

Molecular mechanisms in allergy and clinical immunology

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The role of leukotrienes in airway inflammation

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Cysteinyl leukotrienes (cysLTs) are a class of closely structurally related lipid molecules, originally described as slow-reacting substance of anaphylaxis, with a myriad of biologic functions. These activities include producing smooth muscle contraction and mucus secretion, recruiting allergic inflammatory cells, modulating cytokine production, influencing neural transmission, and altering structural changes in the airway. Administration of cysLTs to animals and human subjects reproduces many features of allergic inflammation and asthma. Leukotriene (LT) blockers have independent efficacy in asthma and improve pulmonary function when added to inhaled steroids. Conversely, blockade of this pathway both in animals and in human subjects results in important reductions in inflammation and its consequences and might reduce structural changes of remodeling. These data collectively make a compelling case for an important role of cysLTs in airway inflammation and asthma. However, the magnitude of effect of anti-LTs is smaller than that of corticosteroids, and there is more variability in benefit of LT blockade than is seen with inhaled steroids. In addition, adding anti-LTs to inhaled steroids in asthmatic patients does not appear to produce added anti-inflammatory benefit. Genetic polymorphisms and environmental factors, such as tobacco smoke exposure, might underlie some of the heterogeneity of response to LT blockers. (J Allergy Clin Immunol 2006;118:789-98.)

Key words: Cysteinyl leukotrienes, asthma, inflammation

Asthma is a lung disease associated with variable bronchoconstriction, airway hyperresponsiveness (AHR), and chronic inflammation in the lower airway, which collectively lead to clinical symptoms of shortness of breath, chest

Abbreviations used

AHR: Airway hyperresponsiveness
ALOX5: Promoter region for the 5-LO gene
BAL: Bronchoalveolar lavage
cysLT: Cysteinyl leukotriene
cysLT1: Cysteinyl leukotriene receptor type 1
cysLT2: Cysteinyl leukotriene receptor type 2
cysLTRA: Cysteinyl leukotriene receptor (type 1) antagonist
DC: Dendritic cell
HpETE: 5(S)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid
ICS: Inhaled corticosteroid
LABA: Long-acting β -agonist
5-LO: 5-Lipoxygenase
LT: Leukotriene
LTM: Leukotriene modifier
LTSI: Leukotriene synthesis inhibitor
5-oxo-ETE: 5-Oxo-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid

tightness, wheezing, and cough. Associated with airway inflammation are hyperresponsiveness, mucous hypersecretion, and airway remodeling. Recently, increasing evidence supports the concept of asthma as a syndrome rather than a single entity. In some patients cysteinyl leukotrienes (cysLTs) might play a significant pathogenic role. In view of the recognized complexity of asthmatic airway inflammation,¹⁻⁶ we will review the role of leukotrienes (LTs) in airway inflammation, its significance, and its clinical relevance.

LT PATHWAY

LTs are potent lipid mediators synthesized through multiple enzymatic steps from nuclear membrane phospholipids.^{3,7-14} Arachidonic acid is cleaved from membrane phospholipid through phospholipase A₂ and subsequently metabolized through several pathways, one of which is the 5-lipoxygenase (LO) pathway.¹⁵ Arachidonic acid is converted to 5(S)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (HpETE) and then to LTA₄ in 2 oxidative steps by 5-LO, with 5-LO-activating protein serving as a necessary presentation molecule. LTA₄ is unstable and

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can hydrolyze or be converted to LTC₄ by means of conjugation to glutathione. Alternatively, LTA₄ can be converted to the hydroxy LT LTB₄ by epoxide hydrolase in neutrophils and other inflammatory cells. LTB₄ is a potent neutrophil chemoattractant and also can cause eosinophil chemotaxis. LTC₄ is exported to the extracellular space and cleaved to LTD₄ and LTE₄. LTC₄, LTD₄, and LTE₄ all contain a cysteine residue and are collectively called cysLTs. They are abundantly produced in mast cells, eosinophils, and alveolar macrophages. CysLTs were previously known as slow-reacting substance of anaphylaxis; these compounds stimulate airway smooth muscle by means of a nonhistaminergic, cysLT receptor type 1 (cysLT1)–dependent mechanism.³ CysLTs are important mediators of inflammation and significantly modulate the inflammatory responses seen in asthma.^{13,16,17} The COX pathway is another possible route for arachidonic acid, and the mediators produced are key regulators of pulmonary inflammation. This critical pathway has recently been reviewed.¹⁸

Another more recently recognized pathway for arachidonic acid metabolism is that of the 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acids (5-oxo-ETEs). In this pathway, 5-HpETE is converted to 5-HETE and subsequently metabolized to 5-oxo-ETEs.¹⁹ 5-oxo-ETEs are chemoattractants for eosinophils and neutrophils and lead to actin polymerization, calcium mobilization, integrin expression, and degranulation. These mediators appear also to be important in asthma but are not a focus of this review.

