



reviews

Cysteinyl Leukotrienes in Allergic Inflammation*

Strategic Target for Therapy

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Systemically bioavailable leukotriene receptor antagonists (LTRAs) can reduce the essential components of allergic inflammation in allergic rhinitis (AR) and asthma by blocking cysteinyl leukotriene (CysLT) activity, resulting in a wide range of clinical effects. CysLTs, mediators, and modulators in the pathophysiology of asthma and AR are a key target for therapy because they modulate production of hemopoietic progenitor cells, survival and recruitment of eosinophils to inflamed tissue, activity of cytokines and chemokines, quantity of exhaled NO, smooth-muscle contraction, and proliferation of fibroblasts. The mechanism of action of LTRAs leads to their effects on systemic allergic inflammatory processes. (CHEST 2005; 127:1312-1326)

Key words: asthma; cytokines; eosinophils; inflammation; leukotrienes; leukotriene receptor antagonists; remodeling; rhinitis

Abbreviations: AR = allergic rhinitis; AUC = area under the FEV₁-vs-time curve; CysLT = cysteinyl leukotriene; GM-CSF = granulocyte-macrophage colony-stimulating factor; ICAM = intercellular adhesion molecule; ICS = inhaled corticosteroid; IL = interleukin; LTC₄ = leukotriene C₄; LTD₄ = leukotriene D₄; LTRA = leukotriene receptor antagonist; NO = nitric oxide; PAF = platelet-activating factor; PC₂₀ = provocative concentration needed to produce a 20% decline in FEV₁; PEF = peak expiratory flow; Th2 = type 2 T helper; TNF = tumor necrosis factor; VCAM = vascular cell adhesion molecule

Asthma and allergic rhinitis (AR) are two of the most common respiratory disorders encountered in clinical practice. They are linked by a unified airway and a common pathophysiology. Asthma is a chronic inflammatory disorder of the lower airway that causes bronchoconstriction, airway edema, mucus secretion, and airway remodeling, leading to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing.¹⁻³ Similarly, AR is a chronic inflammatory disorder of the upper airway that typically causes rhinorrhea, congestion, sneezing, and pruritus.^{4,5}

Cysteinyl leukotrienes (CysLTs) are key mediators and modulators of systemic allergic responses, as well as an important component of the inflammatory responses that lead to the typical symptoms of asthma, including bronchoconstriction, wheezing, increased mucus secretion, and decreased mucociliary clearance.⁶⁻⁸ They are produced by eosinophils, basophils, macrophages, mast cells, and to a lesser extent T cells and endothelial cells.^{9,10} CysLTs cause vasodilation and increased microvascular permeability, both of which lead to tissue edema,¹¹⁻¹³ and can prolong eosinophil survival.¹⁴ Increased CysLT lev-

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els have been detected in the urine,¹⁵⁻¹⁷ BAL fluid,¹⁸ and sputum^{19,20} of patients with asthma; the increased sputum levels correlate with both eosinophil levels²⁰ and symptom severity.¹⁹ Nasal allergen challenge causes a dose-dependent increase in CysLTs that correlates with nasal symptoms.²¹ Consequently, CysLTs likely represent an important strategic target in both asthma and AR.

The effects of CysLTs in asthma and AR are mediated through the CysLT₁ receptor,²² which is a G-protein-coupled receptor expressed in monocytes/macrophages, eosinophils, basophils, mast cells, neutrophils, T cells, B lymphocytes, interstitial cells of the nasal mucosa, and smooth-muscle cells.²²⁻²⁶ The leukotriene receptor antagonists (LTRAs) montelukast and zafirlukast selectively block the CysLT₁ receptor and produce multiple beneficial effects locally in the asthmatic or allergic rhinitic airway and systemically on the allergic inflammatory response. This review examines the wide range of clinical effects produced by LTRAs in asthma and AR as well as the multiple pathophysiologic mechanisms by which LTRAs may alter allergic inflammation.

ADULT PERSISTENT ASTHMA

Pulmonary Function

LTRAs improve pulmonary function, measured as the FEV₁, by 8 to 13% in patients with mild,²⁷

mild-to-moderate,²⁸⁻³⁰ and severe³¹ asthma. Oral montelukast significantly increased baseline FEV₁ by 27%, attaining nearly maximal effect within 2 h.³² Similarly, LTRAs significantly increased peak expiratory flow (PEF), measured by patients at home, in patients with mild-to-moderate asthma^{28-30,33-35} and moderately severe asthma.^{31,36}

When montelukast was compared with the inhaled corticosteroid (ICS) beclomethasone, the mean FEV₁ increase was significantly ($p < 0.05$) greater with beclomethasone (13.1% vs 7.4%³⁷ or 0.38 L vs 0.24 L³⁸). Although the mean responses were significantly different, a large portion of those responding with larger improvements ($\geq 10\%$ change or > 0.4 L) received montelukast. Figure 1 shows the distribution of incremental improvements of FEV₁ for each drug; as shown, there were patients who were better responders and patients who were poorer responders to each drug. Overall, there was an 81% overlap of the responses to each drug.^{38,39} In a 3-year extension study,⁴⁰ montelukast and beclomethasone produced similar overall improvements in FEV₁ during the second and third years.

Airway Hyperresponsiveness

LTRAs have also been found to reduce airway hyperresponsiveness, an indicator of airway inflammation. LTRAs modestly increased the provocative concentration needed to produce a 20% decline in

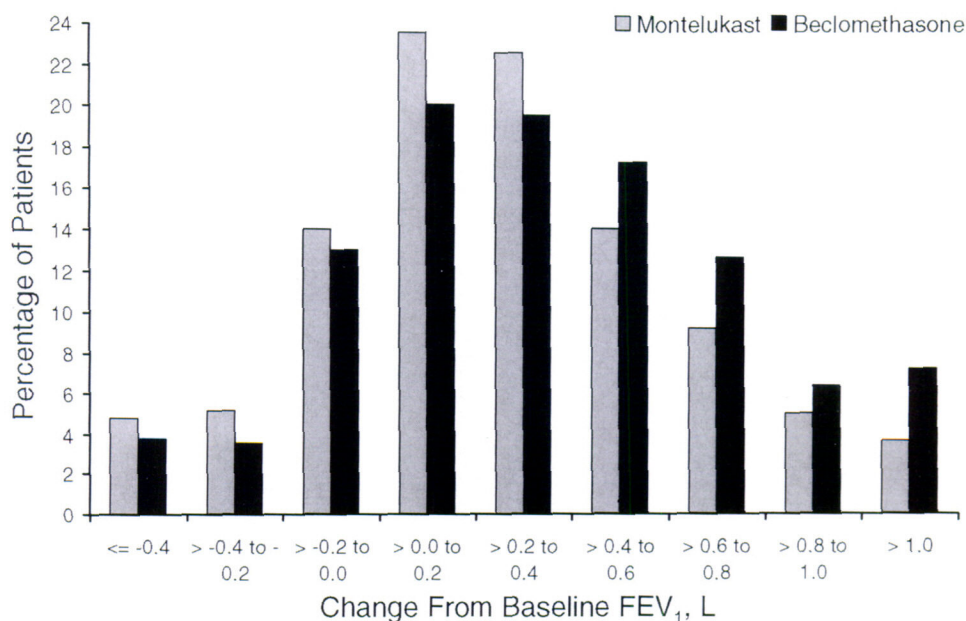


FIGURE 1. Frequency distributions of percentage change from baseline FEV₁ after montelukast (gray bars) and beclomethasone (black bars) treatments. A large portion of individuals responding with larger improvements (> 0.4 L) were patients receiving montelukast. Reproduced with permission from Israel et al.³⁸

FEV₁ (PC₂₀) of methacholine,⁴¹⁻⁴³ histamine,^{43,44} and adenosine monophosphate.⁴⁵ Similarly, adding montelukast or zafirlukast to ICS further increased the PC₂₀ of adenosine monophosphate compared with placebo.^{46,47} Because of its action as a CysLT antagonist, the PC₂₀ for inhaled leukotriene D₄ (LTD₄) increased 66-fold when zafirlukast was added to ICS therapy.⁴⁸

Symptoms and Quality of Life

Montelukast significantly decreased both daytime asthma symptoms and nocturnal awakenings in patients with mild asthma²⁷ and with mild-to-moderate asthma.²⁹ Zafirlukast reduced daytime asthma symptom scores by 26.5%²⁸ and increased symptom-free days by 89%.⁴⁹ Montelukast significantly improved the asthma quality of life (Fig 2) in all four domains (activity, symptoms, emotional function, and exposure to environmental stimuli).^{29,50} In more severe forms of asthma (FEV₁ 45 to 80% of predicted), zafirlukast improved daytime symptom scores by 23 to 26%.^{31,50}

β-Agonist Use

In patients with mild asthma, the LTRA montelukast significantly reduced the need for rescue β-agonist use by 32% and increased the number of rescue-free days by 25%.²⁷ In mild-to-moderate asthma, montelukast or zafirlukast reduced β-agonist

use by 20 to 24%.^{28,29,50} It was also effective when added to high-dose ICS therapy.⁵¹

Eosinophilia

LTRAs significantly decrease eosinophil levels in blood, BAL fluid, and sputum.^{29,52,53}

LTRA as Add-on Therapy

The LTRA montelukast can be added to ICS therapy to obtain better control, as measured by significant improvement of FEV₁, daytime asthma symptom scores, and nocturnal awakenings.⁵⁴ It was as effective as doubling the dose of budesonide.⁵⁵ In patients requiring moderate-to-high doses of ICS, adding montelukast permitted significant tapering of the ICS dose without loss of asthma control.⁵⁶ In chronic asthma inadequately controlled with a high dose of ICS (1,000 to 4,000 μg/d), zafirlukast not only significantly improved pulmonary function and asthma symptom scores^{51,57} but also significantly reduced the risk of an asthma exacerbation by 39%.⁵¹

