

*Series: Tissue-resident immune cells*

# Lung ILC2s link innate and adaptive responses in allergic inflammation

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**How allergens trigger the T helper 2 (Th2) response that characterizes allergic lung inflammation is not well understood. Epithelium-derived alarmins released after an allergen encounter activate the innate immune system, including group 2 innate lymphoid cells (ILC2s) which produce the type 2 interleukins IL-5 and IL-13. It has been recently shown that ILC2-derived cytokines are responsible not only for the innate responses underlying allergic inflammation but also for the initiation of the adaptive Th2 response. We review the role of lung ILC2s in the development of allergic inflammation and, in the context of recent findings, propose a common pathway wherein ILC2s, activated by the epithelium-derived cytokine IL-33, link the innate and the adaptive responses after allergen encounter in the lung.**

## Understanding the drivers of allergic lung inflammation

Allergic lung inflammation is driven by overproduction of type 2 cytokines, including IL-4, IL-5, and IL-13, in response to inhaled allergens. IL-4 stimulates B cells and induces immunoglobulin E (IgE) production. Crosslinking of high-affinity IgE receptors on mast cells and basophils by allergen-IgE immune complexes induces the release of inflammatory mediators including histamine, heparin, and serotonin [1]. These inflammatory mediators are responsible for the recruitment of alternatively activated macrophages (AAMs) and neutrophils, smooth muscle contractility, and mucus hyperproduction. IL-5 recruits and activates eosinophils, which release the lipid mediator leukotriene C4 and platelet-activating factor, and induce mucus production and smooth muscle contraction. IL-13 is a potent inducer of mucus hyperproduction and airway remodeling. Whereas Th2 cells have long been thought to be the crucial source of these type 2 cytokines in allergic inflammation, the recent discovery of group 2 innate lymphoid cells (ILC2s; see [Glossary](#)) in the lung revealed an innate source of type 2 cytokines independent of Th2 cells. ILC2s are a potent source of IL-5 and IL-13 but, although the *Il4* reporter gene is expressed in

ILC2s [2], these cells secrete very little IL-4 protein [3]. ILC2 stimulation results in eosinophil infiltration, mucus hyperproduction, and airway hyper-reactivity, but not IgE production, a hallmark of allergic inflammation. Thus, Th2 cells still remain a crucial source of IL-4 which drives IgE production by B cells [4] in allergic lung inflammation.

Very diverse allergens, ranging from pollen, molds, house dust mites, and proteases to air pollutants, are known to cause allergic lung inflammation [5]. How these diverse allergens, which have no obvious common molecular characteristics, stimulate Th2 cells has been a central question for the field. Inhaled allergens stimulate airway epithelial cells and induce the production of cytokines, including thymic stromal lymphopoeitin (TSLP), IL-25, and IL-33, which have been shown to promote Th2 cell responses [6]. However, the mechanisms by which these cytokines regulate Th2 cells have been unclear. These epithelium-derived cytokines have now been found to be potent stimulators of ILC2s [7]. Furthermore, recent studies have suggested that ILC2s may be a crucial link between allergen-induced epithelial cytokine production and the initiation of Th2 cell-mediated allergic lung inflammation.

## Glossary

**Alarmins:** a loosely defined group of immune-modulating intracellular molecules that are released from damaged and/or stressed cells to directly alert the immune system. Some alarmins are also released by activated leukocytes or epithelia. Alarmins can include the cytokines IL-1, IL-33, IL-25, and TSLP, but a broader definition of alarmins includes HMGB1, heat shock proteins, and uric acid.

**Innate lymphoid cell (ILC):** a family of cytokine-producing innate lymphocytes that do not express antigen-specific receptor but are activated by cytokines. The ILC family is divided into ILC1, ILC2, and ILC3 that produce IFN $\gamma$ , type 2 cytokines, and IL-22/IL-17, respectively. Natural killer (NK) cells are included in the ILC family.

**Type 2 cytokines:** cytokines produced by Th2 cells. They include IL-4, IL-5, IL-6, IL-10, and IL-13. IL-9 was initially considered to be a type 2 cytokine, but recent studies have shown that a IL-9 is produced by a distinct Th subset, termed Th9. Some type 2 cytokines are also produced by non-Th2 cells, including ILC2s, basophils, and NKT cells.

**Type 2 immunity:** immunity mediated by type 2 cytokines and often involves high titer antibody production. It protects against helminth and toxins, and mediates resolution of type 1 inflammation and tissue repair. In addition to Th2 cells, alternatively activated M2 macrophages and ILC2s are known to drive type 2 immunity.

**Type 2 inflammation:** inflammation caused by excessive type 2 immunity and is characterized by eosinophil, rather than neutrophil, recruitment, and by IgE and mucus production. Type 2 inflammation may result in allergy.