LT SYNTHESIS INHIBITOR VERSUS LT RECEPTOR ANTAGONIST

The activity of LT pathway molecules can be blocked in 2 different ways: inhibition of synthetic enzymes and mediator receptor blockade. Zileuton inhibits the catalytic activity of 5-LO and hence is an LT synthesis inhibitor (LTSI).³ An alternative pharmacologic strategy is receptor blockade. Four LT receptors have been cloned: B LT receptor type 1, B LT receptor type 2, cysLT1, and cysLT receptor type 2 (cysLT2). Most of the known actions of cysLTs are mediated through cysLT1.¹⁶ For airway smooth muscle constriction, LTC₄ and LTD₄ are considerably more potent than LTE₄. The cysLT receptor antagonists (cysLTRAs) in clinical practice worldwide selectively block the cysLT1s but have little or no activity for cysLT2. They are montelukast, pranlukast, and zafirlukast.³ Montelukast has been subject to the most extensive trials; both zafirlukast and montelukast have demonstrable efficacy in asthma.^{20–24} There are, at present, neither B LT receptor type 1 nor B LT receptor type 2 receptor antagonists in clinical use.

THESIS: LTs PRODUCE INFLAMMATION

LTs and asthma

The central question is this: Aside from smooth muscle contraction and mucus secretion, what roles do leukotrienes, particularly cysLTs, play in asthma?²⁵ The available

evidence suggests that LTs have several important pathogenic roles in asthma, serve to promote inflammation, and might also contribute to persistent inflammation.²⁵ This information derives from both direct proinflammatory effects of cysLTs on airway inflammation and anti-inflammatory effects of cysLT antagonists.

CysLTs are present in asthma and relate to inflammation. LTs not only produce smooth muscle contraction and bronchoconstriction but also promote eosinophilic inflammation. CysLTs (LTC₄, LTD₄, and LTE₄) are released in asthmatic airways after exposure to allergens and are associated with changes in AHR after the inhalation of allergen.^{14,20} Mondino et al²⁰ found that LTE₄ and LTB₄ are increased in exhaled breath condensate in asthmatic patients. Bronchoalveolar lavage (BAL) fluids, which provide a direct measurement of inflammation from the relevant site, show increased concentrations of LTC₄ in asthmatic subjects after segmental allergen challenge compared with baseline lavage fluids.²⁶ Induced sputum also provides a lower airway sample in which quantitative measurements can be made. Sputum samples from asthmatic subjects also contain high concentrations of eicosanoids. Pavord et al²⁷ showed that sputum LTC₄/LTD₄/LTE₄ concentrations are greater in asthmatic patients than in nonasthmatic control subjects, and subjects with more severe asthma had higher concentrations of cysLTs in sputum. Furthermore, these increased concentrations of cysLTs were not substantially reduced by treatment with inhaled corticosteroids (ICSs), suggesting that the LT pathway is relatively independent of regulation by corticosteroids.²⁸ In addition to the cysLTs, LTB₄ is also known to be increased in acute asthmatic episodes.²⁹ Finally, indirect measures of cysLT participation suggest a more general role for cysLTs in asthma. For example, increased urinary LTE₄ levels can be detected in asthmatic patients compared with control subjects.^{30,31} These increased urinary levels of LTE₄ decrease after asthma is controlled with appropriate treatment.³⁰

LT generation is not limited to allergic stimuli. Cooling and drying of the airways appear also to promote the generation of LTs, which then produce bronchoconstriction. Furthermore, exercise has been linked to the development of inflammation,³² and treatment with zileuton blocks exercise-induced bronchospasm.³³ Hence leukotrienes might participate in the pathogenesis of both exercise-induced and cold-induced asthma.^{34,35}

Collectively, these data show that cysLT levels are increased in patients with asthma, increase with asthma severity, increase after allergen challenge or exercise challenge, and decrease with effective treatment. In aggregate, the data support a close association of cysLTs with asthma and are consistent with a causal role.

