature of asthma, by directly affecting airway epithelial cells and airway smooth muscle cells. Importantly, the inflammation in asthma has been treated for more than 40 years with corticosteroids, in particular, as Th2 cells and eosinophils are exquisitely sensitive to corticosteroids.

However, although Th2 cells, eosinophils, and mast cells can explain many of the features of asthma, over the past 5 years,

it has become very clear that asthma is much more than Th2 cells and eosinophils and that asthma is, in fact, a very heterogeneous and complex trait, with at least several distinct phenotypes [2]. For example, there is an allergic asthma phenotype, with asthma triggered by many different allergens, and perhaps associated with eosinophils in the airways and with adaptive immunity. There is also a nonallergic asthma phenotype, associated with exposure to air pollution, including ozone, cigarette smoke, diesel particles, and with infection [2], and perhaps associated with neutrophils in the airways, with innate immunity, and resistance to corticosteroid therapy. There may also be an intrinsic form of asthma and possibly other forms of asthma as well.

This heterogeneity in asthma suggests that other factors and components of immunity, in addition to Th2 cells, must be involved in regulating and shaping the inflammation in asthma [2]. Among these non-Th2 cells that regulate asthma are ILLs, including NKT cells [3], and a cell type that has been described recently, called ILCs [4].

ILLs

ILLs have rearranged antigen-specific receptors with limited diversity and tissue distribution. Members of this ILL family include iNKTs [5, 6], as well as $\gamma\delta$ T cells [7, 8], marginal zone B cells [9, 10], B1-B cells [11, 12], and mucosal-associated invariant T cells [13, 14]. The antigen receptors on these cells with limited diversity function as PRRs, responding to multiple PAMPs and other antigens. Expression of these PRRs allows these cells to respond rapidly and provide innate-like responses against foreign antigens and pathogens. In particular, iNKT cells have been shown to play an important role in asthma, by enhancing the function of allergen-specific Th2 cells [15] or by responding in the lungs after exposure to ozone [16], a major component of air pollution, or after respiratory infection with Sendai virus [17]. In these instances, iNKT cells appear to become activated by endogenous glycolipids or damage-associated molecular patterns, including

Abbreviations: $^{-/-}$ =deficient, AHR=airway hyper-reactivity, CRTH2=chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes, Id2=inhibitor of DNA binding 2, ILC=innate lymphoid cell, ILL=innate-like lymphocyte, iNKT=invariant NK T, Lin=lineage, LTI=lymphoid tissue inducer, MLN=mesenteric LN, NHC=natural helper cell, NKT=NK T, ROR=retinoic acid receptor-related orphan receptor, Sca-1=stem cell antigen 1, Thy1=thymocyte antigen 1, TSLP=thymic stromal lymphopoietin

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phosphatidylserine [18], but iNKT cells have also been shown to respond to glycolipids from environmental allergens, including house dust, cypress tree pollen, and certain food antigens [19–22]. These results together suggest that immunity mediated by ILLs, including iNKT cells, plays an important role in mucosal surfaces of the lung and gut.

