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An update on the role of leukotrienes in asthma

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Abstract

Purpose of review—Leukotrienes (LT)s are lipid mediators involved in the pathogenesis of asthma. There is significant new information about the actions of LTs in asthma, and the evolving role of anti-LT therapies. We review recent findings on regulation of LT synthesis, biological function of LTs in disease models, and use of LT modifiers in clinical practice.

Recent findings—Our understanding of the regulation of LT synthesis at a molecular level has greatly advanced. Recent evidence indicates that genetic variation in the leukotriene synthetic pathway affects the clinical response to LT modifiers. The participation of LTB_4 in the allergic sensitization process in animal models suggests a larger role for LTB_4 in asthma. Preclinical and *in vitro* models suggest that the cysteinyl LT (CysLT)s are important in airway remodeling. LTs are key mediators of exercise-induced bronchoconstriction (EIB) with recent studies demonstrating that LT modifiers reduce the severity of EIB during short-term and long-term use.

Summary—LTs are clearly involved in airway inflammation and certain clinical features of asthma. Evolving evidence indicates that LTB_4 has an important role in the development of asthma, and that CysLTs are key mediators of the airway remodeling process.

Keywords

Asthma; exercise-induced bronchoconstriction; leukotriene; pharmacogenetics; remodeling; T-cell

Introduction

Leukotriene (LT)s are important lipid mediators involved in asthma, allergic inflammation and innate immunity. Unlike many mediators that are preformed, LTs are synthesized *de novo* by a pathway of oxidative lipid metabolism in response to various stimuli. Substantial progress has been made recently in understanding the regulation of LT biosynthesis at a cellular and molecular level. Recent evidence indicates that genetic variation in the LT synthetic pathway may explain the differences in the pharmacological response to anti-LT therapies in asthma. In addition to a very clear role that the cysteinyl LT (CysLT)s play in asthma, evidence has emerged for a role of LTB_4 especially during sensitization. Although few studies have

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addressed the effects of drugs modifying the LT pathway on remodeling in humans, there is strong pre-clinical evidence that LTs are involved in development of mucus cell hyperplasia, airway smooth muscle thickening, and subepithelial fibrosis. Asthmatics with exercise-induced bronchoconstriction (EIB), have increased levels of LTs in their airways, and response to anti-LT treatments both acutely and during chronic therapy.

Regulation of the leukotriene synthetic pathway

Formation of LTs and other eicosanoids is initiated by release of unesterified arachidonic acid, liberated by hydrolysis at the sn-2 position of membrane phospholipids by phospholipase A_2 (PLA_2). A family of membrane-associated proteins that includes 5-lipoxygenase (5-LO) and 5-lipoxygenase-activating protein (FLAP) acts on free arachidonic acid to form LTs. During activation, a multiprotein complex assembles on the outer and inner nuclear membranes centered on the integral membrane protein FLAP that serves as a scaffold protein for 5-LO [1••]. Arachidonic acid is transferred by FLAP to 5-LO initiating the oxygenation of arachidonic acid to 5(S)-hydroperoxyeicosatetraenoic acid (5S-HpETE), followed by dehydration to the unstable epoxide leukotriene A_4 (LTA_4). The critical enzyme in formation of CysLTs (i.e., LTs C_4, D_4, and E_4) from LTA_4 is LTC_4 synthase (LTC_4S) that is part of a family of membrane-bound proteins involved in eicosanoid and glutathione metabolism including FLAP, microsomal glutathione S-transferase (MGST)s, and microsomal prostaglandin E synthase 1. In contrast with MGSTs, LTC_4S conjugates glutathione with a high degree of substrate selectivity for LTA_4 imparted by the structure of the enzyme [2,3]. The alternative pathway for LTA_4 is conversion to LTB_4 by LTA_4 hydrolase.

