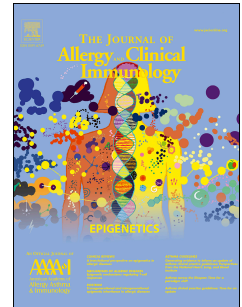


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Innate lymphoid cells in human asthma

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Innate lymphoid cells (ILCs) are innate immune cells that produce high levels of effector cytokines (1). They are defined by their lymphoid morphology, the absence of rearranged antigen receptors and the lack of lineage surface markers. ILCs are divided into three subpopulations based on their distinct patterns of cytokine production: ILC1s produce the T helper 1 (Th1) signature cytokine IFN- γ , ILC2s produce type 2 cytokines, in particular IL-5 and IL-13, and ILC3s produce IL-17 and/or IL-22 (1). Studies performed over the past 10 years have revealed important roles of ILCs in innate immunity, tissue homeostasis and repair, and inflammation at mucosal and barrier surfaces (1).

In this issue of the Journal of Allergy and Clinical Immunology, Kim et al. (2) report that ILC1s, ILC2s and ILC3s were all increased in induced sputum from asthmatic patients (51 newly diagnosed, treatment-naïve patients) when compared with those in healthy control subjects (18 healthy donors). ILC2s counts were significantly increased in patients with eosinophilic asthma (blood eosinophils counts > 300 cells/ μ l) compared to patients with non-eosinophilic asthma. Interestingly, ILC2s percentages were positively correlated with increases in percentages of eosinophils and M2 (alternatively-activated) macrophages, but not M1 (classically activated) macrophages (2). On the other hand, increases in ILC1s and ILC3s percentages in asthmatic airways were positively correlated with increases in percentages of M1 macrophages, but not M2 macrophages or eosinophils.

Kim et al. are the first to describe an increase in ILC1s and ILC3s in the sputum of asthmatic patients. However, increases in ILC2s in asthmatic patients have been previously reported (3-7). Bartemes et al. observed that allergic asthmatics have higher levels of ILC2s in peripheral blood compared with healthy controls and patients with allergic rhinitis (3). Christianson et al. found that ILC2s and their upstream regulator,

the epithelial alarmin IL-33 (8), were both increased in BAL fluids of asthmatic patients (4). Smith et al. showed that type 2 cytokines-producing ILC2s were increased in blood and sputum of patients with severe asthma on high doses of oral corticosteroids (5). Significantly higher numbers of ILC2s expressing IL-5 and IL-13 were detected in the sputum of patients with uncontrolled asthma (> 3% airway eosinophilia), despite normal blood eosinophil numbers (< 300 cells/ μ l). Nagakumar et al. reported for the first time the presence of ILC2s in BAL, induced sputum and peripheral blood from children with severe therapy-resistant asthma (6). Lastly, Chen et al. demonstrated that numbers of IL-5⁺IL-13⁺ ILC2s were significantly increased in sputum of adult asthmatics 24 h after allergen challenge, coincident with a decrease of ILC2s in blood, suggesting trafficking of circulating ILC2s to the lungs (7). Chen et al. observed a correlation between the absolute numbers of IL-5⁺ ILC2s and airway eosinophilia, a finding in accordance with those of Kim et al. (2).

Although the increase of ILC1s and ILC3s in asthmatic airways has not been noticed in previous studies, Kim et al observed that they represented a significant % of ILCs in induced sputum of asthmatic patients. Indeed, they were more abundant than CCR2⁺ ILC2s (2). However, their frequency was variable among patients. The mechanisms responsible for the increase of ILC1s and ILC3s in the airways are currently unknown. ILCs have been shown to exhibit plasticity depending on the cytokine milieu (1). Kim et al observed a negative correlation between ILC1 counts and NKp44⁺ ILC3 counts suggesting some degree of ILC plasticity in the asthmatic airways (2). Conversion of ILC2s to ILC1s and/or ILC3s could also possibly play a role but whether such plasticity really occurs and contributes to human asthmatic disease remains to be determined. Kim et al. studied patients with treatment naïve asthma most of whom were mild asthma (2). In contrast, patients with severe and/or treatment

resistant asthma were analyzed in previous studies (5, 6). It will be thus of great interest to determine if the frequency of ILC1s, ILC2s and ILC3s is modified in severe/treatment resistant asthma. The relative abundance and roles of ILCs in asthma may differ depending on the phenotype, severity and treatment.