CysLTs produce inflammation. Evidence of a proinflammatory role for cysLTs has been provided by means of direct administration of cysLTs to the human airway. Inhaled cysLTs selectively increase the number of eosinophils in the airways of patients with asthma.^{31,36–38} Specifically, LTE₄ inhalation increased the numbers of eosinophils in the airway mucosa, as well as the numbers

of neutrophils and lymphocytes. However, it was not clear from these studies whether LTE_4 *per se* produced eosinophil accumulation or whether it might have induced a second specific chemotactic factor for eosinophils. Endobronchial instillation of LTE_4 into human subjects resulted in the subsequent influx of eosinophils and, to lesser extent, neutrophils into BAL fluid, suggesting the capacity for cysLTs either directly or indirectly to attract leukocytes to initiate inflammatory responses *in vivo*.³⁸ Similarly, in animal models the administration of exogenous LTE_4 produced dose-dependent accumulation of eosinophils, which was blocked by pranlukast.³⁹ The cysLTs might have some ability to promote chemotaxis of eosinophils and also are chemokinetic.^{29,35,40,41} Accordingly, through either direct or indirect mechanisms, administration of cysLTs to animals³⁹ or human subjects³⁸ promotes airway inflammation.

LT blockers reduce inflammation. Several LT antagonists (both LTSIs and cysLTRAs) have been studied for effects on inflammatory responses in the airway. These agents significantly reduce measures of airway inflammation, including fraction of expired nitric oxide, BAL eosinophils, and peripheral blood eosinophils.^{24,42-44} Montelukast exerts anti-inflammatory effects in patients with asthma, as seen in studies by Minoguchi et al²² and Sandrini et al.²⁴ Four weeks of montelukast treatment decreases sputum eosinophils and blood eosinophils, in addition to improving clinical parameters.²³ Montelukast reduces LTC_4 levels and eosinophilic cationic protein concentrations in nasal wash specimens of asthmatic children.²¹ Treatment with zileuton inhibits the antigen-dependent increase in BAL fluid eosinophils after segmental allergen challenge; the study by Kane et al⁴⁵ was the first to demonstrate a measurable anti-inflammatory effect of anti-LT therapy. Moreover, in this same study concentrations of LTs in BAL fluid were reduced by zileuton therapy. In a study of the receptor antagonist zafirlukast, treatment for 7 days reduced the number of basophils and lymphocytes, reduced BAL histamine levels, reduced superoxide production by alveolar macrophages, and blunted the increase in $\text{TNF-}\alpha$ levels in BAL fluid after segmental antigen challenge.^{46,47} Another study showed that LTE_4 -induced persistent eosinophilia and airway obstruction were reversed by zafirlukast in patients with asthma.³¹ Finally, treatment with pranlukast is associated with reduced CD3, CD4, AA1-positive (mast cells), and EG2-positive (activated eosinophils) cells in bronchial mucosal cells. Clearly, cysLTRAs have significant inhibitory effects on the development of allergen-induced bronchial inflammation in patients with mild-to-moderate asthma.^{48,49}

LT interactions with inflammatory pathways

Cells. CysLTs , prostanoids, proteolytic enzymes, and kinins are released into the airways during allergic inflammation. Mast cells can be activated by many factors, such as IgE ligation, hyperosmolarity in exercise-induced bronchoconstriction, and COX-1 inhibition in nonsteroidal anti-inflammatory drug-intolerant asthma. Release of newly synthesized cysLTs is a final common response to

mast cell activation, regardless of the specific route of activation.⁵⁰

Monocytes and macrophages also can produce proinflammatory eicosanoids, such as LTB_4 , LTC_4 , and prostaglandin D_2 . In addition, macrophage functions are modified by cysLTs , with activation, priming, and decreased apoptosis all being reported.⁴⁴ Specifically, LTD_4 primes alveolar macrophages to release macrophage inflammatory protein 1α , $\text{TNF-}\alpha$, and nitric oxide when incubated with LPS.⁵¹

CysLTs induce survival of eosinophils, mast cells, and basophils at multiple stages and contribute to maintaining the inflammatory reaction.^{44,52} CysLTs stimulate progenitor cells to increase eosinophilopoiesis.⁴⁴ Furthermore, cysLTs facilitate eosinophil recruitment by inducing P-selectin and, consequently, reduction in leukocyte rolling velocity.⁵³ They also upregulate β_2 -integrin expression in eosinophils and stimulate vascular endothelial cells to produce platelet activating factor (PAF) and increase leukocyte adhesion. These increases in eosinophil chemotaxis and adhesion molecule expression are blocked by LTSIs and cysLTRAs , and such treatments reduce eosinophil survival.⁴⁴

CysLTs are involved in maturation and migration of dendritic cells (DC). DC migration was substantially attenuated in multidrug resistance-associated protein 1-deficient mice (producing impaired response to arachidonic acid-stimulated inflammation) compared with that observed in wild-type control animals, and the migration defect was corrected by the injection of LTC_4 or LTD_4 .⁵⁴ Multidrug resistance-associated protein 1 is a transporter protein that helps to protect normal cells and tumor cells against the influx of certain xenobiotics, and it is the major transporter for endogenous LTC_4 . LTC_4 produced endogenously by DCs during the initial exposure to antigen is a critical determinant of DC homing to regional lymph nodes. In addition, cysLTs regulate cytokine responses of DCs, which influence the consequential T-cell responses and hence are critically important in regulating adaptive immunity.¹⁶

