Leukotrienes in the Pathogenesis of Asthma*

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Asthma is a chronic inflammatory disease that is associated with widespread but variable airflow obstruction. The mechanisms that lead to airflow obstruction in asthma are bronchoconstriction, mucosal edema, increased secretion of mucus, and an inflammatory-cell infiltrate that is rich in eosinophils. Leukotrienes (LTs) B4, C4, D4, and E4 have been shown experimentally to play a role in each of these inflammatory mechanisms and to mimic the pathologic changes seen in asthma. Inhaled LTC4 and LTD4 are the most potent bronchoconstrictors yet studied in human subjects. LTC4 and LTD4 also may cause migration of inflammatory cells into the asthmatic airway. LTs are derived from the 5-lipoxygenase (5-LO) pathway of arachidonic acid metabolism, and increased production of LTs has been demonstrated in patients who have asthma. Leukotriene receptor antagonists and specific inhibitors of the 5-LO pathway hold great promise as new therapies to treat asthma. Because LTC4, LTD4, and LTE4 appear to interact with a common LTD4 receptor, selective LTD4 receptor antagonists (eg, pranlukast [SB205312/ONO-1078], zafirlukast [ICI 204,219], MK-571, and MK-679), as well as zileuton (A-64077, a direct inhibitor of 5-LO) have been developed as antiasthma agents. Clinical and experimental studies have demonstrated the efficacy of these compounds in reducing not only the symptoms of asthma, but use of β2-agonists and bronchoconstriction induced by exposure to allergens, exercise, aspirin, and cold air.

(Key words: allergen; asthma; bronchoconstriction; exercise; leukotriene; leukotriene receptor antagonist; 5-lipoxygenase; mediator)

The symptoms of asthma—dyspnea, wheezing, coughing, and chest tightness—are caused by airflow obstruction. Another clinical feature of asthma is increased airway responsiveness to various stimuli.1 This means that smaller concentrations of an inhaled bronchoconstrictor agonist are needed to induce narrowing of the airway in subjects who have asthma than are needed in normal subjects. Also, the maximal response to the bronchoconstrictor is greater in subjects with asthma.

Airway hyperresponsiveness, variable airflow obstruction, and the symptoms of asthma are consequences of a characteristic form of cellular inflammation and structural changes in the airway wall of patients with asthma.2 The inflammation consists of the presence of activated eosinophils, lymphocytes, and an increased number of mast cells, which have been identified in both BAL fluid and airway-tissue samples from subjects who have asthma.3-6 The structural changes described in the asthmatic airway, which appear to be characteristic of the disease, are likely caused by persistent inflammation of the airway.7,8 These structural changes include patchy desquamation of the airway’s epithelium, thickening of the reticular collagen layer of the basement membrane,9 and hypertrophy of the airway’s smooth muscle.7

The mechanisms that lead to airflow obstruction in asthma are bronchoconstriction caused by contraction of the airway’s smooth muscle, mucosal edema caused by vascular leakage, increased secretion of mucus, and an inflammatory-cell infiltrate that is rich in eosinophils. Leukotrienes (LTs) have been shown experimentally to play a role in each of these mechanisms. A role in asthma for the cysteinyl LTs, known to consist of LTC4, LTD4, and LTE4,10 stems from studies of slow-reacting substance of anaphylaxis that date back to 1940.11 Slow-reacting substance of anaphylaxis A caused slow onset but very sustained contraction of smooth muscle after immunologic challenge of the lung.11,12

The cysteinyl LTs act on a single smooth muscle receptor in the airway, which has recently been designated the cys-LT1 receptor.13 LTC4 and LTD4

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can contract human airway smooth muscle in vitro and have greater than 1,000 times more potency in this action than does histamine.14,15 The most important cellular sources of the cysteinyl LTs are eosinophils, mast cells, and basophils.16 The cysteinyl LTs have been detected after bronchospasm in the blood, BAL fluid, and urine of patients with asthma.17-20 Inhibitors of LT synthesis and LT receptor antagonists (LTRAs) have demonstrated clinical activity in patients with asthma.21-33

**Dual-Phase Asthmatic Reaction**

In individuals who have atopic asthma, inhalation of a specific allergen results in the immediate degranulation of mast cells; the release of preformed mediators such as histamine and proteases; and the generation of newly formed mediators such as superoxide, platelet-activating factor (PAF), cytokines, prostaglandins (PGs), and cysteinyl LTs.34 The 60 to 75% of persons who develop allergen-induced bronchoconstriction develop bronchoconstriction from 2 to 4 h after inhalation of the allergen, which, if left untreated, progresses over the next 6 to 12 h, a phenomenon that is called the late-phase asthmatic response.35 This late-phase response is associated with increases in airway responsiveness that can last for days.36