ILC family

The second family of non-Th2 cells that regulate immunity in the lungs is called ILCs. ILCs represent an emerging family of non-T and -B effector cells that have crucial roles in tissue remodeling, repair, and innate immunity to pathogenic and nonpathogenic microorganisms [3]. Unlike adaptive immune cells, ILCs lack rearranged antigen-specific receptors but react promptly to a wide range of innate signals. NK cells, which produce large quantities of IFN- γ , express T-bet, or have potent cytotoxic activity, are the prototypical ILC and are called ILC1 or type 1 ILCs [23, 24]. In addition, in the past 3–5 years, several additional ILCs have been described, including LT α i cells, required for lymphoid organogenesis and which produce large amounts of IL-17 and IL-22 [25]; ILC2s (also called NHCs, nuocytes, or type 2 ILCs) [4], producing large quantities of IL-13, IL-5, and IL-9 and involved in the gut for rapid expulsion of helminth; ILC22s (NK22), which produce IL-22, active in the skin, lungs, and gut; and ILC17s, which produce IL-17 and are involved in colitis [26]. LT α i cells, ILC17s, and ILC22s depend on the ROR γ t transcription factor and are now being referred to as type 3 ILCs [27, 28]. Together, ILCs appear to define a universe of cells that is parallel to the universe of CD4-adaptive T cells that includes Th1 cells (comparable with ILC1s/NK cells), Th2 cells (comparable with ILC2s), Th17 cells (comparable with ILC17s), and Th22 cells (comparable with ILC22s). Like CD4 helper cells, which arise from a common precursor cell, ILCs are also thought to arise from a common precursor cell, which is characterized by the expression of the transcription factor Id2 [29–31]. The existence of these parallel universes of adaptive and innate cells, both associated with the production of a wide variety of cytokines, blurs the lines between innate and adaptive immunity.

Among the ILCs, ILC2s have been shown to mediate eosinophilia and goblet-cell hyperplasia at mucosal surfaces, both of which are critical for antihelminth reactions and for allergic disease. Recently, ILC2s have been shown to exist in the lungs and to have a role in the pathophysiology of asthma and allergic inflammation. In the rest of this review, we will discuss how ILC2s are involved in the development of asthma and lung inflammation.

Type 2 ILC2

Several groups have reported the existence of non-T and -B ILCs secreting type 2 cytokines but with slightly different cell-surface markers and function and have called these nuocytes [32, 33], NHCs [34], multipotent progenitor cell [35], or innate helper 2 cells [36]. As all of these cell types secrete large quantities of IL-5 and IL-13, these cells are thought to be variants of a cell type now called ILC2s or type 2 ILCs [4]. In Supplemental Table 1, we summarize the biology of these differ-

ent variants of ILC2s found in different studies in mouse and human systems [36–42]. In general, mouse ILC2s express CD25 (IL-2R α), CD90 (Thy1), variable amounts of CD117 (c-Kit), and CD127 (IL-7R α), depending on the organ, mouse strain, and activation state in which the ILC2s are found. Additionally, ILC2s express variable amounts of CD278 (ICOS), ST2 (IL-33R), and IL-17BR and are IL-25 (IL-17E)- and IL-33-responsive, such that these cells proliferate and expand in response to IL-25 and IL-33 and in response to pathogens, such as helminths, viruses, and fungi [33, 36]. Importantly, they all appear to function by secreting large quantities of cytokines, such as IL-5, IL-13, and IL-9, that are part of the type 2 immunological response underlying asthma and helminth–host defense. ILC2s appear to require IL-7 and IL-33 for their development and the transcription factors Id2, ROR α , and GATA3, as well as Notch signaling [29, 30, 38].

ILC2s play a central role in type 2-like immunity and have been shown by several groups to exist in the lungs and mediastinal LNs of mice during the intestinal helminth infection [43–45]. The role of ILC2s in the lung disease, particularly in the lung inflammation and development of asthma, is discussed in the next sections. In the skin, Roediger et al. [46] demonstrated recently that a phenotypically unique ILC2 subset depended on IL-7 and constitutively produced IL-13 in normal mouse dermis, which they called “dermal ILC2”. This dermal ILC subset interacted specifically with mast cells and when activated with IL-2, led to dermatitis.

ILC2 IN ALLERGIC AIRWAY DISEASE

Allergen-induced innate IL-13 producing ILC2 in lungs

In a mouse model, in which mice were sensitized to OVA and challenged through the airways to induce allergic inflammation, Kearley et al. [47] showed persisting AHR correlated with the continued presence of Th2 cells but not eosinophils in the lungs. In their study, they found that the ST2-IL-33 pathway was important, as treatment with blocking antibodies against ST2 reduced IL-4, IL-13 secretion, and allergic inflammation and AHR. Although they concluded that Th2 cells were important in maintaining AHR and depended on the ST2-IL-33 pathway, it is possible that in addition to adaptive CD4⁺ Th2 cells, innate ILC2s, which are also reliant on the ST2-IL-33 pathway, might participate in type 2 allergic airway inflammation.