PERSISTENT ASTHMA IN PEDIATRIC PATIENTS

Pulmonary Function

Montelukast (approved for children ≥ 2 years old) and zafirlukast (approved for children ≥ 5 years old) significantly improved pulmonary function and re-

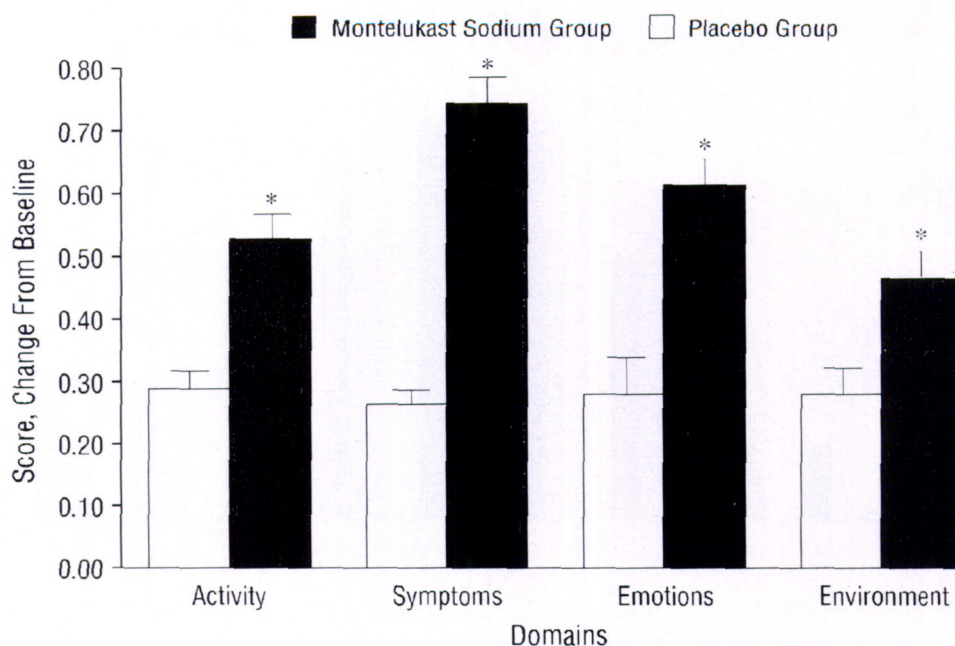


FIGURE 2. Improvement in asthma-specific quality-of-life scores in 681 patients with asthma randomized to 12 weeks of therapy with montelukast or placebo. Bars depict the mean value, and error bars depict the SE. * $p < 0.001$. Reproduced with permission from Reiss et al.²⁹

duced symptoms, irrespective of concomitant ICS use.⁵⁸⁻⁶⁰ In children 6 to 14 years of age who were inadequately controlled by budesonide, the addition of montelukast significantly increased FEV₁ (6.0% vs 4.1% with placebo; $p = 0.01$) and PEF (difference from placebo: morning PEF, 9.7 L/min, $p = 0.023$; evening PEF, 10.7 L/min, $p = 0.012$).⁶¹

In an open-label extension trial⁴⁰ in children 6 to 14 years of age, both montelukast and beclomethasone maintained a 17% improvement of FEV₁ at the end of 68 weeks. Both children and their parents reported significantly more satisfaction with montelukast than with beclomethasone therapy, as they found it more convenient and less difficult to use.⁶²

Symptoms and Quality of Life

Montelukast significantly reduced the percentage of days with daytime asthma symptoms and increased the percentage of asthma-free days in children 2 to 5 years of age with persistent asthma.⁵⁹ Zafirlukast significantly reduced nighttime awakenings in 5- to 11-year-old children.⁶⁰ In a small study⁶³ with very young children (5 to 20 months) with severe virus-induced bronchiolitis poorly responsive to ICS and bronchodilators, montelukast provided clinical improvement within 1 week. In children with respiratory syncytial virus bronchiolitis, 3 months to 3 years of age, montelukast produced symptom-free

days and nights 22% of the time (4% with placebo); cough and the rate of exacerbations were reduced as well.⁶⁴

β -Agonist Use

The LTRAs reduce the need for rescue β -agonists in children by up to 25%.^{59,60} In children 6 to 14 years of age inadequately controlled by budesonide, the addition of montelukast significantly decreased the need for β -agonist.⁶¹ Similarly, montelukast increased the number of rescue-free days per week by 4.5 days.⁶⁵

Eosinophilia

LTRAs significantly decrease eosinophil levels in blood of children with asthma.^{58,59,66}

OTHER ASTHMA PHENOTYPES

Acute Asthma

In adult patients presenting with moderate-to-severe asthma symptoms acutely, IV montelukast (7 or 14 mg) was administered in the emergency department with standard therapy. The FEV₁ significantly improved during the first 20 min (14.8% vs 3.6% with placebo; $p = 0.007$) and lasted > 2 h after

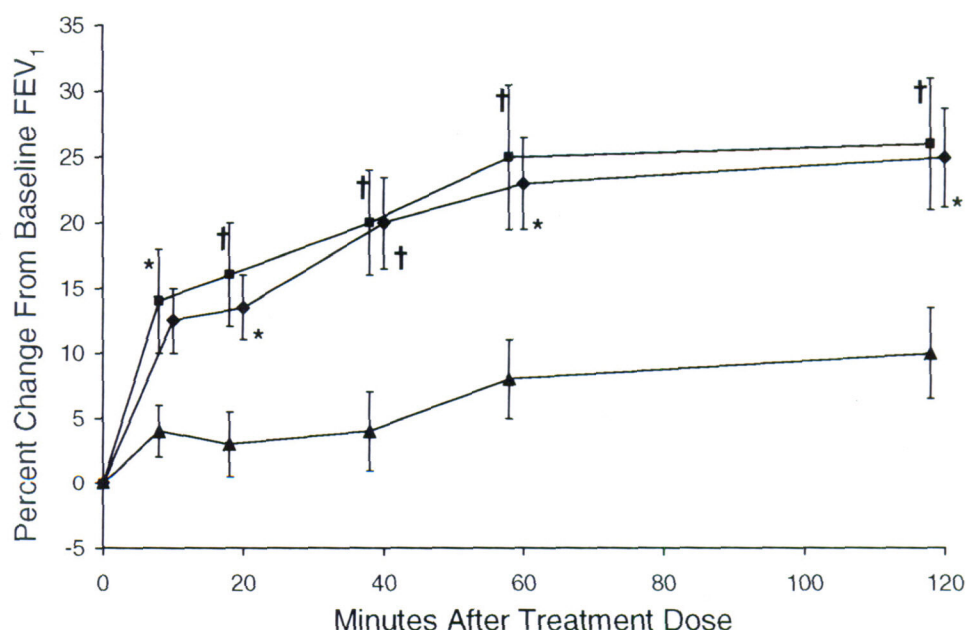


FIGURE 3. Improvement in FEV₁ after IV montelukast in patients with moderate-to-severe acute asthma. Patients received either IV montelukast, 7 mg (diamonds; $n = 66$); IV montelukast, 14 mg (squares; $n = 64$); or IV placebo (triangles; $n = 64$). Data are presented as least square means $\pm$ SE of the percentage change from baseline for each treatment. * $p < 0.05$ vs placebo; † $p < 0.01$ vs placebo. Reproduced with permission from Camargo et al.⁶⁷

infusion (Fig 3). Significantly fewer patients administered montelukast received β -agonist nebulizations or corticosteroids than did patients who received placebo.⁶⁷

Exercise-Induced Bronchoconstriction

After exercise, montelukast produced a 32% reduction of the maximum decrease in FEV₁ after exercise, a 27% reduction of the time to recovery from the maximal decrease in FEV₁, and a 47% inhibition of the area under the FEV₁-vs-time curve (AUC) after exercise. The protection against exercise-induced bronchoconstriction was measured 20 to 24 h after an evening dose.⁶⁸ Zafirlukast significantly attenuated the maximum decrease in FEV₁ after exercise by 57%.⁶⁹

Just as with adults, children experience protective effects of LTRAs after exercise for up to 24 h after the previous nighttime dose.⁷⁰ Zafirlukast, administered 4 h before exercise, significantly reduced the maximum decrease of FEV₁ and the AUC in children 6 to 14 years of age (Fig 4). The time for recovery from exercise was reduced by almost half.⁷¹ In children 7 to 13 years of age with allergic asthma, montelukast significantly reduced the maximum fall in FEV₁ and the AUC when exercise was performed 12 h after dosing.⁷²

Aspirin-Induced Asthma

Nonsteroidal antiinflammatory drugs can dramatically increase the production of CysLTs. In nonste-

roidal antiinflammatory drug-intolerant 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.⁷³

Allergen-Induced Asthma

Montelukast and zafirlukast significantly inhibited inhaled allergen-induced early and late asthmatic responses.⁷⁴⁻⁷⁶ Similarly, in subjects with asthma and cat allergy, montelukast and zafirlukast significantly attenuated the fall in FEV₁ on exposure to high levels of cat antigen.⁷⁷⁻⁷⁹ During the grass pollen season, zafirlukast (20 mg bid) significantly reduced asthma and AR symptoms. The LTRAs inhibited antigen-induced inflammatory cell infiltration and activation, including a reduction in lymphocytes, basophils, and macrophages in the BAL fluid obtained after segmental allergen challenge in allergic subjects with asthma.^{52,80} The number of eosinophils in nasal lavage fluid decreased in patients with both asthma and AR, while neutrophils levels remained unchanged.⁸⁰

