

Clinical reviews in allergy and immunology

Series editors: Donald Y.M. Leung, MD, PhD, and Dennis K. Ledford, MD

Efficacy of leukotriene receptor antagonists and synthesis inhibitors in asthma

Paul M. O'Byrne, MB, FRCP(C), Gail M. Gauvreau, PhD, and Desmond M. Murphy, MB, PhD Hamilton, Ontario, Canada

Cysteinyl leukotrienes are important mediators of asthmatic responses. They are the most potent bronchoconstrictors known; their release is triggered by exposure to inhaled allergens after exercise and after aspirin ingestion by subjects with aspirin-sensitive asthma. The cysteinyl leukotrienes promote inflammatory cell migration into the airways, as well as bone marrow eosinophilopoiesis after allergen inhalation. Leukotriene inhibitors are effective at attenuating asthmatic responses to all of these stimuli and are also effective at treating persistent asthma. These drugs are a viable alternative to low-dose inhaled corticosteroid (ICS) treatment but should be reserved for patients who cannot or will not use ICSs, often because of concerns about potential side effects of ICS treatment, which limits their use, particularly in children. Leukotriene receptor antagonists are also alternatives to long-acting inhaled β_2 -agonists as add-on therapy to ICSs, but their efficacy together with ICSs is less than that of ICS/long-acting inhaled β_2 -agonist combinations. Leukotriene receptor antagonists have an excellent safety profile. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Asthma, treatment, leukotrienes, antagonists, synthesis inhibitors, treatment guidelines

Slow-reacting substance of anaphylaxis was initially identified as a factor with the ability to potently stimulate smooth muscle contraction.¹ The chemical structures of slow-reacting substance of anaphylaxis were identified as 5(S)-hydroxy-6(R)-glutathionyl-7,9-trans-11,14-cis-eico-satetraenoic acid² and its cysteinyl-glycyl and cysteinyl congener. These molecules are now known as the cysteinyl leukotrienes (CysLTs) leukotriene (LT) C₄, LTD₄, and LTE₄, respectively, and were so named because the parent molecule was originally isolated from leukocytes and its carbon backbone is a triene.³

From the Firestone Institute for Respiratory Health, St Joseph's Healthcare, and the Department of Medicine, McMaster University.

Disclosure of potential conflict of interest: P. M. O'Byrne is on advisory boards for AstraZeneca, GlaxoSmithKline, Topigen, Wyeth, and Schering-Plough; has received speakers' honoraria from AstraZeneca and GlaxoSmithKline; and has received research support from AstraZeneca, GlaxoSmithKline, Merck, Wyeth, Schering-Plough, and Alexion. The rest of the authors have declared that they have no conflict of interest.

Received for publication April 27, 2009; accepted for publication May 14, 2009.

Reprint requests: Paul M. O'Byrne, MB, FRCP(C), HSC 3W10, McMaster University, 1200 Main St West, Hamilton, Ontario, Canada, L8N 3Z5. E-mail: obyrd@mcmaster.ca.

0091-6749/\$36.00

© 2009 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2009.05.029

Abbreviations used

CysLT: Cysteinyl leukotriene
CysLT_{ER}: Cysteinyl leukotriene receptor E
EIB: Exercise-induced bronchoconstriction
ICS: Inhaled corticosteroid
LABA: Long-acting inhaled β_2 -agonist
5-LO: 5-Lipoxygenase
LT: Leukotriene
LTRA: Leukotriene receptor antagonist

The oxidative pathway of lipid metabolism, known as the 5-lipoxygenase (5-LO) pathway, required for the production of the CysLTs has also been elucidated.⁴ The catalytic action of 5-LO on arachidonic acid yields LTA₄, which is unstable. In 3 cell types, eosinophils, mast cells, and alveolar macrophages, LTA₄ can be converted to LTC₄ catalyzed by LTC₄ synthase. LTC₄ is then exported to the extracellular space through a specific transmembrane transporter.⁵ In the extracellular space LTC₄ is converted into LTD₄, which in turn is cleaved by extracellular dipeptidases to form LTE₄, which is excreted in bile and urine. In neutrophils LTA₄ is converted to LTB₄, which is a dihydroxy as opposed to a CysLT, by the action of LTA₄ epoxide hydrolase.⁶

The CysLTs are the most potent constrictors of human airway smooth muscle yet described both *in vitro* and *in vivo*.^{7,8} In addition, they can cause tissue edema⁹ and eosinophil migration,¹⁰ can stimulate airway secretions,¹¹ and promote cell cycling and proliferation of airway smooth muscle¹² and hematopoietic cells.¹³ Two subtypes of the receptors for CysLT₁ (for which LTD₄ has the highest affinity) and CysLT₂ (for which LTC₄ and LTD₄ have equal affinity) have had their molecular structures identified.^{14,15} A third receptor with a preferential response to LTE₄ has now been described,¹⁶ but its structure has not yet been reported. There are 2 known receptors for LTB₄, a high-affinity receptor, which is a 7-transmembrane-spanning receptor known as the BLT receptor 1,¹⁷ and a low-affinity receptor, which is known as BLT receptor 2.¹⁸

Many specific and selective antagonist drugs that block the binding of LTs to CysLT₁ receptors have been identified. This class of molecules has been given the generic suffix -lukast, and drugs based on these are known as leukotriene receptor antagonists (LTRAs). Three of these, together with 1 LT synthesis inhibitor, have been approved for use in the treatment of asthma, and 1 has been approved for allergic rhinitis.

