Innate Immune Cells to the Help

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A study by Halim and Steer (2014) in this issue of *Immunity* shows that innate lymphoid cells type 2 (ILC2s) are crucial for inducing adaptive T helper 2 immunity by providing interleukin-13. Another study by van Dyken et al. (2014) shows that ILC2s control eosinophilia and alternative activation of macrophages.

Chronic asthma is an inflammatory disease of the airway wall that leads to bronchial hyperreactivity and variable airway obstruction. Eosinophils are the predominant myeloid cells infiltrating the airway wall. Airway eosinophilia was long believed to be controlled by allergen-specific T helper 2 (Th2) lymphocytes producing the interleukin-5 (IL-5) necessary for eosinophil development, IL-4 driving Th2 cell polarization and immunoglobulin E (IgE) synthesis, and IL-13 driving goblet cell metaplasia and bronchial hyperreactivity. The precise signals that control the development of Th2 lymphocytes upon first encounter with allergens are not precisely understood. Upon inhalation of allergens containing enzymes or TLR agonists, airway dendritic cells and epithelial cells communicate via innate pro-Th2 cytokines like granulocyte-macrophage colony-stimulating factor (GM-CSF), thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 (see Figure 1). Dendritic cells (DCs) then migrate to the nodes and induce Th2 cell polarization but do not produce the prototypical cytokine IL-4 that is necessary to drive Th2 cell polarization. Therefore, induction of Th2 immunity has remained somewhat of an enigma.

The dogma that eosinophilic inflammation in asthma is exclusively controlled by adaptive immune cells has been recently challenged because tissue eosinophilia can also be controlled by innate lymphocytes that lack antigen specificity (Neill et al., 2010). Innate lymphoid cells type 2 (ILC2; also known as natural helper cells or nuocytes) accumulate in the lungs shortly after allergen exposure (Barlow et al., 2012; Halim et al., 2012). These ILC2s share many features with Th2 cells: they produce IL-5 and IL-13 and express the GATA3 transcription factor and chemokine receptors CCR4, CCR8, and CRTH2. The precise contribution of ILC2s to asthma pathogenesis is currently unclear. Most studies describing a role for ILC2s have been performed by comparing RAG1-deficient mice that lack T cells and B cells, with *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice that lack T and B cells and all ILCs. This might have overestimated the contribution of ILC2s in asthma. Also it is unclear whether ILC2s would influence generation of adaptive Th2 immunity or the function of DCs in mice with a fully functional immune system and could help explain the allergenicity of certain antigens.

In this issue of *Immunity*, Halim and Steer have studied C57Bl/6 mice reconstituted with RORα-deficient bone marrow from "staggerer" mice (*Rora*$^{sg/sg}$ mice) (Halim and Steer, 2014). These mice lack ILC2s, yet they have a fully functional immune system and can develop normal Th2 cell responses in vitro (Halim et al., 2012). After inhalation of papain, ILC2s were rapidly recruited to the lungs of wild-type (WT) mice. When challenged a few weeks later with a second series of papain exposures, mice mounted an even more robust eosinophilia, accompanied by heightened IgE responses, resulting from a GATA3^{+} CD4^{+} Th2 adaptive immune response to papain. Whereas the early eosinophilia was intact, the heightened challenge response was abolished in *Rag1*$^{-/-}$ mice lacking T and B cells. In *Rora*$^{sg/sg}$ mice, both the early innate and late heightened eosinophilic and IgE responses were abolished. As only ILC2s are lacking in these mice, this pointed to an enforcing role for ILC2s in adaptive Th2 immunity. The most striking observation of the paper is that ILC2s promote Th2 immunity not through IL-4 (which they hardly produce) but by provision of IL-13. IL-4 and IL-13 can use the same IL-4Rα chain to signal to T cells, and several models have illustrated that Th2 immunity can occur independently of IL-4. Papain is the first model to depend exclusively on IL-13 from ILC2s. In support, Halim and Steer show that Th2 immunity to papain and coadministered OVA in *Rora*$^{sg/sg}$ mice is restored by WT ILC2s, but not by IL-13-deficient ILC2s. What remains to be shown is whether IL-13 directly affects naive T cells or indirectly affects other cells like DCs. The authors do show that IL-13 stimulates migration of activated DCs to the lung draining nodes. An experiment in which T cells or DCs selectively lack the IL-4Rα chain could answer this question.

In a second article, van Dyken et al. have studied how ILCs contribute to allergenicity of chitin (van Dyken et al., 2014). Chitin is a polysaccharide found in the exoskeleton of parasites and arthropods and the hyphal cell wall of fungi. When inhaled, it immediately causes eosinophil-rich airway infammation and accumulation of alternatively activated macrophages (AAMs) that typically produce arginase and express chitinases under the influence of IL-4 and/or IL-13. The authors injected 50-70 μm chitin particles into the trachea, which led to an innate lung immune response within the first 24 hr that was dependent on IL-5 and IL-13. As shown with *Il5*^{RFP} reporter mice and *Il13*^{smart} mice (that report on IL-13 protein expression without affecting endogenous IL-13 levels), the source of these cytokines was shown to be ILC2s exclusively. Whereas the eosinophilia was dependent on ILC2-derived IL-5 and IL-13, the AAM phenotype was