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## Review

We review recent findings on the mechanisms underlying ILC2 activation, focusing on the role of ILC2 in allergen-induced type 2 inflammation in the lung. We discuss the significance of ILC2s during the innate response to allergens, and examine recent data on how ILC2-derived type 2 cytokines promote Th2 cell differentiation. Based on recent evidence, we suggest that ILC2 activation by allergen-induced alarmin IL-33 is a common pathway in the initiation of allergic lung inflammation.

### The innate lymphoid cell (ILC) family and ILC2s

ILC2s are members of the ILC family which have lymphoid morphology and express the lymphoid marker CD127 (IL-7 receptor  $\alpha$ ). ILCs are found in mice deficient for recombina-*nase-activating gene* (*Rag*), but are absent in mice deficient for both *RAG* and the IL-2 receptor  $\gamma$ -chain (*Rag2*<sup>-/-</sup>/*Il2rg*<sup>-/-</sup>) [2,3,8–10]. ILCs are divided into three groups that mirror their T cell counterparts [11] – ILC1s are non-cytotoxic and produce IFN $\gamma$ , ILC2s produce type 2 cytokines, and ILC3s are further divided into IL-22-producing and IL-17-producing cells. Common progenitors characterized by the expression of the transcription factors Id2 [12] and PLZF [13] that give rise to all ILCs, but not to other lymphocytes, have recently been identified indicating that ILCs belong to a distinct lymphoid lineage. ILC2 and ILC3 development is dependent on two members of the retinoic acid receptor-related orphan receptor (ROR) family: ILC2 development requires ROR $\alpha$  [14,15] whereas ILC3 development is ROR $\gamma$ t-dependent [16,17]. ROR $\alpha$  deficiency affects the neurological system and the liver; however, transplantation of bone marrow (BM) cells from ROR $\alpha$ -deficient mice into lethally irradiated mice generates ILC2-deficient mice, and these have been invaluable for the studies of ILC2s *in vivo*.

ILC2s can be identified by using combinations of cell surface markers, namely lineage marker negative (Lin<sup>-</sup>), Thy1<sup>+</sup> (CD90<sup>+</sup>), CD127<sup>+</sup>, CD25<sup>+</sup>, Sca-1<sup>hi</sup>, cKit<sup>lo</sup>, ST2<sup>+</sup> (IL-33R<sup>+</sup>), KLRG1<sup>+</sup>, and ICOS<sup>+</sup> – and these cells have been found in the lung, skin, gut, and adipose tissues of naive mice [7,11]. The surface phenotypes of ILC2s in different tissues can vary; for example, ST2 expression on small intestine ILC2s is very low compare to lung ILC2s [3,18], whereas dermal ILC2s uniquely express CD103 [19]. Nevertheless, ILC2s share similar functions, namely the secretion of type 2 cytokines IL-5, IL-13, and also IL-9 upon activation. The importance of ILC2s is underlined by their role in several pathological and non-pathological conditions (Table 1). In the gut, ILC2s help to expel parasites by producing IL-13, which promotes goblet cell hyperplasia, mucus secretion, and smooth muscle contraction [10]. ILC2s also protect and repair the respiratory epithelia during an influenza infection by releasing amphiregulin, a member of the epidermal growth factor (EGF) family that promotes epithelial cell growth [20].

Constitutive or stimulated ILC2-derived IL-5 controls the eosinophil population in visceral adipose tissue (VAT), maintaining fat homeostasis and preventing the development of obesity and diabetes [21]. ILC2 activation, cytokine production, and consequently tissue eosinophil accumulation are regulated by circadian rhythm and nutrient intake, demonstrating a role in metabolic cycling [22].

Recently, ILC2s were found to respond to environmental cold via IL-33, and ILC2 and eosinophil-derived cytokines were shown to induce beige fat biogenesis which support thermogenesis, suggesting a role in thermal homeostasis as well [23].

By contrast, ILC2 stimulation may also lead to allergic inflammation in the lung and the skin after allergen exposure [3,19]. In the following sections we discuss how ILC2s are activated in the lung and their role in allergic inflammation.

### ILC2 stimulation by cytokines

The characterization of IL-25 as a new member of the IL-17 family led to the discovery of its role in type 2 immunity [8]. *Nippostrongylus brasiliensis* infection and *Aspergillus* treatment induced IL-25 expression in the gut and lung, respectively, and intranasal administration of IL-25 induced eosinophilia in the airways, which required IL-5 and IL-13. The production of these cytokines was T cell and IL-4-independent [8]. The cells producing IL-5 and IL-13 were later characterized as c-kit<sup>+</sup> Fc $\epsilon$ R1<sup>-</sup> cells [9]. In 2010, Moro *et al.* isolated ILC2s, referred to as natural helper cells, as Lin<sup>-</sup> c-Kit<sup>+</sup> Sca-1<sup>+</sup> cells from fat-associated lymphoid clusters in the peritoneal cavity, and showed that they produced high amounts of IL-5 and IL-13 upon stimulation by combinations of IL-2 plus IL-25 or IL-33 [10].