LT INHIBITORS IN MODELS OF PROVOKED ASTHMA

Studies with provoked models of asthma, such as exercise-induced bronchoconstriction (EIB) or allergen-induced asthma,

have been helpful in determining a pathophysiologic role for the CysLTs in asthma. CysLTs have been demonstrated to be produced after these challenges in asthmatic subjects,^{19,20} but the most convincing evidence of a causative role of LTs in asthma comes from studies of the effectiveness against asthma of drugs that inhibit the action or formation of LTs. These studies have also helped in establishing the efficacy of the anti-LT drugs.

EIB is a common feature of asthma and a real-life stimulus that can occur in patients with current asthma.²¹ All of the currently approved LTRAs attenuate EIB. The earliest study showing benefit was conducted with MK-571,²² but subsequently, other LTRAs have shown benefit, including zafirlukast²³ and montelukast.²⁴ These drugs inhibit the maximum bronchoconstrictor response after exercise by 30% to 70% (Fig 1). In addition, when these drugs are administered before exercise, they greatly shorten the time to recovery of normal lung function. Treatment with the 5-LO inhibitor zileuton had similar effects in patients in whom bronchoconstriction was induced by means of either cold-air hyperventilation²⁵ or exercise.²⁶ These studies indicate that cooling and drying of the airways together promote the generation of LTs, which results in bronchoconstriction. However, the degree of protection achieved with LT modifiers varies among patients; administration of an LTRA affords complete protection in some but very little in others.²⁷ Comparisons have been made between the protection afforded by LTRAs and that afforded by long-acting inhaled β_2 -agonists (LABAs) in their respective ability to protect against EIB with regular use.²⁸ This study demonstrated that tolerance develops with the regular use of salmeterol over time, but this did not occur with montelukast, which was therefore superior to salmeterol by 8 weeks of treatment.

Allergen-induced asthma

The inhalation of an allergen by a patient with allergic asthma has several consequences. The first is acute bronchoconstriction that develops within 15 minutes and usually resolves within 2 hours (the early asthmatic response). In up to 70% of patients, a late asthmatic response occurs, which begins 3 to 4 hours after inhalation and lasts up to 24 hours.²⁹ The late asthmatic response is associated with an increase in airway inflammatory cells (predominantly eosinophils, mast cells, and lymphocytes)³⁰ and airway hyperresponsiveness to a variety of inhaled agents; the hyperresponsiveness can last from days to weeks.³¹

Two types of data indicate that the CysLTs have an important role in the pathogenesis of allergen-induced responses. First, urinary excretion of LTE_4 increases after the early asthmatic response.¹⁹ Second, several LT inhibitors, including the LTRAs zafirlukast³² and MK-571³³ and the biosynthesis inhibitors MK-591³⁴ and BAYx1005,³⁵ significantly reduce bronchoconstriction during the early and late asthmatic responses (Fig 2). Pretreatment with a combination of an antihistamine and an LTRA eliminates the residual bronchoconstriction during the early and late allergen responses³⁶; this observation indicates that histamine and the LTs are the major mediators of these responses. LTRAs are also effective at attenuating allergen-induced airway hyperresponsiveness measured 24 hours after administration of allergen,^{37,38} although they are not as effective in this regard as inhaled corticosteroids (ICSs).³⁸

CysLTs are also important in causing allergen-induced airway inflammation. Inhaled LTE_4 ,^{10,39} but not inhaled LTD_4 ,³⁹ increases the number of eosinophils in the airways of patients with asthma (Fig 3). A possible explanation for this discrepancy

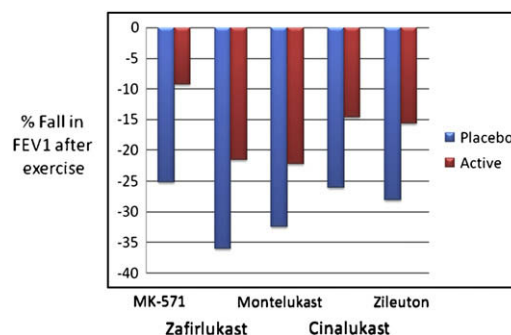


FIG 1. Mean maximal decrease in FEV₁ after exercise after treatment with placebo or an LT inhibitor (summary of data from references 22-24, 26, and 27).

is provided by the discovery of a selective receptor for LTE_4 (CysLT receptor E), the discovery of which is reviewed in the supplement by Austen et al.⁴⁰ In addition, treatment with the LTRAs or the 5-LO inhibitor zileuton reduced the number of airway inflammatory cells after allergen challenge.^{38,41,42} Also, montelukast has been demonstrated to attenuate allergen-induced dendritic cell trafficking⁴³ and bone marrow eosinophilopoiesis.¹³

Aspirin-induced asthma

In up to 8% of patients with asthma, ingestion of aspirin or other COX-1 inhibitors caused profound and sometimes life-threatening bronchoconstriction and naso-ocular, dermal, and gastrointestinal responses.⁴⁴ These patients have an increase in LTC_4 synthase activity,⁴⁵ perhaps as a result of a mutation in the promoter region of the LTC_4 synthase gene.⁴⁶ Pretreatment with a variety of LTRAs have been shown to prevent bronchoconstrictor responses after the inhalation of lysine aspirin,^{47,48} whereas pretreatment with the 5-LO inhibitors inhibited the bronchoconstriction after oral administration of aspirin.^{49,50} Furthermore, LTRAs improved lung function in subjects with aspirin-sensitive asthma in the absence of aspirin challenge.⁵¹ These results indicate that LT inhibitors are the treatment of choice for patients with aspirin-induced asthma.