This idea is supported by studies of Barlow et al. [41], using IL4⁺/cGFP/IL13⁺/Tomato dual reporter mice, which showed that OVA-induced nuocytes (ILC2) provide the major innate source of IL-13 in the lung. They also found that CD4⁺ T cells infiltrating the lung almost exclusively express only IL-13 but not IL-4 and that IL-4-producing T cells were restricted to the draining LNs. Thus, they concluded that T cells and nuocytes in the allergic lung are highly biased toward IL-13 production. Furthermore, they showed that intranasally administered rIL-25 or rIL-33 induced the expansion of an ILC2 population recovered from the lungs, BAL, and mediastinal LNs [41]. Intranasal IL-33 or IL-25 was able to drive airway eosinophilia and the production of lung IL-5 and IL-13 in WT and

Rag1^{-/-} mice, as was reported by several groups [38, 48, 49]. Transfers of WT ILC2s into IL-13^{-/-} mice showed that they were sufficient to restore IL-25-induced or IL-33-induced AHR [41]. Additionally, ILC2s infiltrate into the lungs in the challenge phase of an OVA-alum model and were found to be a substantial population within lung IL-13⁺ cells [41]. In another study, Wolterink et al. [50] showed that ILC2s and Th2 cells produce large amounts of IL-5 and IL-13 that contribute to the house dust mite- or OVA-induced allergic asthma. However, whether ILC2s are required for the development of allergen-induced AHR is not fully established.

Protease allergen-induced airway inflammation

As many strong allergens have protease activity, Halim et al. [51] examined the administration of papain to mice and showed that ILC2s were required for eosinophilia and mucus secretion via their production of IL-5 and IL-13 during allergic airway disease induced by papain or house dust mite. They identified Lin⁻Sca-1⁺c-kit^{+/lo}CD25⁺CD127⁺ cells in the lungs of naive B6 mice that rapidly produce large amounts of Th2 cell-type cytokines and are critical for induction of eosinophilic lung inflammation by protease allergens. They proposed that protease activity damaged airway epithelial cells that released IL-33, which in turn, activated ILC2s. As ILC2s are present in the Rag1^{-/-} mice but not in the Rag2^{-/-}Il2rg^{-/-} mice, they showed that intranasal administration of papain to Rag1^{-/-} mice but not Rag2^{-/-}Il2rg^{-/-} mice rapidly caused lung eosinophilia, mucus hypersecretion, and elevation in BAL IL-5 and IL-13 levels. Furthermore, depletion of ILC2s in Rag1^{-/-} mice by CD25 mAb injection significantly reduced lung eosinophilia and mucus secretion upon papain administration, and adoptive transplant of ILC2s into Rag2^{-/-}Il2rg^{-/-} mice reconstituted these symptoms. Indeed, ILC2s are the main source of IL-5 and IL-13 in Rag1^{-/-} and WT lung-explant cultures treated with papain. They suggested that ILC2s were a critical, early source of IL-5 and IL-13 in protease allergen-induced lung inflammation and that this occurred in a T cell-independent manner.

