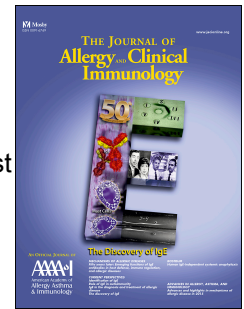


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A New Spin on Mast Cells and Cysteinyl Leukotrienes:**Leukotriene E₄ Activates Mast Cells *in vivo*****Lora G Bankova, MD¹, Joshua A Boyce, MD¹**

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Cysteinyl leukotrienes (cysLTs) are derived from arachidonic acid generated upon activation of hematopoietic cells. 5-lipoxygenase (5-LO) oxidizes arachidonic acid from the outer nuclear membrane in the presence of 5-LO activating protein (FLAP) to leukotriene A₄ (LTA₄), which is subsequently converted to LTC₄ by leukotriene C₄ synthase (LTC₄S). LTC₄ is exported and sequentially converted to LTD₄ and LTE₄. LTE₄ is stable, and is hence the most abundant cysLT in vivo, being detectable in the bronchoalveolar lavage and urine in patients with asthma exacerbations.¹ While each cysLT can elicit airway constriction and vascular leak in vivo, LTE₄ can also induce eosinophil and basophil accumulation in the bronchial mucosa¹, and enhances the sensitivity of both human and guinea pig airways to subsequent histamine-induced contractions.¹ Hence, the mechanisms by which LTE₄ elicits physiologic responses are of significant potential biologic importance.

CysLTs exert their effects through three G-protein coupled receptors. CysLT₁R has high affinity for LTD₄ (K_d ~ 1 nM), and mediates cysLT-driven smooth muscle constriction.¹ It binds LTC₄ with lesser affinity, and can transduce signals to LTE₄ as well. CysLT₂R, which is resistant to montelukast and other CysLT₁R antagonists, binds LTC₄ and LTD₄ at equimolar concentrations.¹ CysLT₃R (OXGR1 or GPR99) is a high affinity receptor for LTE₄.² The CysLT₁R and CysLT₂R receptors are broadly expressed by structural and hematopoietic cells, including MCs.¹ CysLT₃R is dominantly an epithelial cell receptor.³ Hence the full spectrum of cysLT effects in general, and LTE₄ effects in particular, likely reflect their actions at several receptors and cellular targets. Moreover, these actions are likely to be influenced not only by the levels of cysLTs present, but also by the numbers of cysLT-responsive cells that may change because of disease.

LTE₄ has activity in patients with asthma that distinguishes it from its precursor cysLTs.

LTC₄ and LTD₄ both induce airflow obstruction at doses 100-1000-fold lower than histamine in both asthmatic and non-asthmatic subjects. LTE₄ is only ~40-fold more potent than histamine, but asthmatic subjects are ~10-fold more sensitive to LTE₄-induced bronchoconstriction than non-asthmatic subjects, and subjects with AERD exhibit even higher sensitivity to LTE₄ than do aspirin tolerant controls.¹ As noted earlier, inhaled LTE₄ (but not LTD₄) can induce the accumulation of eosinophils and basophils in the bronchial mucosa of subjects with mild asthma¹, and can enhance their reactivity to histamine.¹ Notably, the potentiation of histamine responsiveness induced by LTE₄ inhalation can be blocked by the administration of indomethacin.⁴ Moreover, indomethacin and a selective antagonist of the T prostanoid (TP) receptor prevented the LTE₄-induced potentiation of histamine contractility of human bronchi and guinea pig trachea ex vivo.¹ The increased sensitivity of asthmatic airways to LTE₄ could reflect a change in receptor expression or a specific effect on recruited cells not present in non-asthmatics. Additionally, at least some of the effects of LTE₄ involved secondary generation of cyclooxygenase (COX) products, implying (a) unique cellular target(s) and mechanisms.

Stimulation of human cord blood-derived MCs with LTD₄ can elicit strong calcium fluxes, accompanied by their production of several cytokines and chemokines that are blocked by CysLT₁R antagonists.⁵ When stimulated with LTE₄, LAD2 cells (a human MC line) upregulate their expression of COX-2 and generate substantial amounts of prostaglandin D₂ (PGD₂), a COX product with strong bronchoconstricting properties (through TP receptors) that is also a selective chemoattractant for eosinophils and basophils (through CRTH2)⁵. Curiously, this response to LTE₄ was blocked by the selective antagonist of CysLT₁R MK-571, but was resistant to knockdown of CysLT₁R, implying the potential presence of a second MK-571-sensitive receptor. In patients with aspirin exacerbated respiratory disease (AERD), aspirin causes idiosyncratic

reactions with a surge in cysLT production accompanied by increases in MC-derived mediators (histamine, tryptase, and PGD₂ metabolites) detected in biological fluids indicative of MC activation. The administration of the 5-LO inhibitor zileuton prevented the increase in nasal lavage fluid tryptase levels in response to challenge of subjects with AERD using topical lysine aspirin.⁶ Collectively, these studies imply that endogenously generated cysLTs can drive MC activation, at least in patients with AERD, and that cysLTs (particularly LTE₄) can elicit the production of contractile COX products by MCs as a theoretical downstream effector.