TREATMENT OF PERSISTENT ASTHMA

Patients with persistent asthma have symptoms of asthma and require rescue β_2 -agonist use on most days and often have nocturnal symptoms; also, their lung function might be abnormal.^{52,53} Clinical trials evaluating either LTRAs or 5-LO inhibitors in patients with persistent asthma have demonstrated improved asthma control, as measured based on improved airway function, decreased need for rescue treatment with β_2 -agonists, improved asthma symptoms, and decreased frequency of exacerbations of asthma requiring oral corticosteroid therapy.

Acute bronchodilator effects

LTRAs improve airway function in patients with persistent asthma who have airflow obstruction at baseline and demonstrated responsiveness to β_2 -agonist bronchodilators within 1 to 3 hours.⁵⁴⁻⁵⁶ The magnitude of the improvement in FEV₁ varied from 8% to 20% and was present irrespective of whether patients were taking concomitant ICSs.⁵⁷ The bronchodilator effect was greater in patients with more substantial degrees of

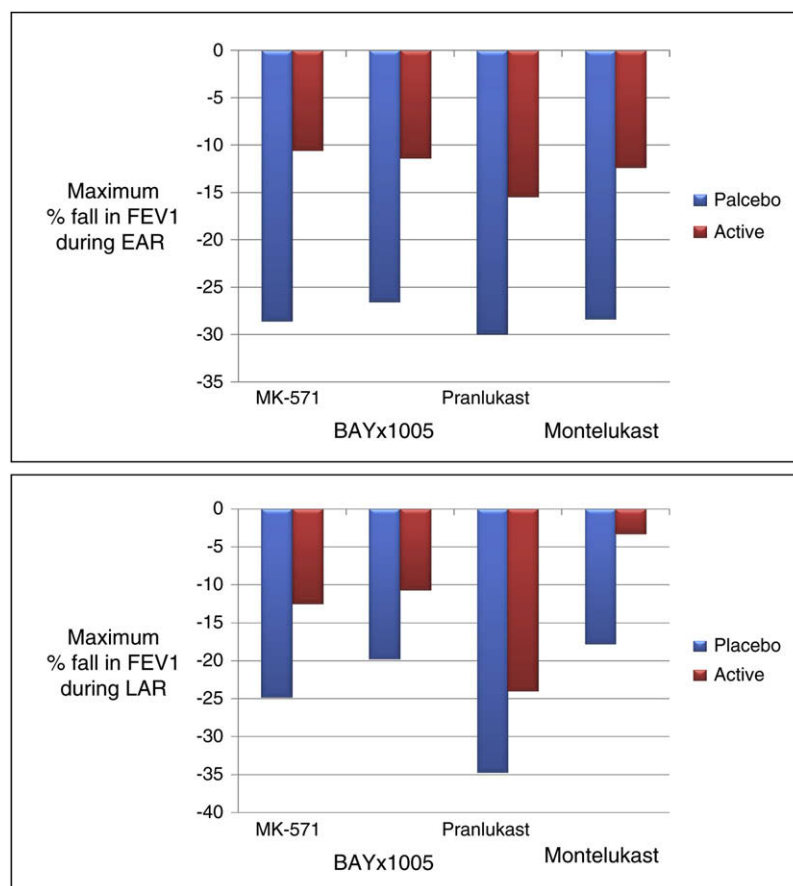


FIG 2. Effect of LT inhibitors on allergen-induced early (EAR; top panel) and late (LAR; bottom panel) asthmatic responses (summary of data from references 33, 35, 37, and 38).

airway obstruction, and its magnitude is about half that of the response to β -adrenergic agonists. The LTRA montelukast has also been evaluated in a small study,⁵⁸ which has shown benefit in the management of acute severe asthma in the emergency department.

Improved asthma control

Over the past 3 years, asthma treatment guidelines have focused on targeting asthma control as the parameter to determine treatment needs.^{52,53} This approach is both logical (because achieving good asthma control is the main outcome objective of treatment) and easy to grasp. The tools for the assessment of control have been published and validated.^{59,60} The management of an asthmatic patient becomes an iterative process in which asthma control is assessed by the health care practitioner at an initial visit and a decision is made on the treatment needed. The patient is then evaluated at the next appointment to decide whether asthma control has been achieved. If so, then treatment is continued or reduced; if not, then treatment is increased. The best outcome for the patient is to achieve current (day-to-day) asthma control, during which the patient is asymptomatic all of the time, has normal lung function, and has no limitations in activities.

Another component of overall asthma control is the reduction of future risks in asthmatic patients. These risks consist of acute severe exacerbations, progressive decrease in lung function, and side effects from medications.⁵³

The potential benefit of LTRAs or 5-LO inhibitors on overall asthma control has not been formally evaluated, but all aspects of asthma control have been shown to benefit from treatment with LTRAs and 5-LO inhibitors. These drugs have been demonstrated to reduce symptoms and the need for rescue β_2 -agonist use and improve lung function in clinical trials.^{54,56,61,62} In addition, both classes of LT inhibitors reduce the risks of severe asthma exacerbations.⁶¹⁻⁶³ There are, however, no data currently available to suggest that LT inhibitors alter the decrease in lung function over time that is observed in a subset of asthmatic patients.⁶⁴

Comparisons with ICSs

ICSs are the current mainstay of treatment in patients with persistent asthma. They improve symptoms, lung function, and airway hyperresponsiveness and reduce asthma exacerbations. ICSs have potent anti-inflammatory effects in asthmatic airways and, in particular, reduce eosinophilic airway inflammation. Several studies have directly compared the efficacy of low-dose ICSs and LT inhibitors in patients with persistent asthma and have demonstrated overall greater efficacy of low-dose ICSs for most outcomes of asthma control.⁶⁵⁻⁶⁷ However, there is variability in the individual responses of asthmatic patients to these classes of anti-asthma drugs, with higher bronchodilator use, bronchodilator responsiveness, exhaled nitric oxide levels, and eosinophil cationic protein levels; greater airway hyperresponsiveness; and pulmonary function values being associated with a greater response to ICS treatment in children.⁶⁶