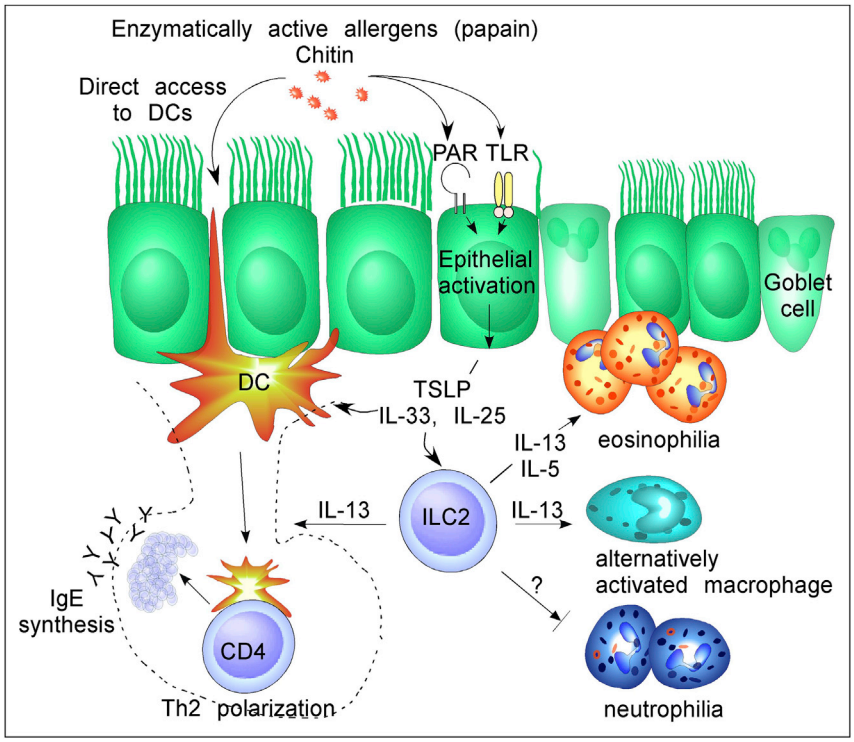

Figure 1. Early Recognition of Inhaled Allergens by Innate Immune Cells and Epithelial Cells
When allergens like the enzyme papain or the polysaccharide chitin are inhaled, they are recognized by epithelial cells and dendritic cells. Epithelial cells produce pro-Th2 cell cytokines like IL-25, TSLP, and IL-33 that activate ILC2s. The ILC2s then produce IL-13, which can activate DCs, so that these start inducing adaptive CD4 Th2 cell immunity (and IgE production) after migration to the draining nodes. ILC2s also produce IL-5 and IL-13, which drive eosinophila and alternative activation of arginase-producing macrophages. The ILCs also suppress the development of neutrophilic airway inflammation by unclear mechansims, involving suppression of γδ T cell-derived IL-17.

dependent exclusively on ILC2-derived IL-13. By using dual photon microscopy to image deep in the lung tissue, the authors found ILC2s in close vicinity to blood vessels, explaining how IL-5 could also be released in the bloodstream to affect bone-marrow output of eosinophils. To delete ILC2s in mice with a functional immune system, the authors cleverly made use of Cre-expressing mice in which ILCs get killed as soon as they commit to IL-5 or IL-13 cytokine production. ILC deletion led to reductions in early eosinophilia and AAM induction, comparable to levels seen in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice. Unexpectedly, ILC2 deletion also led to increased production of tumor necrosis factor alpha (TNF-α), IL-1β, and IL-23 and increased production of IL-17 by innate γδ T cells, causing increased airway neutrophilia. These suppressive effects of ILC2s on neutrophil and γδ T cell activation were not due to defective IL5 and/or IL-13 production but to an unidentified mechanism of suppression.

Barrier epithelial cells are ever more implicated in asthma pathogenesis, because they are able to sense exposure to allergens via pattern recognition receptors (PRRs) and activate DCs and other innate immune cells, through the secretion of TSLP, GM-CSF, IL-1, IL-33, and IL-25 (Lambrecht and Hammad, 2012). ILC2s express receptors for TSLP, IL-33, and IL-25. Chitin inhalation induced these pro-Th2 cytokines in the lung, but the combined absence of all three cytokine receptors did not affect ILC2 number, merely their activation status and production of IL5 and IL-13.

In conclusion, these papers help unravel some of the mysteries surrounding induction of acute type 2 immunity to inhaled model allergens. However, they do not yet answer the important question of whether ILC2s can be solely responsible for causing full-blown and persistent asthmatic airway inflammation to common environmental allergens, often accompanied by goblet cell metaplasia, airway wall remodeling, and bronchial hyperreactivity, so typical of chronic asthma. ILC2-driven asthma in the absence of Th2 cell immunity could be responsible for causing asthma in nonallergic asthmatics. Many of these patients also have chronic rhinosinusitis and nasal polyposis, known to contain many CRTH2^{+} GATA3^{+} ILC2s (Mjösberg et al., 2011). Overproduction of epithelial cytokines TSLP, IL-33, or IL-25 might intrinsically activate the ILC2 system, thus causing salient features of asthma and polyposis without the accompanying IgE induction so typical of adaptive Th2 cell immunity. Another point that needs to be addressed is how ILCs communicate with DCs and epithelial cells to promote Th2 cell immunity. Some of the functions of ILC2s (like suppression of Th17 and γδ T cell responses) do not depend on IL-5 and IL-13, and it will important to fully understand how this might work. The technical and conceptual advance offered by the two papers paves the way for these more mechanistic studies.

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