

Type 2 innate lymphoid cells–new members of the “type 2 franchise” that mediate allergic airway inflammation

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Type 2 innate lymphoid cells (ILC2s) are members of an ILC family, which contains NK cells and Ror γ ⁺ ILCs, the latter including lymphoid tissue inducer (LTi) cells and ILCs producing IL-17 and IL-22. ILC2s are dedicated to the production of IL-5 and IL-13 and, as such, ILC2s provide an early and important source of type 2 cytokines critical for helminth expulsion in the gut. Several studies have also demonstrated a role for ILC2s in airway inflammation. In this issue of the *European Journal of Immunology*, Klein Wolterink et al. [Eur. J. Immunol. 2012. 42: 1106–1116] show that ILC2s are instrumental in several models of experimental asthma where they significantly contribute to production of IL-5 and IL-13, key cytokines in airway inflammation. This study sheds light over the relative contribution of ILC2s versus T helper type 2 cells (Th2) in type 2 mediated allergen-specific inflammation in the airways as discussed in this commentary.

Keywords: Lung inflammation • Innate immunity • Asthma



See accompanying article by Klein Wolterink et al.

Type 2 innate lymphoid cells (ILC2s) are members of a larger family of cells that include Ror γ ⁺ ILCs, producing the cytokines IL-22 and IL-17 and lymphoid tissue inducer (LTi) cells, critical for the formation of lymphoid structures (reviewed in [1]). To this family also belong ILC subsets that develop independently of Ror γ , namely conventional NK cells, and more recently the type 2 ILCs, the latter dedicated to the production of the Th2 cytokines IL-5 and IL-13. These cell types are considered to belong to the same family because they all depend on the transcriptional repressor Id2 and the common γ -chain (γ c) of the IL-2 receptor and share a lymphoid morphology.

Innate type 2 cytokine-producing lymphoid cells were already described in 2001 when it was shown that the administration of IL-25, a member of the IL-17 family of cytokines, to mice triggered release of high levels of IL-5 and IL-13 from a population

of RAG-independent, γ -common chain-dependent, non-T, non-B ILCs in the gut [2]. Almost a decade later, several groups [3–5] reported on a similar population of cells and defined them as Lineage negative (Lin[−]) Sca-1⁺IL7R⁺Thy1⁺T1/ST2⁺, the latter marker constituting a component of the receptor for IL-33. One group located them in mesenteric fat-associated lymphoid clusters and showed that they were instrumental in helminth expulsion by mediating goblet cell hyperplasia and self-renewal of B1 cells in the gut [3]. Two other groups used IL-13 reporter mice to pin down an IL-13-producing ILC type. These cells were induced by *Nippostrongylus brasiliensis* infection or the administration of IL-25 and IL-33, and constituted a major source of IL-13 and IL-5 [4, 5]. However, only a fraction of the ILC2s produced IL-4, which sets them apart from Th2 cells and basophils that do produce IL-4. The various groups gave different names to these cells: natural helper cells [3], nuocytes [4], and innate type 2 helper cells [5]. For the sake of simplicity, we use the name ILC2 throughout this commentary to emphasize the relationship of these cells with other ILC types.

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Given the potency of IL-5 and IL-13 to drive Th2-mediated immunity, it is plausible that ILC2s would also play a role in Th2-mediated immune pathologies, including allergy and asthma. Indeed, the paper of Klein Wolterink et al. [6] in this issue of the *European Journal of Immunology* and several other reports support this contention [7–13]. Also in humans, there is evidence for a pathogenic role, as ILC2s are enriched in nasal polyps of patients with chronic rhinosinusitis, a typical type 2 mediated disease [14].

In mice, it had already been shown that the administration of IL-25 or IL-33, the latter belonging to the IL-1 cytokine family, induced type 2 cytokine production and airway pathology and that blockage of IL-25 could prevent OVA-induced airway hyperreactivity (AHR) [2, 15]. However, the cellular sources of the type 2 cytokines in the IL-25/IL-33 triggered asthma were obscure at that time. A number of subsequent reports provided the first direct evidence that ILC2s could actually mediate airway inflammation in response to IL-25 and IL-33. First, in a mouse model of virus-induced AHR, ILC2s were shown to mediate inflammation following influenza virus infection [8]. In this model, ILC2s were activated by the IL-33 secreted by virus-triggered macrophages, a mechanism very similar to the one described for glycolipid-induced asthma [11]. In the latter, the glycolipid α -galactosylceramide-activated NKT cells, which induced alveolar macrophages to secrete IL-33 that in turn stimulated the accumulation of, and type 2 cytokine production from, ILC2s. Depletion of ILC2s using a Thy1 antibody significantly reduced influenza-induced AHR. Furthermore, adoptive transfer of ILC2s to IL13^{-/-} mice restored AHR in both glycolipid and virus-induced asthma, showing that even when ILC2s are the only sources of IL-13, they are capable of mediating lung pathology.

