Role of Leukotrienes in Asthma Pathophysiology

Hans Bisgaard, MD, Dr Med, Sci*

Summary. Inflammation is an essential component of asthma pathophysiology. While $\beta_2$-agonists are often used for short-term relief of acute bronchospasm, anti-inflammatory agents are required for the long-term management of chronic inflammation in this disease. Corticosteroids have emerged as the first-line anti-inflammatory therapy for asthma management. However, in some patients, especially children, the high doses of corticosteroids that may be required to control features of hyperresponsiveness, including exercise-induced asthma, raise safety concerns. Thus, there is a need for complementary anti-inflammatory, steroid-sparing agents in asthma therapy. Several inflammatory mediators have been targeted in an attempt to thwart this inflammatory process, but so far with little success.

The cysteinyl leukotrienes (CysLT), LTC$_4$, LTD$_4$, and LTE$_4$, have been shown to be essential mediators in asthma, making them obvious targets for therapy. These cysteinyl leukotrienes, previously known as the slow-reacting substance of anaphylaxis (SRS-A), mediate many of the features of asthma, including bronchial constriction, bronchial hyperreactivity, edema, and eosinophilia. Data show that selective cysteinyl leukotriene receptor antagonists (CysLTRAs) effectively reverse these pathologic changes. Corticosteroids do not inhibit the production of CysLTs in vivo, suggesting that CysLTRAs and corticosteroids affect different targets. The bronchodilator properties of CysLTRAs seem to be additive to those of $\beta_2$-agonists and corticosteroids.

These data suggest that CysLTs are important therapeutic targets in the management of inflammation in asthma. Pediatr Pulmonol. 2000; 30:166–176. © 2000 Wiley-Liss, Inc.

Key words: cysteinyl leukotriene; asthma; cysteinyl leukotriene receptor antagonists; montelukast; inflammation.

ASTHMA: MORE THAN BRONCHOCONSTRICTION

The focus in asthma therapy is shifting from the short-term relief of acute bronchoconstriction to long-term management of chronic inflammation. The inflammatory process of asthma involves recruitment and activation of eosinophils and release of cytokines, resulting in changes in airway morphology such as increased smooth muscle mass, subepithelial fibrosis, edema, and epithelial cell damage. These pathologic changes, which ultimately are manifested as bronchial hyperresponsiveness and bronchoconstriction, must be addressed to manage the disease effectively. Asthma therapies developed to block the activities of various inflammatory mediators in an attempt to thwart the inflammatory process have been relatively unsuccessful until recently. This paper will review the studies that implicate the cysteinyl leukotrienes (CysLTs) as key inflammatory mediators in asthma. In addition, it will discuss the growing body of clinical evidence that the CysLT receptor antagonists (CysLTRAs), which can block the pathologic activities of CysLTs, are effective tools for management of asthma.

Defining the Mediator Soup

Development of specific agents to block an inflammatory process is a challenging endeavor, since inflammation is a multistep process with many mediators to be considered. A close look at asthma reveals a complex interaction of an apparently ever-increasing number of mediators in the inflammatory process. The term “mediator soup” has been adopted by some to describe the large collection of mediators involved.

While the inflammatory process in asthma is complex, some key mediators can be discerned. If exposure to an individual mediator results in the pathologic changes associated with the disease, and it can be found in increased quantities during the disease, then the mediator is likely a key component of the process. In addition, antagonism of the mediator’s activities that results in prevention or reversal of the pathologic changes is further evidence of a central role for the mediator. Evidence relating to the biochemistry, pathophysiology, and antagonism of the CysLTs suggests that these mediators play a key role in the pathophysiology of asthma.

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LEUKOTRIENES: GENERAL BIOCHEMISTRY

Leukotrienes are produced from membrane phospholipids via the 5-lipoxygenase pathway of the arachidonic acid (AA) cascade. AA metabolites are short-range inflammatory mediators that exert varied effects locally.4 The types of mediators produced from AA metabolism vary in different tissues, depending on enzyme pathways present in various cell types. AA is incorporated into the membrane phospholipids from the essential fatty acid, linoleic acid. AA is subsequently cleaved from these phospholipids.

