Published in final edited form as:

J Allergy Clin Immunol. 2013 July ; 132(1): 214–216. doi:10.1016/j.jaci.2013.05.019.

Activation of group 2 innate lymphoid cells: A new role for cysteinyl leukotrienes

Nora A. Barrett, MD and Joshua A. Boyce, MD

Department of Medicine, Division of Rheumatology, Immunology and Allergy, Jeff and Penny Vinik Center for Allergic Disease Research, Brigham and Women's Hospital, Boston, Mass

Keywords

Cysteinyl leukotrienes; group 2 innate lymphoid cells; asthma

The pathology of allergic inflammation in patients with asthma and allergic gastrointestinal disease is dominated by eosinophil infiltration and the local generation of a characteristic cassette of cytokines (IL-4, IL-5, and IL-13).¹ Although these cytokines can be generated by TH2 cells, studies in both human subjects² and rodents^{3–5} have implicated innate cell sources of the same cytokines. In 2010, several laboratories simultaneously reported novel innate lymphoid cells in the mesenteric fat and gut-associated lymphoid tissues that expanded and generated IL-5 and IL-13 in vivo in response to exogenous IL-25 and IL-33 or to helminth infection.^{6–8} A similar population was later found in mouse liver, spleen, and lung tissue.^{9,10} As such, these studies suggested that cells of the innate immune system could potentially initiate or amplify eosinophilic inflammation at mucosal sites in response to stimuli, such as tissue injury, viral infection, or pathogen-associated molecular patterns, without the requirement for antigen specificity. Shortly thereafter, a cell population with similar characteristics was identified in human intestine, lung, and nasal polyp tissue that expresses CRTH2, the chemoattractant receptor for prostaglandin D2.² A recent consensus has reclassified these cells in both mice and human subjects as group 2 innate lymphoid cells (ILC2s).¹¹

ILC2s arise from a common lymphoid progenitor that expresses the transcriptional repressor inhibitor of DNA binding 2. They are related to group 1 innate lymphoid cells (ILC1s; which include natural killer cells and IFN- γ -generating ILC1s) and group 3 innate lymphoid cells (which generate IL-17 and IL-22). Although ILC2s share the developmental requirement for IL-7 and IL-2 with ILC1s and group 3 innate lymphoid cells, they differ from these other innate lymphoid cells in their expression of retinoic acid receptor–related orphan receptor α and GATA3, a transcription factor also linked to the development of conventional TH2 cells.^{12,13} The role of ILC2s in human disease is speculative at this stage; however, mouse models suggest that this cell population might provide effector cytokines at

Corresponding author: Joshua A. Boyce, MD, Department of Medicine, Division of Rheumatology, Immunology and Allergy, Jeff and Penny Vinik Center for Allergic Disease Research, Brigham and Women's Hospital, 1 Jimmy Fund Way, Smith Research Bldg, Rm 616C, Boston, MA 02115. jboyce@rics.bwh.harvard.edu.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

levels sufficient to eliminate helminths,⁸ amplify antigen-induced airway hyperreactivity,¹⁴ trigger eosinophilic pulmonary inflammation, and promote both airway hyperreactivity and tissue healing after influenza virus infection.^{15,16} In each instance their activation is thought to occur principally through IL-33, IL-25, and/or thymic stromal lymphopoietin generated by the perturbed barrier cells.

Cysteinyl leukotrienes (CysLTs) are arachidonic acid–derived lipid mediators and the most potent known constrictors of smooth muscle.¹⁷ Leukotriene (LT) C₄, the parent CysLT, is generated through the 5-lipoxygenase and LTC₄ synthase pathway^{18,19} in activated hematopoietic cells, including eosinophils, mast cells, basophils, macrophages, myeloid dendritic cells, and platelet-granulocyte complexes.^{20–25} LTC₄ is enzymatically converted to the short-lived but powerful smooth muscle constrictor LTD₄²⁶ and then to the stable metabolite LTE₄.²⁷ CysLTs are generated during type 1 hypersensitivity reactions as the product of IgE cross-linking. However, CysLTs are also generated by dendritic cells or macrophages in response to innate signaling pathways initiated by zymosan, peptidoglycan, IgG-opsonized microbes, and house dust mites.^{23,28–30} All 3 CysLTs are active in vivo, generating vascular leak, bronchoconstriction, mucus hypersecretion, and eosinophil accumulation.³¹ These functions reflect the capacity of CysLTs to induce signaling through at least 3 G protein–coupled receptors: CysLT₁R (a high affinity receptor for LTD₄),³² CysLT₂R (a lower-affinity receptor for LTC₄ and LTD₄),³³ and the recently identified CysLT₃R with a binding preference for LTE₄.³⁴ CysLTs are produced at high levels in human patients with allergic diseases, especially in patients with aspirin-exacerbated respiratory disease.³⁵ Their importance as disease effectors is validated by the clinical efficacy of drugs that block their synthesis³⁶ or their capacity to induce signaling through CysLT₁R.³⁷ Recent studies in rodent models have expanded the function of the CysLTs, including a prominent role in priming dendritic cells to induce TH₂-type immunity to dust mite allergens.^{23,38} Moreover, the broad expression of CysLT receptors on cells of both the innate³⁹ and adaptive⁴⁰ immune systems suggests additional functions and cellular targets to be identified.