More recently, IL-33 has been shown to be a more potent activator of lung ILC2s than IL-25. Barlow *et al.* demonstrated that IL-33 is more acutely prevalent (by 1000-fold) than IL-25 and is more potent in inducing ILC2s upon allergen challenge in the lung, suggesting a crucial role for IL-33 in an acute allergic lung response [24]. Accordingly, administration of the protease papain induced high IL-33 mRNA expression levels in the lung [25]. We and others have shown that intranasal injection of IL-33 directly stimulates ILC2s to proliferate and produce IL-13 and IL-5 [26,27]. However, *in vitro* experiments revealed that IL-33 alone does not stimulate highly purified ILC2s from naive mouse lungs [3]. Similarly, TSLP, IL-25, IL-7, or IL-2 alone had no effect on ILC2s. Interestingly, a combination of IL-33 with IL-2, IL-7, or TSLP induced production of high amounts of IL-5 and IL-13, whereas IL-33 plus IL-25 did not stimulate ILC2s [3]. By contrast, IL-25 stimulated type 2 cytokine production in lung explant cultures, which is likely mediated by lung ILC2s. A potential explanation for this observation is that IL-25 promotes stromal cell production of TSLP or IL-7 [28], which stimulates ILC2s in the presence of IL-33 [3]. IL-33 was constitutively produced in lung explant cultures, presumably due to mechanical stress. Kakkar *et al.* demonstrated that IL-33 responds to biomechanical strain placed on living cells as well as undergoing necrosis-mediated release [29]. Thus, IL-33 is required for but not sufficient for ILC2 activation because it needs TSLP, IL-2, or IL-7.

In addition to the receptors for IL-33, IL-2, IL-7, and TSLP, lung ILC2s also express the receptor for IL-4 (IL-4R). IL-4 and IL-33 have synergistic effects on cytokine production and proliferation of purified ILC2s *in vitro*, and IL-33-stimulated basophils have been shown to produce IL-4, which promotes cytokine production and proliferation of lung ILC2s *in vivo* [30]. In line with these findings,