In another study, Halim et al. [38] reported that ILC2s express ROR α but not ROR γ t. Thus, ROR α ^{-/-} but not ROR γ t^{-/-} mice lacked ILC2s in all tissues, whereas all other lymphocytes, including ROR γ t⁺ ILCs, were unaffected. They showed that the ILC2^{-/-} chimeric mice, generated by transplantation of ROR α bone marrow into irradiated recipients, had normal Th2 cell responses but failed to develop acute lung inflammation in response to protease allergen, thus confirming the essential role of ILC2 in allergic lung inflammation. They also identified ROR α -dependent ILC2 progenitors in the bone marrow. Thus, they concluded that all ILC2s belong to a unique ROR α -dependent cell lineage separate from other lymphoid cell lineages. Their finding with ROR α ^{-/-} mice confirmed a report by Wong et al. [31] that showed that ROR α is critical for ILC2 development. In Wong's study [31], ILC2s identified in MLNs as Lin⁻ICOS⁺ST2⁺ cells that produced IL-5 and IL-13 upon stimulation and were therefore likely identical to MLN ILC2s observed in Halim's study [38].

Wilhelm et al. [52] had found earlier in a papain-induced model the presence of IL-9-producing ILC2s. Indeed, IL-2 and

IL-33 (but not IL-25) were required to induce IL-9 by ILC2 in this model, whereas blockade of IL-9, using neutralizing antibodies, resulted in reduced expression of IL-5 and IL-13, suggesting that ILC2s may produce IL-9 initially and then mature to produce IL-5 and IL-13.

Fungal allergen (*Alternaria alternata*)-induced innate type 2 lymphoid cells in lungs

In addition to house dust-mite allergen, a recent reported by Bartemes et al. [39] has shown that inhalation of the clinically relevant fungal allergen *A. alternata* was able to induce IL-33, the expansion of lung Lin⁻CD25⁺CD44^{hi} ILC2, the subsequent production of IL-5 and IL-13, and eosinophilia, a chain of events not observed in ST2^{-/-} mice. This suggests that the ST2-IL-33 pathways and ILC2s also participate in the fungal allergen-induced airway inflammation. Fungal antigens often express protease activity that may damage airway epithelial cells, which then release IL-33 and IL-25.

ILC2 IN NONALLERGIC ASTHMA

Although eosinophils and allergen-specific Th2 cells typify the inflammation observed in many patients with allergic asthma, nonallergic forms of asthma, triggered by environmental factors, such as air pollutants (e.g., smoke, diesel particles, and ozone), stress, obesity, and viral infection, appear to develop independently of Th2 cells [16, 17, 53, 54]. In particular, respiratory microbial infections, such as with viruses, precipitate asthma symptoms in virtually all patients with asthma, regardless of the presence of allergy, but the precise pathophysiological mechanisms have not been fully understood.

Influenza virus infection

The asthma symptoms that occur with viral infection are often severe and frequently result in hospitalization as a result of a failure of conventional asthma therapies, such as corticosteroids, which effectively limit the function of eosinophils and Th2 cells in allergic asthma. Viral respiratory tract infections, for example, with rhinovirus infection, have been modeled in mice and shown to worsen allergen-specific Th2 responses and increase lung inflammation and AHR [55]. However, viral infections in patients frequently cause asthma, regardless of the presence of allergy. Although rhinovirus is the most common cause of virus-associated asthma exacerbations, infection with influenza virus is also extremely common and is associated with significant morbidity and mortality in patients with asthma, as observed during the 2009 H1N1 influenza A virus pandemic [56]. Recently, two studies in mouse models—one conducted by our group [37] and another by Monticelli et al. [31]—have demonstrated the important roles for ILC2s in influenza virus infection. In our study [37], we showed that acute infection of mice with H3N1 influenza virus rapidly induced AHR, which depended on IL-33, its receptor ST2 (*Il1rl1*), and ILC2s expressing c-Kit, Sca-1, Thy1.2 (CD90), and ST2. This occurred in the absence of adaptive immunity and NKT cells, as influenza A virus could induce AHR in Rag2^{-/-} and in NKT cell *Cd1d*^{-/-} mice. Influenza-induced AHR how-

