

## REVIEW ARTICLE

## MECHANISMS OF DISEASE

## Leukotrienes

Marc Peters-Golden, M.D., and William R. Henderson, Jr., M.D.

LEUKOTRIENES (“LEUKO,” FROM WHITE BLOOD CELLS; AND “TRIENES,” THREE conjugated double bonds) comprise a family of products of the 5-lipoxygenase pathway of arachidonic acid metabolism. The cysteinyl leukotrienes C<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub> account for the biologic activity that was previously termed “slow-reacting substance of anaphylaxis,” and the efficacy of antagonists to type 1 cysteinyl leukotriene receptor (CysLT<sub>1</sub>) in asthma validates the importance of cysteinyl leukotrienes and CysLT<sub>1</sub> in this disease.<sup>1</sup> This article reviews both established understanding and recent advances in our knowledge about leukotrienes.

## SYNTHESIS OF LEUKOTRIENES

The synthesis of leukotrienes from substrate arachidonic acid is initiated by 5-lipoxygenase in concert with 5-lipoxygenase-activating protein (FLAP)<sup>2</sup> (Fig. 1). Although FLAP does not have enzymatic activity, it enhances the ability of 5-lipoxygenase to interact with its substrate. Leukotriene A<sub>4</sub> (LTA<sub>4</sub>) is converted by LTA<sub>4</sub> hydrolase to leukotriene B<sub>4</sub> (LTB<sub>4</sub>), or it can be conjugated with reduced glutathione by leukotriene C<sub>4</sub> (LTC<sub>4</sub>) synthase to yield LTC<sub>4</sub>. LTB<sub>4</sub> and LTC<sub>4</sub> are exported from the cell by specific transporter proteins; the released LTC<sub>4</sub> is converted to leukotriene D<sub>4</sub> (LTD<sub>4</sub>), which undergoes conversion to leukotriene E<sub>4</sub> (LTE<sub>4</sub>) by sequential amino acid hydrolysis. The capacity to generate large amounts of leukotrienes from arachidonate is largely confined to leukocytes; however, the amounts of LTB<sub>4</sub> and cysteinyl leukotrienes that various types of leukocytes produce depend on the distal enzymes LTA<sub>4</sub> hydrolase and LTC<sub>4</sub> synthase, respectively (Table 1 and Glossary).

Although nonleukocyte cells generally do not have sufficient 5-lipoxygenase and FLAP to synthesize appreciable amounts of leukotrienes from arachidonate, such cells expressing distal LTA<sub>4</sub>-metabolizing enzymes can take up leukocyte-derived LTA<sub>4</sub> and metabolize it into bioactive leukotrienes, a process that is termed “transcellular biosynthesis.”<sup>3</sup> The interaction between neutrophils and endothelial cells is an example of this phenomenon: a neutrophil (the donor cell) containing 5-lipoxygenase provides LTA<sub>4</sub> to the endothelial cell (the acceptor cell), which lacks 5-lipoxygenase but expresses LTC<sub>4</sub> synthase and can thereby metabolize the donated LTA<sub>4</sub> to LTC<sub>4</sub>. Products of the 5-lipoxygenase pathway besides leukotrienes (5-hydroxyeicosatetraenoic acid, 5-oxo-eicosatetraenoic acid,<sup>4</sup> and lipoxins<sup>5</sup>) are not considered in this review.

The output of the leukotriene synthetic pathway is regulated by the amount of free arachidonate that phospholipase A<sub>2</sub> releases from cell-membrane phospholipids,<sup>6,7</sup> the level of each of the proteins in the 5-lipoxygenase pathway, the catalytic activity per enzyme molecule (e.g., modulated by protein kinase-directed phosphorylation<sup>8</sup>), and the availability of small molecules (e.g., ATP, nitric oxide,<sup>9</sup> and reactive oxygen intermediates) that modulate 5-lipoxygenase activity.

Another variable that influences leukotriene synthesis is the intracellular localization of 5-lipoxygenase. In resting leukocytes, this enzyme can reside in the cytoplasm or the nucleoplasm, and it shuttles between them by regulated nuclear import and

From the Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Health System, Ann Arbor (M.P.-G.); and the Center for Allergy and Inflammation, Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington, Seattle (W.R.H.). Address reprint requests to Dr. Peters-Golden at the Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Health System, 6301 MSRB III, 1150 W. Medical Center Dr., Ann Arbor, MI 48109-5642, or at [petersm@umich.edu](mailto:petersm@umich.edu).

N Engl J Med 2007;357:1841-54.

Copyright © 2007 Massachusetts Medical Society.

export processes. When 5-lipoxygenase is activated, it relocates to the outer or inner nuclear membrane.<sup>10</sup> The movement of 5-lipoxygenase from the nucleoplasm to the inner nuclear membrane is associated with a maximal synthesis of LTB<sub>4</sub>.<sup>11</sup>

Heritable deficiencies of enzymes in the leukotriene synthetic pathway are rare,<sup>12</sup> but there are allelic variants of the coding and promoter regions of the genes for 5-lipoxygenase,<sup>13</sup> FLAP,<sup>14</sup> LTA<sub>4</sub> hydrolase,<sup>15</sup> and LTC<sub>4</sub> synthase.<sup>16</sup> Moreover, the transcription of these genes can be regulated by cytokines,<sup>17,18</sup> transforming growth factor  $\beta$ , leptin, endothelin, vitamin D<sub>3</sub>, endotoxin,<sup>19</sup> and corticosteroids. Expression of LTC<sub>4</sub> synthase, for example, is up-regulated by interleukin-4<sup>18</sup> and down-regulated by endotoxin.<sup>19</sup>

#### LEUKOTRIENE RECEPTORS

Leukotrienes act by binding to specific heptahelical receptors of the rhodopsin class that are located on the outer plasma membrane of structural and inflammatory cells.<sup>20,21</sup> Once ligated by the

leukotriene, these receptors interact with G proteins in the cytoplasm, thereby eliciting increases in intracellular calcium and reductions in intracellular cyclic AMP. These proximal signals activate downstream kinase cascades in ways that alter various cellular activities, ranging from motility to transcriptional activation. Table 1 lists the major types of cells that express leukotriene receptors.

Type 1 cysteinyl leukotriene receptor (CysLT<sub>1</sub>)<sup>22</sup> mediates sustained bronchoconstriction, mucus secretion, and edema in the airways. Selective antagonists of CysLT<sub>1</sub> that are approved for the treatment of asthma block the proasthmatic effects of CysLT<sub>1</sub> (Fig. 1). Experiments in mice that are deficient in type 2 cysteinyl leukotriene receptor (CysLT<sub>2</sub>)<sup>23</sup> or that overexpress CysLT<sub>2</sub> in the lungs<sup>24</sup> indicate that CysLT<sub>2</sub> does not mediate bronchoconstriction but, rather, contributes to inflammation, vascular permeability, and tissue fibrosis. There are no known specific antagonists of CysLT<sub>2</sub>. Certain reported actions of cysteinyl leukotrienes are not readily explained by either