AR

Montelukast significantly reduced daytime nasal symptoms (congestion, rhinorrhea, pruritus, and sneezing), daytime eye symptoms, nighttime symp-

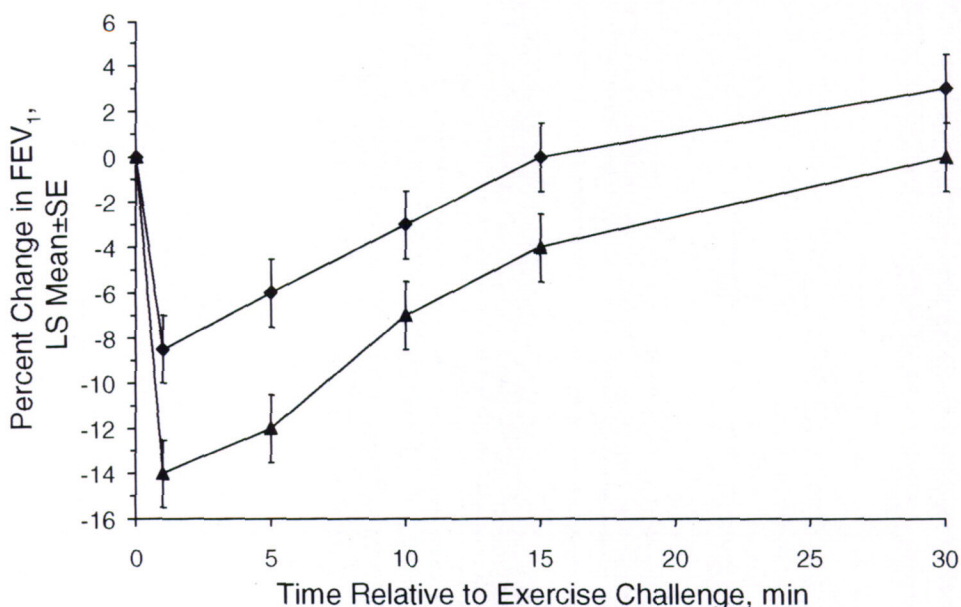


FIGURE 4. Percentage change in FEV₁ after exercise challenge in children with exercise-induced bronchoconstriction. Data are presented as least-square (LS) means $\pm$ SE. The reduction of fall in FEV₁ after zafirlukast, 10 mg, was significantly less than after placebo ($p = 0.01$). The time to recovery within 5% of the preexercise value was also significantly shorter with zafirlukast compared with placebo ($p \leq 0.05$); it was about half the time of the placebo group. Adapted from Pearlman et al.⁷¹

toms (difficulty sleeping, nighttime awakenings, and congestion on awakening), and peripheral eosinophilia.⁸¹⁻⁸³ Montelukast also significantly improved the rhinoconjunctivitis-specific quality-of-life score,⁸¹⁻⁸³ which included significant improvement in domains such as activity, ocular symptoms, practical problems, and emotional symptoms (Fig 5).⁸³⁻⁸⁵ Zafirlukast significantly improved nasal airway resistance by 68% in patients with AR⁸⁰ and reduced nasal congestion in patients allergic to cats.⁷⁸ Likewise, the number of eosinophils in nasal lavage fluid were decreased.⁸⁰

INFLAMMATION IN ALLERGIC RESPIRATORY DISEASE

Evidence of a Systemic Response

Allergic respiratory disorders are the result of a pathophysiologic process that extends well beyond the local, antigen-exposed airway. Inflammation in one part of the airway often manifests itself in another. For example, 21 to 58% of patients with AR have concomitant asthma,^{86,87} and 85 to 95% of patients with asthma have concomitant AR.^{86,88} Moreover, isolated allergen challenge in either the upper or lower airway produces demonstrable inflammatory changes in the nonchallenged site.

In a randomized, double-blind, crossover evaluation⁸⁹ of 10 subjects previously sensitized to grass

pollen but free of nasal and respiratory symptoms, isolated nasal provocation with grass extract induced not only a marked nasal allergic reaction but also a significant increase in bronchial hyperresponsiveness to methacholine, at both 30 min ($p = 0.011$) and 4.5 h ($p < 0.001$), compared with placebo. Separately, in nine subjects with seasonal AR but not asthma, isolated nasal allergen challenge, outside of their allergy season, resulted in significant ($p \leq 0.05$) eosinophil influx into bronchial epithelium and lamina propria in addition to the nasal epithelium and lamina propria.⁹⁰ Furthermore, this influx of cells correlated with detectable increases of intercellular adhesion molecule (ICAM)-1, vascular-cell adhesion molecule (VCAM)-1, and E-selectin expression in vessels in nasal and bronchial tissues.

Similarly, segmental bronchial challenge significantly increased eosinophil and basophil levels in nasal lamina propria as well as interleukin (IL)-5 expression in blood and nasal epithelium ($p < 0.05$),⁹¹ in addition to increases in eosinophil and basophil concentrations in bronchial mucosa ($p < 0.05$).⁹² Moreover, even in the absence of rhinitis, patients with asthma have increased eosinophil levels in their nasal mucosa ($p < 0.01$), and these levels correlate with bronchial eosinophil values ($r = 0.851$; $p < 0.001$).⁹³ Finally, clinical signs and symptoms of sinusitis are often detected during acute exacerbations of asthma; conversely, aggressive treat-

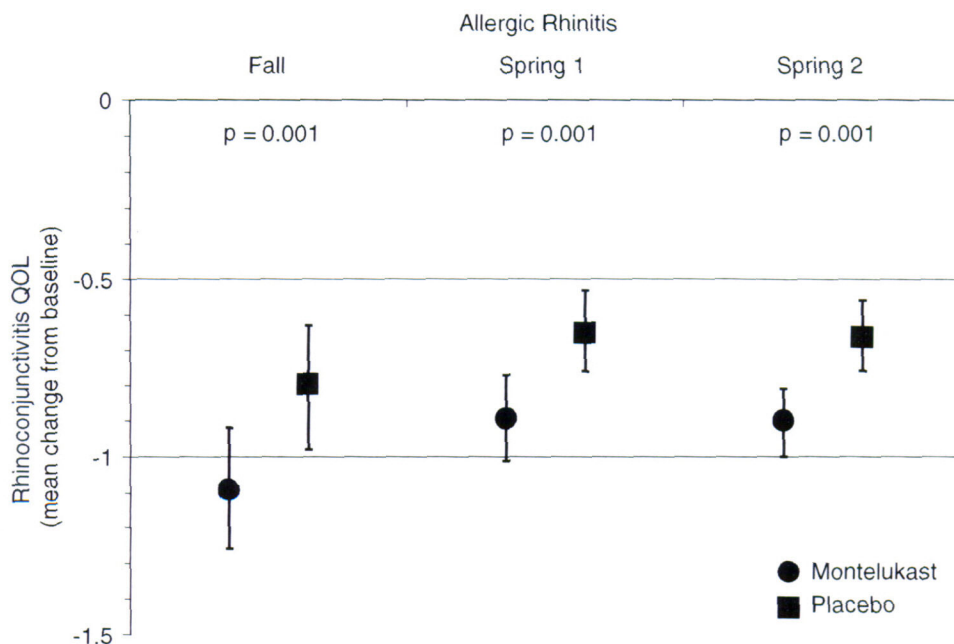


FIGURE 5. Change in overall rhinoconjunctivitis quality-of-life (QOL) score (0 = best, 6 = worst) in patients with fall or spring AR randomized to treatment with montelukast or placebo during their allergy season. Symbols represent the mean change (decrease in score represents improvement in symptoms), and error bars represent the 95% confidence intervals. Adapted from Nayak et al,⁸⁴ Philip et al,⁸⁵ and van Adelsberg et al.⁸³

ment of sinus disease has been shown to decrease medication requirements needed to control concomitant asthma.¹

Systemic Immune Response

The basis of these comorbidities that involve a remote airway response needs to be viewed in the context of the immune response. In atopic individuals, initial antigen exposure leads to the synthesis of antigen-specific IgE, which binds to high-affinity receptors on the surface of tissue mast cells and circulating basophils as well as to low-affinity receptors on the surface of circulating lymphocytes, eosinophils, platelets, and macrophages.⁹⁴ Reexposure to antigen produces molecular bridging of these IgE-receptor complexes, activating the cell to release preformed and newly synthesized mediators.⁹⁴

Located in the mucosa and submucosa of the airway, mast cells are the first cells to be activated on antigen reexposure,^{9,94} causing the release of mediators, including histamine, CysLTs, prostaglandins, platelet-activating factor (PAF), and cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, granulocyte-macrophage colony-stimulating factor [GM-CSF], and tumor necrosis factor [TNF]- α).^{5,9,94,95} These mediators produce the early phase reaction and are responsible for the initial acute symptoms (bronchoconstriction in asthma and sneezing, pruritus, rhinorrhea, and to some extent congestion in AR) of antigen exposure.^{29,96} Furthermore, these mediators initiate the processes leading to the infiltration into local tissues by circulating inflammatory cells, including eosinophils, basophils, T cells, and macrophages. It is proposed that these inflammatory cells then become activated and release their mediators, which act to sustain and enhance the inflammatory response, thus producing the chronic or late-phase response.