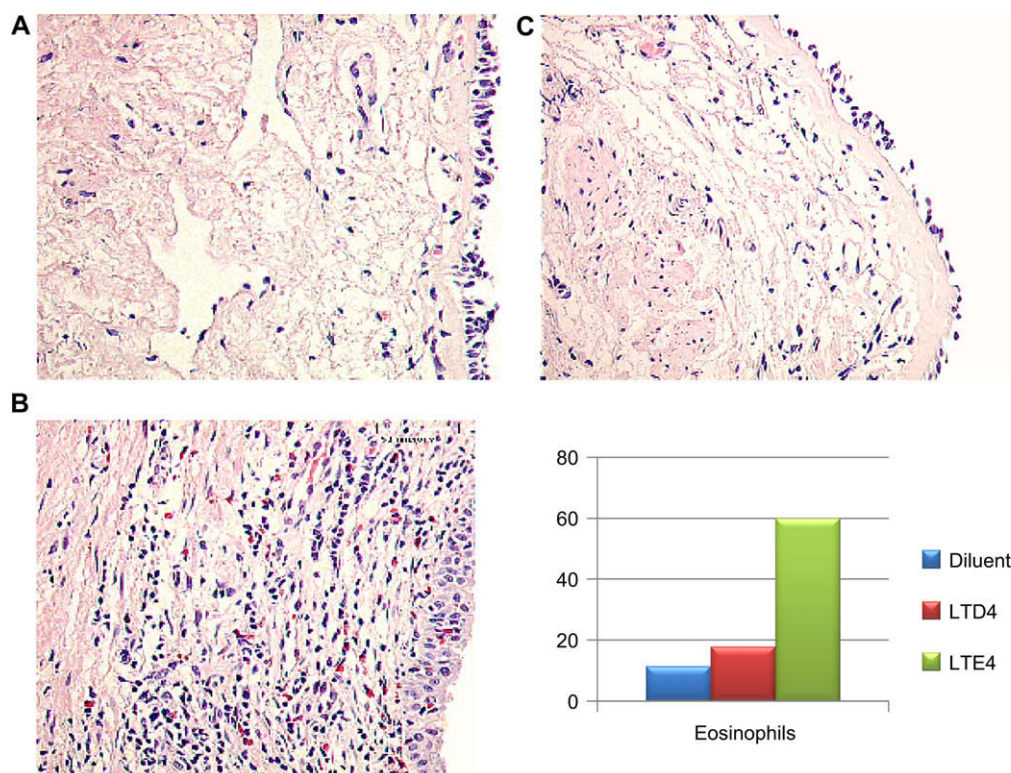


FIG 3. Airway eosinophils (red staining) in airway biopsy specimens 4 hours after inhalation of diluent (A), LTD₄ (B), and LTE₄ (C). The graph shows the mean values for 6 asthmatic subjects (drawn from data in reference 39).

ICSs improve airway responsiveness to inhaled methacholine,^{68,69} which is the most widely used method of measuring airway hyperresponsiveness in patients with asthma. LTRAs do not provide any improvement in this outcome.⁶⁹ In addition, ICSs improve some of the measures of airway remodeling in patients with asthma,⁷⁰⁻⁷² whereas this has not yet been demonstrated for the LTRAs.

Zileuton has broader actions than the LTRAs because it inhibits the formation of both the CysLTs and LTB₄, whereas the receptor antagonists only block the action of the CysLTs. However, to date, there are no published studies directly comparing the 2 classes of drugs. Zileuton has not been widely used to treat asthma because of the initial need to take it 4 times daily and concerns about liver toxicity (see below).

Add-on therapy to ICSs

Asthma treatment guidelines recommend adding a second treatment to ICSs rather than increasing to a high dose of ICS when low-dose ICSs are not providing adequate asthma control.^{52,53} LTRAs have been compared with LABAs in this regard in adult patients. These studies have consistently found clinical benefit when an LTRA is added to an ICS, but this benefit has been less than that demonstrated with LABAs as the add-on treatment.^{73,74}

Safety

An idiosyncratic syndrome similar to the Churg–Strauss syndrome, with circulating eosinophilia, cardiac failure, and associated eosinophilic vasculitis, has been reported in a few patients

treated with zafirlukast⁷⁵ or montelukast.⁷⁶ Almost all these patients had been receiving high-dose ICSs or oral corticosteroids and were able to reduce the dose as a consequence of treatment with the LTRA. It is unclear whether the increased reports of this rare syndrome are due to increased case findings among patients with asthma receiving a new drug, whether the syndrome is unmasked as a result of the reduction in the corticosteroid dose, or whether it represents an idiosyncratic effect of LT antagonists or LT modifiers in general.

An asymptomatic increase in hepatic aminotransferase levels occurred in 4.4% of patients receiving high-dose zileuton compared with 1.6% of patients receiving placebo.⁷⁷ Most of the high values occurred during the first 3 months of therapy, and they resolved with either continued therapy or discontinuation of treatment. It is therefore recommended that serum alanine aminotransferase concentrations should be measured when zileuton therapy is initiated, monthly for 3 months, and periodically thereafter.