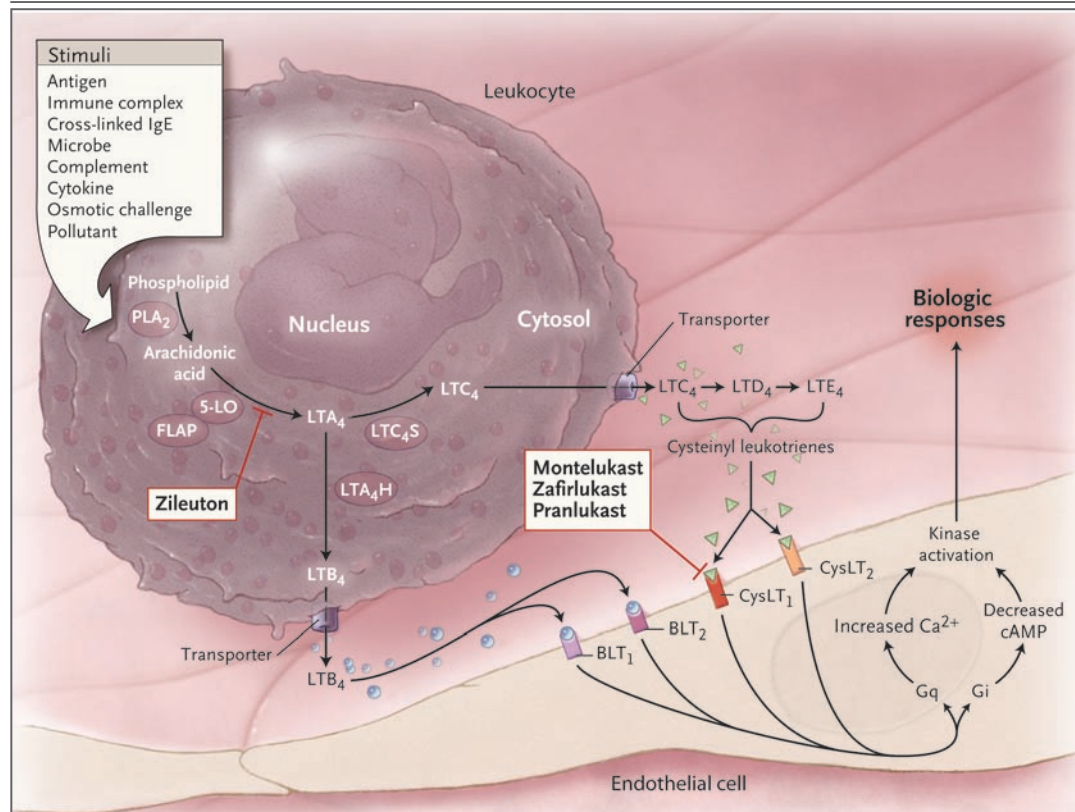
#### Glossary

<b>BLT<sub>1</sub>:</b> B leukotriene receptor 1, a G protein–coupled receptor recognizing leukotriene B <sub>4</sub> with high affinity.
<b>BLT<sub>2</sub>:</b> B leukotriene receptor 2, a G protein–coupled receptor recognizing leukotriene B <sub>4</sub> and a variety of other lipoxygenase metabolites of arachidonic acid with low affinity.
<b>CysLT<sub>1</sub>:</b> Cysteinyl leukotriene receptor 1, a G protein–coupled receptor that recognizes cysteinyl leukotrienes in a descending order of affinity (first, leukotriene D <sub>4</sub> ; second, either leukotriene C <sub>4</sub> or leukotriene E <sub>4</sub> ); the target of antileukotriene agents such as montelukast, zafirlukast, and pranlukast.
<b>CysLT<sub>2</sub>:</b> Cysteinyl leukotriene receptor 2, a G protein–coupled receptor that recognizes cysteinyl leukotrienes in a descending order of affinity (first, either leukotriene C <sub>4</sub> or leukotriene D <sub>4</sub> ; second, leukotriene E <sub>4</sub> ).
<b>Cysteinyl leukotrienes:</b> Class includes leukotriene C <sub>4</sub> , leukotriene D <sub>4</sub> , and leukotriene E <sub>4</sub> , all of which contain the amino acid cysteine conjugated to the lipid backbone.
<b>5-Lipoxygenase:</b> The enzyme that initiates leukotriene synthesis from arachidonic acid.
<b>FLAP:</b> 5-Lipoxygenase–activating protein, a helper protein for 5-lipoxygenase that is thought to bind arachidonic acid and present it to 5-lipoxygenase to facilitate its catalytic function.
<b>LTA<sub>4</sub>:</b> Leukotriene A <sub>4</sub> , the unstable product of 5-lipoxygenase action on substrate arachidonic acid and the precursor of the bioactive leukotriene B <sub>4</sub> and cysteinyl leukotrienes.
<b>LTA<sub>4</sub> hydrolase:</b> Leukotriene A <sub>4</sub> hydrolase, an enzyme that hydrolyzes leukotriene A <sub>4</sub> to leukotriene B <sub>4</sub> .
<b>LTB<sub>4</sub>:</b> Leukotriene B <sub>4</sub> , a product of the action of leukotriene A <sub>4</sub> hydrolase on leukotriene A <sub>4</sub> and a potent leukocyte chemoattractant and activator.
<b>LTC<sub>4</sub>:</b> Leukotriene C <sub>4</sub> , a product of the action of leukotriene C <sub>4</sub> synthase on leukotriene A <sub>4</sub> and a precursor of the other cysteinyl leukotrienes.
<b>LTC<sub>4</sub> synthase:</b> Leukotriene C <sub>4</sub> synthase, an enzyme that conjugates glutathione to leukotriene A <sub>4</sub> to form leukotriene C <sub>4</sub> .
<b>LTD<sub>4</sub>:</b> Leukotriene D <sub>4</sub> , a metabolite of leukotriene C <sub>4</sub> and the most potent of the cysteinyl leukotrienes in contracting airway smooth muscle by ligation of CysLT <sub>1</sub> .
<b>LTE<sub>4</sub>:</b> Leukotriene E <sub>4</sub> , a metabolite of leukotriene D <sub>4</sub> and end product of all cysteinyl leukotrienes that can be measured in the urine.
<b>Rhodopsin:</b> A retinal photoreceptor that is the prototypic heptahelical G protein–coupled receptor and that resembles a leukotriene receptor.

CysLT<sub>1</sub> or CysLT<sub>2</sub>, raising the possibility of the presence of CysLT<sub>1</sub>–CysLT<sub>2</sub> heterodimers or additional receptors.<sup>25</sup> One candidate is G protein–coupled receptor 17 (GPR17), a dual-uracil nucleotide–cysteinyl leukotriene receptor.<sup>26</sup> B leukotriene receptor 1 (BLT<sub>1</sub>) is the high-affinity receptor for LTB<sub>4</sub> that mediates most, if not all, of its chemoattractant and proinflammatory action.<sup>20</sup> B leukotriene receptor 2 (BLT<sub>2</sub>) is a lower-affinity receptor for LTB<sub>4</sub> that also binds other lipoxygenase products; little is known about its physiological function.

The expression of CysLT<sub>1</sub> can be influenced at the transcriptional level by type 2 helper T (Th2)-cell-type cytokines.<sup>27</sup> This effect probably explains why CysLT<sub>1</sub> is overexpressed in patients with asthma or chronic rhinosinusitis who have aspirin sensitivity and why levels of the receptor return to normal after aspirin desensitization.<sup>28</sup>

In addition to the actions of cysteinyl leukotrienes in the airway, they join LTB<sub>4</sub> in exerting other biologic actions. Some of the actions of cysteinyl leukotrienes and LTB<sub>4</sub> are distinctive (e.g., smooth-muscle contraction for the former and



**Figure 1. Leukotriene Synthesis, Receptors, and Signaling.**

Leukotriene synthesis can be activated in a cell (e.g., a leukocyte) by a variety of stimuli. The enzymatic machinery for phospholipase A<sub>2</sub> (PLA<sub>2</sub>)–catalyzed arachidonate hydrolysis and leukotriene synthesis is localized primarily at or near the nuclear membrane, necessitating that leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and leukotriene C<sub>4</sub> (LTC<sub>4</sub>) be transported by carrier proteins out of the cell; the LTC<sub>4</sub> transporter is multidrug resistance protein 1; the LTB<sub>4</sub> transporter is unknown. In the extracellular milieu, LTC<sub>4</sub> is converted to leukotriene D<sub>4</sub> (LTD<sub>4</sub>) and LTD<sub>4</sub> to leukotriene E<sub>4</sub> (LTE<sub>4</sub>). Collectively, these molecules make up the cysteinyl leukotrienes. Leukotrienes act on target cells, which may be leukocytes, epithelial cells, smooth-muscle cells, or endothelial cells, by interacting with one or both classes of their cognate receptors. B leukotriene receptor 1 (BLT<sub>1</sub>) is expressed primarily on leukocytes and is a high-affinity receptor, whereas B leukotriene receptor 2 (BLT<sub>2</sub>) is expressed more ubiquitously, has a somewhat lower affinity for LTB<sub>4</sub>, and can bind other lipids. The two cysteinyl leukotriene receptors have a broad distribution. All leukotriene receptors activate the Gq class of G proteins, resulting in increased intracellular calcium, the Gi class, resulting in decreased intracellular cyclic AMP (cAMP), or both. These effects, which activate downstream protein kinases, culminate in myriad cellular and tissue responses. The sites of action of antileukotriene drugs (5-lipoxygenase [5-LO] for zileuton and CysLT<sub>1</sub> for montelukast, zafirlukast, and pranlukast) are shown. FLAP denotes 5-lipoxygenase–activating protein.