Eosinophils characterize and reflect the systemic nature of this late phase (Fig 6). Their development is under the regulatory control of cytokines IL-3, IL-5, and GM-CSF.^{5,94} IL-5, released from cells as part of the allergic response, is proposed to circulate to the bone marrow, where it induces the terminal differentiation of immature eosinophils.⁹⁴ These eosinophils then enter the circulation and bind to selectins and adhesion molecules VCAM-1 and ICAM-1 located on vascular endothelial surfaces near inflammatory sites.^{94,95} Migrating through the endothelial surface into the inflammatory tissue, eosinophils become activated to synthesize and release additional inflammatory mediators, including CysLTs, prostaglandin E₁, PAF, IL-3, IL-5, IL-8, and GM-CSF, and to reactive oxygen intermediates, major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil peroxi-

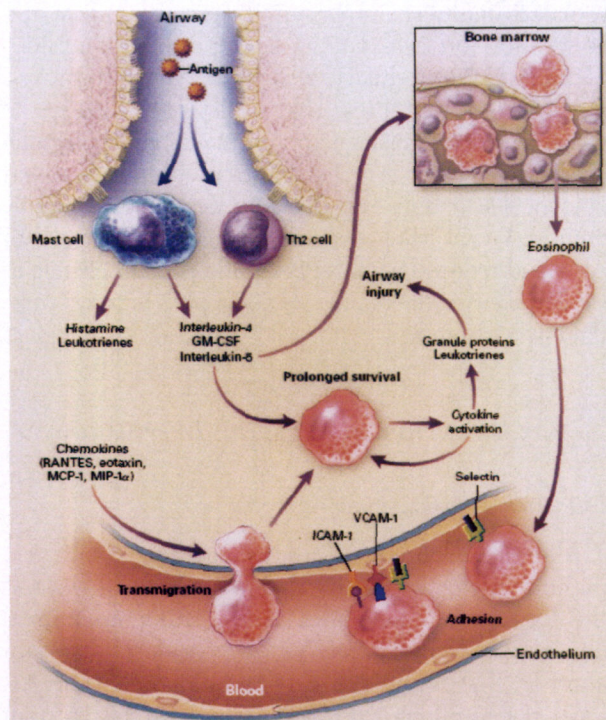


FIGURE 6. Eosinophils in allergic respiratory disorders. In response to antigen activation, mast cells and Th2 cells located in the airway release inflammatory mediators, such as histamine and leukotrienes, and cytokines, such as IL-4, IL-5, and GM-CSF. IL-5 travels to the bone marrow, where it stimulates the terminal differentiation of eosinophils. These eosinophils enter the circulatory system and, through interactions with selectins and adhesion proteins (VCAM-1 and ICAM-1), ultimately migrate to and infiltrate regions of inflammation. Once activated in the local tissue, these eosinophils release inflammatory mediators such as leukotrienes and granule proteins, leading to airway injury. In addition, they generate GM-CSF, which, along with IL-5, reduces apoptosis, prolonging their survival and contributing to persistent airway inflammation. MCP = monocyte chemotactic protein, MIP = macrophage inflammatory protein. RANTES = regulated upon activation, normal T-cell expressed and secreted. Reproduced with permission from Busse and Lemanske.⁹⁴

dase.^{1,5,94,95} Furthermore, GM-CSF, IL-5, and CysLTs reduce apoptosis, prolonging eosinophil survival in inflammatory tissue, likely sustaining their mediator release, and resulting in ongoing tissue damage and inflammation.^{5,94}

Similar to eosinophils, basophils, T cells, especially type 2 T helper (Th2) cells, and macrophages migrate to the inflammatory site, become activated, and release their mediators.^{1,5,88,95} Basophils release CysLTs, IL-4, and IL-13 and appear to be the primary source of histamine in the late phase of allergic reactions.^{5,9} Th2 cells are one of the principal cells that regulate and coordinate IgE-mediated allergic inflammation⁵; they release IL-3, IL-4, IL-5, IL-9, IL-10, IL-13, and GM-CSF on activation.^{5,9} Finally, macrophages release similar cytokines plus lysosomal enzymes and histamine-releasing factors, which ap-

pear to act on mast cells and basophils to perpetuate the allergic response independent of repeated allergen exposures.⁹

This allergen-induced up-regulation of inflammatory cell function leads to migration and infiltration of these cells into all susceptible tissues. Consequently, tissue sites far removed from the inciting focus may manifest clinical or subclinical inflammatory activity, further illustrating the systemic nature of this response.^{1,88}

Role of CysLTs in the Systemic Inflammatory Response

Progenitor Cells: In the bone marrow, CysLTs act synergistically with either GM-CSF or IL-5 to facilitate the growth and subsequent differentiation of progenitor cells. The CysLT₁ receptor has been detected on CD34⁺ progenitor cells, eosinophils, monocytes, macrophages, and some lymphocytes, and a model has been proposed in which CysLTs could prime progenitor cells to differentiate into mature blood cells (Fig 7).²⁴ This model was subsequently confirmed when it was demonstrated that adding LTD₄ (0.1 μmol/L) to GM-CSF caused a small but significant ($p < 0.001$) increase in the proliferation of eosinophil hemopoietic progenitor cells in peripheral blood and that adding LTD₄ to IL-5 significantly ($p = 0.01$) increased the proliferation of eosinophil hemopoietic progenitor cells in bone marrow.⁹⁷ Moreover, adding montelukast (1 μmol/L) suppressed both of these (peripheral blood

suppression, $p = 0.01$; bone marrow suppression, $p = 0.001$).⁹⁷

Recruitment: CysLTs facilitate eosinophil recruitment. Leukocyte rolling is a prerequisite for vascular adhesion and subsequent migration into inflammatory tissues.⁹⁸ The CysLT LTD₄ induces P-selectin-dependent increases of leukocyte rolling flux and a reduction in leukocyte rolling velocity.⁹⁸ CysLTs also increase eosinophil adhesion by up-regulating β₂-integrin expression in eosinophils (Fig 8)^{99,100} and stimulate vascular endothelial cells to produce PAF, a phospholipid known to increase leukocyte adhesion by activating β₂-integrin.⁹⁸ Finally, CysLTs are chemoattractants that induce eosinophil migration (Fig 8).^{99,101} Conversely, LTRAs significantly inhibit both the up-regulation of adhesion molecule expression and eosinophil chemotaxis.^{99,100}

Apoptosis: Once eosinophils migrate into the inflammatory site, CysLTs prolong their survival, contributing to maintenance of the inflammatory reaction. LTD₄ (1 μmol/L) was as effective as GM-CSF (5 ng/mL) or fibronectin (400 ng/mL) in promoting the survival of peripheral blood eosinophils obtained from asthmatic subjects.¹⁴ Moreover, inhibition of CysLTs with either leukotriene synthesis inhibitors or LTRAs reduced eosinophil survival both under basal conditions and following stimulation with GM-CSF or fibronectin, demonstrating that CysLTs play a central role in this survival.¹⁴

Cytokine Production: In addition to their modu-

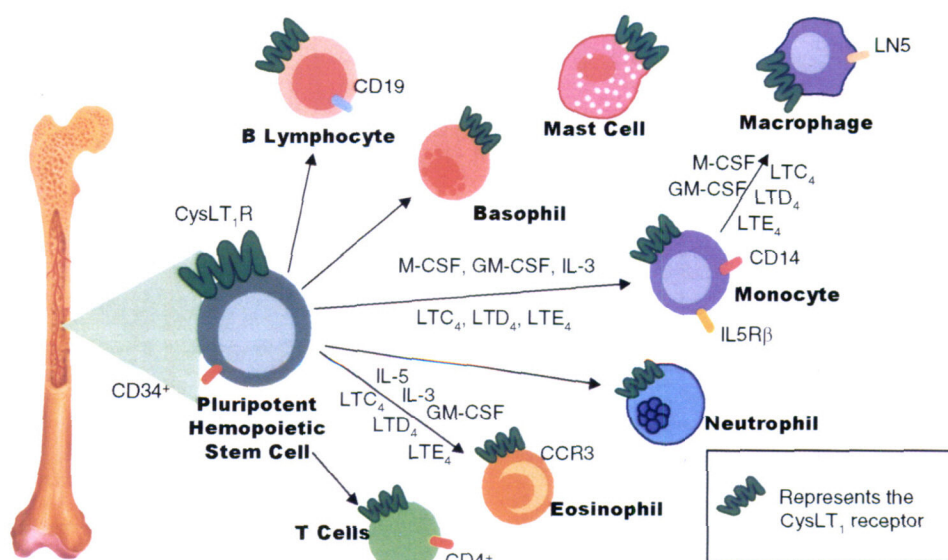


FIGURE 7. Proposed model of how CysLT₁ receptor expression in CD34⁺ progenitor cells and peripheral blood cells promotes the maturation of these cells. LTE₄ = leukotriene E₄; M-CSF = macrophage-colony stimulating factor. Reproduced with permission from Figueroa et al.²⁴