Because expression of 5-LO is largely restricted to myeloid cells, the majority of LT synthesis is restricted to leukocytes; however, arachidonic acid and intermediates such as LTA_4 are permeable across cell membranes, allowing for transcellular metabolism of eicosanoids. Eicosanoid production in leukocytes is increased when the leukocyte is co-cultured with a structural cell such as an epithelial cell [4]. In important recent work, 5-LO deficient mice transplanted with immune cells deficient in either LTA_4 hydrolase or LTC_4S were able to make near normal quantities of LTB_4 and normal quantities of CysLTs respectively, demonstrating that 5-LO-containing immune cells transfer intermediates that restore LT synthetic capacity by transcellular metabolism and that structural cells play an important role in LT synthesis [5•].

Significant advances have led to a better understanding of the regulation of arachidonic acid release from membrane phospholipids by PLA_2 that may be the rate-limiting step in eicosanoid formation. Although cytosolic $PLA_2\alpha$ ($cPLA_2\alpha$) plays a key role in eicosanoid formation and is co-localized with membrane associated 5-LO during activation, recent studies have identified a family of secreted PLA_2 ($sPLA_2$)s that may be upregulated by inflammatory stimuli, and are involved in the generation of arachidonic acid. Groups V and X $sPLA_2$ (i.e., $sPLA_2$-V and $sPLA_2$-X) are of unique functional importance because they initiate cellular eicosanoid synthesis at the outer plasma membrane rich in phosphatidyl choline and sphingomyelin [6]. Early studies showed that $sPLA_2$ activity was released into bronchoalveolar lavage (BAL) and nasal lavage fluid in patients with asthma and rhinitis respectively following allergen challenge. More recent work demonstrates an increase in $sPLA_2$ activity in BAL fluid of subjects with asthma compared to normal controls [7•] and an increase in $sPLA_2$-X protein in induced sputum following exercise challenge and an increase in immunostaining for this enzyme in epithelial cells and macrophages following challenge [8]. In a murine model of asthma, genetic deficiency of $sPLA_2$-X inhibits the development of airway inflammation, formation of LTs, airway hyperresponsiveness (AHR), and structural remodeling of the lung [9]. Either genetic deficiency of $sPLA_2$-V or a blocking antibody against murine $sPLA_2$-V in a murine asthma model prevents the development of allergen-induced airway inflammation

and AHR [10]. Further research is needed to understand the roles that $sPLA_2$s play in regulation of LT synthesis and diseases such as asthma.

CysLTs and LTB_4 function through distinct sets of receptors termed CysLT and the BLT receptors respectively. CysLTs have a clearly defined role in asthma, perpetuating airway inflammation, leading directly to airflow obstruction through effects on vascular permeability, mucus production, and smooth muscle constriction, and contribute to airway remodeling in murine asthma models. CysLT receptors are G-protein-coupled receptors that mediate cellular function. Two CysLT receptors have been have been characterized in detail and are designated the $CysLT_1$ and $CysLT_2$ receptors. The $CysLT_1$ receptor has the highest affinity for LTD_4, is increase in the bronchial mucosa of asthmatics, and clearly mediates many of the pathophysiological effects of CysLTs in asthma [11]. The $CysLT_2$ receptor, which has an equal affinity for LTC_4 and LTD_4, is found on leukocytes, smooth muscle cells, and endothelial cells, and may have a role in fibrosis and vascular injury [12,13]. Earlier studies had shown that LTE_4 has distinct functions that can be differentiated from the effects of LTC_4 and LTD_4 [14]. Consistent with this observation, inhalation of LTE_4, but not LTD_4 initiates a cellular influx into the airways in patients with asthma suggesting that LTE_4 plays an important and distinct pathobiological role in cellular inflammation in asthma [15]. A third CysLT receptor, designated the $CysLT_E$ receptor, was identified with high specificity for LTE_4 in mice lacking both $CysLT_1$ or $CysLT_2$ receptors [16••]. The effects of LTE_4 are also mediated by the adenosine diphosphate-reactive purinergic $P2Y_{12}$ receptor that may complex with another receptor to recognize LTE_4 [17••]. This LTE_4-mediated potentiation of airway eosinophilia and goblet cell metaplasia is abrogated in mice lacking the $P2Y_{12}$ receptor, but not in mice lacking either the $CysLT_1$ or $CysLT_2$ receptors [17]. The effects of LTE_4 in this model are antagonized by administration of the $P2Y_{12}$-selective antagonist clopidogrel, or by antibody-mediated platelet depletion [17]. The $CysLT_1$ receptor is antagonized montelukast, pranlukast, and zafirlukast. Selective antagonists for $CysLT_2$ and $CysLT_E$ receptors have not been developed. Inhibitors of 5-LO (e. g., zileuton, ZD2138, and MK-0633) and FLAP (e.g., MK-886, MK-591, BAY X1005, ABT-080, AM103, and AM803) prevent the synthesis of LTs.