**Table 1. Leukotriene Synthesis and Receptor Expression in Leukocyte Subgroups.\***

Type of Cell	Relative Synthetic Capacity		Receptor Expression			
	LTB <sub>4</sub>	Cysteinyl Leukotrienes	BLT <sub>1</sub>	BLT <sub>2</sub>	CysLT <sub>1</sub>	CysLT <sub>2</sub>
Neutrophil	+++	–	+	+	±	±
Macrophage or monocyte	++	++	+	+	+	+
Eosinophil	–	+++	+	+	+	+
Basophil	–	+++	+	–	+	+
Mast cell	+	+++	+	+	+	+
B lymphocyte	–	–	ND	+	+	ND
CD4 T lymphocyte	–	–	+	+	+	ND
CD8 T lymphocyte	–	–	+	+	ND	ND
Dendritic cell	++	+	+	+	+	ND
Hematopoietic progenitor cell	–	–	ND	+	+	ND

\* Relative synthetic capacity is expressed by the number of plus (+) signs; a minus sign (–) denotes no or negligible synthetic capacity. Receptor expression is classified as positive (+), negative (–), minimal (±), or not determined (ND). With respect to cells for which discrepancies exist in reports on leukotriene synthesis or receptor expression, the table lists the best available information taken from primary cells, particularly those that are human in origin.

neutrophil chemotaxis for the latter), whereas other actions (e.g., promotion of allergic responses) are not. Leukotrienes promote the movement into tissues and function of virtually all subgroups of leukocytes<sup>29–32</sup> (Fig. 2). Also important is their role in amplifying inflammatory responses mediated by Th2 cells.<sup>33–35</sup> The ability of a CysLT<sub>1</sub> antagonist to reduce serum levels of IgE in children with asthma is indicative of the effects of cysteinyl leukotrienes on systemic immune responses.<sup>36</sup>

#### BLOCKADE OF LEUKOTRIENE SYNTHESIS AND LEUKOTRIENE RECEPTORS

Commonly used antiinflammatory medications do not predictably interfere with the synthesis of<sup>37</sup> or responses to<sup>38</sup> leukotrienes. Nonsteroidal antiinflammatory drugs can actually increase the production of leukotrienes,<sup>39</sup> and corticosteroids can increase the expression of BLT<sub>1</sub> on neutrophils.<sup>40</sup>

CysLT<sub>1</sub> antagonists (Fig. 1) include montelukast, zafirlukast, and pranlukast (the last available only in Japan). The only other antileukotriene drug that has been approved by the Food and Drug Ad-

ministration (FDA) is zileuton, which directly inhibits 5-lipoxygenase; this effect blocks production of cysteinyl leukotrienes and LTB<sub>4</sub>. Zileuton inhibits an estimated 26 to 86% of endogenous leukotriene production.<sup>41</sup> The clinical use of zileuton is limited by the need to monitor hepatic enzyme levels and to administer the drug four times daily. A twice-daily sustained-release form of zileuton has recently received FDA approval. Other candidate 5-lipoxygenase inhibitors also have hepatotoxicity. The hepatic injury caused by zileuton appears to be a direct toxic effect that is unrelated to inhibition of 5-lipoxygenase.<sup>42</sup>

An alternative way of blocking leukotriene synthesis is to inhibit FLAP. The crystal structure of this molecule has recently been reported,<sup>43</sup> and this information should prove useful in drug design. One FLAP inhibitor, DG031 (veliflapon, DeCode Genetics), is being reformulated for use in phase 3 trials for the prevention of myocardial infarction. Selective BLT<sub>1</sub> antagonists that have been developed up to now have been tested in animal models of disease,<sup>44</sup> but none have emerged for clinical use.

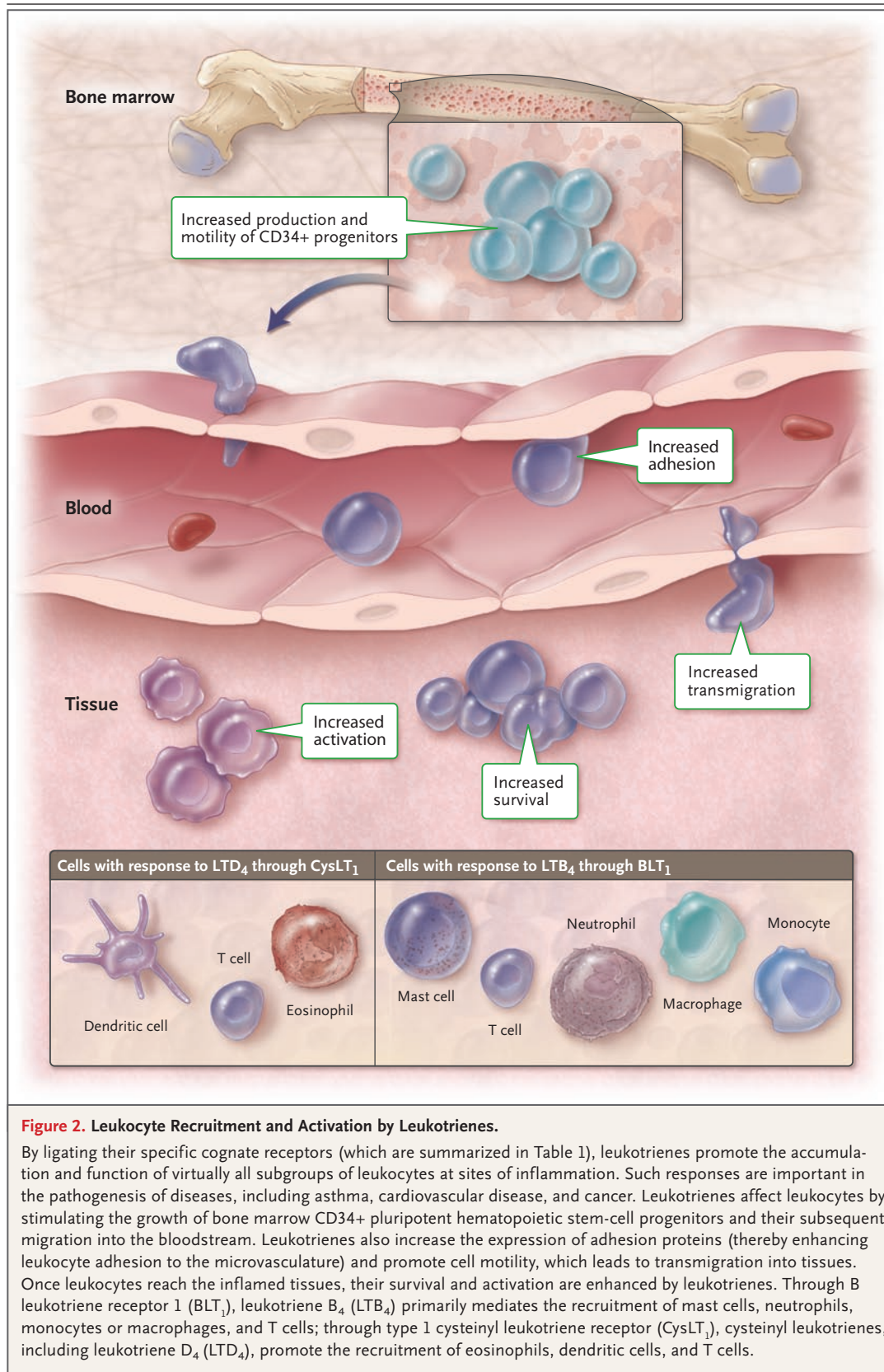
#### LEUKOTRIENES IN DISEASE

Leukotrienes have a multitude of biologic actions (Table 2) and have been suggested as factors in numerous disease processes (Table 3). The role of leukotrienes has been validated in clinical trials of antileukotriene agents for asthma and only a few of the other diseases listed in Table 3.