Cytosolic phospholipase A2 (PLA2), which is activated by mechanical, chemical, or physical stimuli, translocates to the membrane to liberate membrane-bound arachidonic acid (Fig. 1).4 Free AA can be converted by the cyclooxygenase pathway to form the prostaglandins (PG) PGI2, PGE2, PGF2, PGD2, and thromboxane (TBX). Alternatively, AA can be converted by the 5-lipoxygenase (5-LO) pathway to form leukotrienes.5 The 5-LO enzyme acts on AA to form 5-hydroperoxyeicosatetraenoic acid (5 HPETE), which it then converts to the nonpeptide leukotriene LTA4.6 For activation, the 5-LO enzyme must bind to the membrane-bound 5-lipoxygenase-activating protein (FLAP).7 LTA4 can then be converted to the nonpeptide leukotriene LTB4 or to the cysteinyl leukotriene (CysLT) LTC4. LTC4 is enzymatically converted to LTD4, and subsequently LTE4.3,8,9 Although LTB4 has important functions in cellular recruitment, its role in asthma has not been well defined, and this leukotriene will not be discussed further in this review.

LEUKOTRIENES: PATHOPHYSIOLOGY

History

Although the chemical characterization of the CysLT mediators is relatively recent, the effects of these agents have been known for a long time. In the late 1930s and early 1940s, the laboratory of Feldberg and Kellaway noted that a material released from cobra venom-perfused guinea-pig lungs caused the contraction of smooth muscle. They proposed that this substance contributes to anaphylaxis-induced contraction of smooth muscle independently of histamine.10–12 They named this material “slow-reacting muscle-stimulant substance” because it caused a more delayed muscle contraction than did histamine.10 This substance was later named slow-reacting substance of anaphylaxis (SRS-A) when its role in anaphylaxis-induced bronchoconstriction was better defined.13 It was not until the late 1970s and early 1980s that the CysLTs were identified as the active component of SRS-A and that their metabolic pathways were defined.14

Sources/Sites of Action

The CysLTs are produced predominantly by eosinophils, mast cells, and macrophages, which are cells present or recruited to the lung in large numbers in asthmatics.5 The production of CysLTs can be induced by a number of stimuli that activate the tissue 5-LO enzyme. These include antigen challenge of sensitized tissues or exposure to the inflammatory mediator PAF, the complement anaphylatoxins, or calcium signals.15–19 Several studies suggest that elevated levels of CysLTs are markers of the asthmatic process. Blood leukocytes from patients with asthma release more CysLTs than do those of controls,20 and the plasma levels of LTE4 correlate with disease severity in asthmatics.21 In addition, LTE4 levels are elevated in the urine of patients with spontaneous asthma attacks,22,23 in patients with exercise-induced bronchospasm,24 in patients with nocturnal exacerbations of asthma,25 and in patients with mild-to-moderate asthma (compared with those in controls).26 It is not known, however, whether the urinary levels of CysLTs reflect the local production of CysLTs in the lung or a more systemic response to allergens.27

In humans, a single cell membrane receptor (CysLTR1)8 modulates the activity of both LTC4 and LTD4.28 CysLT receptors are located in the plasma membranes of smooth muscle cells in the airway, as well as on other cell types.29,30 A CysLTR2, which is resistant to blockade, has been identified on the human pulmonary vasculature.8

Pathophysiologic Effects

While the above studies do not establish causality between CysLTs and asthma, other studies have shown that the CysLTs mediate, either directly or indirectly, several of the pathophysiologic changes of asthma (Fig. 2), as discussed below.