Pharmacogenetics of the LT pathway

Recent studies have assessed the relationship between genetic variation within the leukotriene synthetic pathway, susceptibility to asthma, and pharmacological response to asthma treatment (i.e., pharmacogenetics). Studies identifying associations between asthma susceptibility and genetic variation within the LT pathway generally require further replication, and have identified effects that are small in magnitude suggesting that genetic variation in this pathway has a relatively minor influence on asthma susceptibility (reviewed in [18]). In contrast, good evidence exists that genetic variation in this pathway affects the response to asthma therapy among asthmatics. An early study found that variants in the number of Sp1 binding motifs in the 5-LO gene (i.e., ALOX5) promoter other than the wild-type were associated with reduced gene transcription of 5-LO *in vitro* and a reduced clinical response to the 5-LO inhibitor ABT-761 [19]. Similarly in a study evaluating the treatment response to the $CysLT_1$ receptor antagonist montelukast, subjects with a least one copy of the wild-type 5-LO promoter polymorphism had improved FEV_1 and fewer exacerbations compared to the alternative genotypes [20]. Out of 28 polymorphisms in 5 LT pathway genes, polymorphisms located in intron 2 of the 5-LO gene, and in the ATP-binding cassette family member ABCC1 (multidrug resistance protein 1 [MRP1]) gene involved in transport of LTC_4 to the extracellular space, were both associated with the change in FEV_1 during treatment with montelukast [21]. In another study that assessed many of these genes, the same two polymorphisms in the 5-LO gene and ABCC1 were also associated with the treatment response to the 5-LO inhibitor zileuton [22]. In a pharmacokinetic study, a non-synonymous polymorphism rs12422149 in

the solute carrier organic anion transporter family member B1 (SLCO2B1) gene that is involved in the absorption of montelukast was associated with reduced plasma concentration of this CysLT1 receptor antagonist in patients with the variant genotype, and lack of a symptom reduction during montelukast treatment [23•].

Evolving role of leukotriene B_4 in allergic disease

Although much of the focus on LTs in asthma is on the CysLTs, recent studies have defined an important regulatory role of LTB_4 in models of asthma. LTB_4 is formed from LTA_4 by the enzyme LTA_4 hydrolase in leukocytes. High-affinity and low-affinity receptors for LTB_4 designated BLT1 and BLT2 respectively are G-protein-coupled receptors found on the cell surface [24,25]. The expression of the BLT1 receptor is restricted to leukocytes including effector T-cells and is highly specific for LTB_4, while the BLT2 receptor is ubiquitously expressed. Recently, both the BLT1 and BLT2 receptors were identified on human airway smooth muscle cells [26]. The endogenous ligand for BLT2 is 12(S)-hydroxyheptadeca-5Z, 8E, 10E-trienoic acid (12*S*-HHT), which like other hydroxyeicosanoids have significantly stronger bindings affinities for BLT2 than LTB_4 [27•,28].