#### ASTHMA

##### *Antileukotriene Drugs as Controller Agents*

The benefits of antileukotriene therapy (i.e., 5-lipoxygenase inhibition by zileuton<sup>45</sup> and CysLT<sub>1</sub> blockade by montelukast or zafirlukast<sup>46,47</sup>) in children and adults with asthma are improved pulmonary function, decreased daytime and nocturnal symptoms, a reduced need for short-acting rescue  $\beta_2$  agonists, fewer exacerbations of asthma, and an increased quality of life. Inhaled corticosteroids are more potent than antileukotriene agents<sup>48</sup> and hence are favored as first-line treatment; however, antileukotriene therapy can be used initially in a patient who cannot or will not take corticosteroids.<sup>49</sup> Antileukotriene agents have an additive benefit in patients whose disease is not adequately controlled by inhaled corticosteroids,<sup>50</sup> perhaps reflecting the inability of corticosteroids



**Table 2.** Effects of Leukotrienes on the Biologic Actions of Cells Associated with Asthma, Cardiovascular Disease, and Cancer.\*

Type of Cell	Asthma	Cardiovascular Disease	Cancer
Leukocyte	Increases recruitment of T cells,† eosinophils,† and mast cells‡ Increases Th2 responses, cytokines or chemokines, and reactive oxygen species†	Increases monocyte and T-cell recruitment‡ Increases differentiation of macrophages or foam cells‡ Increases chemokines (e.g., MCP-1 and MIP-1α) and proteases†	Increases recruitment of monocytes‡ Increases cytokines or chemokines and reactive oxygen species†
Dendritic cell	Increases cell recruitment and activation§	NA	NA
Epithelial cell	Increases mucus release and goblet cells§	NA	NA
Fibroblast or myofibroblast	Increases collagen release§	NA	NA
Smooth-muscle cell	Increases contractility and proliferation§	Increases contractility and proliferation§	NA
Endothelial cell	Increases vascular permeability§	Increases vascular permeability§ Increases intimal hyperplasia† Increases chemokines (e.g., MIP-2)† Increases thrombosis§	Increases vascular permeability and angiogenesis§
Malignant cell	NA	NA	Increases proliferation (e.g., kinase or β-catenin signaling)† Increases transcriptional activity of oncogenic genes‡ Increases expression of adhesion molecules† Decreases apoptosis (by increasing Bcl-2) to increase tumor-cell survival†

\* Th2 denotes type 2 helper T cell, MCP-1 monocyte chemoattractant protein 1, MIP-1α macrophage inflammatory protein 1α, MIP-2 macrophage inflammatory protein 2, and NA not applicable.

† Biologic action is most closely attributable to both leukotriene B<sub>4</sub> and cysteinyl leukotrienes.

‡ Biologic action is most closely attributable to leukotriene B<sub>4</sub>.

§ Biologic action is most closely attributable to cysteinyl leukotrienes.

to inhibit leukotriene pathways. Although the weight of evidence from randomized, controlled studies indicates that antileukotriene agents are inferior to long-acting β<sub>2</sub> agonists as add-on therapy to inhaled corticosteroids (particularly with respect to improving lung function<sup>51</sup>), some studies have shown that montelukast is similar to long-acting β<sub>2</sub> agonists in reducing symptoms and exacerbations of asthma.<sup>52,53</sup>

Some patients with asthma are particularly good candidates for antileukotriene therapy. One example is the patient with allergic rhinitis. The nasal congestion and rhinorrhea in allergic rhinitis are reduced by montelukast to a degree similar to that with antihistamines but inferior to that with topical corticosteroids.<sup>54–56</sup> Since allergic rhinitis is often present with asthma and complicates its management, a leukotriene modifier could im-

prove upper- and lower-airway symptoms and signs.<sup>57</sup> However, trials of the use of montelukast to reduce the need for other rhinitis medications in patients with both asthma and allergic rhinitis have had divergent results.<sup>58,59</sup>

Montelukast also provides protection against exercise-induced asthma. A single oral dose of montelukast is as effective as inhaled salmeterol, a long-acting β<sub>2</sub> agonist, in preventing exercise-induced asthma,<sup>60</sup> and its regular use during a 2-month period was not associated with the development of tachyphylaxis, as occurs with the use of salmeterol.<sup>61</sup>

Antileukotriene agents are beneficial in patients with aspirin-sensitive asthma, a condition in which production of very high levels of cysteinyl leukotrienes is typical.<sup>62,63</sup> However, even among such patients, the drugs are inconsistent in their abil-

ity to attenuate responses to aspirin challenge.<sup>64</sup> Applications of antileukotriene therapy that remain under investigation are the treatment of persistent respiratory symptoms in children after respiratory syncytial virus infection<sup>65</sup> and the treatment of acute asthma exacerbations in children<sup>66</sup> and adults.<sup>67</sup>

#### *Effects of Antileukotriene Agents on Airway Remodeling*

Structural alterations referred to as “airway remodeling,” consisting of increases in the number of airway goblet cells and smooth-muscle mass, as well as deposition of subepithelial collagen (Table 2), contribute to the progressive loss of lung function in patients with chronic asthma.<sup>68</sup> For this reason, a desirable feature of controller therapy for asthma is its ability to ameliorate airway remodeling. Airway remodeling appears to be resistant to inhaled corticosteroids.<sup>69</sup> However, in a mouse model of chronic allergic asthma, CysLT<sub>1</sub> blockade with montelukast reversed all the histologic features of airway remodeling.<sup>70</sup> Myofibroblasts, which are mesenchymal cells with features of both fibroblasts and smooth-muscle cells, have been implicated in airway remodeling because of their high capacity for collagen synthesis. In atopic asthma, the allergen-induced increase in the number of myofibroblasts in the airway wall is limited by montelukast treatment,<sup>71</sup> but whether antileukotriene drugs prevent or ameliorate airway remodeling in patients with asthma is unknown.

#### *Responsiveness to Antileukotriene Agents*

Since asthma involves multiple mediators, it is not surprising that the efficacy of agents that target only components of the 5-lipoxygenase pathway is limited and variable. Why are antileukotriene drugs effective in some patients and ineffective in others?

One explanation for the efficacy of these drugs is that CysLT<sub>1</sub> signaling contributes to many aspects of asthma pathogenesis besides constriction of bronchial smooth muscle. For example, CysLT<sub>1</sub> influences systemic immune responses and has bidirectional interactions with cytokines. The interplay between the Th2 cytokine interleukin-13 and cysteinyl leukotrienes and CysLT<sub>1</sub> exemplifies such relationships (Fig. 3).

Variations in responses of patients with asthma to corticosteroids and  $\beta_2$  agonists were illuminated by studies of an antileukotriene agent.<sup>72</sup> In randomized, controlled trials with these drugs,

**Table 3. Diseases That Have a Possible Association with Leukotrienes.**

Allergic diseases
Asthma*
Allergic rhinitis*
Rhinosinusitis
Atopic dermatitis†
Urticaria†
Fibrotic diseases
Airway remodeling in asthma
Bronchiolitis obliterans after lung transplantation
Idiopathic pulmonary fibrosis‡
Scleroderma
Asbestosis
Other pulmonary syndromes
Acute lung injury or adult respiratory distress syndrome
Viral bronchiolitis†
Obstructive sleep apnea†
Chronic obstructive pulmonary disease†‡
Cystic fibrosis§ and other forms of bronchiectasis
Bronchopulmonary dysplasia
Other local inflammatory diseases
Arthritis (including osteoarthritis and gout)
Glomerulonephritis
Interstitial cystitis†
Psoriasis
Inflammatory bowel disease¶
Systemic inflammatory diseases
Rheumatoid arthritis†
Vasculitides (systemic lupus erythematosus,† Churg–Strauss syndrome, Henoch–Schönlein purpura)
Transplant rejection
Cancer
Solid tumors (including melanoma, mesothelioma, and pancreatic, lung,‡ esophageal, prostate, and colon cancers)
Leukemias
Lymphomas
Cardiovascular disease
Atherosclerosis†
Aortic aneurysm
Sickle cell crisis
Ischemia–reperfusion injury
Pulmonary arterial hypertension
Sepsis

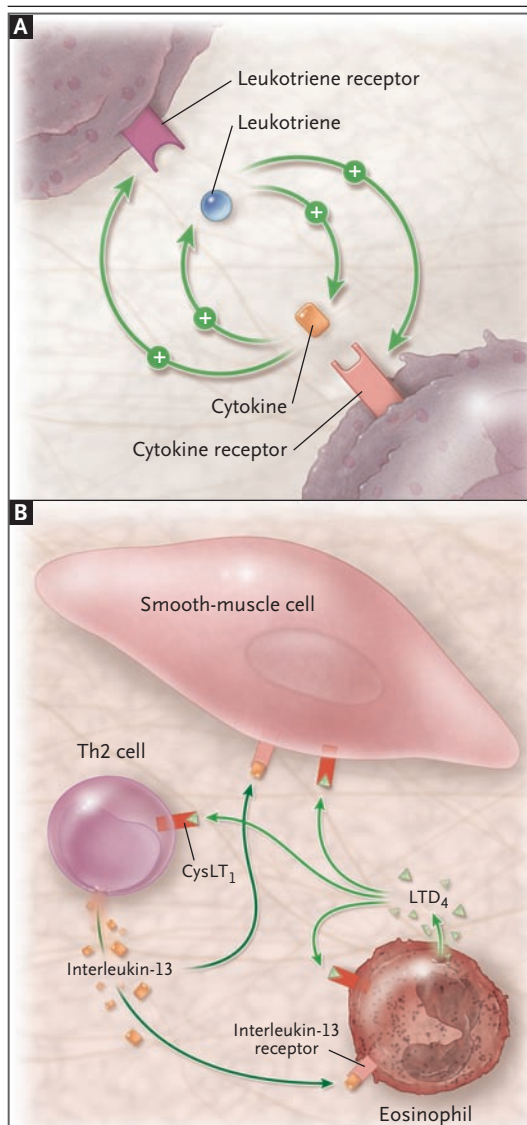
\* An association has been established in large-scale randomized, double-blind, placebo-controlled clinical trials, and the Food and Drug Administration has approved an antileukotriene intervention.