The study by Lazarinis et al. in this issue of the Journal finds that LTE₄-elicited bronchoconstriction in subjects with mild asthma is associated with sharp increases in the urinary levels of metabolites of the MC-derived mediator PGD₂, as well as thromboxane A₂, and several other COX pathway products (PGF₂α, PGE₂).⁷ Furthermore, both LTE₄-induced bronchoconstriction and LTE₄-elicited COX products (with the noteworthy exception of PGE₂) were entirely abolished by montelukast pre-treatment for 7 days. This is the first direct demonstration that LTE₄ elicits CysLT₁R-dependent activation of MCs, and confirms that LTE₄ can induce the formation of several COX products, at least some of which likely derived from MCs.

There are several pathobiologic implications of the study by Lazarinis and colleagues. First, the findings are consistent with the sensitivity of LTE₄-mediated histamine hyperresponsiveness to indomethacin, which blocks the formation of all COX products.¹ Second, each of the COX pathway products elicited by LTE₄ inhalation (PGD₂, TXA₂, PGF₂, and PGE₂) can contract human airways by a mechanism that is blocked by TP receptor antagonists.⁸ Thus, at least some of the contractile effects of LTE₄ could be secondary to direct actions of prostanoids. Third, PGD₂ (acting at CRTH2) and LTE₄ (acting at a montelukast-responsive

receptor) synergize to promote the generation of the pathogenic cytokines IL-4, IL-5, and IL-13 by human Th2 cells and ILC2s.⁹ Although the Lazarinis study did not identify a difference in sputum eosinophil counts between the placebo and montelukast arms of the study, it did demonstrate a rapid LTE₄-induced decline in blood eosinophils that was montelukast-resistant. The ability of LTE₄ to promote recruitment of eosinophils and basophils in vivo may reflect both CysLT₁R-dependent (i.e., MC-derived PGD₂) and -independent actions. Moreover, the COX products induced by LTE₄ could broadly effect the functions of airway cells that express receptors for prostanoids, even if they lack receptors for cysLTs. These effects are likely to be most prominent in settings such as AERD, where local levels of LTE₄ are especially high, and are coupled with high end-organ LTE₄ sensitivity.

The Lazarinis study also leaves some important questions yet to be answered. Whether LTE₄ elicits bronchoconstriction directly or by downstream prostaglandins remains to be clarified. Indeed, the previously identified shifts in the doses of LTE₄ required to elicit bronchoconstriction in subjects with asthma and (especially) with AERD could be due to increments in the numbers and/or function of LTE₄-responsive MCs or other cell types that generate smooth muscle active prostaglandins. Additionally, while the findings clearly implicate a montelukast-sensitive receptor (likely CysLT₁R) as being essential for MC activation and bronchoconstriction in response to LTE₄, they do not mitigate against the involvement of other receptors and cell targets. In mouse studies, LTE₄ strongly induces MC activation and COX pathway products by a pathway requiring epithelial cell-derived IL-33; this mechanism is montelukast-sensitive.¹⁰ LTE₄ also induces epithelial cell mucin release by acting exclusively at CysLT₃R/GPR99, rather than at CysLT₁R, when administered intranasally to naïve mice.³ The inability of montelukast to prevent the LTE₄-induced production of PGE₂, which is generated

abundantly by epithelial cells, could reflect actions at CysLT₃R/GPR99 (or CysLT₂R) as another relevant target in LTE₄-induced airway biology. Carefully conducted pharmacologic and physiologic human studies such as the Lazarinis study, aided by biologics and antagonists of other cysLT receptors in development, can ultimately integrate our understanding of the full range of mechanisms underlying the role of LTE₄, the cysLT receptors, and their cellular targets, as well as their therapeutic potential.

Figure legend: LTE₄ activates MCs in a montelukast-dependent fashion through CysLT₁R for production of PGD₂ and TXA₂. LTE₄-elicited MC-derived COX products may activate the bronchial smooth muscle through the TP receptors leading to bronchoconstriction. LTE₄ might also contribute to this effect through direct activation of CysLT₁R. LTE₄ also elicits montelukast-independent epithelial cell activation for production of PGE₂ and possibly other epithelial cell cytokines. The combined effects of PGD₂, LTE₄ and epithelial cell cytokines lead to LTE₄-elicited inflammation that is only partially suppressed by montelukast.

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