Increased levels of LTB_4 have been identified in asthmatics, but whether LTB_4 plays an important role has remained controversial because BLT1 antagonists (e. g. LY293111) decreased neutrophil recruitment to the airways, but did not inhibit the early or late asthmatic response in patients with asthma [29]. In mice, the expression of BLT1 is induced on $CD4^+$ and $CD8^+$ T cells, including antigen-specific effector $CD4^+$ T cells during ovalbumin (OVA) sensitization, and effector T cell trafficking was substantially diminished in mice lacking the expression of BLT1 [30]. Differences in early granulocyte recruitment were identified only during the first 2 days of airway challenge following sensitization in BLT1-deficient mice, suggesting that BLT1 is important in the early phase of allergen-induced granulocyte recruitment to airways [30,31]. Further, BLT1-deficient mice developed significantly lower AHR, goblet cell hyperplasia, and IL-13 production than wild-type mice, which could be fully restored by adoptive transfer of T cells from allergen-sensitized BLT1-sufficient mice [32, 33]. Using adoptive transfer of *in vivo* primed BLT1 sufficient and deficient $CD8^+$ T cells into CD8-deficient mice, BLT1 was shown to have a critical role in the development of Th2 cytokine (i.e., IL-13)-mediated airway inflammation and AHR [34]. The BLT1 receptor is also important in LTB_4-mediated migration of mast cells [35]. In a mast cell-dependent murine model established by passive sensitization with anti-OVA IgE followed by airway challenge with OVA, the development of AHR was restored in CD8-deficient mice by adoptive transfer of allergen-primed $CD8^+$ T cells from BLT1-sufficient mice [36]. Treatment of $CD8^+$ T cells with the corticosteroid dexamethasone prior to adoptive transfer increases the expression of BLT1 and the activation state of the CD8 cells, and increases AHR in CD8-deficient mice adoptively transferred these cells [37•]. These findings may be of importance to human asthma since the number of $CD8^+$ T cells in the airway wall is associated with the lung function decline and reticular basement membrane thickness in asthmatics treated with inhaled corticosteroids [38]. In humans, $BLT1^+$ T cells express the effector cytokines IFNγ and IL-4 and inflammatory chemokine receptors, CCR1, CCR2, CCR6, and CXCR1 [39]. Normally, T cells expressing BLT1 make up a minority of peripheral blood T cells, but the number of such cells is increased in airways of subjects with allergic asthma [39]. Overall, these findings indicate that BLT1 plays an important role in early effector T cell recruitment and effector cell function regulating development of cellular airway inflammation and AHR in models of human asthma.

There is relatively little information about the role of BLT2 in asthma. Murine bone marrow-derived mast cells migrate towards the BLT2 ligand 12-HHT, in cells from BLT2-sufficient, but not cells from BLT2-deficient mice [27]; these mast cells also migrate towards another BLT2 ligand, 12(S)-hydroxyeicosotetraenoic acid (12*S*-HETE) [35]. In a murine asthma

model, expression of BLT2 is increased in lungs after sensitization and challenge, and antisense oligonucleotide inhibition of BLT2 expression or pharmacological inhibition of BLT2 with LY255283 attenuated airway inflammation and AHR [40]. In this model, inhibition of BLT2 reduced generation of reactive oxygen species (ROS) and expression of the key inflammatory transcription factor NF-kB [40]. Similarly in an oncogene-transformed fibroblast cell line, BLT2 is involved in generation of ROS [41]. There is little known about the potential role of BLT2 in human asthma; however, there is evidence of increased expression of BLT2 in airway biopsies of a small number of asthmatic samples, with expression being similar in mild and moderate asthmatics [40].