GUIDELINE RECOMMENDATIONS FOR THE USE OF LT INHIBITORS IN ASTHMA

Asthma treatment guidelines now recommend maintenance treatment with drugs that reduce airway inflammation and improve overall asthma control for all patients with persistent asthma.^{52,53} The mainstay of maintenance asthma treatment are low-dose ICSs. This is because they demonstrate superior efficacy for almost all clinical outcomes when compared with other treatments, including LTRAs. However, the guidelines also recognize that other maintenance treatments are also effective and might play an important role in asthma management. Thus for patients who are being

initially treated with maintenance asthma treatment, LT inhibitors are recommended as an alternative to low-dose ICSs for patients who either will not or cannot take ICS therapy. This is often because of concerns about potential side effects of ICS treatment, which, although generally unfounded, limit their use, particularly in children. Also, LT inhibitors are alternatives to LABAs as an add-on to an ICS when low-dose ICS therapy is not providing optimal asthma control, but again, their efficacy together with an ICS is less than that of ICS/LABA combinations.

There are 2 situations in which LT inhibitors might have particular advantages over other drugs for asthma treatment. The first is EIB. The long-lasting protection of LTRAs without the development of tolerance, which occurs with regular β_2 -agonist use, might be of value for children who want to exercise at school without having to use an intermediate-acting inhaled β -adrenergic agonist or for adults whose jobs require exercise under atmospheric conditions likely to induce an asthmatic episode.

The second situation is aspirin-sensitive asthma. Virtually all the physiologic effects of aspirin-sensitive asthma are due to CysLTs.⁷⁸ Also, the magnitude of improvement in asthma control appears to be greater in subjects with aspirin-sensitive asthma when compared with those who do not.⁷⁹ Thus all subjects with aspirin-sensitive asthma should have a therapeutic trial with LTRAs.

CONCLUSIONS

CysLTs are important mediators of a variety of asthmatic responses. They remain the most potent bronchoconstrictors known and are released after exposure to inhaled allergens, after exercise, and after aspirin (or other COX-1 inhibitor) ingestion by subjects with aspirin-sensitive asthma. The CysLTs also cause eosinophil and dendritic cell migration into the airways, as well as bone marrow eosinophilopoiesis after allergen inhalation in asthmatic subjects. LT inhibitors are effective at attenuating asthmatic responses to all of these stimuli and are also effective in treating persistent asthma. These drugs are a viable alternative to low-dose ICS treatment but should be reserved for patients who cannot or will not use ICSs. Also, LTRAs are alternatives to LABAs as an add-on treatment to ICSs when low-dose ICS therapy alone is not providing optimal asthma control but with the caveat that their efficacy together with ICSs is less than that of ICS/LABA combinations. LTRAs have an excellent safety profile.

Despite LT inhibitors being available for more than 10 years for the treatment of asthma, some issues still need to be resolved about the use of inhibitors of this pathway. To date, little is known about the importance of the CysLTs in the initiation of the atopic diathesis or in the onset of asthma in individuals at risk (particularly atopic children). The central role of the CysLTs in all aspects of allergen-induced responses in patients with established asthma, together with the epidemiologic evidence of the importance of environmental allergens in causing asthma,⁸⁰ suggests a plausible hypothesis that their inhibition might be a useful secondary prevention measure in atopic children. In addition, the long-term benefits of LT inhibitors on airway remodeling or the accelerated decrease in lung function, which are features of asthma, have not been established.

There is also a paucity of information concerning the roles of the CysLT₂ receptor (for which LTC₄ is the primary ligand) or the CysLT_ER (for which LTE₄ is the primary ligand) in patients with asthma or other allergic diseases. This is particularly relevant for the CysLT_ER because LTE₄ appears to be particularly important

in causing eosinophil recruitment in human airways and eosinophils are now recognized as a critical effector cell in asthma.^{81,82} Resolution of these issues awaits the availability of selective antagonists of these receptors. Finally, the value of using LT synthesis inhibitors, which prevent production of both LTB₄ and the CysLTs when compared with an LTRA, particularly in patients with severe asthma, and can be associated with an increase in airway neutrophils, has not been established.

What do we know?

- CysLTs are important mediators of asthmatic responses and promote airway inflammation.
- LT inhibitors markedly attenuate allergen-induced and aspirin-induced asthmatic responses and EIB.
- LTRAs are administered orally either once (montelukast) or twice (zafirlukast) daily and are selective antagonists of the CysLT₁ receptor.
- LT inhibitors are effective therapy for persistent asthma but less so than low-dose ICS therapy.
- LT inhibitors are effective when added to ICS therapy but less so than LABAs.
- LTRAs have an excellent safety profile.

What is still unknown?

- The effects of CysLTs on airway remodeling or the decrease in lung function in patients with asthma.
- The benefits of LTRAs as an effective secondary prevention strategy for asthma in high-risk children.
- The role of CysLT₂ receptors in patients with asthma.
- The role of receptors for CysLT_ER in asthma and, in particular, whether blockade of these receptors attenuates airway eosinophilia.
- The efficacy of LT synthesis inhibitors in severe neutrophil-associated asthma.

REFERENCES

1. Kellaway CH, Trethewie ER. Liberation of slow-reacting smooth muscle stimulating substance in anaphylaxis. *QJM* 1940;30:121-45.
2. Hammarstrom S, Samuelsson B, Clark DA, Goto G, Marfat A, Mioskowski C, et al. Stereochemistry of leukotriene C-1. *Biochem Biophys Res Commun* 1980; 92:946-53.
3. Samuelsson B, Hammarstrom S. Nomenclature for leukotrienes. *Prostaglandins* 1980;19:645-8.
4. Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science* 1983;220:568-75.
5. Lam BK, Xu K, Atkins MB, Austen KF. Leukotriene C4 uses a probenecid-sensitive export carrier that does not recognize leukotriene B4. *Proc Natl Acad Sci U S A* 1992;89:11598-602.
6. Radmark O, Malmsten C, Samuelsson B, Clark DA, Goto G, Marfat A, et al. Leukotriene A: stereochemistry and enzymatic conversion to leukotriene B. *Biochem Biophys Res Commun* 1980;92:954-61.
7. Dahlen SE, Hedqvist P, Hammarstrom S, Samuelsson B. Leukotrienes are potent constrictors of human bronchi. *Nature* 1980;288:484-6.
8. Weiss JW, Drazen JM, Coles N, McFadden ER Jr, Weller PF, Corey EJ, et al. Bronchoconstrictor effects of leukotriene C in humans. *Science* 1982;216:196-8.
9. Dahlen SE, Bjork J, Hedqvist P, Arfors KE, Hammarstrom S, Lindgren JA, et al. Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response. *Proc Natl Acad Sci U S A* 1981;78:3887-91.
10. Laitinen LA, Laitinen A, Haahtela T, Vilkkä V, Spur BW, Lee TH. Leukotriene E4 and granulocytic infiltration into asthmatic airways. *Lancet* 1993;341:989-90.