Finally, LTB_4 is a potent chemoattractant for neutrophils, macrophages, and other inflammatory cells and induces chemokinesis and adhesion of these cells to the vascular endothelium.^{29,41}

Cytokines/chemokines. In addition to the key role of airway smooth muscle in regulating airway tone, this tissue also has an important role in regulating inflammation. Airway smooth muscle can enhance airway inflammation by secreting chemokines and cytokines.^{52,55-57} CysLTs selectively promote the generation of $\text{T}_\text{H}2$ cytokines and hence might enhance allergic responses.⁵⁸⁻⁶⁰ Blockade of cysLTs by MK-571 or inhibition of endogenous cysLT production by MK-886 significantly attenuates the generation of IL-5 and $\text{TNF-}\alpha$ by mast cells activated by Fc ϵ RI cross-linking.^{60,61} $\text{TNF-}\alpha$ production is enhanced by LTC_4 and LTD_4 .⁶² IL-13 upregulates cysLT1 on human lung fibroblasts, leading to enhanced eotaxin production in a concentration-dependent fashion; furthermore, this effect is inhibited by cysLTRAs .⁵⁹ High doses of montelukast modulate the production of IL-6,

TNF- α , and macrophage chemotactic protein 1 through the inhibition of nuclear factor κ B activation.⁶³

Conversely, many of these mediators, including IL-4, IL-5, IL-13, and GM-CSF, augment cysLT production. IL-4 upregulates LTC₄ synthase gene expression and cysLT1 gene expression.^{58-60,64,65} IL-4 also enhances macrophage chemotactic protein 1 (CCL2) production.⁶⁶ Accordingly, there is the potential for a positive feedback loop between cysLTs and T_H2 cytokines.

In the bone marrow cysLTs act with GM-CSF or IL-5 to facilitate the growth and subsequent differentiation of progenitor cells. CysLTs could prime progenitor cells to differentiate into mature blood cells. GM-CSF-dependent neutrophil survival is mediated by LTB₄ acting at surface receptors. GM-CSF also causes a small increase in the proliferation of eosinophil hematopoietic progenitor cells in peripheral blood, and LTD₄ enhances proliferation of eosinophil hematopoietic progenitor cells in bone marrow. Adding montelukast suppressed both peripheral blood and bone marrow proliferation.⁵⁹ GM-CSF produces a modest increase in neutrophil survival *in vitro*, and this is blocked by the LTB₄ receptor antagonist SB-201146 but not by the cysLT1 antagonist pobilukast.⁴⁴

TGF- β expression by airway smooth muscle is enhanced by cysLTs. Injury to epithelial monolayers results in increased release of fibroproliferative and profibrogenic growth factors, including TGF- β , insulin-like growth factor, and platelet-derived growth factor. Because both TGF- β production and epithelial growth factor receptor expression are refractory to corticosteroids, and cysLT production is also relatively unaffected by corticosteroids, it is plausible to suggest that cysLTs might contribute to incomplete resolution of inflammatory airway responses with therapeutic doses of ICSs.

Mediators. CysLTs might affect other important mediators of allergic responses. CysLTs increase expression of histamine receptors, potentially augmenting responses to this important mediator of inflammation.⁶⁷ In contrast, blocking cysLT1 with 4 weeks of montelukast therapy does not alter basophil histamine release but can result in small decreases in cysLT production.⁶⁸

CysLTs induce surface expression of endothelial P-selectin through a mechanism that might be independent of cysLT1.⁶⁹ Whether activation of cysLT2 was involved in these responses was not evaluated, in that specific cysLT2 antagonists were, and are, not available.

Lipid mediators are potent regulators of airway tone and inflammation. PAF, a lipid mediator that has been shown to be increased in asthma, also appears to increase the subsequent release of LTB₄ from macrophages.⁷⁰ Unlike proinflammatory lipid mediators, lipoxins promote resolution of acute inflammation.⁷¹ In an intriguing study Levy et al⁷¹ studied concentrations of lipoxin A₄ in BAL fluids in patients with severe asthma and compared these with concentrations in patients with mild-to-moderate asthma. They observed decreased concentrations of lipoxin A₄ in patients with severe asthma and suggested that reduced lipoxin A₄ concentrations might contribute to persistent inflammation in severe asthma.