Vascular effects

Net fluid transport across a vascular bed reflects the net hydrostatic and osmotic pressures as well as the in-

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>5-LO</td>
<td>5-lipoxygenase</td>
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<tr>
<td>5-HPETE</td>
<td>5-hydroperoxyeicosatetraenoic acid</td>
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<tr>
<td>AA</td>
<td>Arachidonic acid</td>
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<tr>
<td>CysLT</td>
<td>Cysteinyl leukotrienes</td>
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<td>CysLTR1</td>
<td>Cyst LT membrane receptor</td>
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<tr>
<td>CysLTRA</td>
<td>Cysteinyl leukotrine receptor antagonists</td>
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<tr>
<td>FLAP</td>
<td>5-lipoxygenase-activating protein</td>
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<tr>
<td>PLA2</td>
<td>Cytosolic phospholipase A2</td>
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<tr>
<td>SRS-A</td>
<td>Slow-reacting substance of anaphylaxis</td>
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<td>TBX</td>
<td>Thromboxane</td>
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trinastic permeability of the vessel wall. Increasing the permeability of the vessels and potentiating this effect by increasing blood flow through capillary recruitment may cause edema. Our group found that LTD₄ increases the vascular permeability and interstitial transport of macromolecules in human skin.³¹ LTD₄ also induces increased cutaneous blood flow in human skin as potently as does histamine.³² In addition, LTD₄ induces a dose-related increase in nasal mucosal blood flow.³³ Thus, increased blood flow (resulting in increased hydrostatic pressure) combined with increased vascular permeability result in net fluid extravasation and edema. An increased amount of plasma proteins escapes into the tissues, providing the source for potent plasma-protein-derived inflammatory mediators including the kinins, complement components, and clotting factors. These mediators may form mucus
plugs, inhibit mucociliary clearance, and fuel the inflammatory process.\textsuperscript{34,35}

**Cellular recruitment**

Cellular recruitment is a key step in pulmonary inflammation. Eosinophils contribute to the amplification of the inflammatory process in asthma and can release mediators that damage tissues directly.\textsuperscript{36} LTD\textsubscript{4} inhalation has been shown to recruit eosinophils into the airways of guinea pigs.\textsuperscript{37} LTD\textsubscript{4} inhalation also recruits eosinophils into the lungs of atopic asthmatic patients, as measured in a differential cell count of a sample of induced sputum taken 4 hr after the challenge.\textsuperscript{38} Laitinen et al.\textsuperscript{39} also found that LTE\textsubscript{4} inhalation increases the number of eosinophils and neutrophils in airway mucosa 4 hr after challenge in asthmatic patients.

The mechanism by which CysLTs induce chemotraction is not well defined. While some studies showed that LTD\textsubscript{4} and LTB\textsubscript{4} possess chemotactant activity,\textsuperscript{40,41} other in vitro studies have generally not found CysLTs to be eosinophil chemotactants.\textsuperscript{42–44} White blood cell recruitment might occur in the absence of a direct chemotactant effect of the CysLTs, since several of the edema-inducing properties of CysLT may encourage eosinophil infiltration. For example, the increased venopermeability caused by these agents may allow more white blood cells to extravasate. Alternatively, CysLT may induce endothelial cells to secrete PAF, a mediator that can increase cellular adhesion.\textsuperscript{45}

**Bronchoconstriction**

CysLTs are potent contractile agonists of both peripheral and central airway smooth muscle. Drazen et al.\textsuperscript{46} found that LTD\textsubscript{4} and LTC\textsubscript{4} were more potent contractile agonists than histamine in guinea pig bronchial and tracheal smooth muscle strips studied in vitro. These bronchoconstricting effects are also found in humans in vivo. We conducted a study in 33 nonasthmatics to determine the bronchoconstricting properties of inhaled LTD\textsubscript{4}. While LTD\textsubscript{4} caused a dose-dependent reduction in flow measures of small airways in these nonasthmatics, it resulted in only minor changes in forced expiratory vol-