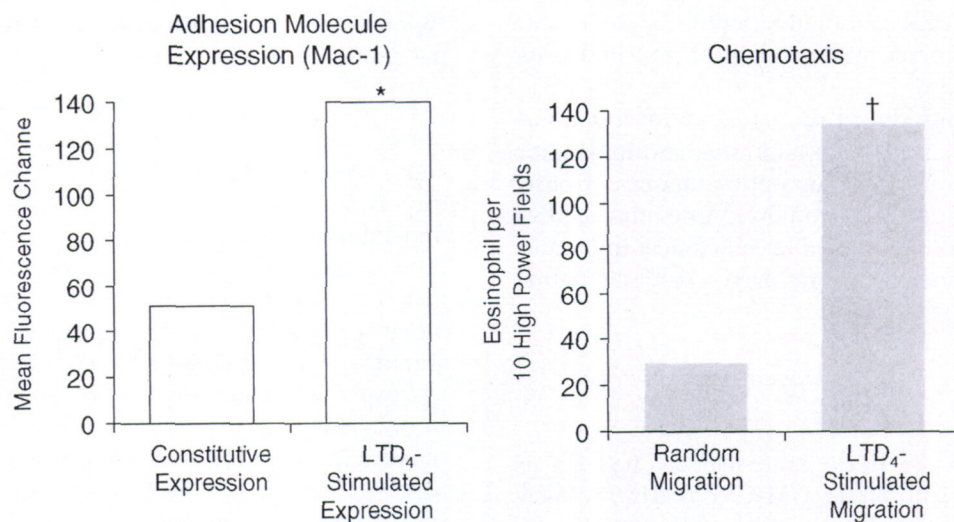


FIGURE 8. Effects of LTD₄ on eosinophil adhesion molecule expression and eosinophil chemotaxis *ex vivo*. Blood eosinophils from 32 atopic patients were stimulated with LTD₄ (10 nmol/L). Significant up-regulation of adhesion molecule expression (* $p < 0.01$ compared with constitutive expression) was measured as constitutive expression of macrophage activated protein-1 (Mac-1), expressed as mean fluorescence channel. Significant chemotaxis († $p < 0.001$ compared with random migration) was measured as cellular migration, expressed as cells counted in 10 high-power fields. Adapted from Fregonese et al.⁹⁹

lating effects on cytokine activity, CysLTs also modulate cytokine production and the production of other inflammatory mediators and markers. Leukotriene C₄ (LTC₄) and LTD₄ have been shown to induce messenger RNA expression, stimulating the production of IL-5 (Fig 9), TNF- α , and macrophage inflammatory protein-1 β in mast cells derived from human cord blood and primed with IL-4.¹⁰² Moreover, an LTRA inhibited not only this exogenous CysLT-induced cytokine production but also the production of IL-5 (Fig 9) and TNF- α in response to IgE receptor cross-linking, implying a positive feed-

back loop involving endogenous CysLTs.¹⁰² Similarly, in sensitized mice, high-dose IV montelukast for 3 days prior to antigen inhalation significantly blunted antigen-induced increases in IL-5 and total IgE levels in serum; IL-4, IL-5, and eotaxin levels in BAL fluid; and IL-4, IL-5, and IL-13 protein expression in the total lung.¹⁰³ Likewise, in allergic asthmatic patients, pranlukast suppressed antigen-induced increases in IL-3, IL-4, IL-5, and GM-CSF in peripheral blood mononuclear cells,¹⁰⁴ and zafirlukast suppressed an antigen-induced increase in TNF- α in BAL fluid.⁵²

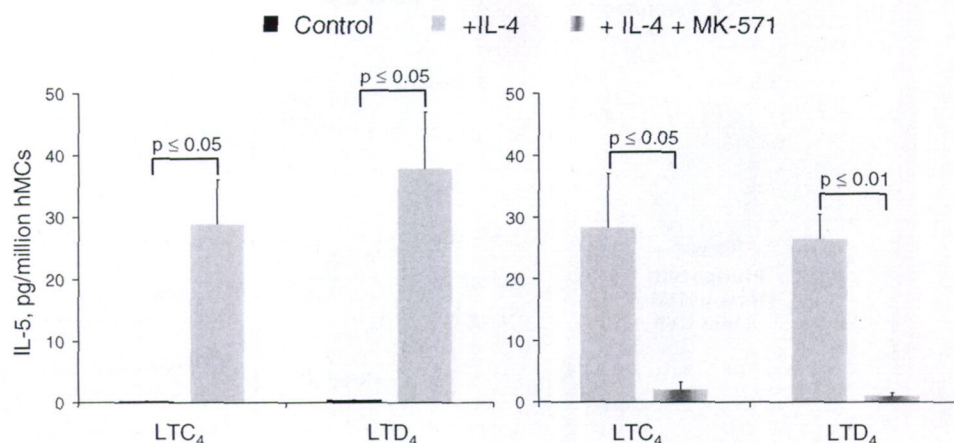


FIGURE 9. LTC₄ and LTD₄ caused an increase of IL-5 in human mast cells (hMCs) primed with IL-4 *in vitro*. The increase of IL-5 was blocked with 1 μ mol/L MK-571, an LTRA, demonstrating that this increase was specific to CysLTs and not the result of IL-4. Adapted from Mellor et al.¹⁰²

Nitric Oxide: Exhaled nitric oxide (NO), a surrogate marker of airway inflammation, is elevated in asthmatic patients, and LTC₄ causes an increase of NO in human polymorphonuclear leukocytes *in vitro*¹⁰⁵ (Fig 10). In two separate evaluations of school-age children with mild-to-moderate asthma, exhaled NO was elevated at baseline compared with age-matched nonasthmatic control subjects ($p < 0.001$) and significantly reduced by 20% ($p = 0.02$)¹⁰⁶ and 30% ($p < 0.01$)¹⁰⁷ following 2 to 4 weeks of montelukast therapy.

Smooth Muscle: Epithelial and smooth-muscle cells also generate CysLTs in the lower airway, where they produce both acute and chronic adverse structural changes.¹⁰⁸ Acutely, CysLTs are potent bronchoconstrictors.^{6,108} This effect is enhanced in small, clinically significant bronchi (inner diameter, 0.5 to 2 mm), where they are 30-fold more potent than in larger bronchi (inner diameter, 4 to 6 mm).¹⁰⁹ LTRAs decrease this bronchoconstriction.^{109,110} In small bronchi isolated from macroscopically normal sections of human lung removed during carcinoma surgery, montelukast and its two main metabolites produced parallel rightward (antagonist) shifts in the concentration-response curves for LTC₄, LTD₄, and leukotriene E₄-induced bronchoconstriction.¹⁰⁹

Remodeling: The chronic effects of CysLTs on airway remodeling may be even more important than acute bronchoconstriction. CysLTs enhanced the proliferation of lung fibroblasts to known

mitogens.^{108,111} In cultures of murine peritoneal macrophages, LTC₄ stimulated a dose-dependent increase in macrophage-derived fibroblast growth factor; conversely, this reaction was inhibited by an LTRA.¹¹² The stimulation response depended on the local balance between CysLTs and prostaglandins.¹¹¹ In cultures of normal human skin fibroblasts, LTC₄ and LTD₄ caused dose-dependent cell proliferation when 50 $\mu\text{mol/L}$ indomethacin was added to the medium.¹¹¹ Moreover, this proliferation could be reproduced by the addition of other cyclooxygenase inhibitors, such as ibuprofen and aspirin, and was abolished by the addition of prostaglandin E₂.¹¹¹ Of note, a reduction in prostaglandin E₂ synthesis by respiratory epithelium has been reported in both an equine model of asthma and in patients with aspirin-sensitive asthma.¹⁰⁸ In addition, CysLTs promoted pulmonary fibrosis by stimulating fibroblasts to increase collagen synthesis.¹⁰⁸ In cultured rat lung fibroblasts, LTC₄ produced a dose-dependent increase in collagen synthesis (85% at 1 nmol/L; $p < 0.02$) that was independent of fibroblast proliferation; this increase was suppressed by an LTRA (FPL-55712).¹¹³ Additionally, CysLT levels were increased fivefold in wild-type mice, but were undetectable in 5-lipoxygenase gene knockout mice, following intratracheal instillation of 0.5 U of bleomycin; this difference was associated with decreased histologic evidence of collagen deposition and a 60% reduction ($p < 0.05$) in lung hydroxyproline, 14 days following the intratracheal instillation, in knockout compared with wild-type

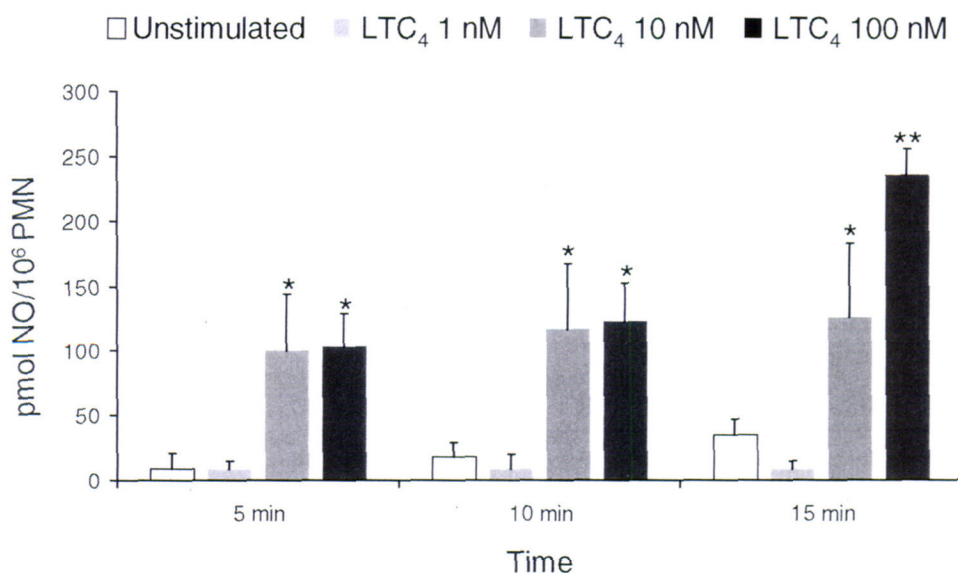


FIGURE 10. LTC₄ caused an increase of NO in human polymorphonuclear leukocytes (PMN) *in vitro*. Reproduced from Lärfaars et al.¹⁰⁵