Effects of leukotrienes on airway remodeling

Airway remodeling refers to long-term changes in the airway walls of patients with asthma including thickening of the reticular basement membrane, mucus cell metaplasia, and increased smooth muscle mass, and deposition of extracellular matrix (ECM) proteins such as collagen, tenascin, and laminin. LTs are implicated in airway remodeling through *in vitro* and *in vivo* studies. There is evidence that the level of CysLTs in exhaled breath is correlated with reticular basement membrane thickening [42], but little information on the effectiveness of anti-LT drugs on this remodeling process in humans. In a mouse model of chronic asthma, the $CysLT_1$ receptor antagonist montelukast reduced goblet cell metaplasia, airway smooth muscle mass, and subepithelial collagen deposition, while treatment with the corticosteroid dexamethasone only reduced goblet cell metaplasia [43]. Several recent studies demonstrate that CysLTs induce mucin gene expression and gel-forming mucin release in cultured epithelial cells [44,45]. In human airway smooth muscle that contains both BLT1 and BLT2 receptors, LTB_4 mediates migration and proliferation via the BLT1 receptor [26]. LTD_4 induces gene transcription for TGF-β_1 and conditioned media from LTD_4-treated cells transfected with $CysLT_1$ receptor causes airway smooth muscle proliferation in a TGF-β_1-dependent manner [46]. Also in cultured airway smooth muscle cells, the key remodeling cytokine IL-13 induces proliferation of these cells through a LT-dependent mechanism [47]. In an epithelial cell line that expresses both $CysLT_1$ and $CysLT_2$ receptors, LTD_4 and LTE_4 increased the expression of tenascin, while LTD_4 but not LTE_4 induced the β2 chain of laminin that was $CysLT_1$ receptor-dependent [48]. The TNF-α-induced expression of matrix metalloproteinase-9 (MMP-9), which is implicated in ECM remodeling, is augmented in macrophages by LTC_4 and LTD_4 [49]. The role of the $CyslT_2$ receptor in airway remodeling is largely unknown; however, in a bleomycin model of lung fibrosis, genetic deficiency of the $CysLT_2$ receptor reduces lung fibrosis [12].

Role of leukotrienes in exercise-induced bronchoconstriction

An important role of leukotrienes has been identified in exercise-induced bronchoconstriction (EIB), a clinical condition characterized by bronchoconstriction lasting 30–90 minutes after a short period of exercise. Among asthmatics, ~ 40–50% of patients will have EIB when tested with exercise challenge [50]. Asthmatics with EIB have increased concentrations of epithelial cells shed into induced sputum, and increased levels of CysLTs in induced sputum and exhaled breath condensate [51,52]. Although several different eicosanoids are released into airways following exercise challenge, CysLT release is particularly prominent as evidenced by a sustained increase in their levels 0.5–6 hr after exercise challenge in asthmatics with EIB [53,54]. Airway LTB_4 release has been identified following exercise challenge in some [54], but not all studies [53]. In animal models, the effects of LTs on hyperpnea-induced bronchoconstriction are mediated through tachykinins produced by sensory nerves [55,56]. Recent *in vitro* studies have demonstrated that LTs both increase excitability of sensory nerves and reduce excitation threshold for sensory nerves [57•]. These findings may explain the finding that MUC5AC release into airways following exercise challenge is associated with the

CysLT level, which in turn is associated with the neurokinin A level, since secretion of tachykinins is thought to be an important event in goblet cell degranulation [58]. Another important finding is the increase in $sPLA_2$-X in the airways following challenge, since this enzyme may be an important regulator of LT generation in response to exercise challenge [8].