In this issue of the Journal, Doherty et al⁴¹ report an entirely novel function for the CysLTs, namely the activation and expansion of ILC2s. The fungal allergen species *Alternaria* can induce the rapid release of IL-33 from epithelial cells and cause the accumulation of eosinophils independently of the adaptive immune system.⁴² Doherty et al⁴¹ found that *Alternaria* challenge of naive mice caused the production of high CysLT levels, as detected by ELISA from bronchoalveolar lavage fluid obtained 12 hours after a single dose of *Alternaria* extract. Using flow cytometry and real-time PCR, the investigators demonstrated that *Alternaria* expanded the population of lung ILC2s (identified as lineage-negative, Th₁.2-positive cells in the lymphocyte gate) and that ILC2s in both naive and *Alternaria*-challenged mouse lung tissue express CysLT₁R protein and transcripts, respectively. This expression did not depend on signal transducer and activator of transcription 6 or the presence of an adaptive immune system. As expected, ILC2s isolated and cultured from the lungs of *Alternaria*-challenged mice generated abundant quantities of IL-5 and IL-13 when stimulated with IL-33. Surprisingly, LTD₄ induced similar quantities of IL-5 and IL-13 from ILC2s, as did IL-33. It also induced the generation of large quantities of IL-4, which was not elicited by IL-33. The intrapulmonary administration of LTC₄, LTD₄, and LTE₄ to

naive mice each increased the percentages of ILC2s in the lung that expressed IL-5, as determined by using intracellular cytofluorographic staining. Importantly, pretreatment of the mice with the CysLT1R antagonist montelukast blocked IL-5 generation by ILC2s in response to LTC4 and LTD4, whereas the effects of LTE4 were completely resistant to montelukast treatment. Finally, the intrapulmonary administration of LTD4 did not elicit proliferation of ILC2s when given to naive mice but did potentiate proliferation in the setting of *Alternaria* challenge.

The findings of Doherty et al.⁴¹ carry many implications. First, the fact that LTD4 was as potent as IL-33 for inducing ILC2s to generate IL-5 and IL-13 suggests that endogenous CysLTs can amplify the production of effector cytokines through ILC2s, promoting eosinophilia and airway reactivity. Second, the capacity of LTD4, but not IL-33, to elicit the generation of large quantities of IL-4 by ILC2s suggests a potential link to the TH2priming functions of the CysLTs in mouse models of allergen-induced pulmonary inflammation.^{23,38} Third, CysLTs generated by tissue-resident innate immune cells (dendritic cells, mast cells, and macrophages) in response to natural allergens might contribute not only to breaking tolerance and activating the adaptive immune response but also to driving pathology that is independent of adaptive immunity. Lastly, the fact that LTE4, the most stable and abundant of the CysLTs, can drive IL-5 production through a mechanism that is resistant to CysLT1R antagonists is potentially highly significant. LTE4 induces pulmonary eosinophilia through a pathway that depends on the P2Y12 purinergic receptor⁴³ and induces cutaneous vascular leak in vivo through a novel CysLT3R.³⁴ Both P2Y12 and CysLT3R resist blockade by CysLT1R antagonists, and it is tempting to speculate that one or both receptors might also be expressed by ILC2s and might account for the montelukast-resistant effects of LTE4 identified in the study by Doherty et al.⁴¹ It also seems possible that high levels of LTE4 found in biologic fluids from patients with aspirin-exacerbated respiratory disease could drive effector responses from ILC2s. This would be consistent with the persistent tissue eosinophilia, airway remodeling, and nasal polyposis, frequently with no sensitization to allergens, that characterize this disease.

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