The early-phase asthmatic airway response, which occurs shortly after antigen challenge, is most likely caused by the action of bronchoconstrictor mediators (LTs, PGD₂, thromboxane A₂, and histamine) that are released by human lung cells as a result of immunoglobulin (Ig)E-mediated degranulation. Elevated levels of LTC₄, PGD₂, thromboxane B₂, and histamine are found in BAL fluid from subjects after endobronchial-allergen challenge.17-19 Similarly, LTE₄ and thromboxane B₂ are recovered in the urine of subjects 2 h after allergen challenge.20 In contrast, the late-phase asthmatic response is characterized by an inflammatory response consisting of airway perivascular edema, mucus plugging, and infiltration that is characterized by eosinophils and other inflammatory cells, such as neutrophils and monocytes, in the airways.37 However, the late-phase asthmatic response also results from release of LTs and other products from cells infiltrating the airways.

**Endogenous Production of LTs**

IgE-mediated activation of airway mast cells leads to activation of phospholipase A₂ and hydrolysis of arachidonic acid from the cell membrane phospholipid. Arachidonic acid released in free form undergoes oxidation by the cyclooxygenase pathway in mast cells to form PGs and thromboxane (Fig 1).38,39 Although arachidonic acid is also oxygenated by 12-lipoxygenase or 15-lipoxygenase enzymes,40 its oxygenation by the 5-lipoxygenase (5-LO) enzyme pathway is what appears to be crucial for the development of allergic inflammatory reactions in the lung (Fig 1).

As a result of mast-cell stimulation, 5-LO is translocated from its cytosolic location to the nuclear membrane, where it is fully activated by the membrane protein 5-LO-activating protein.41-44 These enzymes catalyze the oxygenation of arachidonic acid to form the unstable hydroperoxyeicosatetraenoic acid (HPETE), which is the precursor of LTA₄. LTA₄ then is transformed enzymatically by the cytochemical enzyme LT₄ hydrolase to form LTD₄, a dihydroxy acid,41,45 which is inactivated by α-oxidation to 20-OH- and 20-COOH-metabolites.38,46 Alternatively, LTA₄ may be converted by the action of LTC₄ synthase to the cysteinyl LTC₄,47 LTC₄ is actively transported out of the cell, where it is metabolized further to LTD₄ and LTE₄ (formed by the sequential removal of glutamic acid and glycine from LTC₄ by dipeptidases). LTE₄ is either excreted unchanged in the urine and bile or metabolized further to a number of biologically inactive intermediates, which also are excreted in the urine and bile. The measurement of LTE₄ in urine has been used as a convenient estimate of LT production.

**LT Production in Patients Who Have Asthma**

Although the most important cellular sources of the LTs in mediation of lower and upper airway

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**Figure 1.** Schematic representation of the arachidonic acid cascade. LTC₄ is generated by the action of 5-LO on cell membrane-derived arachidonic acid. It is rapidly converted to the equipotent LTD₄ and then to the stable excretory product LTE₄.

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inflammation are eosinophils, mast cells, and basophils, each of these cells releases a distinct profile of LTs. The principal product of human eosinophils and lung mast cells is LTC₄, and LTB₄ is the major product of alveolar macrophages and neutrophils. These cells also have the potential to amplify the inflammatory response by releasing 5-LO products. In inflammatory sites, cells such as platelets, endothelial cells, and erythrocytes, which do not normally release 5-LO but do contain LTC₄ synthase, can metabolize exogenous LTA₄ to LTC₄ and LTB₄.

The production of LTs appears to be increased in patients who have asthma. A threefold to fivefold increase in production of LTB₄ and LTC₄ has been shown ex vivo in leukocytes from patients who have asthma, compared with those from healthy volunteers. Increased levels of LTC₄ and LTD₄ have also been found in BAL fluid after endobronchial allergen challenge in volunteers with asthma and in nasal secretions during reaction to aspirin in aspirin-sensitive patients with asthma. Further, urinary excretion of LTE₄ has been shown to be increased in patients with asthma who were undergoing allergen challenge, aspirin-induced asthma, nocturnal asthma, and exercise-induced asthma. In one study, increases in urinary LTE₄ correlated significantly with the magnitude of bronchoconstriction (Fig 2).

It is interesting that no significant increases in urinary LTE₄ could be demonstrated during the allergen-induced late asthmatic response, even though the magnitude of the bronchoconstriction was similar to that during the early response. Increases in urinary LTE₄ have also been demonstrated during acute spontaneous asthma. These increases decline as the asthma is treated and the bronchoconstriction resolves, a finding that suggests that the LTs are involved in the development of spontaneous symptomatic asthma.