ever, required the IL-33-ST2 signaling pathway and did not occur in ST2^{-/-} mice. IL-33 was induced upon viral infection during the first 24 h of the response, and it appeared that alveolar macrophages were a major cellular source of this cytokine. Virally induced AHR was dependent on IL-13, and adoptive transfer experiments demonstrated that only IL-13-secreting ILC2s were capable of restoring AHR in IL-13^{-/-} hosts. Furthermore, we showed that depletion of ILC2s by treatment of Rag2^{-/-} mice, which include the ILC2 population, with anti-Thy1.2 (CD90) mAb abolished the H3N1-induced AHR response. This reliance on IL-13 is similar to the situation observed in models of allergic asthma, although in allergic asthma, the IL-13 was produced by allergen-specific Th2 cells and by NKT cells [57].

In the study conducted by Monticelli et al. [31], the authors did not examine AHR but rather, the role of ILC2s in restoring lung-tissue homeostasis after influenza H1N1 infection. Interestingly, by depleting CD90.2-expressing cells in Rag1^{-/-} mice, it was found that ILC2s play a key role in the maintenance of epithelial integrity and lung function following viral infection. Furthermore, adoptive transfer of allotypic CD90.1⁺ ILC2 restored lung repair. This was independent of IL-13 and IL-22, although a more recent study suggests that IL-22 is essential for lung epithelial repair following influenza infection [58]. Instead, Monticelli et al. [31] found, using a gene-expression profiling approach, that amphiregulin, a member of the epidermal growth factor family, was an important product of lung ILC2s and critical for lung repair. Moreover, administration of amphiregulin was sufficient to restore epithelial cell integrity in influenza-infected mice, suggesting that ILC2s play an important role in tissue repair during viral infection.

These studies thus show that ILC2s can promote lung-inflammatory disease and also restore airway epithelial cell integrity. Whereas these two functions of ILC2s may sound contradictory, these roles may exemplify the homeostatic versus the pathological role of ILC2s, similar to the opposing roles that have been observed with many other cell types. For example, Th1 cells, Th2 cells, and Th17 cells each have evolutionarily conserved salutary effects (in resistance to intracellular, helminth, and fungal infections, respectively), as well as pathological effects (in augmenting autoimmunity, mediating allergy, or autoimmunity, respectively). Therefore, like with these other cell types, the context in which ILC2s are activated will determine whether their function is beneficial (enhancing epithelial cell integrity) or dysregulated and detrimental (causing airway inflammation and AHR). During influenza infection, ILC2s are activated rapidly and cause airway inflammation and AHR. However, when influenza infection persists and causes severe airway injury, as in Rag^{-/-} mice, which cannot clear the infection, amphiregulin production may help to repair the damage. On the other hand, in the context of helminth infection, ILC2s are also activated rapidly, are essential for the initial response that evolves into an effective adaptive Th2 response associated with eosinophilia, and increase mucus production of worm expulsion. In this setting, the production of amphiregulin by ILC2s may be effective in enhancing the recovery from the helminth infection and may explain the synchronized production of proinflammatory Th2 cytokines

and amphiregulin. Alternatively, it is possible that subsets of ILC2s may exist, such that some ILC2 subsets produce more IL-13 and less amphiregulin and cause airways disease, whereas others produce more amphiregulin and less IL-13 and reverse airway injury. Further investigation into the characteristics of ILC2s and possible subsets will help to resolve this issue.