11. Coles SJ, Neill KH, Reid LM, Austen KF, Nii Y, Corey EJ, et al. Effects of leukotrienes C4 and D4 on glycoprotein and lysozyme secretion by human bronchial mucosa. *Prostaglandins* 1983;25:155-70.
12. Cohen P, Noveral JP, Bhala A, Nunn SE, Herrick DJ, Grunstein MM. Leukotriene D4 facilitates airway smooth muscle cell proliferation via modulation of the IGF axis. *Am J Physiol Lung Cell Mol Physiol* 1995;269:L151-7.
13. Parameswaran K, Watson R, Gauvreau GM, Sehmi R, O'Byrne PM. The effect of pranlukast on allergen-induced bone marrow eosinophilopoiesis in subjects with asthma. *Am J Respir Crit Care Med* 2004;169:915-20.
14. Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS, et al. Characterization of the human cysteinyl leukotriene 2 receptor. *J Biol Chem* 2000;275:30531-6.
15. Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM, et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 1999;399:789-93.
16. Maekawa A, Kanaoka Y, Xing W, Austen KF. Functional recognition of a distinct receptor preferential for leukotriene E4 in mice lacking the cysteinyl leukotriene 1 and 2 receptors. *Proc Natl Acad Sci U S A* 2008;105:16695-700.
17. Masuda K, Yokomizo T, Izumi T, Shimizu T. cDNA cloning and characterization of guinea-pig leukotriene B4 receptor. *Biochem J* 1999;342:79-85.
18. Yokomizo T, Kato K, Terawaki K, Izumi T, Shimizu T. A second leukotriene B(4) receptor, BLT2. A new therapeutic target in inflammation and immunological disorders. *J Exp Med* 2000;192:421-32.
19. Manning PJ, Rokach J, Malo JL, Ethier D, Cartier A, Girard Y, et al. Urinary leukotriene E4 levels during early and late asthmatic responses. *J Allergy Clin Immunol* 1990;86:211-20.
20. Kikawa Y, Miyanomae T, Inoue Y, Saito M, Nakai A, Shigematsu Y, et al. Urinary leukotriene E4 after exercise challenge in children with asthma. *J Allergy Clin Immunol* 1992;89:1111-9.
21. Anderson SD, Daviskas E. The mechanism of exercise-induced asthma is.... *J Allergy Clin Immunol* 2000;106:453-9.
22. Manning PJ, Watson RM, Margolskee DJ, Williams VC, Schwartz JI, O'Byrne PM. Inhibition of exercise-induced bronchoconstriction by MK-571, a potent leukotriene D4-receptor antagonist. *N Engl J Med* 1990;323:1736-9.
23. Finnerty JP, Wood-Baker R, Thomson H, Holgate ST. Role of leukotrienes in exercise-induced asthma. Inhibitory effect of ICI 204219, a potent leukotriene D4 receptor antagonist. *Am Rev Respir Dis* 1992;145:746-9.
24. Leff JA, Busse WW, Pearlman D, Bronsky EA, Kemp J, Hendeles L, et al. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. *N Engl J Med* 1998;339:147-52.
25. Israel E, Dermarkarian R, Rosenberg M, Sperling R, Taylor G, Rubin P, et al. The effects of a 5-lipoxygenase inhibitor on asthma induced by cold, dry air. *N Engl J Med* 1990;323:1740-4.
26. Meltzer SS, Hasday JD, Cohn J, Bleecker ER. Inhibition of exercise-induced bronchospasm by zileuton: a 5-lipoxygenase inhibitor. *Am J Respir Crit Care Med* 1996;153:931-5.
27. Adelroth E, Inman MD, Summers E, Pace D, Modi M, O'Byrne PM. Prolonged protection against exercise-induced bronchoconstriction by the leukotriene D4-receptor antagonist cinalukast. *J Allergy Clin Immunol* 1997;99:210-5.
28. Edelman JM, Turpin J, Bronsky EA, Grossman J, Kemp JP, Ghannam AF, et al. Oral montelukast compared with inhaled salmeterol to prevent exercise-induced bronchoconstriction. A randomized, double-blind trial. Exercise Study Group. *Ann Intern Med* 2000;132:97-104.
29. O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic responses. *Am Rev Respir Dis* 1987;136:740-51.
30. Gauvreau GM, Watson RM, O'Byrne PM. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. *Am J Respir Crit Care Med* 1999;160:640-7.
31. Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. *J Allergy Clin Immunol* 1982;70:170-7.
32. Taylor IK, O'Shaughnessy KM, Fuller RW, Dollery CT. Effect of cysteinyl-leukotriene receptor antagonist ICI 204.219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. *Lancet* 1991;337:690-4.
33. Rasmussen JB, Eriksson LO, Margolskee DJ, Tagari P, Williams VC, Andersson KE. Leukotriene D4 receptor blockade inhibits the immediate and late bronchoconstrictor responses to inhaled antigen in patients with asthma. *J Allergy Clin Immunol* 1992;90:193-201.
34. Diamant Z, Timmers MC, van d V, Friedman BS, De SM, Depra M, et al. The effect of MK-0591, a novel 5-lipoxygenase activating protein inhibitor, on leukotriene biosynthesis and allergen-induced airway responses in asthmatic subjects in vivo. *J Allergy Clin Immunol* 1995;95:42-51.
35. Hamilton AL, Watson RM, Wylie G, O'Byrne PM. Attenuation of early and late phase allergen-induced bronchoconstriction in asthmatic subjects by a 5-lipoxygenase activating protein antagonist, BAYx 1005. *Thorax* 1997;52:348-54.