**Table 1. ILC2 activation in lung disease and infection models<sup>a</sup>**

| Disease/infection  | Model                                                                     | ILC2-activating cytokines                                                                                          | Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Refs                  |
|--------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Allergy            | Cysteine proteases<br>• Papain<br>• Bromelain<br>• HDM (Der p 1; Der f 1) | Epithelium-derived IL-25, IL-33, and TSLP released through epithelial damage and PARs mediated by airway uric acid | <ul style="list-style-type: none"> <li>• Eosinophilia</li> <li>• Mucus hyperproduction</li> <li>• Increased serum IgE</li> <li>• Increased BAL infiltrates</li> <li>• Th2 cell mediated immunity (IL-4, IL-13, IL-5 secretion)</li> </ul>                                                                                                                                                                                                                                                                                                                       | [3,26,27,30,42,48]    |
|                    | Fungal extracts from <i>Alternaria alternata</i>                          | Mostly IL-33; low levels of IL-25 and TSLP                                                                         | <ul style="list-style-type: none"> <li>• Proliferation of ILC2s and basophils</li> <li>• Increased BAL infiltrates</li> <li>• Epithelial hyperplasia</li> <li>• Eosinophilia</li> <li>• TH2 cell mediated immunity</li> </ul>                                                                                                                                                                                                                                                                                                                                   | [55,56]               |
|                    | Chitin                                                                    | IL-25, IL-33, and TSLP                                                                                             | <ul style="list-style-type: none"> <li>• All cytokines induce ILC2-derived IL-13 and IL-5</li> <li>• IL-33 and TSLP increase numbers of ILC2s, eosinophils, and AAMs</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                 | [32]                  |
|                    | Cytokines                                                                 | rmIL-33<br><br>rmIL-25                                                                                             | <ul style="list-style-type: none"> <li>• Direct activation of ILC2s in wild type, <i>Rag1</i><sup>-/-</sup>, and <i>Rag2</i><sup>-/-</sup></li> <li>• Combined with OVA, results in Th2 activation</li> </ul> <p>Into <i>Rag1</i><sup>-/-</sup>:</p> <ul style="list-style-type: none"> <li>• Eosinophilia</li> <li>• ILC2 production of IL-5, IL-13</li> <li>• Mucus production</li> </ul> <p>Into wild type:</p> <ul style="list-style-type: none"> <li>• No increase in BAL cellularity</li> <li>• Little eosinophilia</li> <li>• Low ILC2 number</li> </ul> | [3,26,27]<br><br>[27] |
| Virus              | H1N1 PR8-GP33 virus                                                       | IL-33                                                                                                              | <ul style="list-style-type: none"> <li>• Following infection: ILC2-derived amphiregulin-dependent restoration of epithelial integrity (tissue repair)</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                | [20]                  |
|                    | Influenza A virus (strain Mem/71 (H3N1))                                  | IL-33 (produced by AMs)                                                                                            | <ul style="list-style-type: none"> <li>• Early in infection: induces AHR, mucus secretion</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                            | [57]                  |
|                    | Rhinovirus (administrated to neonatal mice)                               | Epithelium-derived IL-25 expression increases in neonatal but not adult mice                                       | <ul style="list-style-type: none"> <li>• Asthma-like pathophysiological changes</li> <li>• IL-13 expression</li> <li>• Mucus metaplasia</li> <li>• AHR</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                               | [58]                  |
| Helminth           | <i>Nippostrongylus brasiliensis</i>                                       | IL-25 and IL-33                                                                                                    | <ul style="list-style-type: none"> <li>• Production of IL-13 by ILC2s</li> <li>• Th2-mediated immunity</li> <li>• Helminth expulsion: eosinophilia, goblet cell hyperplasia, mucus secretion, IgE production</li> </ul>                                                                                                                                                                                                                                                                                                                                         | [18]                  |
| Asthma             | Leukotriene D4                                                            | Activation via CysLT1R on ILC2s                                                                                    | <ul style="list-style-type: none"> <li>• IL-4, IL-13, and IL-5 production by ILC2s</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | [59]                  |
| Pulmonary fibrosis | <i>Schistosoma mansoni</i> egg- induced pulmonary granuloma model         | IL-25 expression significantly upregulated                                                                         | <ul style="list-style-type: none"> <li>• High numbers of IL-13-secreting ILC2s</li> <li>• Less granuloma formation</li> <li>• Pulmonary collagen deposition</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                          | [60]                  |
|                    | Bleomycin (cancer chemotherapeutic agent)                                 | Airway epithelial cells produce full-length IL-33 and AMs process it into mIL-33 by neutrophil proteases           | <ul style="list-style-type: none"> <li>• High numbers of IL-13-producing ILC2s</li> <li>• AAM activation via IL-33 and IL-13</li> <li>• Activated, proliferating fibroblasts</li> <li>• Increased collagen synthesis</li> </ul>                                                                                                                                                                                                                                                                                                                                 | [61]                  |

<sup>a</sup>AAM, alternatively activated macrophages; AHR, airway hyper-reactivity; BAL, bronchoalveolar lavage; CysLT1R, cysteinyl leukotriene receptor 1; full-IL-33, full-length IL-33; HDM, house dust mite; IgE, immunoglobulin E; mIL-33, mature IL-33; OVA, ovalbumin; PAR, protease activated receptor; rm, recombinant protein; TSLP, thymic stromal lymphopoietin.

Kim *et al.* have reported that basophil-derived IL-4 stimulates ILC2s in the skin. It should be noted that IL-4 alone had little effect on ILC2s in both studies [31]. Thus, the stimulation of naive ILC2s appears to require two stimuli, namely IL-33 and either IL-2, IL-7, TSLP, or IL-4. IL-33 alone is able to stimulate ILC2s *in vivo* likely due to IL-4

production by IL-33-stimulated basophils or constitutive production of IL-7 by stromal cells and epithelial cells. Because IL-4-deficient mice treated with the protease allergen papain developed normal lung eosinophilia [26], IL-4 deficiency seems to be readily compensated for by other cytokines in ILC2 activation (Figure 1).

### ILC2 stimulation by allergens and T cell independent type 2 lung inflammation

Previously, T cells were thought to be the main mediators for type 2 immune response to allergens. However, Oboki *et al.* showed that inhalation of the protease allergen papain or recombinant IL-33 induced type 2 lung inflammation in T and B cell deficient mice, indicating that innate cells rather than Th2 cells were crucial [25]. Indeed, we found that intranasal injection of papain stimulated lung ILC2s, induced IL-5 and IL-13 production, and caused eosinophil infiltration and mucus hyperproduction [3]. Intranasal administration of other allergens, including house dust mite (HDM) and *Aspergillus* protease, also induced ILC2-dependent type 2 lung inflammation [26]. The fundamental role of ILC2s in the papain-induced lung inflammation was shown using ILC2-deficient mice generated by transplantation of ROR $\alpha$ -deficient mouse BM into irradiated host mice. Briefly, no eosinophil infiltration was observed when ILC2-deficient mice received intranasal papain injection, and inflammation was only seen upon ILC2 transplantation. Similar results were obtained by depleting ILC2s in *Rag1*<sup>-/-</sup> mice by anti-CD25 antibody injection before papain treatment of the mice [3,14].