Bacterial infection

Components of some bacteria, specifically glycolipids, have also been shown to activate iNKT cells, which then interact with ILC2s and alveolar macrophages in the lungs, resulting in AHR [40]. In this model of experimental asthma, AHR was induced rapidly by treatment with glycolipid antigens, including glycolipids from *Sphingomonas* species and α -galactosylceramide that directly activate iNKT cells, even in the MHCII^{-/-} mice, which lack conventional CD4⁺ T cells. The activated iNKT cells directly induced alveolar macrophages and DCs to produce IL-33, which in turn, activated ILC2s and further activated iNKT cells producing IL-13. Furthermore, this glycolipid-induced AHR pathway required not only IL-13 but also IL-33 and its receptor ST2, as it was blocked by an anti-ST2 mAb and was greatly reduced in ST2^{-/-} mice. With the use of transfers of WT iNKT cells or ILC2s into IL-13^{-/-} mice, it was found that either cell population was sufficient to restore glycolipid-induced AHR [39]. As plant pollens, house dust, and some bacteria contain glycolipids that can activate iNKT cells directly, these studies, taken together, suggest that innate-immune mechanisms, involving the IL-33-ST2-IL-13 axis, ILC2s, and iNKT cells, can work together to greatly enhance the development of AHR and asthma.

Taken together, a newly described immunological pathway for asthma that is dependent on IL-33-IL13 axis and ILC2s is illustrated in **Figure 1**.

ILC2S IN HUMANS

Increased IL-25 and IL-33 expression in allergic rhinitis and asthmatic patients

In 2001, Oshikawa et al. [59] reported an increase level of soluble human ST2 protein in sera of asthmatic patients. Furthermore, increased serum and tissue expression of IL-33 and ST2 has been noted in rhinitis patients [60], and epithelial and airway smooth muscle cells appear to represent two major sources of IL-33 in asthmatics [61]. In addition to IL-33, another innate type 2 cytokine, IL-25, has been shown to be associated with increases in IL-25R expression after allergen challenge in asthmatics [62, 63]. Although when reported, the role of ILC2s had not been investigated in humans, the important role of IL-33 and IL-25 in the regulation and expansion of ILC2 in mice suggested that innate ILC2 or ILC2-like cells might be present in the human asthmatics, particularly as the *IL-33* and *ST2* genes have both been identified as very important asthma-susceptibility genes in humans in genome-wide association studies [64]. Based on the murine studies of ILC2s in asthma and given the potential of ILC2s to produce IL-13