Neural interactions. CysLTs also interact with neural control mechanisms. In guinea pig isolated trachea and bronchus, endogenous 5-LO activity leads to the production of cysLTs that amplify action potential-dependent release of tachykinins from airway afferent nerve fibers. Accordingly, cysLTs might amplify neurogenic inflammation. McAlexander et al⁷² have demonstrated that the cysLT1 antagonist pobilukast is an effective inhibitor of smooth muscle contraction and plasma extravasation induced by tachykinins in isolated tracheas of guinea pigs. Similar observations have been made in human subjects as well. Pretreatment of asthmatic subjects with montelukast elicits significant protection against neurokinin A-induced bronchoconstriction, as shown by Crimi et al.⁷³ Collectively, these data support the concept that cysLTs can amplify the effects of tachykinins in the airway.

Of note, cysLTs appear to modulate β -receptor function. By using human tracheal rings that were sensitized with IgE and triggered to activate mast cells, the effects of the water-soluble cysLTRA irlukast were evaluated. Smooth muscle contraction dose-response curves to albuterol were constructed. In a dose-dependent fashion, irlukast enhanced the effects of albuterol on smooth muscle relaxation, without altering maximal contraction or relaxation. In analogous experiments blockade of histamine effects with cetirizine was without effect, arguing that the effect on β -receptor function was cysLT specific and was not shared by other mediators of allergic inflammation. These intriguing data suggest that in addition to their potent effects on smooth muscle contraction, cysLTs might also augment β -receptor dysfunction during allergic inflammation.⁷⁴

Remodeling. Recent evidence suggests that cysLTs have a key regulatory role in remodeling, the structural changes of the airway that are nearly universally seen in asthma.^{52,57,75} CysLTs promote collagen synthesis and release by mitogen-stimulated lung fibroblasts.⁵² Moreover, LTs appear to be essential for pulmonary fibrosis to develop, suggesting a role of LTs in fibroproliferative processes, such as airway wall remodeling.⁷⁶ LTC₄ stimulates a dose-independent increase in macrophage-derived fibroblast growth factor, an important structural cell regulator. This increase is inhibited by treatment with cysLTRA. CysLTs also increase collagenase expression by smooth muscle cells and augment growth factor-induced proliferation of airway smooth muscle cells.^{52,57} LTD₄ potentiated the effects of either epidermal growth factor or thrombin on DNA synthesis and cell proliferation in human tracheal smooth muscle cells.^{52,56}

In a series of elegant experiments in a murine model of allergic airway inflammation, Henderson et al⁵⁷ evaluated the effects of montelukast on airway histology using measures of both airway inflammation and structural changes of remodeling. Repeated administration of allergen resulted in inflammation, goblet cell hyperplasia, increases in smooth muscle, and increases in subepithelial fibrosis. Treatment with montelukast, but not vehicle, reduced the measures of inflammation, airway eosinophilia, and hyperplasia of goblet cells and smooth muscle and

returned the airway fibrosis score to that observed in mice not treated with allergen. Also noted was that montelukast also inhibited the presence of Charcot-Leyden-like crystals in airway macrophages and the increased IL-4 and IL-13 mRNA expression in lung tissue and protein in BAL fluid.

In recently published subsequent experiments, the same group has shown that cysLTs have an important role not only in the development of the airway structural changes but also in their maintenance. Allergen-induced trafficking of eosinophils into the BAL fluid and lung interstitium and airway goblet cell metaplasia were reduced by either montelukast or dexamethasone. Increases in airway smooth muscle cell mass and subepithelial fibrosis were reversed by montelukast but not by dexamethasone. In ovalbumin-treated mice, cysLT1 expression was found to be significantly increased in airway smooth muscle cells, which was reduced by montelukast but not by dexamethasone. These findings indicate the important role of cysLTs in the airway remodeling process that is not modulated by corticosteroids.⁷⁷ An intriguing next step would be to investigate this role of cysLTs clinically in asthmatic patients.

Effect of steroids on LT production

In vitro systems demonstrate that glucocorticosteroids at high doses can block LT synthesis by inhibiting phospholipase-dependent liberation of arachidonic acid from the cellular membranes.⁷⁸ However, *in vivo* studies have suggested that cysLT production is relatively independent of steroid regulation.^{27,55} In short-term studies oral prednisone does not decrease LT levels in the BAL fluid or the urine of healthy or asthmatic subjects. Long-term studies showed some inhibition of the LT pathway in asthmatic patients treated with steroids.^{79,80} Steroid therapy also seems to be capable of suppressing LT synthesis in alveolar macrophages but not in neutrophils and monocytes. Other studies have shown that leukotriene biosynthesis remains unaffected by inhaled or oral glucocorticosteroids.^{78,81} Furthermore, there is evidence that some enzymes in the leukotriene cascade are upregulated by corticosteroids.⁸² LT synthesis and action appear to be relatively resistant to suppression by glucocorticoids.

