Eosinophils Express LTA4 Hydrolase and Synthesize LTB4: Important for Asthma Pathogenesis?

Although inflammation in asthma is often characterized as type 2 in nature as a result of the presence of T-helper cell type 2 (Th2) lymphocytes, group 2 innate lymphoid cells (ILC2), eosinophils, and mast cells in the airway and lung, many patients with asthma, particularly those with more severe disease, have substantial airway neutrophilia (1). The mechanisms by which this airway neutrophilia occurs and the role of neutrophils in asthma pathogenesis are not well defined. Recently, there has been a marked interest in the contribution of the Th17 cytokines IL-17A and IL-17F to the recruitment of neutrophils to the airway in asthma; however, in one study, antagonism of this pathway did not have an impact on asthma outcomes (2). These results raised questions as to whether the subjects with asthma were properly phenotyped to examine the role of IL-17 antagonism, whether the antagonist used was one that could affect Th17 inflammation, and whether there may be a different pathway that leads to neutrophilic airway inflammation. Leukotriene B_4 (LTB4) is a potent neutrophil chemoattractant (3), and this mediator might be an alternative to the Th17 axis as a potential mechanism leading to airway neutrophils. In this issue of the *Journal*, Pal and colleagues (pp. 413–419) present the possibility that eosinophils are a source of LTB4 that might explain the mixed Th2/neutrophilia seen in some subjects with asthma (4).

A brief review of leukotriene biology provides context to this work (5). Arachidonic acid is converted to LTA4 by 5-lipoxygenase (5-LO), and LTA4 may be metabolized through one of two distinct pathways. LTA4 can be converted by LTA4 hydrolase into LTB4. LTB4 signals through the receptors BLT_1 and BLT_2. LTB4 signaling has a range of effects on different cell types that drive inflammatory responses. Most importantly, LTB4 signaling through BLT_1 promotes neutrophil chemotaxis. Alternatively, LTA4 may be metabolized to LTC4 by LTC4 synthase, which can be converted sequentially into LTD4 and LTE4. LTC4, LTD4, and LTE4 are known as the cysteinyl leukotrienes, and one or more of these lipids may signal through the individual cysteinyl leukotriene receptors $CysLT_1R$, $CysLT_2R$, and $CysLT_3R$ (GPR99) (6). Montelukast, a $CysLT_1R$ antagonist, is approved for asthma treatment (7). Strategies to inhibit LTB4 signaling could include blocking the activity of either 5-LO or LTA4 hydrolase, or preventing LTB4 binding to its receptors. The 5-LO inhibitor zileuton is used clinical practice, but it also reduces the generation of cysteinyl leukotrienes, so its specific effect on LTB4 inhibition in asthma pathogenesis is unknown. There are currently no LTA4 hydrolase or LTB4 receptor antagonists used in clinical practice, so data on the contribution of LTB4 signaling on airway neutrophilia are unavailable. Traditionally, neutrophils, mononuclear phagocytes, and epithelial cells have been regarded as the cell types responsible for LTA4 hydrolase expression and the production of LTB4; therefore, the possibility that eosinophils express LTA4 hydrolase and synthesize LTB4, and modulate neutrophilia through this mechanism is novel.

Pal and colleagues use a multipronged approach to show that eosinophils express LTA4 hydrolase and produce LTB4, and, most importantly, they carefully eliminate the possibility that contaminating cells may confound their results. They identified LTA4 hydrolase via immunofluorescence assays in eosinophils taken from both BAL fluid and blood from subjects with and without asthma. They further demonstrate that eosinophils express LTA4 hydrolase by flow cytometry, and they minimize the possibility that their eosinophil population was contaminated by finding that only 0.7% of their presumed eosinophil population expresses CD16b (FcγRIII), a cell surface receptor not found on eosinophils. They show that roughly two-thirds of their eosinophil population express 5-LO, and of those 5-LO–expressing cells, over 99% express both LTA4 hydrolase and LTC4 synthase. This study further substantiates LTA4 expression in purified blood eosinophils via qPCR. In addition, the authors report that eosinophils not only express LTA4 hydrolase but also release LTB4. Peripheral blood eosinophils cultured *in vitro* produced LTB4 when unstimulated, and they released significantly elevated levels of LTB4 when stimulated with aspirin-lysine, PMA, and platelet-activating factor. Taken together, these results clearly demonstrate that eosinophils express LTA4 hydrolase and release LTB4. This discovery is significant, as it identifies yet another cell type that produces LTB4. In addition to neutrophils, epithelial cells, and certain phagocytes, we can now classify eosinophils as contributors to the generation of this inflammatory mediator. These results do, however, raise some important questions.

First, does LTB4 produced by eosinophils contribute significantly to asthma pathophysiology? The authors compare the levels of LTA4 hydrolase mRNA in blood eosinophils isolated from both patients with asthma and control subjects. Although these data show that LTA4 hydrolase is indeed expressed by eosinophils, there is no difference in the quantity of LTA4 hydrolase mRNA between patients with asthma and control subjects. If LTB4 of eosinophilic origin made a meaningful contribution to asthma, one might expect that eosinophils from subjects with asthma would have greater expression of the enzyme responsible for LTB4 production. Surprisingly, *in vitro* release of LTB4 in stimulated and unstimulated peripheral blood eosinophils was lower in those obtained from patients with asthma compared with healthy control subjects. The authors did not report a statistically significant difference between LTB4 produced from eosinophils from patients with asthma and control subjects. The question of whether eosinophil-produced LTB4 plays a significant role in asthma pathophysiology was again raised by the finding that the BAL fluid from patients with severe neutrophilic asthma had >10-fold more LTB4 than the BAL fluid from patients with eosinophilic asthma. This difference calls into question the overall contribution of eosinophils to airway LTB4.

Considering these new data, it is important to evaluate the therapeutic potential of targeting LTB4/BLT signaling in asthma.

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Multiple clinical trials in the past have evaluated the efficacy of various pharmacologic agents targeting this signaling axis in the context of asthma. Of note, a clinical trial examining an LTA4 hydrolase inhibitor (JNJ 40929837) found no effect on forced expiratory volume in 1 second (8, 9). The lack of success with specific targeting of LTB4 and the BLT receptors calls into question this pathway's potential for therapeutic investigation. It will be interesting to determine whether the IL-5 antagonist biologics reduce airway neutrophils, as these drugs target the cytokine that promotes eosinophil differentiation and survival. If they do not, this would be further evidence that eosinophil production of LTB4 may not be responsible for airway neutrophils in severe asthma. Although their therapeutic utility might be limited, the results of this study are nonetheless paradigm shifting in that the authors have carefully shown that eosinophils produce and release LTB4, contributing to the ever-expanding cache of inflammatory mediators that we can attribute to these cells. ■

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Mark Rusznak, B.A.
R. Stokes Peebles, Jr., M.D.
Division of Allergy, Pulmonary, and Critical Care Medicine
Vanderbilt University School of Medicine
Nashville, Tennessee

ORCID ID: 0000-0002-1429-7875 (R.S.P.).

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