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**Fig. 2. Role of CysLTs in asthma.** Potential sites and effects of CysLTs relevant to a pathophysiological role in asthma. CysLTs mediate several of the hallmark processes of asthma including edema, contraction and proliferation of smooth muscle, ciliary paralysis, increased mucus secretion (?), and eosinophil recruitment.
Increased mucus secretion/decreased clearance

The presence of thick, tenacious mucus plugs in the airway lumina is a frequent finding in patients with severe asthma. Mucus may accumulate owing to increased production or inefficient elimination caused by ciliary dysfunction. LTC4 and LTD4 are potent airway mucus secretagogues. Using cultured human airways, Marom et al. found that LTC4 and LTD4 dose-dependently increased the production of mucus in vitro. In animal models, CysLTs increased mucus production from both submucosal glands and epithelial goblet cells. However, these data were apparently contradicted by our findings that LTD4 is involved in altering nasal mucosal blood flow and does not increase nasal secretions. Also, none of the studies on the effects of inhaled CysLT reported an increased airway secretion in the study patients.

Our group also found that CysLTs diminish the activity of human respiratory cilia, an effect that may contribute to the impaired clearance of mucus in the lower airway.

In summary, the above evidence indicates that CysLTs mediate many of the pathophysiologic changes in asthma.

POTENTIAL ANTI-INFLAMMATORY TARGETS IN ASTHMA

Targeting the Mediators

Histamine was one of the first asthma mediators discovered, and like others, it has been targeted in an attempt to thwart the inflammatory process of asthma. However, antihistamines have not been shown effective in the treatment of asthma. Prostaglandins have also been potential targets, but agents directed against this group of mediators have been disappointing. Subsequent investigations of antagonists of numerous other potent mediators have also provided disappointing results.

The mast cell stabilizing agents, cromolyn and nedocromil, inhibit inflammatory cell action and mediator release, bronchoconstriction, and airway responsiveness. However, these agents have been insufficient for disease control, and there is no direct evidence for their having an anti-inflammatory effect. Inhaled corticosteroids effectively alter the production of inflammatory cytokines and reduce the infiltration of inflammatory cells in the bronchial mucosa. These agents have moved into place as first-line controller treatment for mild persistent asthma. High doses of inhaled corticosteroids are required to control asthma symptoms in some pediatric patients. Symptoms of bronchial hyperreactivity, particularly exercise-induced asthma, require relatively high doses of corticosteroids, and this may be cause for concern in some children. Thus, there is a need for comple-
mentary treatment that can be used along with glucocorticoids to lower the dose of glucocorticoid required (steroid-sparing agents). Preferably, such treatment should supplement the anti-inflammatory effect of the glucocorticoids.

**Targeting the CysLTs**

**The role of fish oil diet supplementation and corticosteroids**

Several steps in the AA cascade are potential targets for CysLT inhibition. Supplementation of the diet with fish oils can make available alternative fatty acid substrates that can be incorporated into membrane phospholipids. Such substances, when metabolized, might yield products with less inflammatory activity. Ingestion of fish oil enriched in eicosapentaenoic acid (an omega-3 fatty acid) results in altered AA structure, and studies suggest that these changes may affect phospholipase, 5-LO, or LTB₄ hydrolyase functions or alter the structure of the leukotriene products formed. A study in mice found that a diet rich in fish oil protected against hypoxia-induced bowel necrosis. In rats, a diet rich in fish oil suppressed the increase in CysLT production in bronchoalveolar lavage (BAL) fluid and neutrophil accumulation in the lung associated with endotoxemia.

The potential role of eicosapentaenoic acid in human asthma is not clear. The response in pulmonary mechanics (decrease in dynamic compliance) to infusion of antigen in sensitized guinea pigs was actually enhanced by a diet enriched in marine lipids compared to that in animals fed a control diet. In such instances, ingestion of fish oils may actually shift the balance of products from the cyclooxygenase pathway to the 5-LO pathway, incurring the 5-LO pathway and CysLT activity have been investigated in vitro. Sebaldt et al. found that prednisone administration did not alter LTE₄ excretion in the urine of healthy volunteers. One animal study did show an in vivo effect. Treatment of rats with dexamethasone at both 2 and 14 hr before ovalbumin challenge resulted in reduction of N-acetyl-LTE₄ production in bile. However, the drug given only once at 1 hr before challenge had little effect on LTE₄ production.