IL-33 is crucial for the stimulation of lung ILC2s by intranasal injection of papain. IL-33-deficient mice showed

impaired ILC2 responses to papain, and intranasal injection of IL-33 strongly stimulated lung ILC2s and induced severe eosinophilic lung inflammation in wild type, but not in ILC2-deficient mice [26]. Recently, Van Dyken *et al.* showed a crucial role for IL-33, TSLP, and IL-25 in ILC2-mediated lung inflammation induced by intranasal administration of the non-protease allergen chitin, a polysaccharide from arthropods, parasites, and fungi. Chitin-induced IL-5 and IL-13 production by ILC2s, and eosinophilia was partially impaired in mice deficient for the receptors for TSLP, IL-25, or IL-33, whereas combined deficiency of all three cytokine receptors completely inhibited chitin-induced ILC2 activation and allergic responses [32]. These results highlight the importance of the epithelial cell derived cytokines, particularly IL-33, for allergen-induced ILC2 activation and type 2 lung inflammation.

IL-33 is a member of the IL-1 family of cytokines that includes IL-1 $\alpha$ , IL-1 $\beta$ , and IL-18. It is constitutively expressed in the nuclei of airway epithelial and endothelial cells [33]. Similarly to other members of the IL-1 family, full length IL-33 does not have a leader sequence for secretion, suggesting that IL-33 is not secreted via the same pathways as other cytokines. Although several cell types can release IL-33, alveolar epithelial type II cells are the major source of IL-33 in the lung [34]. It is thought that,