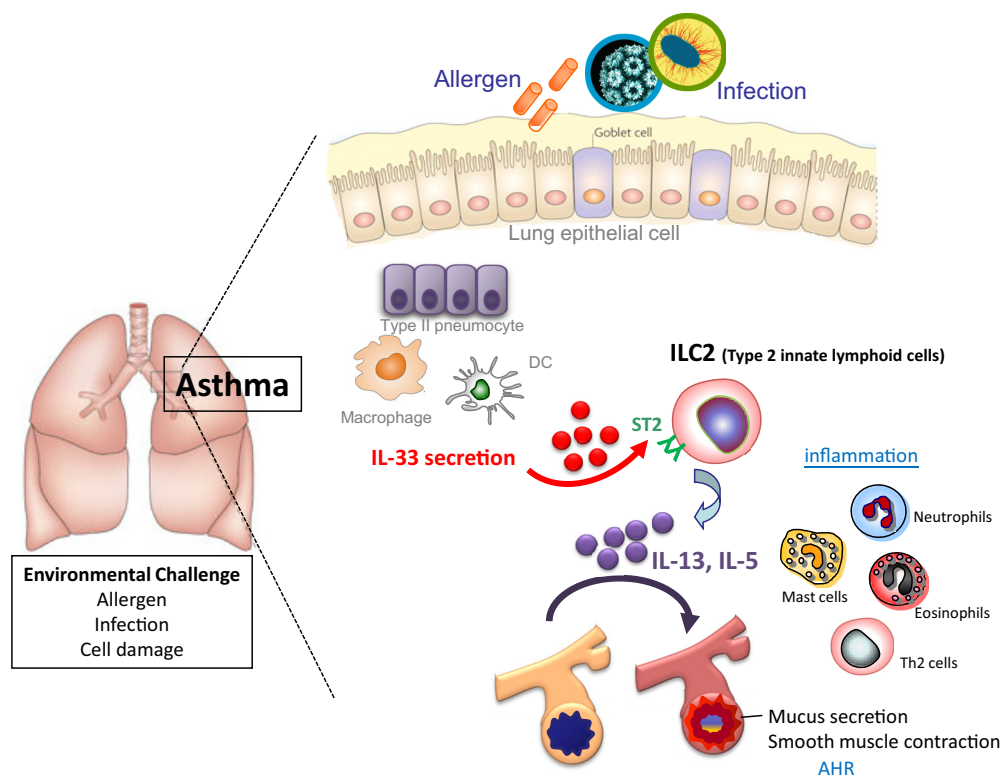


Figure 1. An immunological pathway for asthma that is dependent on IL-33-IL13 axis and ILC2s. Some environmental challenges, such as allergen, infections or cell damage, induce the rapidly secretion or release from lung cells (epithelial cells, type II pneumocyte, macrophage, dendritic cells). IL-33 production in turn activates type 2 innate lymphoid cells (ILC2) through ST2 receptors. This interaction leads to type 2 cytokines IL-5 and IL-13 production in ILC2. The IL-5 and IL-13 then induce inflammatory cells (Neutrophils, Mast cells, Eosinophils, Th2 cells) infiltration, drive mucus secretion by airway epithelial cells and the development of airway hyper-reactivity, which cause asthma.

and IL-5, it is reasonable to believe that ILC2s may play an important role in human asthma.

Human sputum

Several years ago, Allakhverdi et al. [65] reported that non-T and -B cells producing IL-13 and IL-5, similar to type 2 ILCs, were present in the sputum of asthmatic subjects but not in normal subjects. In their study, they found that the non-T and -B CD34⁺ lymphoid cells expressed receptors for TSLP and IL-33 and responded to these cytokines by rapidly producing high levels of proinflammatory Th2-like cytokines and chemokines. In addition, the number of these cells increased in response to specific allergen-inhalation challenge. These cells were also activated in a TSLP-dependent manner by the supernatants from activated, primary human small airway epithelial cells and from nasal explants of patients with chronic rhinosinusitis. Allakhverdi et al. [65] suggested that non-T and -B CD34⁺ cells, in addition to being progenitors, may act as pro-inflammatory effector cells and directly contribute to the allergic inflammation. Although Allakhverdi et al. [65] did not further characterize this subset and although it is not known whether CD34, a stem cell marker, is the proper marker to distinguish this population, their findings nevertheless suggest that ILC2s may play an important role in asthma.

Human fetal and adult lung and gut

Recently, Mjosberg et al. [42] conducted a more detailed study to characterize human type 2 innate subsets in human lung, as well as in gut, nasal tissues, and peripheral blood. They identified a human Lin⁻CD127⁺ ILC population, charac-

terized by expression of the Th2 marker CRTH2, with similarities and differences compared with mouse ILC2s. These human ILCs expressed CD161 and the lymphoid marker CD7, were of lymphoid size, and lacked the mast cell and basophil markers FcεR1 and the IL-3R (CD123). Like mouse ILC2s, these human CRTH2⁺ cells responded to IL-25 and IL-33 by producing IL-13 and IL-5 and were therefore, thought to represent human ILC2s. Interestingly, in human patients with chronic rhinosinusitis, a disease dominated by eosinophils, increased numbers of ILC2 were observed in nasal polyp tissue but not in uninfamed nasal tissue of nonallergic control subjects [42].