ILC2s and mRNAs of type 2 cytokines IL-5 and IL-13 were significantly increased in patients with eosinophilic asthma. In contrast, no significant differences between patients with eosinophilic asthma and non-eosinophilic asthma were found for ILC1s, ILC3s and mRNAs of their signature cytokines, IFN- γ and IL-17. ILC1s and/or ILC3s could represent important sources of IFN- γ or IL-17, responsible for the upregulation of these two cytokines in subsets of patients with severe asthma (2). However, Kim et al used flow cytometry to define ILC1s (CD45⁺Lineage-IL-7R⁺CRTH2⁻ckit⁻NKp44⁻ cells) and ILC3s (CD45⁺Lineage-IL-7R⁺CRTH2⁻ckit⁺ cells) rather than intracellular cytokine staining. It will be thus important in future studies to further characterize the capacity of ILC1s and ILC3s to produce IFN- γ and IL-17 in the asthmatic airways and to determine their relative importance compared to other sources of these cytokines. A previous study by Kim et al. identified IL-17-producing ILCs in the lungs of patients with asthma (9). Although the study was limited to ten patients and the phenotype of the cells was not precisely characterized, it is interesting to note that these ILC3-like cells producing IL-17 were more frequent in BAL fluids of patients with severe asthma than patients with mild asthma (9).

As mentioned above, the frequencies of ILC1s/ILC3s and ILC2s in asthmatic airways were correlated to the frequencies of M1 and M2 macrophages, respectively. The authors thus performed co-culture studies to determine whether ILCs can promote macrophage polarization. They found that co-culture of alveolar macrophages with ILC1s and ILC3s significantly induced mRNA expression of M1 macrophage-related

genes, whereas co-culture with ILC2s significantly induced mRNA expression of M2 macrophage-related genes (2). Kim et al. suggested that specific types of ILCs regulate the polarization of alveolar macrophages by secretion of cytokines (IFN- γ or IL-17 for ILC1s/ILC3s, IL-4/IL-13 for ILC2s) and cell-cell contact. However, these studies were performed *ex vivo* using co-cultures of mouse ILCs and alveolar macrophages isolated from the lungs of C57BL/6 female mice, and it is not yet known whether the findings of Kim et al. can be extrapolated to the human situation. Additional studies will be required to determine the nature of innate immune crosstalk in the airways of asthmatic patients.

Kim et al. found that M1 macrophage counts were significantly increased in patients with non-eosinophilic asthma (2). In addition, they observed that the frequencies of M1 macrophages and neutrophils were correlated in induced sputum. Therefore, although ILC1s and ILC3s were not specifically increased in non-eosinophilic asthma, these later observations suggested that ILC1s/ILC3s that are correlated to M1 macrophages could play a role in non-allergic inflammation in asthmatic patients. This would not exclude a role for ILC1s/ILC3s and their effector cytokines (e.g. IL-17) in the severity of eosinophilic asthma, in synergy with ILC2s that are strongly linked to allergic inflammation in asthmatic patients.

The genes encoding the major cytokine regulators of lung ILC2s, IL-33 and its receptor ST2 (*IL1RL1*), and TSLP, were among the most replicated loci in large scale genome-wide association studies of asthma susceptibility (10). These observations accelerated the clinical development of therapeutic agents targeting these critical regulators of ILC2s, and clinical trials with antibodies targeting TSLP (Tezepelumab, Phase III), IL-33 (Etokimab and REGN3500, Phases II) and ST2 (RG6149, GSK3772847, Phases II) are currently ongoing in patients with severe asthma. It

remains to be seen whether targeting upstream regulators of ILC1s and ILC3s or their effector cytokines could also be useful in the future for some patients with severe uncontrolled asthma.

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Figure legend**Figure 1. Innate lymphoid cells in asthmatic airways**

ILC2s are believed to be induced and/or maintained by epithelium-derived cytokines, such as IL-33 and TSLP that are released upon tissue damage or cellular stress following exposure to allergens or infection with viruses. In this issue (2), Kim et al. demonstrate that the three subpopulations of innate lymphoid cells (ILCs), ILC1s, ILC2s and ILC3s, are present in asthmatic airways. They found that ILC2s were significantly increased in patients with eosinophilic asthma, and correlated with M2 macrophages, whereas ILC1s and ILC3s were increased in both eosinophilic and non-eosinophilic asthma, and correlated with M1 macrophages. Production of IFN- γ and IL-17 cytokines by ILC1s and ILC3s (dotted arrows) in asthmatic airways has not yet been shown. Kim et al. found a role of ILC1s/ILC3s and ILC2s in M1 and M2 macrophage polarization, respectively (2). However, these later experiments were performed *ex vivo* with cultured mouse cells, and it remains to be seen whether similar mechanisms operate in human asthmatic airways. Other immune cells with important roles in asthmatic airways (T cells, mast cells,...) are not shown in this figure for simplification purposes. BEC, bronchial epithelial cell; SMC, smooth muscle cell.