In line with these findings, Klein Wolterink et al. [6] now show in this issue of the *European Journal of Immunology* that cells with a characteristic phenotype of Lin⁻ Sca1⁺ IL7R⁺ T1/ST2⁺ are found in the lungs and mediastinal lymph nodes of naïve mice. The finding that Rag2/Il2rg double-deficient mice lack these cells is consistent with earlier studies and clearly identifies these lung-residing cells as ILC2s. Intranasal administration of IL-33, and to a lesser extent also IL-25, causes airway inflammation as characterized by increased pulmonary cellularity including the accumulation of eosinophils, T cells, and T1/ST2⁺ ILC2s. Compared with Th2 cells and other sources of IL-5 and IL-13, ILC2s turn out to be the major producers of these cytokines. Similar findings were reported by Barlow et al. [7], who used IL-13 reporter mice to reveal ILC2s as a major source of IL-13 in IL-33-induced asthma. Supporting the idea that ILC2s and not Th2 cells are the main players in airway inflammation, Klein Wolterink et al. [6] demonstrate that IL-25- and IL-33-induced asthma and ILC2 activation occur also in RAG2^{-/-} mice, which lack T cells, a finding also supported by others [12].

A recent study has shed light on the origin of ILC2s. ILC2s were demonstrated to develop from a bone marrow derived Lin⁻ IL7R α ⁺ Flt3⁺ common lymphoid progenitor (CLP) under the influence of Notch1, IL-7, and IL-33 signaling [10]. The ILC2 progenitor depended on the transcription factor retinoic acid receptor-related orphan receptor alpha (ROR α , also known as TR1F1), as ROR α -deficient mice lacked ILC2s. In line with a crucial

role for ILC2s in type 2 mediated immunity, ROR α -deficient mice demonstrated a severely impaired capacity to handle intestinal *N. brasiliensis* infection.

In addition to a significant role for ILC2s in IL-25- and IL-33-induced asthma, the study by Klein Wolterink et al. [6] shows a significant contribution of type 2 cytokines from ILC2s in an allergen-specific setting of allergic airway inflammation. ILC2s are activated in house dust mite (HDM)-induced asthma and again, as observed for IL-25- and IL-33-induced asthma, comprise a significant source of IL-5 and IL-13 as compared with Th2 cells. These data raise the question about the trigger of ILC2s in this setting. HDM extracts contain thousands of molecules, including not only a number of characterized allergens, but also microbial components such as LPS (reviewed in [16]). It remains unknown if HDM directly can activate ILC2s, for example, through stimulation of proteinase-activated receptors (PARs) and pathogen-recognition receptors (PRRs, including Toll-like receptors) (reviewed in [16]). Recently, HDM-induced asthma was shown to depend on LPS activation of TLR4 on respiratory epithelium. This led to increased secretion of IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) [17], suggesting that ILC2s might be activated by these stroma-derived cytokines in HDM-induced asthma. A recent study of asthma induced by the protease allergen papain supports this idea [13]. Hence, the role for ILC2s in asthma seems to be as an early translator of stromal-derived signals upon allergen encounter, which subsequently induces type 2 cytokine production from ILC2s (Fig. 1). This might be the early trigger of type 2 mediated pathology, which can be subsequently propagated to include the adaptive immune system. However, further research is needed to reveal the exact mechanisms behind the role of ILC2s in allergen-induced asthma, also in the human setting.

Interestingly, in the current study by Klein Wolterink et al. [6], even in OVA-induced asthma, which relies on the specific T-cell recognition of OVA peptides, ILC2s are the major producers of IL-5, and produce similar amounts of IL-13 as Th2 cells. This is an intriguing observation, which is supported by a study from the group of Andrew McKenzie [7]. Using IL-13 reporter mice, IL-13⁺ ILC2s were shown to accumulate in the lungs following OVA-induced asthma [7]. One explanation for this could be that OVA-activated T cells trigger ILC2s. Indeed, activated T cells produce IL-2, a growth factor for both T cells and ILC2s. Hence, in situations of T-cell activation, such as in OVA-induced asthma, ILC2s might work downstream from T cells to amplify the type 2 inflammation. A recent paper supports this theory. Intranasal administration of papain induced airway inflammation and a concomitant appearance of a population of ILCs transiently producing IL-9 [18]. IL-9 production depended on IL-2 from the adaptive immune system as RAG1^{-/-} mice expressed less IL-9, which was restored by the intranasal administration of IL-2. Much effort is now needed to address the question as to how this newly found source of type 2 cytokines could be used as targets for therapy of allergy and asthma.