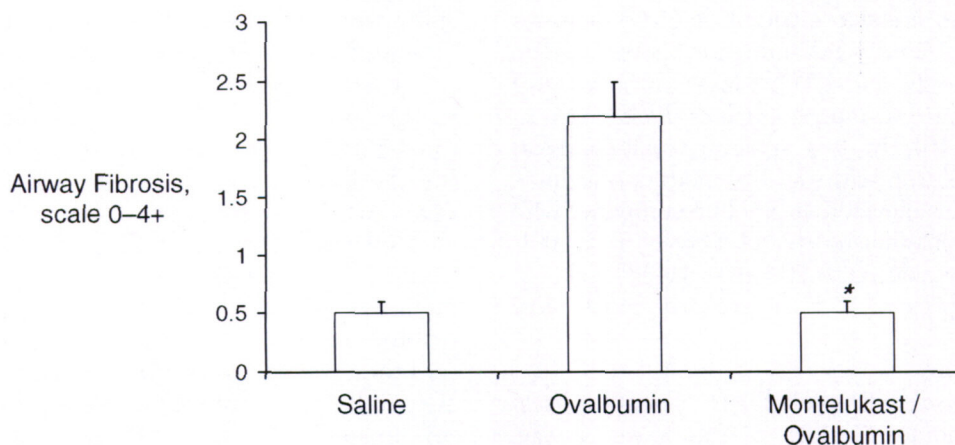


FIGURE 11. Ovalbumin-treated mice (14 days of intraperitoneal ovalbumin followed by intranasal ovalbumin on days 14 to 75) were used as a model of human asthma. Using morphometric analysis, on a scale of 0 to 4+, the mean airway fibrosis score was 2.2 in ovalbumin-treated mice compared with 0.55 in saline solution-treated mice (* $p < 0.0001$). The airway fibrosis score was reduced by 98.8% by treatment with the LTRA montelukast. Reproduced from Henderson et al.¹¹⁵

mice.¹¹⁴ Similarly, in ovalbumin-sensitized/challenged mice, montelukast markedly reduced collagen deposition in lung interstitium, decreasing the airway fibrosis score by 98.8% ($p < 0.001$) compared with ovalbumin control (Fig 11).¹¹⁵ Through use of electron microscopy, montelukast appeared to reduce the thickness and number of collagen bundles in the lung interstitium.

Proliferation of Smooth Muscle: Finally, CysLTs augment growth factor-induced proliferation of airway smooth-muscle cells.¹⁰⁸ In human tracheal smooth-muscle cells obtained from lung transplant donors, LTD₄ potentiated the effects of either epidermal growth factor or thrombin on DNA synthesis leading to cell proliferation.¹¹⁶ This potentiation was abolished by pranlukast but unaffected by zafirlukast.¹¹⁶ Consistent with this finding, in sensitized mice, ovalbumin challenge produced a 2.1-fold increase in the thickness of the smooth-muscle layer surrounding the airway ($p < 0.001$); montelukast therapy reduced this proliferative response by 80.1% ($p < 0.001$).¹¹⁵ The relevance and relationship of these *in vitro* observations to airway remodeling in asthma awaits further study.

CONCLUSION

In summary, allergic respiratory disorders are the result of a systemic inflammatory process that involves the complex interaction of numerous cell types and mediators. In many of these interactions, CysLTs are key mediators and modulators. Locally, CysLTs increase mucus secretion, promote tissue edema, enhance inflammatory cell survival, and

modulate cytokine production and activity. In the bone marrow and peripheral blood, CysLTs facilitate the production and maturation of inflammatory cells. In the vascular system, these products increase adhesion molecule expression, enhance recruitment of these inflammatory cells and, in the airway, promote tissue fibrosis, hypertrophy, hyperplasia, and constriction of smooth-muscle cells.

LTRAs have high systemic bioavailability and, consequently, are able to target various sites of allergic reactions to produce disease control. Data from both animal and human studies⁷⁴⁻⁷⁹ demonstrate that these agents inhibit the early phase allergic reaction, suppress progenitor cell proliferation, reduce eosinophil survival, decrease cytokine production, and inhibit airway remodeling. Consequently, LTRAs are designed to modify airway tissue responses to CysLTs and thus provide effective treatment of asthma or AR.

REFERENCES

- 1 Lemanske RF Jr, Busse WW. Asthma. JAMA 1997; 278: 1855-1873
- 2 National Asthma Education and Prevention Program. Expert panel report: guidelines for the diagnosis and management of asthma update on selected topics-2002. J Allergy Clin Immunol 2002; 110:S141-S219
- 3 National Asthma Education and Prevention Program. Guidelines for the diagnosis and management of asthma: expert panel report 2. Bethesda, MD: National Institutes of Health/National Heart, Lung, and Blood Institute, 1997
- 4 Lierl MB. Allergy of the upper respiratory tract. In: Lawlor GJ, Fischer TJ, Adelman DC, eds. Manual of allergy and immunology. Boston, MA: Little, Brown and Company, 1995; 95-111
- 5 Bousquet J, van Cauwenberge P, Khaltaev N. Allergic

- rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001; 108:S147–S334
- 6 Barnes NC, Piper PJ, Costello JF. Comparative effects of inhaled leukotriene C₄, leukotriene D₄, and histamine in normal human subjects. *Thorax* 1984; 39:500–504
 - 7 Busse WW. Leukotrienes and inflammation. *Am J Respir Crit Care Med* 1998; 157:S210–S213; discussion S247–S218
 - 8 Johnson HG, McNeel ML. Secretagogue responses of leukotriene C₄, D₄: comparison of potency in canine trachea in vivo. *Prostaglandins* 1983; 25:237–243
 - 9 Hamid Q, Tulic MK, Liu MC, et al. Inflammatory cells in asthma: mechanisms and implications for therapy. *J Allergy Clin Immunol* 2003; 111:S5–S12; discussion S12–S17
 - 10 Salvi SS, Krishna MT, Sampson AP, et al. The anti-inflammatory effects of leukotriene-modifying drugs and their use in asthma. *Chest* 2001; 119:1533–1546
 - 11 Bisgaard H, Olsson P, Bende M. Effect of leukotriene D₄ on nasal mucosal blood flow, nasal airway resistance and nasal secretion in humans. *Clin Allergy* 1986; 16:289–297
 - 12 Hay DW, Torphy TJ, Udem BJ. Cysteinyl leukotrienes in asthma: old mediators up to new tricks. *Trends Pharmacol Sci* 1995; 16:304–309
 - 13 Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science* 1983; 220: 568–575
 - 14 Lee E, Robertson T, Smith J, et al. Leukotriene receptor antagonists and synthesis inhibitors reverse survival in eosinophils of asthmatic individuals. *Am J Respir Crit Care Med* 2000; 161:1881–1886
 - 15 Taylor GW, Taylor I, Black P, et al. Urinary leukotriene E₄ after antigen challenge and in acute asthma and allergic rhinitis. *Lancet* 1989; 1:584–588
 - 16 Drazen JM, O'Brien J, Sparrow D, et al. Recovery of leukotriene E₄ from the urine of patients with airway obstruction. *Am Rev Respir Dis* 1992; 146:104–108
 - 17 Reiss TF, Hill JB, Harman E, et al. Increased urinary excretion of LTE₄ after exercise and attenuation of exercise-induced bronchospasm by montelukast, a cysteinyl leukotriene receptor antagonist. *Thorax* 1997; 52:1030–1035
 - 18 Wenzel SE, Larsen GL, Johnston K, et al. Elevated levels of leukotriene C₄ in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. *Am Rev Respir Dis* 1990; 142:112–119
 - 19 Pavord ID, Ward R, Woltmann G, et al. Induced sputum eicosanoid concentrations in asthma. *Am J Respir Crit Care Med* 1999; 160:1905–1909
 - 20 Macfarlane AJ, Dworski R, Sheller JR, et al. Sputum cysteinyl leukotrienes increase 24 hours after allergen inhalation in atopic asthmatics. *Am J Respir Crit Care Med* 2000; 161:1553–1558
 - 21 Miadonna A, Tedeschi A, Leggieri E, et al. Behavior and clinical relevance of histamine and leukotrienes C₄ and B₄ in grass pollen-induced rhinitis. *Am Rev Respir Dis* 1987; 136:357–362
 - 22 Lynch KR, O'Neill GP, Liu Q, et al. Characterization of the human cysteinyl leukotriene CysLT₁ receptor. *Nature* 1999; 399:789–793
 - 23 Evans J, Figueroa DJ. Cloning and expression of leukotriene receptors. *Clin Exp Allergy Rev* 2001; 1:142–144
 - 24 Figueroa DJ, Breyer RM, Defoe SK, et al. Expression of the cysteinyl leukotriene 1 receptor in normal human lung and peripheral blood leukocytes. *Am J Respir Crit Care Med* 2001; 163:226–233
 - 25 Mellor EA, Maekawa A, Austen KF, et al. Cysteinyl leukotriene receptor 1 is also a pyrimidinergic receptor and is expressed by human mast cells. *Proc Natl Acad Sci U S A* 2001; 98:7964–7969
 - 26 Shirasaki H, Kanaizumi E, Watanabe K, et al. Expression and localization of the cysteinyl leukotriene 1 receptor in human nasal mucosa. *Clin Exp Allergy* 2002; 32:1007–1012
 - 27 Barnes N, Wei LX, Reiss TF, et al. Analysis of montelukast in mild persistent asthmatic patients with near-normal lung function. *Respir Med* 2001; 95:379–386
 - 28 Fish JE, Kemp JP, Lockey RF, et al. Zafirlukast for symptomatic mild-to-moderate asthma: a 13-week multicenter study. The Zafirlukast Trialists Group. *Clin Ther* 1997; 19:675–690
 - 29 Reiss TF, Chervinsky P, Dockhorn RJ, et al. 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; 158:1213–1220
 - 30 Barnes NC, Pujet JC. Pranlukast, a novel leukotriene receptor antagonist: results of the first European, placebo controlled, multicentre clinical study in asthma. *Thorax* 1997; 52:523–527
 - 31 Kemp JP, Minkwitz MC, Bonuccelli CM, et al. Therapeutic effect of zafirlukast as monotherapy in steroid-naïve patients with severe persistent asthma. *Chest* 1999; 115:336–342
 - 32 Dockhorn RJ, Baumgartner RA, Leff JA, et al. Comparison of the effects of intravenous and oral montelukast on airway function: a double blind, placebo controlled, three period, crossover study in asthmatic patients. *Thorax* 2000; 55:260–265
 - 33 Zhang J, Yu C, Noonan G, et al. Effect of montelukast, a once-daily leukotriene receptor antagonist, on peak expiratory flow variability. *Clin Ther* 2002; 24:574–582
 - 34 Ishioka S, Hozawa S, Haruta Y, et al. Effects of pranlukast, a cysteinyl leukotriene antagonist, on bronchial responsiveness to methacholine in aspirin-intolerant asthmatics treated with corticosteroids. *Hiroshima J Med Sci* 2000; 49:105–108
 - 35 Obase Y, Shimoda T, Tomari S, et al. Efficacy and safety of long-term treatment of asthmatic patients with pranlukast, a cysteinyl-leukotriene-receptor antagonist: four-year follow-up study. *Ann Allergy Asthma Immunol* 2001; 87:43–47
 - 36 Ferreira MB, Santos AS, Pregal AL, 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; 33:315–318
 - 37 Malmstrom K, Rodriguez-Gomez G, Guerra J, et al. Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma: a randomized, controlled trial. Montelukast/Beclomethasone Study Group. *Ann Intern Med* 1999; 130: 487–495
 - 38 Israel E, Chervinsky PS, Friedman B, et al. Effects of montelukast and beclomethasone on airway function and asthma control. *J Allergy Clin Immunol* 2002; 110:847–854
 - 39 Baumgartner RA, Martinez G, Edelman JM, et al. Distribution of therapeutic response in asthma control between oral montelukast and inhaled beclomethasone. *Eur Respir J* 2003; 21:123–128
 - 40 Williams B, Noonan G, Reiss TF, et al. Long-term asthma control with oral montelukast and inhaled beclomethasone for adults and children 6 years and older. *Clin Exp Allergy* 2001; 31:845–854
 - 41 Riccioni G, Vecchia RD, D'Orazio N, et al. Comparison of montelukast and budesonide on bronchial reactivity in subjects with mild-moderate persistent asthma. *Pulm Pharmacol Ther* 2003; 16:111–114
 - 42 Carratu P, Morelli N, Freire AX, et al. Effect of zafirlukast on methacholine and ultrasonically nebulized distilled water challenge in patients with mild asthma. *Respiration* 2003; 70:249–253
 - 43 Yoshida S, Ishizaki Y, Shoji T, et al. Effect of pranlukast on