ANTITHESIS: LTs ARE INCONSEQUENTIAL CAUSES OF AIRWAY INFLAMMATION

The controversy

There remains controversy in the literature regarding the anti-inflammatory effects of LT blockade because (1) the magnitude of the anti-inflammatory effect is generally small relative to that achieved with corticosteroids⁸³ and (2) the anti-inflammatory effects are not consistently observed. Despite their clear effects on inflammation (*vide supra*), LT blockers have not proved effective in inhibiting allergen-induced AHR (5-LO-activating protein antagonists MK-591, MK-886, and BAYx1005), to the extent that ICSs do. Moreover, the extent of suppression of

eosinophilic inflammation with anti-LTs ranges from 30% to 50%, whereas near-total suppression of eosinophils is commonly observed with ICSs.^{78,84-86} These observations then raise the questions of whether the magnitude of the anti-inflammatory effect of LT modifiers (LTMs) is insufficient to be clinically meaningful and whether reducing airway eosinophilia *per se* is sufficient to reduce AHR. Affirmative insight into the latter question has been provided by a clinical trial of neutralizing IL-5 antibody, which eliminated airway eosinophils but did not alter AHR.⁸⁷ Furthermore, anti-IL-5 therapy markedly reduced BAL fluid and circulating eosinophils but reduced eosinophils in the airway walls by only about 50%.⁸⁸

Effects of LTMs and ICSs on lung function and measures of inflammation

The addition of licensed doses of anti-LTs to add-on therapy to inhaled glucocorticoids brings modest improvement in lung function.⁸⁹ Although addition of anti-LTs to inhaled glucocorticoids appears comparable with increasing the dose of inhaled steroids for lung function measures, the power of the reviews is insufficient to confirm the equivalence of both treatment options.⁹⁰ Another Cochrane study showed that in asthmatic adults whose symptoms were inadequately controlled with a low dose of inhaled steroids, the addition of a long-acting β -agonist (LABA) is superior to cysLTRA for preventing exacerbations requiring systemic steroids and for improving lung function, symptoms, and use of rescue β_2 -agonists.⁹¹ In neither of these reviews were measures of airway inflammation summarized. However, this question has been addressed experimentally. One well-designed study has shown no added anti-inflammatory benefit of adding montelukast to inhaled budesonide.⁹² In this study investigators used sputum eosinophils as an index of airway inflammation and evaluated the effects of budesonide and montelukast, alone or in combination, on airway function, airway responsiveness, and airway inflammation. Either budesonide or montelukast was associated with reduced sputum eosinophils before allergen challenge and with a similar blunting of airway inflammation 7 and 24 hours after challenge. However, there was no added benefit of the combination on airway inflammation. Only budesonide, alone or in combination, blunted development of AHR after allergen challenge. These data contrasted to the lung function data, in which montelukast alone partially blocked the late decrease in FEV₁ after allergen challenge, and budesonide, alone or in combination, completely blocked late responses.

Clinical trial evidence: Childhood Asthma Research and Education Network—Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid (CLIC) trial

Szeffler et al,⁹³ reporting for the National Heart, Lung, and Blood Institute–funded Childhood Asthma Research

and Education Network conducted a study comparing ICSs and cysLTRAs in 6- to 17-year-old children with mild-to-moderate persistent asthma. Subjects were randomized to 2 crossover sequences, 8 weeks of an ICS and 8 weeks of montelukast, and response was assessed on the basis of improvement in FEV₁ and asthma-associated biomarkers. Of note, the majority (55%) of these subjects did not achieve as much as a 7.5% improvement in FEV₁ with either medication. More children responded to fluticasone alone (23%) than montelukast alone (5%), and a small subset responded to both (17%). Children with low pulmonary function or high levels of markers associated with allergic inflammation, such as a high fraction of expired nitric oxide, blood eosinophil count, eosinophil cationic protein, and serum IgE, appeared to have preferential response to ICSs. Favorable response to montelukast alone was associated with higher urine LTE₄ levels, younger age, and shorter disease duration. These data suggest that control of inflammation might be better achieved with steroids than with LTMs.