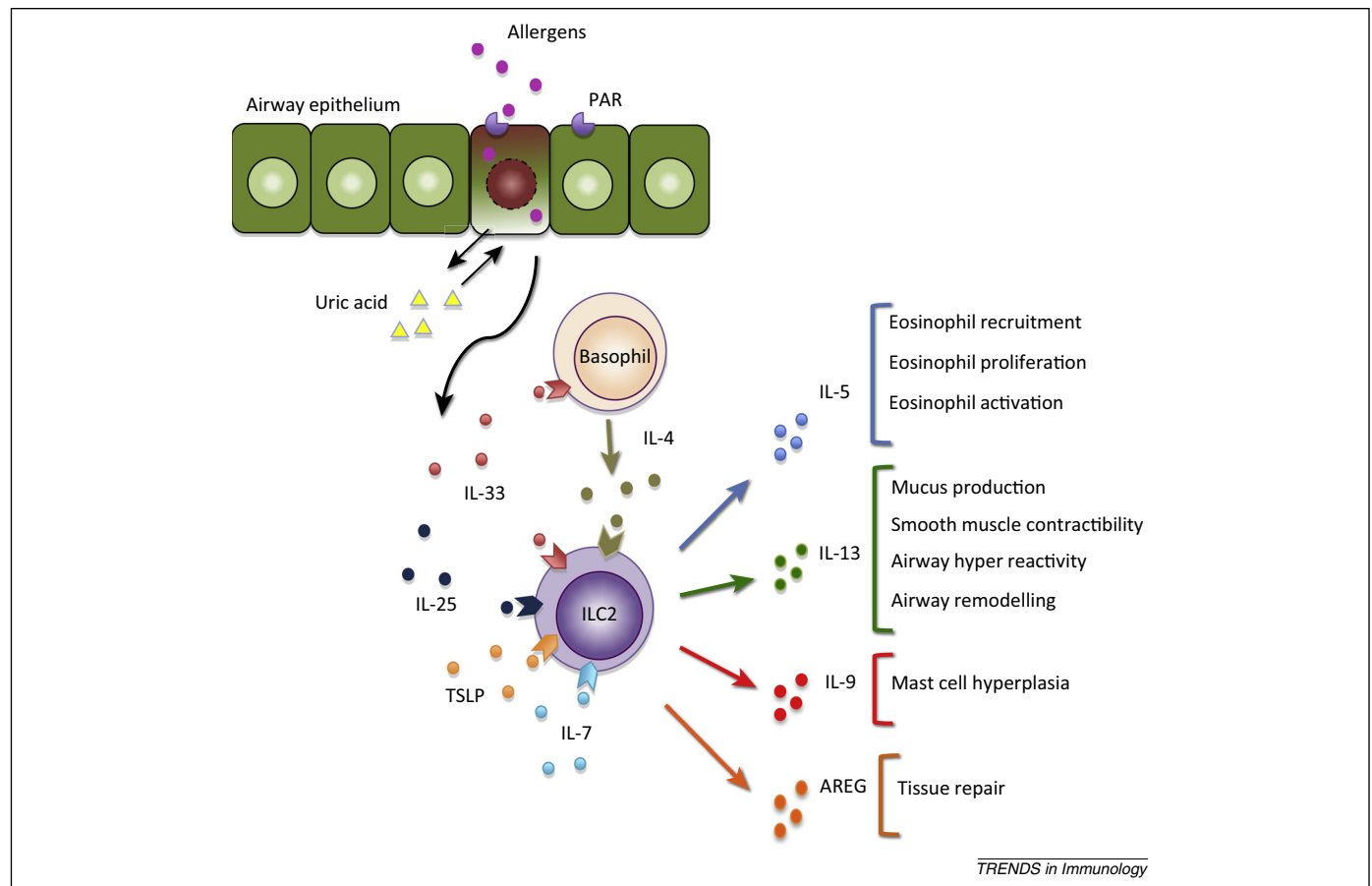


**Figure 1.** Stimulation of group 2 innate lymphoid cells (ILC2s) by epithelium-derived cytokines leading to innate type 2 immunity. Inhaled allergens induce the release of the alarmins IL-33 and uric acid as well as the secretion of IL-25 and TSLP by the lung epithelium. IL-33 release may be promoted by PAR activation or by uric acid. Epithelial cells also produce IL-25, TSLP, and IL-7. IL-33 stimulates basophils to produce IL-4. Lung ILC2s are stimulated by combinations of IL-33, TSLP, IL-7, and IL-4 and produce type 2 cytokines including IL-5, IL-13, IL-9, and amphiregulin. These cytokines act on different types of cells promoting type 2 lung inflammation characterized by eosinophilia, mucus production, smooth muscle contraction, and airway hyper-reactivity. Abbreviations: AREG, amphiregulin; IgE, immunoglobulin E; IL, interleukin; PAR, protease-activated receptor; TSLP, thymic stromal lymphopoietin.

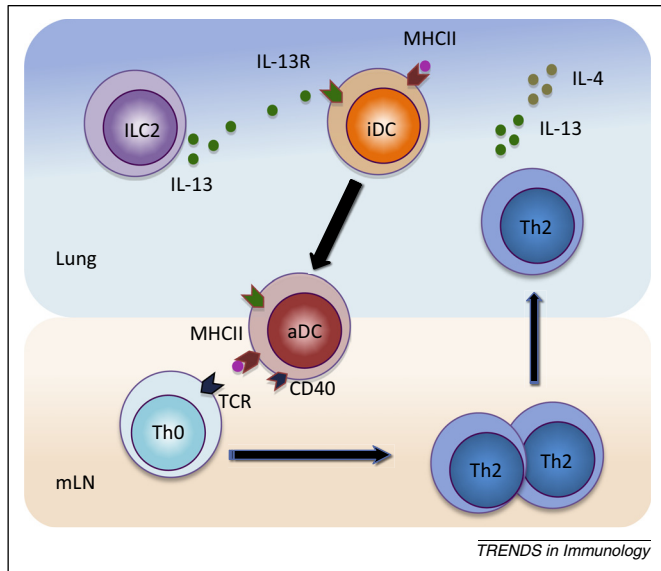


**Figure 2.** Lung group 2 innate lymphoid cells (ILC2s) promote Th2 cell differentiation. Upon allergen activation, lung ILC2s produce IL-13, which promotes activated CD40<sup>+</sup> dendritic cell (DC) migration to the draining mediastinal lymph node (mLN). DCs present antigen to naive CD4<sup>+</sup> T cells and drive their differentiation into Th2 cells. Th2 cells then migrate to the lung and produce type 2 cytokines such as IL-4, IL-5, and IL-13 and induce allergic lung inflammation. Abbreviations: aDC, activated dendritic cell; iDC, immature dendritic cell; IL, interleukin; MHCII, major histocompatibility complex class II; mLN, mediastinal lymph node; TCR, T cell receptor; Th2, T helper type 2.

upon necrosis or cell damage, IL-33 is released passively [35]. Thus, IL-33 can be considered to be an ‘alarmin’ that alerts the immune system to tissue damage. It is still unclear whether protease allergens induce IL-33 release simply by damaging epithelial cells, or whether protease-activated receptors (PARs [36]) on epithelial cells also play a role [37]. PAR2-deficient mice treated with HDM allergens showed decreased allergic lung inflammation, suggesting a role for PARs [38]. IL-33 activity may also be regulated by proteases released during inflammation, such as mast cell derived tryptase and neutrophil-derived cathepsin G, which cleave full-length IL-33 to generate a highly active mature form of IL-33 [39].