† An association has been established in small-scale or nonrandomized, placebo-controlled trials indicating that leukotrienes may play a role in disease pathogenesis, but larger randomized clinical trials are required.

‡ Clinical trials are ongoing or contemplated.

§ Adverse events (e.g., increased infectious exacerbations) that were associated with antileukotriene therapy have been reported.

¶ Results of a clinical trial involving patients with ulcerative colitis were negative.



**Figure 3. Cross-Talk between Leukotrienes and Cytokines.**

As shown in Panel A, leukotrienes and cytokines can regulate each other. This process is shown in Panel B. Interleukin-13, a product derived from type 2 helper T (Th2) lymphocytes that participates in the development of asthma, can up-regulate both the leukocyte biosynthesis of leukotriene D<sub>4</sub> (LTD<sub>4</sub>) and the expression of cellular type 1 cysteinyl leukotriene receptors (CysLT<sub>1</sub>). Moreover, LTD<sub>4</sub> can up-regulate production of interleukin-13, as well as the expression of its receptor. The result of this cross-talk is a self-perpetuating circuit of inflammation and smooth-muscle contraction in which interleukin-13 and its receptor mediate some of the actions of LTD<sub>4</sub> and LTD<sub>4</sub>-CysLT<sub>1</sub> mediates some of the actions of interleukin-13.

symptoms were reduced as compared with baseline in 60 to 80% of patients, whereas improvements in lung function were noted in only 35 to 50% of such patients.<sup>73</sup> This discordance in outcome measures may be due to differences among patients in leukotriene biology, genotype, or clinical characteristics.

After spontaneous episodes of bronchospasm in patients with asthma or after challenge with allergen, exercise, or aspirin in susceptible patients, levels of cysteinyl leukotrienes increase in airway fluids and urine.<sup>74,75</sup> Urinary LTE<sub>4</sub>, a measure of whole-body synthesis of cysteinyl leukotrienes,<sup>76</sup> is a possible predictor of drug responsiveness, and significant associations between high urinary LTE<sub>4</sub> levels and responsiveness to antileukotriene agents have been reported.<sup>77</sup> Other studies, however, have shown either no such relationship<sup>63</sup> or an inverse relationship.<sup>78</sup> It seems that technical limitations and spontaneous variations in the level of urinary LTE<sub>4</sub> reduce its value as a robust predictor of the response to antileukotriene therapy in individual patients. Leukotriene synthesis by cultured leukocytes is also an unreliable predictor of the clinical response to pranlukast.<sup>79</sup>

In patients with asthma who were treated with a 5-lipoxygenase inhibitor, polymorphisms in the promoter of the 5-lipoxygenase gene (*ALOX5*) were associated with diminished improvement in airflow.<sup>80</sup> However, since these genetic variants occur in less than 10% of patients with asthma, they can explain only a fraction of the unresponsiveness to leukotriene modifiers. By contrast, an A-to-C transversion in the LTC<sub>4</sub> synthase promoter region occurs in approximately 20 to 40% of patients with asthma<sup>16</sup> and is associated with increased synthesis of cysteinyl leukotrienes. A positive relation between this variant allele and the clinical response to various CysLT<sub>1</sub> antagonists was reported in two studies<sup>81,82</sup> but not in a third.<sup>83</sup> A variant allele in the coding region of the gene encoding CysLT<sub>2</sub> has recently been reported to be associated with an enhanced response to montelukast.<sup>84</sup> It is likely that these pharmacogenetic associations vary according to the geographic origin of the population under analysis. Since levels of leukotrienes and their receptors are greatly influenced by substances such as cytokines, analysis of responsiveness to antileukotriene therapy must take into account the genes for

molecules that reside outside the leukotriene pathway.

Several characteristics of patients have been associated with responsiveness to antileukotriene drugs. A benefit is more likely in children than in adults<sup>85</sup> and in younger children than in older children.<sup>86</sup> Moreover, acquired factors such as obesity and smoking may be relevant in understanding the clinical effects of antileukotriene agents. Although in one study the effect of inhaled beclomethasone on asthma control declined with increasing body-mass index, no such decline was observed for montelukast,<sup>87</sup> suggesting that the relative benefit of montelukast may have been greater in more obese patients. Likewise, patients with asthma who smoked had a greater response to montelukast than did their nonsmoking counterparts.<sup>88</sup>

#### *Therapeutic Trials of Antileukotriene Agents*

Since it is impossible to predict responsiveness to antileukotriene therapy in an individual patient, a therapeutic trial in the patient may be necessary. Improvements in symptoms or a reduced need for rescue bronchodilators can be seen with antileukotriene therapy as early as the first day of treatment, since cysteinyl leukotrienes increase bronchial tone.<sup>46,89</sup> Reductions in levels of exhaled nitric oxide<sup>90</sup> and bronchial hyperresponsiveness<sup>91</sup> occur within 1 week and 2 weeks after the initiation of therapy, respectively, and improvements in lung function and symptoms occur over a period of weeks or months.<sup>52,73</sup> A trial period of 1 to 2 months is recommended.

Montelukast is the most commonly used inhibitor of the leukotriene pathway because of its ease of use, good safety profile, and once-daily regimen. However, use of this CysLT<sub>1</sub> antagonist ignores possible contributions of CysLT<sub>2</sub> to effects mediated by cysteinyl leukotrienes. Montelukast also does not inhibit other products of the 5-lipoxygenase pathway — most notably, LTB<sub>4</sub>. The lack of an effect on LTB<sub>4</sub> could be important, because this leukocyte chemoattractant and activator is probably involved in severe asthma and asthma exacerbations. A 5-lipoxygenase inhibitor might therefore have better efficacy than a CysLT<sub>1</sub> antagonist. Although retrospective data support the possibility that zileuton is more effective in severe asthma than in mild asthma,<sup>92</sup> there are

no data from large-scale, prospective comparisons of an inhibitor of leukotriene synthesis with a CysLT<sub>1</sub> antagonist.

#### **CARDIOVASCULAR DISEASE**

The role of leukotrienes in cardiovascular disease has been the subject of intense investigation (Tables 2 and 3). Atherosclerotic vascular lesions express the entire cassette of leukotriene biosynthetic proteins (5-lipoxygenase, FLAP, LTA<sub>4</sub> hydrolase, and LTC<sub>4</sub> synthase) and receptors (CysLT<sub>1</sub>, CysLT<sub>2</sub>, BLT<sub>1</sub>, and BLT<sub>2</sub>). Moreover, levels of 5-lipoxygenase correlate with the severity of the atherosclerotic lesion<sup>93</sup> and plaque instability.<sup>94</sup> Studies in animals and in vitro suggest that both LTB<sub>4</sub> and cysteinyl leukotrienes participate in the development of atherosclerotic lesions. LTB<sub>4</sub>, by promoting BLT<sub>1</sub>-mediated intracellular signaling, contributes to recruitment of monocytes and foam-cell differentiation,<sup>95</sup> as well as intimal hyperplasia.<sup>96</sup> Cysteinyl leukotrienes — probably involving signaling mediated by both CysLT<sub>1</sub> and CysLT<sub>2</sub> — enhance the recruitment of leukocytes into the arterial wall and contribute to thrombosis and vascular remodeling<sup>97,98</sup> (Fig. 2 and Table 2).