- bronchial inflammation in patients with asthma. *Clin Exp Allergy* 2000; 30:1008–1014
- 44 Westbroek J, Pasma HR. Effects of 2 weeks of treatment with fluticasone propionate 100 mcg b.d. by comparison with zafirlukast 20 mg b.d. on bronchial hyper-responsiveness in patients with mild to moderate asthma. *Respir Med* 2000; 94:112–118
 - 45 Rorke S, Jennison S, Jeffs JA, et al. Role of cysteinyl leukotrienes in adenosine 5'-monophosphate induced bronchoconstriction in asthma. *Thorax* 2002; 57:323–327
 - 46 Dempsey OJ, Wilson AM, Sims EJ, et al. Additive bronchoprotective and bronchodilator effects with single doses of salmeterol and montelukast in asthmatic patients receiving inhaled corticosteroids. *Chest* 2000; 117:950–953
 - 47 Dempsey OJ, Fowler SJ, Wilson A, et al. Effects of adding either a leukotriene receptor antagonist or low-dose theophylline to a low or medium dose of inhaled corticosteroid in patients with persistent asthma. *Chest* 2002; 122:151–159
 - 48 Smith LJ, Hanby LA, Lavins BJ, et al. A single dose of zafirlukast reduces LTD₄-induced bronchoconstriction in patients on maintenance inhaled corticosteroid therapy. *Ann Allergy Asthma Immunol* 1998; 81:43–49
 - 49 Suissa S, Dennis R, Ernst P, et al. Effectiveness of the leukotriene receptor antagonist zafirlukast for mild-to-moderate asthma: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1997; 126:177–183
 - 50 Nathan RA, Bernstein JA, Bielory L, et al. Zafirlukast improves asthma symptoms and quality of life in patients with moderate reversible airflow obstruction. *J Allergy Clin Immunol* 1998; 102:935–942
 - 51 Virchow JC, Prasse A, Naya I, et al. Zafirlukast improves asthma control in patients receiving high-dose inhaled corticosteroids. *Am J Respir Crit Care Med* 2000; 162:578–585
 - 52 Calhoun WJ, Lavins BJ, Minkwitz MC, et al. Effect of zafirlukast (Accolate) on cellular mediators of inflammation: bronchoalveolar lavage fluid findings after segmental antigen challenge. *Am J Respir Crit Care Med* 1998; 157:1381–1389
 - 53 Pizzichini E, Leff JA, Reiss TF, et al. Montelukast reduces airway eosinophilic inflammation in asthma: a randomized, controlled trial. *Eur Respir J* 1999; 14:12–18
 - 54 Laviolette M, Malmstrom K, Lu S, et al. Montelukast added to inhaled beclomethasone in treatment of asthma. *Am J Respir Crit Care Med* 1999; 160:1862–1868
 - 55 Price DB, Hernandez D, Magyar P, et al. Randomised controlled trial of montelukast plus inhaled budesonide versus double dose inhaled budesonide in adult patients with asthma. *Thorax* 2003; 58:211–216
 - 56 Lofdahl C-G, Reiss TF, Leff JA, et al. Randomised, placebo controlled trial of effect of a leukotriene receptor antagonist, montelukast, on tapering inhaled corticosteroids in asthmatic patients. *BMJ* 1999; 319:87–90
 - 57 Centanni S, Santus P, Casanova F, et al. Evaluation of the effects of zafirlukast 40 mg b.i.d. in addition to preexisting therapy of high-dose inhaled steroids on symptomatic patients with reversible respiratory obstruction: preliminary data. *Drugs Exp Clin Res* 2000; 26:133–138
 - 58 Knorr B, Matz J, Bernstein JA, et al. Montelukast for chronic asthma in 6- to 14-year-old children: a randomized, double-blind trial. Pediatric Montelukast Study Group. *JAMA* 1998; 279:1181–1186
 - 59 Knorr B, Franchi LM, Bisgaard H, et al. Montelukast, a leukotriene receptor antagonist, for the treatment of persistent asthma in children aged 2 to 5 years. *Pediatrics* 2001; 108:E48
 - 60 Pearlman DS, Lampl KL, Dowling PJ Jr, et al. Effectiveness and tolerability of zafirlukast for the treatment of asthma in children. *Clin Ther* 2000; 22:732–747
 - 61 Simons FE, Villa JR, Lee BW, et al. Montelukast added to budesonide in children with persistent asthma: a randomized, double-blind, crossover study. *J Pediatr* 2001; 138: 694–698
 - 62 Maspero JF, Duenas-Meza E, Volovitz B, et al. Oral montelukast versus inhaled beclomethasone in 6- to 11-year-old children with asthma: results of an open-label extension study evaluating long-term safety, satisfaction, and adherence with therapy. *Curr Med Res Opin* 2001; 17:96–104
 - 63 Ng DK, Law AK, Chau KW, et al. Use of montelukast in the treatment of early childhood wheezing from clinical experience with three cases. *Respirology* 2000; 5:389–392
 - 64 Bisgaard H. A randomized trial of montelukast in respiratory syncytial virus postbronchiolitis. *Am J Respir Crit Care Med* 2003; 167:379–383
 - 65 Phipatanakul W, Greene C, Downes SJ, et al. Montelukast improves asthma control in asthmatic children maintained on inhaled corticosteroids. *Ann Allergy Asthma Immunol* 2003; 91:49–54
 - 66 Stelmach I, Jerzynska J, Kuna P. A randomized, double-blind trial of the effect of treatment with montelukast on bronchial hyperresponsiveness and serum eosinophilic cationic protein (ECP), soluble interleukin 2 receptor (sIL-2R), IL-4, and soluble intercellular adhesion molecule 1 (sICAM-1) in children with asthma. *J Allergy Clin Immunol* 2002; 109:257–263
 - 67 Camargo CA Jr, Smithline HA, Malice MP, et al. A randomized controlled trial of intravenous montelukast in acute asthma. *Am J Respir Crit Care Med* 2003; 167:528–533
 - 68 Leff JA, Busse WW, Pearlman D, et al. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. *N Engl J Med* 1998; 339:147–152
 - 69 Dahlen B, Roquet A, Inman MD, et al. Influence of zafirlukast and loratadine on exercise-induced bronchoconstriction. *J Allergy Clin Immunol* 2002; 109:789–793
 - 70 Kemp JP, Dockhorn RJ, Shapiro GG, et al. Montelukast once daily inhibits exercise-induced bronchoconstriction in 6- to 14-year-old children with asthma. *J Pediatr* 1998; 133:424–428
 - 71 Pearlman DS, Ostrom NK, Bronsky EA, et al. The leukotriene D₄-receptor antagonist zafirlukast attenuates exercise-induced bronchoconstriction in children. *J Pediatr* 1999; 134:273–279
 - 72 Peroni DG, Piacentini GL, Pietrobello A, et al. The combination of single-dose montelukast and loratadine on exercise-induced bronchospasm in children. *Eur Respir J* 2002; 20:104–107
 - 73 Dahlen SE, Malmstrom K, Nizankowska E, 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
 - 74 Diamant Z, Grootendorst DC, Veselic-Charvat M, et al. The effect of montelukast (MK-0476), a cysteinyl leukotriene receptor antagonist, on allergen-induced airway responses and sputum cell counts in asthma. *Clin Exp Allergy* 1999; 29:42–51
 - 75 Roquet A, Dahlen B, Kumlin M, et al. Combined antagonism of leukotrienes and histamine produces predominant inhibition of allergen-induced early and late phase airway obstruction in asthmatics. *Am J Respir Crit Care Med* 1997; 155:1856–1863
 - 76 Hamilton A, Faierman I, Stober P, et al. 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; 102:177–183