Uric acid, another well-characterized alarmin released by injured cells, was shown to stimulate dendritic cells and provide a link between cell injury and immunity [40]. Kool *et al.* found that intranasal administration of HDM allergen resulted in a rapid increase in uric acid in BAL fluid, followed by type 2 lung inflammation, and this was inhibited by uricase treatment [41]. Recently, Hara *et al.* found that uric acid was rapidly released into the airway lumen upon treatment with protease allergens including papain, whereas simultaneous treatment with uricase to remove uric acid strongly inhibited protease-induced type 2 lung inflammation [42]. The treatment of airway epithelial cells with uric acid also promoted IL-33 release *in vitro* whereas intranasal injection of uric acid crystals induced IL-33 release and type 2 lung inflammation *in vivo*. Thus, it appears that allergen-stimulated or damaged epithelial cells release uric acid, which in turn mediates IL-33 release by unknown mechanisms. However, other pathways of IL-33 release that are independent of uric acid may also exist [43]. Because IL-33-stimulated ILC2s produce

amphiregulin, a member of the EGF family, which promotes repair of damaged tissues, it has been suggested that IL-33 release and ILC2 stimulation is a feedback repair mechanism in response to tissue damage [20].

### Regulation of Th2 cells by ILC2s

The mechanisms by which inhaled allergens induce Th2 cell responses have been a key question that has yet to be resolved. It has been well established that the transcription factor GATA-3 is crucial for Th2 cell differentiation, whereas T-bet drives Th1 cell differentiation. However, what dictates the expression of GATA-3 versus T-bet in antigen-stimulated naive CD4<sup>+</sup> T cells has been unclear. It is generally thought that cytokines, particularly IL-12 and IL-4, are crucial for Th1 and Th2 cell differentiation, respectively [44]. However, Th2 cells were efficiently generated in IL-4-deficient mice upon helminth infection [45,46]. Repeated intranasal injections of papain also generated Th2 cells in IL-4-deficient mice [26]. Because both helminth infection and papain treatment are known to stimulate ILC2s, which do not secrete much IL-4, these studies suggested that ILC2s may play an important role in an IL-4-independent pathway of Th2 cell differentiation. Indeed, papain-induced Th2 differentiation and IgE production was impaired in ILC2-deficient mice, and the impairment was reversed by ILC2 transplantation. Therefore, ILC2s are important for effective generation of papain-specific Th2 cell responses. Further analysis of ILC2-deficient mice showed that the differentiation of naive T cells into Th2 cells was impaired, but was rescued by wild type, but not by *Il13*<sup>-/-</sup> ILC2s, indicating that ILC2-derived IL-13 is crucial. IL-13 did not directly act on naive CD4<sup>+</sup> T cells which do not express IL-13R. Instead, DCs expressed IL-13R, and IL-13 stimulated migration of activated DCs from the lung to the draining mediastinal lymph node (mLN) (Figure 2). CD4 T cell stimulation and Th2 cell differentiation in naive mice treated with papain seemed to take place primarily in mLN. Multiple intranasal papain treatment of LN-deficient mice generated by transplanting wild type BM into *Rag1*<sup>-/-</sup>*Il2rg*<sup>-/-</sup> mice resulted in much lower Th2 cell responses and allergic lung inflammation compared to normal mice, highlighting the importance of lung DC migration into the site of Th2 cell differentiation in naive mice [26]. The protease activity of papain is important for the activation of ILC2s, which promote Th2 skewing but are not required for the stimulation of memory Th2 cells [26]. It should be noted that proteases are important components of many allergens including those from fungi, ragweed, cockroach, and house dust mites [47].

Gold *et al.* also found that ILC2s are required for the development of allergic lung inflammation by multiple intranasal injections of HDM using ILC2-deficient mice. By contrast, in the classical ovalbumin (OVA) model of mouse asthma, in which OVA-specific T cells are sensitized by intraperitoneal injection of OVA combined with the Th2-promoting adjuvant alum followed by intranasal OVA challenge, ILC2-deficient mice were found to effectively generate Th2 cell mediated allergic lung inflammation [48]. Thus, ILC2s promote the initiation of Th2 cell

**Box 1. Outstanding questions**

- Is Th2 cell differentiation of naive CD4<sup>+</sup> T cells driven by unique co-stimulatory molecules on DCs or by the cytokine environment in mLN of protease allergen-treated mice?
- Do ILC2s, eosinophils, and basophils that accumulate in mLN of papain-treated mice play a role in allergic lung inflammation?
- Do ILC2s and T cells directly interact in the context of allergic inflammation? If so, do these interactions occur in the LN or in the tissue?
- Is the IL-33–ILC2–DC–Th2 pathway of Th2 cell induction applicable to non-protease allergens?
- What is the role of IL-9 in allergic inflammation? ILC2s are known to produce IL-9 and are stimulated by IL-9. ILC2-specific IL-9 knockout will likely answer the question.
- Does the inhibition or depletion of ILC2s have effects on ongoing allergic inflammation? Does it have therapeutic application in the treatment of allergic diseases?

differentiation whereas they do not seem to be required for the stimulation of memory Th2 cells.