36. Davis BE, Illamperuma C, Gauvreau GM, Watson RM, O'Byrne PM, Deschesnes F, et al. Single dose desloratadine and montelukast and allergen-induced late airway responses. *Eur Respir J* 2009;33:1302-8.
37. Hamilton A, Faiferman I, Stober P, Watson RM, O'Byrne PM. Pranlukast, a cysteinyl leukotriene receptor antagonist, attenuates allergen-induced early- and late-phase bronchoconstriction and airway hyperresponsiveness in asthmatic subjects. *J Allergy Clin Immunol* 1998;102:177-83.
38. Leigh R, Vethanayagam D, Yoshida M, Watson RM, Rerecich T, Inman MD, et al. Effects of montelukast and budesonide on airway responses and airway inflammation in asthma. *Am J Respir Crit Care Med* 2002;166:1212-7.
39. Gauvreau GM, Parameswaran KN, Watson RM, O'Byrne PM. Inhaled leukotriene E(4), but not leukotriene D(4), increased airway inflammatory cells in subjects with atopic asthma. *Am J Respir Crit Care Med* 2001;164:1495-500.
40. Austen KF, Maekawa A, Kanaoka Y, Boyce JA. The leukotriene E4 puzzle: finding the missing pieces and revealing the pathobiological implications. *J Allergy Clin Immunol*. In press.
41. Calhoun WJ, Lavins BJ, Minkwitz MC, Evans R, Gleich GJ, Cohn J. Effect of zafirlukast (Accolate) on cellular mediators of inflammation: bronchoalveolar lavage fluid findings after segmental antigen challenge. *Am J Respir Crit Care Med* 1998;157:1381-9.
42. Kane GC, Pollice M, Kim CJ, Cohn J, Dworski RT, Murray JJ, et al. A controlled trial of the effect of the 5-lipoxygenase inhibitor, zileuton, on lung inflammation produced by segmental antigen challenge in human beings. *J Allergy Clin Immunol* 1996;97:646-54.
43. Parameswaran K, Liang H, Fanat A, Watson R, Snider DP, O'Byrne PM. Role for cysteinyl leukotrienes in allergen-induced change in circulating dendritic cell number in asthma. *J Allergy Clin Immunol* 2004;114:73-9.
44. Stevenson DD, Szczeklik A. Clinical and pathologic perspectives on aspirin sensitivity and asthma. *J Allergy Clin Immunol* 2006;118:773-86.
45. Cowburn AS, Sladek K, Soja J, Adamek L, Nizankowska E, Szczeklik A, et al. Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J Clin Invest* 1998;101:834-46.
46. Sampson AP, Cowburn AS, Sladek K, Adamek L, Nizankowska E, Szczeklik A, et al. Profound overexpression of leukotriene C4 synthase in bronchial biopsies from aspirin-intolerant asthmatic patients. *Int Arch Allergy Immunol* 1997;113:355-7.
47. Christie PE, Smith CM, Lee TH. The potent and selective sulfidopeptide leukotriene antagonist, SK&F 104353, inhibits aspirin-induced asthma. *Am Rev Respir Dis* 1991;144:957-8.
48. Dahlen B, Kumlin M, Margolskee DJ, Larsson C, Blomqvist H, Williams VC, et al. The leukotriene-receptor antagonist MK-0679 blocks airway obstruction induced by inhaled lysine-aspirin in aspirin-sensitive asthmatics. *Eur Respir J* 1993;6:1018-26.
49. Nasser SM, Bell GS, Foster S, Spruce KE, MacMillan R, Williams AJ, et al. Effect of the 5-lipoxygenase inhibitor ZD2138 on aspirin-induced asthma. *Thorax* 1994;49:749-56.
50. Israel E, Fischer AR, Rosenberg MA, Lilly CM, Callery JC, Shapiro J, et al. The pivotal role of 5-lipoxygenase products in the reaction of aspirin-sensitive asthmatics to aspirin. *Am Rev Respir Dis* 1993;148:1447-51.
51. Dahlen B, Margolskee DJ, Zetterstrom O, Dahlen SE. Effect of the leukotriene receptor antagonist MK-0679 on baseline pulmonary function in aspirin sensitive asthmatic subjects. *Thorax* 1993;48:1205-10.
52. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, Fitzgerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008;31:143-78.
53. Expert Panel Report 3 (EPR-3): guidelines for the diagnosis and management of asthma—summary report 2007. *J Allergy Clin Immunol* 2007;120(suppl):S94-138.
54. Barnes NC, Pujet JC. Pranlukast, a novel leukotriene receptor antagonist: results of the first European, placebo controlled, multicentre clinical study in asthma. *Thorax* 1997;52:523-7.
55. Gaddy JN, Margolskee DJ, Bush RK, Williams VC, Busse WW. Bronchodilation with a potent and selective leukotriene D4 (LTD4) receptor antagonist (MK-571) in patients with asthma. *Am Rev Respir Dis* 1992;146:358-63.
56. Spector SL, Smith LJ, Glass M. Effects of 6 weeks of therapy with oral doses of ICI 204,219, a leukotriene D4 receptor antagonist, in subjects with bronchial asthma. ACCOLATE Asthma Trialists Group. *Am J Respir Crit Care Med* 1994;150:618-23.
57. Reiss TF, Sorkness CA, Stricker W, Botto A, Busse WW, Kundu S, et al. Effects of montelukast (MK-0476); a potent cysteinyl leukotriene receptor antagonist, on bronchodilation in asthmatic subjects treated with and without inhaled corticosteroids. *Thorax* 1997;52:45-8.
58. Ferreira MB, Santos AS, Pregal AL, Michelena T, Alonso E, de Sousa AV, et al. Leukotriene receptor antagonists (montelukast) in the treatment of asthma crisis: preliminary results of a double-blind placebo controlled randomized study. *Allerg Immunol (Paris)* 2001;33:315-8.

59. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;14:902-7.
60. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004;113:59-65.
61. Israel E, Cohn J, Dube L, Drazen JM. Effect of treatment with zileuton, a 5-lipoxygenase inhibitor, in patients with asthma. A randomized controlled trial. Zileuton Clinical Trial Group. *JAMA* 1996;275:931-6.
62. Reiss TF, Chervinsky P, Dockhorn RJ, Shingo S, Seidenberg B, Edwards TB. Montelukast, a once-daily leukotriene receptor antagonist, in the treatment of chronic asthma: a multicenter, randomized, double-blind trial. Montelukast Clinical Research Study Group. *Arch Intern Med* 1998;158:1213-20.
63. Johnston NW, Mandhane PJ, Dai J, Duncan JM, Greene JM, Lambert K, et al. Attenuation of the September epidemic of asthma exacerbations in children: a randomized, controlled trial of montelukast added to usual therapy. *Pediatrics* 2007;120:e702-12.
64. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 1998;339:1194-200.
65. Busse W, Raphael GD, Galant S, Kalberg C, Goode-Sellers S, Srebro S, et al. Low-dose fluticasone propionate compared with montelukast for first-line treatment of persistent asthma: a randomized clinical trial. *J Allergy Clin Immunol* 2001;107:461-8.
66. Szeffler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF, Strunk RC, et al. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. *J Allergy Clin Immunol* 2005;115:233-42.
67. Bleecker ER, Welch MJ, Weinstein SF, Kalberg C, Johnson M, Edwards L, et al. Low-dose inhaled fluticasone propionate versus oral zafirlukast in the treatment of persistent asthma. *J Allergy Clin Immunol* 2000;105:1123-9.
68. Juniper EF, Kline PA, Vanzieleghe MA, Ramsdale EH, O'Byrne PM, Hargreave FE. Long-term effects of budesonide on airway responsiveness and clinical asthma severity in inhaled steroid-dependent asthmatics. *Eur Respir J* 1990;3:1122-7.
69. Kannies F, Richter K, Bohme S, Jorres RA, Magnussen H. Montelukast versus fluticasone: effects on lung function, airway responsiveness and inflammation in moderate asthma. *Eur Respir J* 2002;20:853-8.
70. Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a beta 2-agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomized, double-blind, parallel-group controlled trial. *J Allergy Clin Immunol* 1992;90:32-42.
71. Hoshino M, Takahashi M, Takai Y, Sim J, Aoike N. Inhaled corticosteroids decrease vascularity of the bronchial mucosa in patients with asthma. *Clin Exp Allergy* 2001;31:722-30.
72. Trigg CJ, Manolitsas ND, Wang J, Calderon MA, McAulay A, Jordan SE, et al. Placebo-controlled immunopathologic study of four months of inhaled corticosteroids in asthma. *Am J Respir Crit Care Med* 1994;150:17-22.
73. Ram FS, Cates CJ, Ducharme FM. Long-acting beta2-agonists versus anti-leukotrienes as add-on therapy to inhaled corticosteroids for chronic asthma. *Cochrane Database Syst Rev* 2005 (1):CD003137.
74. Nelson HS, Busse WW, Kerwin E, Church N, Emmett A, Rickard K, et al. Fluticasone propionate/salmeterol combination provides more effective asthma control than low-dose inhaled corticosteroid plus montelukast. *J Allergy Clin Immunol* 2000;106:1088-95.
75. Wechsler ME, Garpestad E, Flier SR, Kocher O, Weiland DA, Polito AJ, et al. Pulmonary infiltrates, eosinophilia, and cardiomyopathy following corticosteroid withdrawal in patients with asthma receiving zafirlukast. *JAMA* 1998;279:455-7.
76. Wechsler ME, Finn D, Gunawardena D, Westlake R, Barker A, Haranath SP, et al. Churg-Strauss syndrome in patients receiving montelukast as treatment for asthma. *Chest* 2000;117:708-13.
77. Watkins PB, Dube LM, Walton-Bowen K, Cameron CM, Kasten LE. Clinical pattern of zileuton-associated liver injury: results of a 12-month study in patients with chronic asthma. *Drug Saf* 2007;30:805-15.
78. Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis and management. *J Allergy Clin Immunol* 1999;104:5-13.
79. Dahlen SE, Malmstrom K, Nizankowska E, Dahlen B, Kuna P, Kowalski M, et al. Improvement of aspirin-intolerant asthma by montelukast, a leukotriene antagonist: a randomized, double-blind, placebo-controlled trial. *Am J Respir Crit Care Med* 2002;165:9-14.
80. Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD. Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma. *Clin Exp Allergy* 1993;23:941-8.
81. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;360:985-93.
82. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009;360:973-84.