The reasons for the discrepancies between the in vitro and in vivo effects of glucocorticoids on CysLTs are unclear. Crocker et al. and Kurimoto et al. suggested that glucocorticoids may modulate their effects on CysLT indirectly through other mediators. Another possible explanation may be that urinary CysLT levels, frequently measured to determine the effect of an agent on the lipoxygenase pathway, do not reflect the anti-inflammatory effect of glucocorticoids in the lung. If this is the case, glucocorticoids may reduce the production of CysLT in the lung, thereby reducing inflammation, but with no change in overall whole body LT production as measured in urine. Perhaps basal and stimulated urinary LTs arise from different cell populations. Alternatively, glucocorticoids may have a differential effect on basal and stimulated (i.e., allergen) eicosanoid generation. These observations also raise issues about the meaningfulness of measuring urinary CysLT levels to determine what is happening in the lung.

Collectively, these studies suggest that the metabolic effects of corticosteroids on CysLT production are complex and need further elucidation. Most in vivo studies do not show an inhibitory effect of glucocorticoids on CysLT production, and it may be that the inflammatory pathways affected by glucocorticoids and CysLT inhibitors might be somewhat distinct. Therefore, agents that interfere with CysLT activity may work synergistically with glucocorticoids, since they might target a pathway not blocked by glucocorticoids (i.e., a divergent target). Agents blocking CysLT activity are thus likely to work complementary to glucocorticoids.

**5-LO inhibitors and FLAP inhibitors**

Several types of inhibitory therapies specifically targeting the 5-LO pathway and CysLT activity have been developed. These include 5-LO inhibitors and FLAP inhibitors, which block the biosynthetic pathway, and CysLTRAs, which block the receptor binding of CysLTs.
The 5-LO enzyme is a complex enzyme that requires many cofactors for activity; it can be inhibited by trapping radicals, chelation, iron reduction, or by binding at an active or regulatory site. Zileuton (A-64077), an N-hydroxyurea derivative, is probably the most extensively studied 5-LO inhibitor. In humans, it has been found effective in inhibiting bronchoconstriction induced by cold, dry air, and aspirin-induced asthma. Specific 5-LO inhibitors have been developed that have been shown effective in reversing CysLT activity in animal models of inflammation, reducing antigen-induced bronchoconstriction. Interestingly, mediators that bind to FLAP and 5-LO may also cross-react with binding sites on LTC₄ synthase.

FLAP is the target of several agents, including BAYx 1005, MK-886, and MK0591. BAYx 1005 has been shown to attenuate allergen-induced bronchoconstricctor responses. Another FLAP inhibitor, MK-0591, antagonizes FLAP, preventing the translocation of 5-LO to the membrane that is necessary for 5-LO activation. MK-0591 also inhibited urinary LTE₄ production in normal human volunteers and blocked CysLT activity in vitro and in vivo.

The CysLT receptor antagonists

CysLT receptors have been the target of compounds from diverse structural classes. The first-generation CysLTRAs were neither selective, nor potent. However, three selective and potent second-generation CysLTRAs, pranlukast, zafirlukast, and montelukast, have been studied extensively for clinical use. The sites where CysLTRAs block the pathologic changes mediated by CysLTs are illustrated in Figure 2.

Eosinophil recruitment into the lung is an important part of the asthmatic process, and CysLTRAs block this step. Underwood et al. found that pranlukast attenuates eosinophil recruitment following LTD₄ instillation in the guinea pig. Calhoun et al. showed that zafirlukast inhibits the influx of eosinophils and mast cells during segmented-allergen challenge in patients with mild asthma. Montelukast reduced the peripheral eosinophilia of asthmatic children in a large, multicenter clinical trial. In adults with mild uncontrolled asthma, montelukast reduced the number of sputum eosinophils.