**Inhaled LTs and Airway Responsiveness**

To my knowledge, the first report of the effects of inhaled LTC₄ and LTD₄ in vivo was published in 1981 by Holroyde et al., who demonstrated that these mediators caused bronchoconstriction in normal human subjects. Subsequently, inhaled LTC₄ and LTD₄ were shown to be potent bronchoconstrictors in both normal subjects and patients with asthma. LTD₄ has been shown to be at least 1,000 times more potent than histamine in causing smooth muscle contraction (Fig 3) and to have a longer duration of action than inhaled histamine. Inhaled LTC₄ and LTD₄ appear to have similar potencies, but LTE₄ is 30- to 100-fold less potent. The onset of action for LTD₄ and LTE₄ is 4 to 6 min, but the effect of LTC₄ is delayed 10 to 20 min.

In general, the severity of airway hyperresponsiveness is related to the severity of the asthma and to the amount of medication needed for optimal control of the symptoms of asthma. The response to inhaled LTs is exaggerated in persons who have asthma compared with that in healthy volunteers. Airway hyperresponsiveness in subjects who have asthma is nonspecific, so that an asthmatic who is
hyperresponsive to inhaled histamine will also be hyperresponsive to other inhaled mediators of constriction, such as methacholine\textsuperscript{72}, PGD\textsubscript{2},\textsuperscript{73} and PGF\textsubscript{2α},\textsuperscript{74} as well as to exercise\textsuperscript{75} and isocapnic hyperventilation.\textsuperscript{76} However, the relationships between airway responsiveness to inhaled histamine or methacholine and LTC\textsubscript{4} and LTD\textsubscript{4} are more complex. In normal subjects, LTC\textsubscript{4} and LTD\textsubscript{4} are from 1,000 to 10,000 times more potent than methacholine in causing bronchoconstriction.\textsuperscript{55} In subjects who have asthma, the dose-ratios of the concentrations of LTC\textsubscript{4} and LTD\textsubscript{4} needed to cause bronchoconstriction are lower, suggesting that persons who have asthma have tachyphylaxis to the effects of inhaled LT, because of the presence of endogenous LT in the airways.

**LTs and Airway Inflammation**

The mediators responsible for recruiting and activating inflammatory cells in asthmatic airways have not yet been identified. T-cell-derived proinflammatory cytokines may regulate the release of LTs in the bronchi of patients who have atopic asthma. The cytokines interleukin (IL)-3, IL-4, IL-5, and granulocyte-macrophage colony-stimulating factor are important in the propagation of allergic inflammation\textsuperscript{77,78} and are known to prime human basophils, eosinophils, and neutrophils for enhanced release of LTC\textsubscript{4} after stimulation by a second agonist;\textsuperscript{79,80} this may regulate LT release by cells involved in the mediation of allergic pulmonary inflammation.

Chemotaxis is an important step in the migration of inflammatory cells from the circulation to the site of inflammation. LTB\textsubscript{4} is produced mainly by neutrophils, and its predominant effect is one of neutrophil chemotaxis, although it has a lesser chemotactic effect on eosinophils.\textsuperscript{81,82} Increased numbers of neutrophils and eosinophils have been demonstrated in BAL fluid after allergen challenge,\textsuperscript{83} and inhaled LTE\textsubscript{4} also has been shown to cause eosinophils to migrate into the asthmatic air-
way, as measured in airway biopsy specimens.\textsuperscript{84} However, other mediators may be more important chemoattractants than LTD\textsubscript{4}, and the role of LTD\textsubscript{4} in the pathogenesis of asthma remains unclear.\textsuperscript{85}

The role of LTs in increased airway vascular permeability, which leads to edema, has been demonstrated in animal studies, in which LTD\textsubscript{4} was 10-fold and histamine was 100-fold less active than PAF.\textsuperscript{86} Intradermal application of LTC\textsubscript{4}, LTD\textsubscript{4}, and LTE\textsubscript{4} in humans also has been shown to produce a flare-and-wheal reaction.\textsuperscript{87,88} An increased volume of mucus is formed in asthma by submucosal glands; the LTs are some of the most potent mucus secretagogues.\textsuperscript{89,90}

Recently, a CysLT\textsubscript{4}-receptor antagonist, zafirlukast, was reported to attenuate allergen-induced migration of inflammatory cells.\textsuperscript{91} Pranlukast, a selective CysLT\textsubscript{4}-receptor antagonist, also has been shown to reduce markedly (83 to 89\%) LTD\textsubscript{4}-induced microvascular leakage, as measured by extravasation of Evans’ blue dye and eosinophilic influx in guinea pig trachea, main bronchi, and small airways.\textsuperscript{92} Both of these reports provide evidence that the ability of the CysLT\textsubscript{4}-receptor antagonist to attenuate proinflammatory effects of LTD\textsubscript{4} may contribute significantly to their therapeutic effectiveness in asthma. Ultimately, studies of the ability of the CysLT\textsubscript{4}-receptor antagonist to attenuate persistent inflammation in the asthmatic airway are needed.