There is compelling evidence that $CysLT_1$ receptor antagonists and 5-LO inhibitors reduce the severity of EIB during short-term administration, and as a component of chronic therapy for persistent asthma (reviewed in [59]). In 2007, the US Food and Drug Administration (FDA) approved the $CysLT_1$ antagonist montelukast for the prevention of EIB in patients aged 15 years and older based on studies demonstrating efficacy for the prevention of EIB 2 hr after a single 10 mg dose [60,61]. In 51 adult patients with EIB, montelukast significantly reduced the severity of EIB at 2, 12, and 24 hr after a single dose based on maximum decrease in FEV_1 (10.8% montelukast vs. 22.3% placebo at 2 hr), and area under the curve for the percentage decrease in FEV_1, and improved post-exercise recovery from EIB [60]. A second study showed similar efficacy at 2 hr after a single dose of montelukast compared to placebo (11.7% montelukast vs. 17.5% placebo), but failed to show a statistically significant difference at 12 and 24 hr in part because the severity of EIB waned in the placebo group at 12 and 24 hr [61]. In 19 children with EIB who were all using an inhaled corticosteroid (ICS), severity of EIB was reduced relative to placebo at 12 hr after the single dose, but not at 2 or 24 hr [62]. A recent study also showed that the effect of a $CysLT_1$ receptor antagonist is additive to the inhaled β_2-agonist salmeterol in reducing the airways response to isocapnic hyperventilation while breathing frigid air after single doses of these drugs [63]. Another recent study compared effects of a) the inhaled ICS budesonide alone, b) budesonide plus the long-acting β_2-agonist (LABA) formoterol, c) budesonide plus the $CysLT_1$ receptor antagonist montelukast, d) montelukast alone, and e) placebo in children ages 6–18 with EIB, finding the greatest protection from EIB in either of the two groups given the $CysLT_1$ receptor antagonist [64•].

Evolving role of leukotrienes modifiers in asthma therapy

Leukotriene modifiers are an important component of long-term preventative therapy for persistent asthma, either alone in mild persistent asthma as an alternative to an ICS, or in combination with other therapies for asthma of greater severity. A recent study designed to determine if therapy with a combination of a $CysLT_1$ receptor antagonist with a LABA could be used as an alternative to the ICS and LABA combination, demonstrated that therapy with a LTRA and LABA combination was inferior to therapy with a ICS and LABA in moderate persistent asthma [65]. A recent systematic review of 12 and 48 week randomized controlled trials compared the clinical effectiveness and safety of montelukast versus the LABA salmeterol as add-on treatment to constant doses of ICS in adolescents and adults with asthma [66]. Meta-analyses of the 12-week trials found the montelukast/ICS combination clinically inferior to salmeterol/ICS in the proportion of patients with exacerbations and comparable in safety. In contrast, no statistically significant difference was found between the montelukast/ICS and salmeterol/ICS groups with regard to exacerbations, and a significantly higher rate of serious adverse events was seen in the salmeterol-treated group in meta-analyses of the 48-week trials.

Several recent studies in children and adults suggest that tobacco smoke exposure may worsen asthma through a CysLT-mediated pathway, and that such subjects exposed to tobacco smoke may have a greater response to therapy targeting the LT pathway [67,68]. With increasing body mass in patients with asthma, the therapeutic response to ICS decreases, but is maintained to LT receptor blockade [69]. Regarding the safety of $CysLT_1$ receptor antagonists during pregnancy, a retrospective cohort study and a prospective cohort study found that the rate of birth defects was no different between the asthma groups treated with a $CysLT_1$ receptor

antagonist as compared to the alternative treatment which was limited to β-agonist treatment in the retrospective study, and also included ICS treatment in the prospective study [70,71].

Conclusions

A strong biological role for LTs in the pathogenesis of asthma has been identified in studies conducted over the past 50 years. Our understanding of the biological role of LTs in disease continues to evolve with recent advances in understanding the regulation of the LT synthetic pathway, the receptors that mediate the response to LTs, and the cellular and molecular events mediated through these receptors. Genetic variation in enzymes and transport proteins involved in LT synthesis may influence the response to LT modifiers in clinical practice. LTs play a key role in certain clinical features of asthma such as EIB, where therapies targeting this pathway are effective during short-term and long-term use. Collectively, these recent studies represent a major advance in understanding the role of LTs in asthma pathogenesis and provide insights for the potential to select therapy based on patient genotype.

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