The CysLTRAs also block leukotriene-mediated bronchoconstriction. In isolated lung bronchi, pranlukast antagonized LTC₄-induced contractions. De Lepeleire et al. found that montelukast attenuated LTD₄-induced bronchoconstriction in patients with mild asthma. As described below, these agents also protect against bronchoconstriction in clinical asthma. However, CysLTRAs do not induce bronchodilation in normal volunteers. This difference between nonasthmatics and asthmatics suggests that the CysLTRAs reverse the bronchoconstriction or increased basal smooth muscle tone caused by CysLTs, but that they do not exert a direct bronchodilating effect. Several other studies support this premise. Persistent bronchoconstriction in aspirin-sensitive asthmatic subjects may be relieved by CysLTRAs. In one study, baseline airway obstruction actually correlated with the degree of bronchodilation achieved with a CysLTRA. The CysLTRAs may even act to prevent some of the pathologic airway remodeling found in asthma. Panettieri et al. found that pranlukast inhibited LTD₄-induced potentiation of human airway smooth muscle proliferation.

CysLTRAs may reverse other pathologies associated with CysLT activity. ICI 204.219 suppressed the allergen-induced increase in nonspecific bronchial reactivity (hyperresponsiveness) to histamine. Using cultured human airways, Marom et al. found that a selective CysLTRA antagonist reversed the LTC₄⁻ and LTD₄⁻induced production of mucus in vitro.

**CYSTEINYL LT RECEPTOR ANTAGONISTS IN CLINICAL ASTHMA**

Clinical studies suggest that the CysLTRAs will be important therapies for asthma. Use of these agents improves baseline lung function, reduces symptoms, and lessens the requirement for rescue medications. High-dose zafirlukast therapy for 6 weeks significantly improved asthma control over placebo in patients who were refractory to high-dose inhaled corticosteroid therapy. Montelukast has been found effective in improving asthma control in both pediatric and adult patients with chronic asthma.

Several types of observations support an important role for CysLTRAs in asthma control. The early-phase response (most likely caused by acute airway smooth muscle contraction) and the late-phase response (thought to involve edema and inflammatory cell recruitment and activation) of asthma can be blocked by these agents. Taylor et al. found that the CysLTRA ICI 204.219 significantly attenuated the early- and late-phase bronchoconstriction to inhaled allergen and suppressed the allergen-induced increase in nonspecific bronchial reactivity. In animals and humans, montelukast significantly inhibited airway resistance associated with the early and late phase of airway response to allergens. The finding that the CysLTs block both stages suggests that CysLTs contribute more extensively to the asthmatic process than was previously thought. In addition, the beneficial effects of CysLTRAs on acute bronchoconstriction suggest that they protect against events that usually require rescue β-agonist use. Another observation suggests that CysLTRAs maintain asthma control. Exercise-induced bronchoconstriction is likely to occur in patients with uncontrolled...
against exercise-induced bronchoconstriction in both adults and children with asthma. These data suggest that this once-daily CysLTRAs protects the patient against potential asthma challenges throughout the day. Such activity may also protect against other manifestations of poor control, such as progressive airway remodeling.

CONCLUSIONS

CysLTs are key mediators in the pathophysiology of asthma. Inhibition of their action is a promising therapeutic approach for a difficult-to-manage disease. As we begin to tease out the inflammatory processes occurring in individual asthma patients, we should be able to use anti-inflammatory tools such as CysLTRAs to target the pathophysiology leading to bronchoconstriction. These agents may be important long-term therapeutic options for complementary control of inflammation in children and adults with various types of asthma.

REFERENCES


Pizzichini E, Leff JA, Reiss TF, Hendeles L, Boulety L-P, Wei LX, Weinland DE, Hargreave FE. Montelukast reduces airway

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inflammation in asthma; a randomized controlled clinical trial. Europ Respir J 1999;14:12–18.