**LTs in Clinical Models of Asthma**

The best evidence that the LTs play a central role in causing clinical models of asthma such as exercise-, cold air-, and allergen-induced bronchoconstriction is provided by the observations that many different CysLT\textsubscript{4}-receptor antagonists (eg, pranlukast; zafirlukast; MK-571; MK-679) and LT synthesis inhibitors (eg, zileuton) markedly attenuate bronchoconstrictor responses after exposure to exercise, cold air, and allergens. In studies of exercise-induced asthma, MK-571 inhibited exercise-induced bronchoconstriction by 50 to 70\%.\textsuperscript{24} Also, the 5-LO inhibitor zileuton has been shown to attenuate cold-air-induced bronchoconstriction.\textsuperscript{32} In allergen-challenge studies, the early-phase and late-phase bronchoconstrictor responses are reduced by zafirlukast.\textsuperscript{21} In a more recent study, the CysLT\textsubscript{4}-receptor antagonist pranlukast was shown to reduce significantly the changes in total respiratory resistance and FEV\textsubscript{1} in the later part (20 to 60 min after challenge) of immediate airway obstruction after inhaled allergen without any improvement in the early part (first 10 min after challenge) (Fig 4).\textsuperscript{22} Allergen-induced increase in airway responsiveness after allergen challenge is also inhibited by zafirlukast.\textsuperscript{21} In studies of aspirin-induced asthma, SK&F 104,353\textsuperscript{29} and zileuton\textsuperscript{31} inhibited bronchoconstriction. Pranlukast also inhibited the bronchoconstriction during inhalation challenge with the antipyretic drug dipyrone in aspirin-sensitive subjects: bronchoconstriction was completely inhibited in subjects whose plasma pranlukast levels were more than 0.5 \(\mu\)g/mL.\textsuperscript{30}

CysLT\textsubscript{4}-receptor antagonist also improved lung function acutely in subjects with asthma who had experienced bronchoconstriction before beginning treatment with these antagonists.\textsuperscript{75,77,79} These results suggest that the LTs are partially responsible for spontaneous bronchoconstriction in asthma. The magnitude of the resulting improvement in lung function was much less than that achieved with large doses of an inhaled \(\beta_2\)-agonist. However, in both studies that investigated this phenomenon, the magnitude of improvement achieved with concomitant administration of the CysLT\textsubscript{4}-receptor antagonist and the \(\beta_2\)-agonist was significantly greater than that noted with the \(\beta_2\)-agonist alone. In airway hyperre-

![Figure 5. Effect of oral administration of pranlukast on bronchial responsiveness to methacholine in subjects who had asthma. PC\textsubscript{20}FEV\textsubscript{1} = provococation concentration of methacholine that produced a 20\% fall in FEV\textsubscript{1}. Dashed line = patients taking theophylline; solid line = patients not taking theophylline; closed circles = patients who had extrinsic asthma; open circles = patients who had intrinsic asthma (reprinted with permission from Fujimura et al).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21744/ on 06/26/2017)
responsiveness studies, pranlukast suppressed airway hyperresponsiveness to methacholine after 1 week of treatment in a double-blind, randomized, placebo-controlled, two-phase crossover study in subjects whose asthma was stable (Fig 5).23

In clinical studies of asthma, the CysLT1-receptor antagonists pranlukast, zafirlukast, and MK-679 have been shown to be effective at reducing symptom scores, β2-agonist use, and improving results of spirometry.25-28 A recent multicenter, placebo-controlled, double-blind study showed a 15% increase in FEV1 in patients who had moderately severe asthma within 1 h of an initial dose of 600 mg zileuton.33 A statistically significant increase in FEV1, a decrease in the symptoms of asthma, and a decrease in the use of β2-agonists were observed in subjects given the highest dosage of zileuton (2.4 g daily) after 4 weeks of treatment, compared with those given placebo.

CONCLUSION

In recent years, numerous studies have demonstrated the critical role that LTs play in the pathogenesis of asthma and have confirmed the role played by LTC4 and LTD4 in causing bronchoconstriction in asthma. These studies have demonstrated that inhaled LTC4 and LTD4 are the most potent bronchoconstrictors yet studied in human subjects. Further, clinical models have supported the hypothesis that the LTs play an important role in different manifestations of asthma, such as exercise-, allergen-, and aspirin-induced asthma. Moreover, specific inhibitors of the 5-LO pathway and the CysLT1-receptor antagonists do seem to be effective in a number of experimental models of asthma and, in particular, in a number of clinical trials.

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