The incidence of strokes and myocardial infarctions in European, Japanese, and American black populations has been linked to variants of the genes that encode FLAP and LTA<sub>4</sub> hydrolase; these variants result in overproduction of leukotrienes.<sup>14,15,99</sup> In a 4-week pilot study, Icelandic patients with a history of myocardial infarction and one of the variant genes that encodes FLAP or LTA<sub>4</sub> hydrolase who were treated with the investigational FLAP inhibitor DG031 (veliflapon) had a significant reduction in levels of C-reactive protein, a biomarker of inflammation that has been linked to cardiovascular disease.<sup>100</sup> In a population of predominantly white North Americans, the association of variants of the *FLAP* gene with cardiovascular disease is less clear. Two studies in the United States showed no association between variants of *FLAP* and the risk of ischemic stroke,<sup>101,102</sup> and one of the studies also showed no association between these alleles and myocardial infarction.<sup>102</sup> Another U.S.-based study showed an association between *FLAP* variants and ischemic strokes among whites but not blacks.<sup>103</sup>

Other polymorphisms involving the 5-lipoxygenase pathway have been linked to atherosclero-

sis. In women, an A-to-C transversion in the LTC<sub>4</sub> synthase promoter region has been associated with an age-adjusted risk of increased levels of coronary-artery calcium and an increased mean intimal-medial thickness of the carotid artery,<sup>104</sup> a surrogate measure of atherosclerosis. Carotid intimal-medial thickness was also shown to be increased in carriers of two variant alleles of the 5-lipoxygenase promoter in a North American population.<sup>105</sup> The same 5-lipoxygenase promoter polymorphisms were not associated with an increased risk of myocardial infarction in a Spanish population.<sup>106</sup> Taken together, these data suggest a role of leukotrienes in the development of atherosclerotic vascular disease, and genetic studies suggest a population-specific influence of polymorphisms in genes encoding leukotriene biosynthetic enzymes or leukotriene receptors.

#### CANCER

Chronic inflammation can increase the risk of cancer. Cysteinyl leukotrienes and LTB<sub>4</sub> that are released by inflammatory cells infiltrating the mucosa of the gut in inflammatory bowel disease may be mediators of such malignant transformation (Table 2). LTD<sub>4</sub> activates  $\beta$ -catenin signaling, leading to the up-regulation of the antiapoptotic protein Bcl-2 and increased cell survival.<sup>107</sup> As compared with normal intestinal epithelial cells, colorectal adenocarcinomas have increased nuclear localization of CysLT<sub>1</sub>, which may facilitate proliferation of the malignant cells by kinase signaling.<sup>108</sup> LTB<sub>4</sub> signaling has also been associated with cancer-cell proliferation. In colon-cancer tissue and cell lines, there is increased expression of BLT<sub>1</sub>, and when BLT<sub>1</sub> is suppressed in cultured cells by a small interfering RNA, cell proliferation decreases.<sup>109</sup>

Malignant cells in colorectal, esophageal, and pancreatic adenocarcinomas, bronchogenic carcinoma, melanoma, lymphomas, and leukemias express large amounts of 5-lipoxygenase, FLAP, and other leukotriene biosynthetic enzymes.<sup>110,111</sup> In one study, treatment of 5-lipoxygenase-expressing esophageal cancer cell lines with a 5-lipoxygenase inhibitor decreased cell survival.<sup>112</sup> The effects of the inhibitor paralleled reductions in LTB<sub>4</sub> production and could be reversed by exogenous LTB<sub>4</sub>, which indicated that the decreased cell survival was due to interruption of LTB<sub>4</sub> synthesis. Chronic lymphocytic leukemia cells express large amounts of 5-lipoxygenase and BLT<sub>1</sub>, and treat-

ment with an investigational inhibitor of 5-lipoxygenase or FLAP blocks the CD40-dependent activation of these cells; this effect is reversed by the addition of LTB<sub>4</sub> to the cultured cells.<sup>113</sup>

In a mouse model of lung cancer, a FLAP inhibitor that reduces leukotriene synthesis reduced the volume of lung tumors induced by a tobacco-specific carcinogen.<sup>114</sup> In rats with a gastroesophageal reflux disease similar to Barrett's esophagus, an esophageal adenocarcinoma that overexpresses LTA<sub>4</sub> hydrolase developed; inhibition of LTB<sub>4</sub> synthesis by the LTA<sub>4</sub> hydrolase inhibitor bestatin reduced the incidence and volume of such tumors.<sup>115</sup> Administration of both zileuton and bestatin also reduced the incidence of carcinogen-induced oral squamous-cell carcinoma in hamsters.<sup>116</sup> These results are the basis of an ongoing clinical trial sponsored by the National Cancer Institute to test the idea that interruption of leukotriene synthetic pathways or leukotriene receptors reduces the growth of cancer.<sup>114</sup>

---

#### LEUKOTRIENES IN ANTIMICROBIAL DEFENSE

---

Leukotrienes that are synthesized in response to a spectrum of infectious agents enhance the capacities of macrophages and neutrophils to ingest and kill microbes and to produce antimicrobial mediators.<sup>117</sup> LTB<sub>4</sub>-BLT<sub>1</sub> signaling exerts broader and more potent effects in this regard than does signaling between cysteinyl leukotrienes and CysLT<sub>1</sub>. In animal models of infection, genetic or pharmacologic interruption of leukotriene synthesis or signaling impairs local microbial clearance.<sup>117</sup> For example, lungs of 5-lipoxygenase-null mice contain almost 100 times as much *Klebsiella pneumoniae* as do lungs of wild-type animals 48 hours after intrapulmonary bacterial inoculation.

In humans, acquired states of leukotriene deficiency have been described in human immunodeficiency virus (HIV) infection<sup>118</sup> and protein-calorie malnutrition.<sup>119</sup> In HIV infection, leukotriene deficiency is the result of reduced expression of 5-lipoxygenase and, especially, of FLAP. These reductions are a consequence of a deficiency of CD4 T-cell-derived cytokines that stimulate the production of these elements in the leukotriene pathway.<sup>118</sup> Although patients with asthma who are treated with antileukotriene agents do not have an increased risk of infection, a significant in-

crease in the risk of infectious pulmonary exacerbations was reported in patients with cystic fibrosis who were treated with an investigational BLT<sub>1</sub> antagonist (BIIL 284), necessitating early termination of the clinical trial.<sup>120</sup>

## CONCLUSIONS

Today, nearly three decades since leukotrienes were discovered and one decade since drugs targeting this pathway became available, new and often unexpected insights into the biology and clinical importance of these lipid mediators continue to emerge. Beyond the classically recognized and validated participation of cysteinyl leukotrienes in

asthma, it is now evident that leukotrienes are multifunctional mediators that influence many biologic responses and probably play a role in other diseases.

Dr. Peters-Golden reports receiving consulting fees from Critical Therapeutics, Pfizer, Wyeth, and Efficas and lecture fees from Merck and Critical Therapeutics and serving on a respiratory advisory board for Merck. He shares a patent with the University of Michigan for intrapulmonary administration of leukotriene B<sub>4</sub> as a local immunostimulant for respiratory infections. Dr. Henderson reports receiving research support from Merck and Sepracor, consulting fees from Alza (Johnson & Johnson), Amgen, and Critical Therapeutics, and lecture fees from Critical Therapeutics and Merck and serving on a scientific advisory board for Gilead and Icos. No other potential conflict of interest relevant to this article was reported.

We thank Christine Abrass, David Fox, and Teal Hallstrand for their helpful comments and Rachel Norris and Patty Urban for their assistance in the preparation of the manuscript.