In addition to mediating the inflammatory aspects of AHR, ILC2s have also been shown to mediate tissue protection following respiratory viral infection. In a study by Monticelli et al. [9], AHR

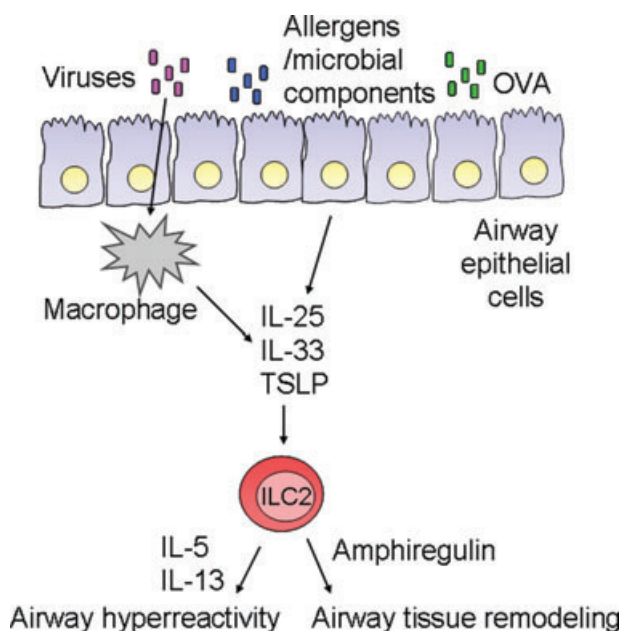


Figure 1. ILC2s constitute a critical and early source of type 2 cytokines in several models of experimental airway inflammation. Influenza virus-induced airway hyperreactivity is mediated by ILC2s, activated by IL-33 produced from virus-triggered macrophages and epithelial cells. In this model, ILC2s also provide tissue protection through production of amphiregulin. In allergen-mediated asthma, epithelial cells provide the initial source of IL-25, IL-33, and TSLP, which in turn activates ILC2s. The mechanism behind ILC2 activation in OVA-induced asthma remains obscure but, in addition to IL-25, IL-33, and TSLP produced by the epithelium, the mechanism might involve IL-2 production from OVA-activated T cells, which induces IL-5 and IL-13 production from ILC2s.

following influenza virus infection was, surprisingly, worsened upon ILC depletion. It was shown that ILC2s produce a factor of the epidermal growth factor family, known as amphiregulin (Fig. 1). Administration of amphiregulin restored epithelial cell integrity, airway remodeling, and lung function. In line with these findings, another study demonstrated an important role for type 2 immunity in controlling lung tissue damage [19]. Infection with the helminth *N. brasiliensis* induces type 2 immunity, also in the lung, through which, the larvae migrate on its way to the small intestine. Chen et al. [19] found that IL-4 receptor signaling is crucial to control the IL-17-mediated inflammation that is simultaneously induced upon *N. brasiliensis* infection. The proposed mechanism involved IL-4-mediated induction of wound-healing molecules such as IL-10, EGF-1, Arg1, and MMP-13 from lung tissue macrophages. The data of Monticelli et al. [9] now suggest that ILCs might be novel sources of such tissue-protective molecules. However, the role for ILC2s in airway tissue protection in the allergen-specific setting remains unknown.

In conclusion, ILC2s are now emerging as critical players in type 2 mediated immune pathology, not only in the gut, as previously acknowledged, but also in the airways. Hence, they can be considered to be part of the “type 2 franchise,” including mast cells, basophils, eosinophils, and Th2 cells that collectively mediate allergy and asthma. In experimental asthma, ILC2s constitute

a significant and early source of type 2 cytokines, and also tissue-protective molecules. As such, ILC2s are new targets for the treatment of allergy and asthma. However, this prompts acquisition of further detailed information about how ILC2s are regulated in the setting of allergic inflammation. In addition, studies unraveling the contribution of ILC2s in allergy and asthma in humans deserve urgent attention.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

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