Clinical trial evidence: Asthma Clinical Research Network–Improving Asthma Control Trial (IMPACT)

In a landmark study by the National Institutes of Health–funded Asthma Clinical Research Network, the comparative benefit of placebo, daily zafirlukast, and daily budesonide was evaluated in adult subjects with mild persistent asthma who had the benefit of a symptom-based action plan and as-needed anti-inflammatory therapy. Boushey et al⁹⁴ studied lung function, markers of airway inflammation, and quality-of-life scores. For the primary outcome, morning peak expiratory flow rate, and most key secondary outcomes, there were not significant differences among the 3 treatments. For markers of airway inflammation, however, daily budesonide was associated with a significant reduction in sputum eosinophils and exhaled nitric oxide, whereas neither as-needed therapy nor daily zafirlukast had an effect on these inflammatory markers. These data support the generally held concept that anti-inflammatory properties of steroids are quantitatively greater than those of anti-LTs.

Benefit of cysLTRA added to combination therapy with ICSs and LABAs

When asthma symptoms are uncontrolled with combination therapy of ICSs and LABAs, current guidelines advocate to add a cysLTRA.^{5,6} However, there are actually few data specifically evaluating this regimen.⁹⁵ Robinson et al⁹⁶ conducted a 4-week study in which supplemental montelukast did not improve peak expiratory flow rates in patients receiving ICSs and second-line therapy (most of which consisted of LABAs). Nathan et al⁹⁷ also investigated the effects of an added cysLTRA (montelukast) to standard therapy in patients with both seasonal allergic rhinitis and persistent asthma and found that the addition of montelukast to ICSs and LABAs did not result

in additional improvement in overall asthma control. Another study did show improvements in AHR and inflammatory biomarkers in the montelukast add-on group compared with an ICS plus salmeterol for mild-to-moderate persistent asthma, but no improvements were seen in FEV₁ and peak expiratory flow rate.⁹⁸ Treatment with a cysLTRA might not confer improvements in airway caliber, especially when patients are already receiving LABAs. The potential additional benefit for AHR remains controversial and inconsistent.

SYNTHESIS: VARIABILITY OF RESPONSE TO LT INHIBITION

Clinical response to anti-LTs is variable, both in terms of airway function and control of inflammation. There are also known to be large variations in therapeutic response in both steroids and LT blockers, as shown in the study by Malmstrom et al.⁹⁹ Furthermore, the responses to anti-LTs are quantitatively smaller than those seen with steroid treatments. What factors account for this variability? Several possible explanations have emerged.

Genetic polymorphisms

Polymorphisms of genes in the LT pathway, including the promoter region of the 5-LO gene^{100,101} and the LTC₄ synthase gene,^{102,103} have been identified and have been demonstrated to have pharmacogenetic associations in asthma.

Mutation in the G-C–rich region of the core promoter that regulates 5-LO (ALOX5) gene transcription is associated with asthmatic patients with diminished response to anti-LT treatment. The wild-type allele is associated with the most robust promoter activity, and the variants have considerably reduced expression of 5-LO and reduced production of 5-LO products. Approximately 6% of asthmatic patients have a mutant allele in the ALOX5 core promoter locus and have minimal improvement in lung function when treated with anti-LTs, presumably as a consequence of the minimal production of LTs in these patients. However, not all patients unresponsive to anti-LTs have the ALOX5 core promoter locus variant, and other gene defects might exist.¹⁰⁴

LTC₄ synthase is present in eosinophils, basophils, macrophages, and platelets and converts LTA₄ to cysLTs. A single-nucleotide transversion of adenine to cytosine 444 bases upstream of the translation start creates a common diallelic variability of the LTC₄ synthase gene and promoter C variant and aspirin-induced asthma is associated.¹⁰² However, van Sambeek et al¹⁰³ did not find a correlation between LTC₄ synthase gene polymorphism, and aspirin-intolerant asthma, in the United States.

Asthma: A syndrome with distinct subsets, some of which are LT responsive

LT inhibition might be more effective in a subset of asthmatic patients in whom LTs are a major contributory factor in causing allergic inflammation. The CLIC trial⁹³

demonstrated that a distinct fraction of patients responded better to LTs than to ICSs, and a higher urinary LTE₄ level was one predictor of preferential benefit of montelukast therapy. Hasday et al¹⁰⁵ showed that zileuton decreased eosinophil counts in BAL fluid after segmental antigen challenge but only in “high LT producers”. Collectively, these data, coupled with the ALOX5 variant data, suggest that one predictor of minimal response to anti-LTs is minimal production of this mediator, either endogenously or in response to allergen challenge.

Aspirin causes bronchoconstriction in patients with aspirin-intolerant asthma by triggering cysLT production, probably by removing prostaglandin E₂-dependent inhibition of leukotriene synthesis.¹⁰² Only in patients with aspirin-intolerant asthma does increased bronchial expression of LTC₄ synthase allow marked overproduction of cysLTs, leading to bronchoconstriction. This field is, however, controversial because a contradictory study by van Sambeek et al¹⁰³ has been published. Clearly, further investigation is warranted to identify biochemical or clinical factors that could predict clinical response to LT blockers.