## REFERENCES

1. Drazen JM, Israel E, O'Byrne PM. Treatment of asthma with drugs modifying the leukotriene pathway. *N Engl J Med* 1999;340:197-206. [Errata, *N Engl J Med* 1999;340:663, 341:1632.]
2. Peters-Golden M, Brock TG. 5-Lipoxygenase and FLAP. *Prostaglandins Leukot Essent Fatty Acids* 2003;69:99-109.
3. Folco G, Murphy RC. Eicosanoid transcellular biosynthesis: from cell-cell interactions to in vivo tissue responses. *Pharmacol Rev* 2006;58:375-88.
4. Powell WS, Rokach J. Biochemistry, biology and chemistry of the 5-lipoxygenase product 5-oxo-EETE. *Prog Lipid Res* 2005;44:154-83.
5. Schwab JM, Serhan CN. Lipoxins and new lipid mediators in the resolution of inflammation. *Curr Opin Pharmacol* 2006;6:414-20.
6. Uozumi N, Kume K, Nagase T, et al. Role of cytosolic phospholipase A<sub>2</sub> in allergic response and parturition. *Nature* 1997;390:618-22.
7. Henderson WR Jr, Chi EY, Bollinger JG, et al. Importance of group X-secreted phospholipase A<sub>2</sub> in allergen-induced airway inflammation and remodeling in a mouse asthma model. *J Exp Med* 2007;204:865-77.
8. Luo M, Jones SM, Phare SM, Coffey MJ, Peters-Golden M, Brock TG. Protein kinase A inhibits leukotriene synthesis by phosphorylation of 5-lipoxygenase on serine 523. *J Biol Chem* 2004;279:41512-20.
9. Coffey MJ, Phare SM, Peters-Golden M. Prolonged exposure to lipopolysaccharide inhibits macrophage 5-lipoxygenase metabolism via induction of nitric oxide synthesis. *J Immunol* 2000;165:3592-8.
10. Peters-Golden M, Brock TG. Intracellular compartmentalization of leukotriene synthesis: unexpected nuclear secrets. *FEBS Lett* 2001;487:323-6.
11. Luo M, Jones SM, Peters-Golden M, Brock TG. Nuclear localization of 5-lipoxygenase as a determinant of leukotriene B<sub>4</sub> synthetic capacity. *Proc Natl Acad Sci U S A* 2003;100:12165-70.
12. Mayatepek E. Leukotriene C<sub>4</sub> synthesis deficiency: a member of a probably underdiagnosed new group of neurometabolic diseases. *Eur J Pediatr* 2000;159:811-8.
13. In KH, Asano K, Beier D, et al. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. *J Clin Invest* 1997;99:1130-7.
14. Helgadottir A, Manolescu A, Thorleifsson G, et al. The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. *Nat Genet* 2004;36:233-9.
15. Helgadottir A, Manolescu A, Helgason A, et al. A variant of the gene encoding leukotriene A<sub>4</sub> hydrolase confers ethnicity-specific risk of myocardial infarction. *Nat Genet* 2006;38:68-74.
16. Sanak M, Simon HU, Szczeklik A. Leukotriene C<sub>4</sub> synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 1997;350:1599-600.
17. Cowburn AS, Holgate ST, Sampson AP. IL-5 increases expression of 5-lipoxygenase-activating protein and translocates 5-lipoxygenase to the nucleus in human blood eosinophils. *J Immunol* 1999;163:456-65.
18. Hsieh FH, Lam BK, Penrose JF, Austen KF, Boyce JA. T helper cell type 2 cytokines coordinately regulate immunoglobulin E-dependent cysteinyl leukotriene production by human cord blood-derived mast cells: profound induction of leukotriene C(4) synthase expression by interleukin 4. *J Exp Med* 2001;193:123-33.
19. Serio KJ, Johns SC, Luo L, Hodulic CR, Bigby TD. Lipopolysaccharide down-regulates the leukotriene C<sub>4</sub> synthase gene in the monocyte-like cell line, THP-1. *J Immunol* 2003;170:2121-8.
20. Tager AM, Luster AD. BLT<sub>1</sub> and BLT<sub>2</sub>: the leukotriene B<sub>4</sub> receptors. *Prostaglandins Leukot Essent Fatty Acids* 2003;69:123-34.
21. Kanaoka Y, Boyce JA. Cysteinyl leukotrienes and their receptors: cellular distribution and function in immune and inflammatory responses. *J Immunol* 2004;173:1503-10.
22. Lynch KR, O'Neill GP, Liu Q, et al. Characterization of the human cysteinyl leukotriene CysLT<sub>1</sub> receptor. *Nature* 1999;399:789-93.
23. Beller TC, Maekawa A, Friend DS, Austen KF, Kanaoka Y. Targeted gene disruption reveals the role of the cysteinyl leukotriene 2 receptor in increased vascular permeability and in bleomycin-induced pulmonary fibrosis in mice. *J Biol Chem* 2004;279:46129-34.
24. Hui Y, Cheng Y, Smalera I, et al. Directed vascular expression of human cysteinyl leukotriene 2 receptor modulates endothelial permeability and systemic blood pressure. *Circulation* 2004;110:3360-6.
25. Yoshisue H, Kirkham-Brown J, Healy E, Holgate ST, Sampson AP, Davies DE. Cysteinyl leukotrienes synergize with growth factors to induce proliferation of human bronchial fibroblasts. *J Allergy Clin Immunol* 2007;119:132-40.
26. Ciana P, Fumagalli M, Trincavelli ML, et al. The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor. *EMBO J* 2006;25:4615-27.
27. Espinosa K, Bossé Y, Stankova J, Rola-Pleszczynski M. CysLT<sub>1</sub> receptor upregulation by TGF-beta and IL-13 is associated with bronchial smooth muscle cell proliferation in response to LTD<sub>4</sub>. *J Allergy Clin Immunol* 2003;111:1032-40.
28. Sousa AR, Parikh A, Scadding G, Cor-