THE MODULATION OF ILC2 AS THERAPY FOR ASTHMA

Currently, the mainstay of therapy for asthma relies heavily on inhaled and systemic corticosteroids, which are very effective for allergic asthma mediated by Th2 cells and eosinophils [66, 67]. Although corticosteroids are very effective for allergic asthma, this approach targeting Th2 cell immunity has not reduced the high rates of hospitalizations for acute asthma, most often caused by nonallergic factors, including viral infections. Because of this, asthma remains a very common medical diagnosis and reason for hospitalization. Corticosteroid treatment fails to abolish TNF-α-induced IL-33 production in human airway smooth muscle cells, suggesting that the ILC2s, but not Th2 cells, may be a major effector cell type in corticosteroids-resistant asthmatic patients. Given that ILC2s produce IL-5 and IL-13 in several models of airway allergic inflamma-

tion and infection and that IL-13 is a major factor underlying the type-2-induced AHR, goblet cell metaplasia, mucus hypersecretion, and inflammatory chemokines that characterize human asthma [57], it is tempting to speculate that modulation of ILC2 development and function may be of use in the clinic. Therefore, in addition to corticosteroids for asthma therapy, treatments targeting innate type 2 cells may be very effective in many forms of asthma.

Targeting IL-25 or IL-33

In murine models, several groups have targeted IL-25 and IL-33 and their receptors, which are the primary cytokines responsible for the expansion of ILC2 in the lung. For example, in an OVA-alum model, blockade of IL-33 with a neutralizing antibody resulted in significantly reduced inflammatory cell infiltrates, BAL IL-4, IL-5, and IL-13 and serum anti-OVA IgE [68]. Another study also showed that anti-IL-33- and anti-ST2-neutralizing mAb were also effective in an *Aspergillus fumigatus* model of airway inflammation. In their study, anti-ST2 mAb treatment resulted in an abrogation of mucus secretion, lung Th2 cytokine mRNA expression, BAL cellular inflammation, and AHR compared with controls [69]. With a similar approach, targeting IL-25 expression in a mouse OVA-alum model with a mAb during sensitization and challenge prevented the development of allergic inflammation, an IgE-immune response, and AHR. Interestingly, neutralization of IL-25, at the time of challenge but not sensitization, had no effect on lung inflammatory cell infiltrations or Ig production. However, anti-IL-25 mAb treatment did result in a significant reduction of AHR [70]. Furthermore, IL-25-neutralizing therapy has also been investigated in pneumonia virus of mice model of respiratory syncytial virus-associated asthma. The results showed that treatment with anti-IL-25 mAb during the challenge phase resulted in significantly reduced lung Th2 cytokine mRNA expression and reduced airway remodeling [71].

Taken together, targeting the cytokines that lie upstream of ILC2 activation appears beneficial, at least in mouse asthma models. Notably, the recent identification of ROR α and GATA3 as key transcription factors involved in ILC2 development raises the possibility of developing therapeutic transcription factor inhibitors that would specifically block the production of ILC2s in lung-inflammatory diseases.

Targeting ILCs by lipoxin A4

Recently, Barnig et al. [72] have identified ILC1s (NK cells) and ILC2s in human lung and peripheral blood from healthy and asthmatic subjects. They found that NK cells were highly activated in severe asthma, were linked to eosinophilia, and interacted with autologous eosinophils to promote their apoptosis. Furthermore, they found the airway epithelial cytokines IL-25 and IL-33 stimulated ILC2s to generate IL-13 in response to the mast cell product PGD₂. In addition, NK cells and ILC2s expressed pro-resolving ALX/formyl peptide receptor 2 receptors, and its ligand lipoxin A4 increased NK cell-mediated eosinophil clearance and decreased IL-13 release by

ILC2s. Their findings suggested that pro-resolving mediators could regulate ILCs in asthma pathobiology.

CONCLUDING REMARKS

The discovery of ILCs over the past several years has changed our understanding of the principles for immune regulation and shown how innate immunity profoundly shapes adaptive immunity. These ILCs appear to be as important as adaptive immune cells and appear to greatly contribute to immune disorders, including allergic disease and asthma. Although the unique cell surface and intracellular markers of ILC2s and their interactions with other cell types are not fully understood, studies of these ILC2s have opened a new area of investigation that may lead to much improved therapies for lung disease.

DISCLOSURES

There are no conflicts of interest.

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