Effect of LTSIs and LTRAs on clinical aspects of asthma

LTMs improve asthma symptoms and quality of life in patients with moderate airway obstruction.¹⁰⁶⁻¹¹² To date, there are no clinical trials directly comparing zafirlukast, zileuton, and montelukast in asthmatic patients. All 3 medications have been shown to be effective in the treatment of asthma when compared with placebo, but the study populations in the respective trials have been different enough to cloud direct comparisons.

Asthmatic patients who smoke might be a subset in which treatment with anti-LTs might be considered. In these smoking patients responses to cysLTRAs are maintained, whereas the response to inhaled steroids is impaired.¹¹³ Preliminary data from the Asthma Clinical Research Network Smoking Modulates Outcomes of Glucocorticoid Therapy (SMOG) trial similarly suggest that smoking blunts physiologic responses to inhaled steroids in asthmatic subjects but might enhance the benefit of montelukast.

LTMs have particular advantages in exercise-induced asthma and aspirin-induced asthma, both in adults and children. For exercise-induced asthma, montelukast and zafirlukast are known to improve FEV₁ after exercise.^{114,115}

Nonsteroidal anti-inflammatory drugs, including aspirin, can dramatically increase the production of cysLTs. In aspirin-sensitive patients, 90% of whom were already receiving moderate-to-high doses of corticosteroids, montelukast significantly improved pulmonary function, asthma-specific quality of life, the need for bronchodilator use, and frequency of exacerbations.^{114,115}

LTMs do not cause relaxation of the airway *per se*, but they do inhibit LT production and its bronchoconstrictive action. As a representative example, Roquet et al¹¹⁶ showed increases in FEV₁ with zafirlukast therapy. In addition, there is some evidence that the response to LTM might be quantitatively larger with higher degrees of

airway obstruction. However, a similar relationship between the degree of obstruction and the degree of response is often made with other classes of asthma therapy.

Interestingly, one study showed that LT antagonists might have some prophylactic effects in viral-induced exacerbation in children between the ages of 2 and 5 years, whereas steroids appeared not to have such effects.¹¹⁷⁻¹¹⁹ Microbial killing effects of LTs are seen in multiple studies as well.^{120,121} Lysosomal enzyme release was stimulated by LTB₄.¹²² LTB₄ also induced the release of the antimicrobial peptide α -defensin by neutrophils.¹²³ Serezani et al¹²⁴ showed that LTs, especially LTB₄, enhance alveolar macrophage microbicidal activity through the protein kinase C δ -dependent activation of reduced nicotinamide adenine dinucleotide phosphate oxidase. These aspects of LT biology seem to be potentially beneficial, and hence LT blockade might be associated with a clinical disadvantage. These kinds of potential adverse effects have not been demonstrated, perhaps because they are uncommon or because compensatory mechanisms of host defense render the blockade of these LT effects clinically unimportant.

LTMs are generally well tolerated and appropriate for use in children and pregnant women. Reported side effects of LTMs are headache, gastrointestinal symptoms, and reversible increases of liver enzyme levels.^{125,126} As for headache and gastrointestinal complaints, these were actually equally seen in the placebo group in most clinical trials. Reversible liver enzyme level increases have been discussed in several studies. Overall, LTMs have a low incidence of mild side effects.

Summary

LTMs, both cysLTRAs and LTSIs, are the first new class of asthma therapy in the past 30 years. The contributions of cysLTs to the pathogenesis of asthma syndromes are many and include airway smooth muscle contraction and bronchoconstriction, mucus secretion, recruitment and activation of eosinophils, upregulation of cytokines that coordinate and amplify the allergic inflammatory response, facilitation of cholinergic neural transmission, inhibition of β -receptor signaling, and promotion of growth and activation of structural cells involved in the remodeling process. Evaluated in isolation, anti-LTs have significant, measurable, and clinically important anti-inflammatory effects in allergic airway diseases. Moreover, this important pathway is relatively independent of regulation by ICSs, and consequently, there is a rational basis for and physiologic evidence supporting the use of combination therapy of asthma with inhaled steroids and LTMs.

However, the magnitude of clinical efficacy has been disappointing based on the compelling data supporting a pathogenic role for cysLTs in asthma. Variability in the response to LTMs is also significant, although heterogeneity in response to other agents (ie, inhaled steroids and β -agonists) is also commonly observed. Finally, there is little evidence that LTMs provide additional anti-inflammatory activity when added to inhaled steroids.

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