Recent studies have shown that cells of the ILC family express MHC class II, suggesting another mechanism through which ILC2s may induce Th2 differentiation [49,50]. Oliphant *et al.* have recently shown that MHCII expression by gut ILC2s is crucial for the efficient expulsion of helminths from the gut [51]. They showed that ILC2s processed and presented antigen, albeit to a lesser extent than professional antigen-presenting cells, but not sufficiently to elicit T cell proliferation *in vitro*; instead, crosstalk between ILC2 and T cells through MHCII–TCR interaction led to IL-2 production by T cells that in turn promoted ILC2 proliferation and IL-13 production. Therefore, it seems there is a reciprocal interaction between ILC2s and T cells: ILC2s indirectly promote Th2 cell differentiation and T cells support ILC2 proliferation and activation. Preliminary data from our group failed to detect significant levels of MHC II expression or presentation of OVA antigen by mLN ILC2s from papain-treated mice (unpublished observations), and thus further studies will be necessary to examine the significance of MHCII–TCR interactions in the context of allergic inflammation.

**Concluding remarks**

Airway epithelial cell derived cytokines, including IL-33, IL-25, and TSLP, have long been known to promote type 2 immunity [52]. The discovery of ILC2s that are stimulated by these cytokines and produce type 2 cytokines has now provided a clue to the longstanding question of how inhaled allergens induce type 2 lung inflammation. Activation of ILC2s can result in innate type 2 inflammation because they produce large amounts of IL-5 and IL-13, which induce lung eosinophilia, mucus hyperproduction, and airway hyper-responsiveness, but not IgE production. IL-9 is another type 2 cytokine associated with lung inflammation. Interestingly, ILC2s are the main source of IL-9 in papain-stimulated airway inflammation [53]. While IL-9 is a known Th2 cytokine, it is still unclear what role ILC2-derived IL-9 plays in allergic inflammation.

While ILC2-mediated type 2 inflammation is T cell independent, ILC2s also play an important role in the initiation of Th2 cell mediated allergic lung inflammation. DCs in the airway epithelium are known to take up allergens and present them to naive CD4<sup>+</sup> T cells to stimulate

their differentiation to Th2 cells. However, the frequency of allergen-specific cells among lung-resident T cells in naive mice is likely extremely low. Indeed, Th2 cell generation in papain-treated naive mice lacking lymph nodes is very inefficient compared to normal mice [26]. Therefore, lung DCs must migrate to the draining mLN, where they can present antigens to circulating T cells. ILC2s are important for the stimulation of naive CD4<sup>+</sup> T cells because ILC2-derived IL-13 promotes the migration of lung DCs to the mLN. In allergen-sensitized mice, macrophages and B cells may present allergens to lung-resident memory T cells, but whether ILC2s play an essential role is unknown. Whereas the mechanisms by which mLN DCs in papain-treated mice stimulate Th2, but not Th1, cell differentiation remains to be elucidated, it should be noted that intranasal papain injection did not induce detectable IL-12 production in the lung. Thus, in papain-treated mice, naive CD4<sup>+</sup> T cells are stimulated by DCs in the absence of IL-12, a key Th1-inducing cytokine. Outstanding questions are listed in (Box 1).

In summary, we propose a common pathway of allergic lung inflammation induced by various allergens. Allergens provoke lung epithelium to release alarmins such as IL-33 which stimulate lung-resident ILC2s. Stimulated ILC2s produce large amounts of type 2 cytokines capable of triggering an allergic response. Moreover, ILC2-derived IL-13 promotes Th2 cell differentiation and therefore the activation of the adaptive response, which involves IgE production.

It has been thought that one of the important functions of type 2 immunity is to repair tissue damage [54]. Damaged airway epithelium release IL-33, IL-25, and TSLP that stimulate ILC2s, and ILC2 stimulation results not only in T cell independent type 2 inflammation but also in the initiation of Th2 cell responses. Indeed, intranasal injection of IL-33 together with OVA, which is inert and does not induce Th2 cell responses on its own, stimulates Th2 cell differentiation of OVA-specific OT-II T cells [26]. Thus, this pathway of type 2 immunity likely applies to a broad range of inhaled allergens that cause airway epithelium damage.

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