- 77 Phipatanakul W, Nowak-Wegrzyn A, Eggleston PA, et al. The efficacy of montelukast in the treatment of cat allergen-induced asthma in children. *J Allergy Clin Immunol* 2002; 109:794–799
- 78 Phipatanakul W, Eggleston PA, Conover-Walker MK, et al. A randomized, double-blind, placebo-controlled trial of the effect of zafirlukast on upper and lower respiratory responses to cat challenge. *J Allergy Clin Immunol* 2000; 105:704–710
- 79 Corren J, Spector S, Fuller L, et al. Effects of zafirlukast upon clinical, physiologic, and inflammatory responses to natural cat allergen exposure. *Ann Allergy Asthma Immunol* 2001; 87:211–217
- 80 Piatti G, Ceriotti L, Cavallaro G, et al. Effects of zafirlukast on bronchial asthma and allergic rhinitis. *Pharmacol Res* 2003; 47:541–547
- 81 Nayak AS, Philip G, Lu S, et al. Efficacy and tolerability of montelukast alone or in combination with loratadine in seasonal allergic rhinitis: a multicenter, randomized, double-blind, placebo-controlled trial performed in the fall. *Ann Allergy Asthma Immunol* 2002; 88:592–600
- 82 Philip G, Malmstrom K, Hampel FC, et al. Montelukast for treating seasonal allergic rhinitis: a randomized, double-blind, placebo-controlled trial performed in the spring. *Clin Exp Allergy* 2002; 32:1020–1028
- 83 van Adelsberg J, Philip G, LaForce CF, et al. Randomized controlled trial evaluating the clinical benefit of montelukast for treating spring seasonal allergic rhinitis. *Ann Allergy Asthma Immunol* 2003; 90:214–222
- 84 Nayak AS, Philip G, Lu S, et al. Efficacy and tolerability of montelukast alone or in combination with loratadine in seasonal allergic rhinitis: a multicenter, randomized, double-blind, placebo-controlled trial performed in the fall. *Ann Allergy Asthma Immunol* 2002; 88:592–600
- 85 Philip G, Malmstrom K, Hampel FC, et al. Montelukast for treating seasonal allergic rhinitis: a randomized, double-blind, placebo-controlled trial performed in the spring. *Clin Exp Allergy* 2002; 32:1020–1028
- 86 Greisner WA 3rd, Settipane RJ, Settipane GA. Co-existence of asthma and allergic rhinitis: a 23-year follow-up study of college students. *Allergy Asthma Proc* 1998; 19:185–188
- 87 Mullarkey MF, Hill JS, Webb DR. Allergic and nonallergic rhinitis: their characterization with attention to the meaning of nasal eosinophilia. *J Allergy Clin Immunol* 1980; 65:122–126
- 88 Togias A. Rhinitis and asthma: evidence for respiratory system integration. *J Allergy Clin Immunol* 2003; 111:1171–1183
- 89 Corren J, Adinoff AD, Irvin CG. Changes in bronchial responsiveness following nasal provocation with allergen. *J Allergy Clin Immunol* 1992; 89:611–618
- 90 Braunstahl GJ, Overbeek SE, Kleinjan A, et al. Nasal allergen provocation induces adhesion molecule expression and tissue eosinophilia in upper and lower airways. *J Allergy Clin Immunol* 2001; 107:469–476
- 91 Braunstahl GJ, Kleinjan A, Overbeek SE, et al. Segmental bronchial provocation induces nasal inflammation in allergic rhinitis patients. *Am J Respir Crit Care Med* 2000; 161:2051–2057
- 92 Braunstahl GJ, Overbeek SE, Fokkens WJ, et al. Segmental bronchoprovocation in allergic rhinitis patients affects mast cell and basophil numbers in nasal and bronchial mucosa. *Am J Respir Crit Care Med* 2001; 164:858–865
- 93 Gaga M, Lambrou P, Papageorgiou N, et al. Eosinophils are a feature of upper and lower airway pathology in non-atopic asthma, irrespective of the presence of rhinitis. *Clin Exp Allergy* 2000; 30:663–669
- 94 Busse WW, Lemanske RF. Asthma. *N Engl J Med* 2001; 344:350–362
- 95 Kay AB. Allergy and allergic diseases: first of two parts. *N Engl J Med* 2001; 344:30–37
- 96 Naclerio R. Clinical manifestations of the release of histamine and other inflammatory mediators. *J Allergy Clin Immunol* 1999; 103:S382–S385
- 97 Braccioni F, Dorman SC, O'Byrne PM, et al. The effect of cysteinyl leukotrienes on growth of eosinophil progenitors from peripheral blood and bone marrow of atopic subjects. *J Allergy Clin Immunol* 2002; 110:96–101
- 98 Kanwar S, Johnston B, Kubes P. Leukotriene C₄/D₄ induces P-selectin and sialyl Lewis(x)-dependent alterations in leukocyte kinetics *in vivo*. *Circ Res* 1995; 77:879–887
- 99 Fregonese L, Silvestri M, Sabatini F, et al. Cysteinyl leukotrienes induce human eosinophil locomotion and adhesion molecule expression via a CysLT₁ receptor-mediated mechanism. *Clin Exp Allergy* 2002; 32:745–750
- 100 Nagata M, Saito K, Tsuchiya K, et al. Leukotriene D₄ upregulates eosinophil adhesion via the cysteinyl leukotriene 1 receptor. *J Allergy Clin Immunol* 2002; 109:676–680
- 101 Spada CS, Krauss AH, Nieves AL, et al. Effects of leukotrienes B₄ (LTB₄) and D₄ (LTD₄) on motility of isolated normodense human eosinophils and neutrophils. *Adv Exp Med Biol* 1997; 400B:699–706
- 102 Mellor EA, Austen KF, Boyce JA. Cysteinyl leukotrienes and uridine diphosphate induce cytokine generation by human mast cells through an interleukin 4-regulated pathway that is inhibited by leukotriene receptor antagonists. *J Exp Med* 2002; 195:583–592
- 103 Wu AY, Chik SC, Chan AW, et al. Anti-inflammatory effects of high-dose montelukast in an animal model of acute asthma. *Clin Exp Allergy* 2003; 33:359–366
- 104 Tohda Y, Nakahara H, Kubo H, et al. Effects of ONO-1078 (pranlukast) on cytokine production in peripheral blood mononuclear cells of patients with bronchial asthma. *Clin Exp Allergy* 1999; 29:1532–1536
- 105 Larfars G, Lantoine F, Devynck MA, et al. Activation of nitric oxide release and oxidative metabolism by leukotrienes B₄, C₄, and D₄ in human polymorphonuclear leukocytes. *Blood* 1999; 93:1399–1405
- 106 Bisgaard H, Loland L, Oj JA. NO in exhaled air of asthmatic children is reduced by the leukotriene receptor antagonist montelukast. *Am J Respir Crit Care Med* 1999; 160:1227–1231
- 107 Bratton DL, Lanz MJ, Miyazawa N, et al. Exhaled nitric oxide before and after montelukast sodium therapy in school-age children with chronic asthma: a preliminary study. *Pediatr Pulmonol* 1999; 28:402–407
- 108 Holgate ST, Peters-Golden M, Panettieri RA, et al. Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodeling. *J Allergy Clin Immunol* 2003; 111:S18–S34; discussion S34–S16
- 109 Mechiche H, Naline E, Candenas L, et al. Effects of cysteinyl leukotrienes in small human bronchus and antagonist activity of montelukast and its metabolites. *Clin Exp Allergy* 2003; 33:887–894
- 110 Ruck LM, Rizzo CA, Anthes JC, et al. Synergistic antiallergic activity of combined histamine H₁- and cysteinyl leukotriene 1-receptor blockade in human bronchus. *Life Sci* 2001; 68:2825–2834
- 111 Baud L, Perez J, Denis M, et al. Modulation of fibroblast proliferation by sulfidopeptide leukotrienes: effect of indomethacin. *J Immunol* 1987; 138:1190–1195
- 112 Phan SH, McGarry BM, Loeffler KM, et al. Regulation of macrophage-derived fibroblast growth factor release by arachidonate metabolites. *J Leukoc Biol* 1987; 42:106–113
- 113 Phan SH, McGarry BM, Loeffler KM, et al. Binding of leukotriene C₄ to rat lung fibroblasts and stimulation of colla-

- gen synthesis *in vitro*. *Biochemistry* 1988; 27:2846–2853
- 114 Peters-Golden M, Bailie M, Marshall T, et al. Protection from pulmonary fibrosis in leukotriene-deficient mice. *Am J Respir Crit Care Med* 2002; 165:229–235
- 115 Henderson WR, Tang LO, Chu SJ, et al. A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. *Am J Respir Crit Care Med* 2002; 165:108–116
- 116 Panettieri RA, Tan EM, Ciocca V, et al. Effects of LTD₄ on human airway smooth muscle cell proliferation, matrix expression, and contraction *in vitro*: differential sensitivity to cysteinyl leukotriene receptor antagonists. *Am J Respir Cell Mol Biol* 1998; 19:453–461