- rigan CJ, Lee TH. Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. *N Engl J Med* 2002;347:1493-9.
29. Ott VL, Cambier JC, Kappler J, Marack P, Swanson BJ. Mast cell-dependent migration of effector CD8+ T cells through production of leukotriene B<sub>4</sub>. *Nat Immunol* 2003;4:974-81.
30. Tager AM, Bromley SK, Medoff BD, et al. Leukotriene B<sub>4</sub> receptor BLT1 mediates early effector T cell recruitment. *Nat Immunol* 2003;4:982-90.
31. Lee E, Lindo T, Jackson N, et al. Reversal of human neutrophil survival by leukotriene B<sub>4</sub> receptor blockade and 5-lipoxygenase and 5-lipoxygenase activating protein inhibitors. *Am J Respir Crit Care Med* 1999;160:2079-85.
32. Lee E, Robertson T, Smith J, Kilfeather S. Leukotriene receptor antagonists and synthesis inhibitors reverse survival in eosinophils of asthmatic individuals. *Am J Respir Crit Care Med* 2000;161:1881-6.
33. Robbiani DF, Finch RA, Jäger D, Muller WA, Sartorelli AC, Randolph GJ. The leukotriene C<sub>4</sub> transporter MRP1 regulates CCL19 (MIP-3b, ELC)-dependent mobilization of dendritic cells to lymph nodes. *Cell* 2000;103:757-68.
34. Okunishi K, Dohi M, Nakagome K, Tanaka R, Yamamoto K. A novel role of cysteinyl leukotrienes to promote dendritic cell activation in the antigen-induced immune responses in the lung. *J Immunol* 2004;173:6393-402.
35. Parameswaran K, Liang H, Fanat A, Watson R, Snider DP, O'Byrne PM. Role for cysteinyl leukotrienes in allergen-induced change in circulating dendritic cell number in asthma. *J Allergy Clin Immunol* 2004;114:73-9.
36. Stelmach I, Bobrowska-Korzeniowska M, Majak P, Stelmach W, Kuna P. The effect of montelukast and different doses of budesonide on IgE serum levels and clinical parameters in children with newly diagnosed asthma. *Pulm Pharmacol Ther* 2005;18:374-80.
37. Dworski R, FitzGerald GA, Oates JA, Sheller JR. Effect of oral prednisone on airway inflammatory mediators in atopic asthma. *Am J Respir Crit Care Med* 1994;149:953-9.
38. Gyllfors P, Dahlén SE, Kumlin M, Larsson K, Dahlén B. Bronchial responsiveness to leukotriene D<sub>4</sub> is resistant to inhaled fluticasone propionate. *J Allergy Clin Immunol* 2006;118:78-83.
39. Montuschi P, Macagno F, Parente P, et al. Effects of cyclo-oxygenase inhibition on exhaled eicosanoids in patients with COPD. *Thorax* 2005;60:827-33.
40. Stankova J, Turcotte S, Harris J, Rolapleszczynski M. Modulation of leukotriene B<sub>4</sub> receptor-1 expression by dexamethasone: potential mechanism for enhanced neutrophil survival. *J Immunol* 2002;168:3570-6.
41. Dubé LM, Swanson LJ, Awani W, Zileuton, a leukotriene synthesis inhibitor in the management of chronic asthma: clinical pharmacokinetics and safety. *Clin Rev Allergy Immunol* 1999;17:213-21.
42. Beierschmitt WP, McNeish JD, Griffiths RJ, Nagahisa A, Nakane M, Amacher DE. Induction of hepatic microsomal drug-metabolizing enzymes by inhibitors of 5-lipoxygenase (5-LO): studies in rats and 5-LO knockout mice. *Toxicol Sci* 2001;63:15-21.
43. Ferguson AD, McKeever BM, Xu S, et al. Crystal structure of inhibitor-bound human 5-lipoxygenase-activating protein. *Science* 2007;317:510-2.
44. Griffiths RJ, Pettipher ER, Koch K, et al. Leukotriene B<sub>4</sub> plays a critical role in the progression of collagen-induced arthritis. *Proc Natl Acad Sci U S A* 1995;92:517-21.
45. Israel E, Rubin P, Kemp J, et al. The effect of inhibition of 5-lipoxygenase by zileuton in mild-to-moderate asthma. *Ann Intern Med* 1993;119:1059-66.
46. Knorr B, Matz J, Bernstein JA, et al. Montelukast for chronic asthma in 6- to 14-year-old children: a randomized, double-blind trial. *JAMA* 1998;279:1181-6.
47. Suissa S, Dennis R, Ernst P, Sheehy O, Wood-Dauphinee S. Effectiveness of the leukotriene receptor antagonist zafirlukast for mild-to-moderate asthma: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1997;126:177-83.
48. Ducharme FM, Hicks GC. Anti-leukotriene agents compared to inhaled corticosteroids in the management of recurrent and/or chronic asthma. *Cochrane Database Syst Rev* 2000;3:CD002314.
49. Peters SP, Anthonisen N, Castro M, et al. Randomized comparison of strategies for reducing treatment in mild persistent asthma. *N Engl J Med* 2007;356:2027-39. [Erratum, *N Engl J Med* 2007;357:728.]
50. Laviolette M, Malmstrom K, Lu S, et al. Montelukast added to inhaled beclomethasone in treatment of asthma. *Am J Respir Crit Care Med* 1999;160:1862-8.
51. Ducharme FM, Lasserson TJ, Cates CJ. Long-acting beta2-agonists versus anti-leukotrienes as add-on therapy to inhaled corticosteroids for chronic asthma. *Cochrane Database Syst Rev* 2006;4:CD003137.
52. Ilowite J, Webb R, Friedman B, et al. Addition of montelukast or salmeterol to fluticasone for protection against asthma attacks: a randomized, double-blind, multicenter study. *Ann Allergy Asthma Immunol* 2004;92:641-8.
53. Bjermer L, Bisgaard H, Bousquet J, et al. Montelukast and fluticasone compared with salmeterol and fluticasone in protecting against asthma exacerbation in adults: one year, double blind, randomised, comparative trial. *BMJ* 2003;327:891.
54. 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;32:1020-8.
55. Peters-Golden M, Henderson WR Jr. The role of leukotrienes in allergic rhinitis. *Ann Allergy Asthma Immunol* 2005;94:609-18.
56. Nayak A, Langdon RB. Montelukast in the treatment of allergic rhinitis: an evidence-based review. *Drugs* 2007;67:887-901.
57. Price DB, Swern A, Tozzi CA, Philip G, Polos P. Effect of montelukast on lung function in asthma patients with allergic rhinitis: analysis from the COMPACT trial. *Allergy* 2006;61:737-42. [Erratum, *Allergy* 2006;61:1153.]
58. Virchow JC, Bachert C. Efficacy and safety of montelukast in adults with asthma and allergic rhinitis. *Respir Med* 2006;100:1952-9.
59. Stempel DA, Stanford RH, Carranza Rosenzweig JR, McLaughlin TP. The use of rhinitis medications in children receiving initial controller therapy for asthma. *Curr Med Res Opin* 2006;22:2279-85.
60. Coreno A, Skowronski M, Kotaru C, McFadden ER Jr. Comparative effects of long-acting beta2-agonists, leukotriene receptor antagonists, and a 5-lipoxygenase inhibitor on exercise-induced asthma. *J Allergy Clin Immunol* 2000;106:500-6.
61. Edelman JM, Turpin JA, Bronsky EA, et al. Oral montelukast compared with inhaled salmeterol to prevent exercise-induced bronchoconstriction: a randomized, double-blind trial. *Ann Intern Med* 2000;132:97-104.
62. Dahlén B, Nizankowska E, Szczeklik A, et al. Benefits from adding the 5-lipoxygenase inhibitor zileuton to conventional therapy in aspirin-intolerant asthmatics. *Am J Respir Crit Care Med* 1998;157:1187-94.
63. Dahlén SE, Malmström 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.
64. Volkman JA, Pontikes PJ. Leukotriene modifiers to prevent aspirin-provoked respiratory reactions in asthmatics. *Ann Pharmacother* 2002;36:1457-61.
65. Bisgaard H. A randomized trial of montelukast in respiratory syncytial virus postbronchiolitis. *Am J Respir Crit Care Med* 2003;167:379-83.
66. Harmanci K, Bakirtas A, Turktas I, Degim T. Oral montelukast treatment of preschool-aged children with acute asthma. *Ann Allergy Asthma Immunol* 2006;96:731-5.
67. Camargo CA Jr, Smithline HA, Malice MP, Green SA, Reiss TF. A randomized controlled trial of intravenous montelukast in acute asthma. *Am J Respir Crit Care Med* 2003;167:528-33.
68. Bergeron C, Boulet LP. Structural

- changes in airway diseases: characteristics, mechanisms, consequences, and pharmacologic modulation. *Chest* 2006;129:1068-87.
69. Covar RA, Spahn JD, Murphy JR, Szefler SJ. Progression of asthma measured by lung function in the Childhood Asthma Management Program. *Am J Respir Crit Care Med* 2004;170:234-41.
  70. Henderson WR Jr, Chiang GK, Tien YT, Chi EY. Reversal of allergen-induced airway remodeling by CysLT1 receptor blockade. *Am J Respir Crit Care Med* 2006;173:718-28.
  71. Kelly MM, Chakir J, Vethanayagam D, et al. Montelukast treatment attenuates the increase in myofibroblasts following low-dose allergen challenge. *Chest* 2006;130:741-53.
  72. Malmstrom K, Rodriguez-Gomez G, Guerra J, et al. Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma. *Ann Intern Med* 1999;130:487-95.
  73. Israel E, Chervinsky PS, Friedman B, et al. Effects of montelukast and beclomethasone on airway function and asthma control. *J Allergy Clin Immunol* 2002;110:847-54.
  74. Wenzel SE, Trudeau JB, Kaminsky DA, Cohn J, Martin RJ, Westcott JY. Effect of 5-lipoxygenase inhibition on bronchoconstriction and airway inflammation in nocturnal asthma. *Am J Respir Crit Care Med* 1995;152:897-905.
  75. Hallstrand TS, Moody MW, Aitken ML, Henderson WR Jr. Airway immunopathology of asthma with exercise-induced bronchoconstriction. *J Allergy Clin Immunol* 2005;116:586-93.
  76. Frölich JC, Fauler J, Tsikas D. Assessment of cysteinyl leukotriene synthesis in man. *J Lipid Mediat Cell Signal* 1994;9:75-8.
  77. Cai C, Yang J, Hu S, Zhou M, Guo W. Relationship between urinary cysteinyl leukotriene E<sub>4</sub> levels and clinical response to antileukotriene treatment in patients with asthma. *Lung* 2007;185:105-12.
  78. Tanaka H, Saito T, Kurokawa K, et al. Leukotriene (LT)-receptor antagonist is more effective in asthmatic patients with a low baseline ratio of urinary LTE<sub>4</sub> to 2,3-dinor-6-keto-prostaglandin (PG)F<sub>2</sub>α. *Allergy* 1999;54:489-94.
  79. Terashima T, Amakawa K, Matsumaru A, Yamaguchi K. Correlation between cysteinyl leukotriene release from leukocytes and clinical response to a leukotriene inhibitor. *Chest* 2002;122:1566-70.
  80. Drazen JM, Yandava CN, Dubé L, et al. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. *Nat Genet* 1999;22:168-70.
  81. Sampson AP, Siddiqui S, Buchanan D, et al. Variant LTC<sub>4</sub> synthase allele modifies cysteinyl leukotriene synthesis in eosinophils and predicts clinical response to zafirlukast. *Thorax* 2000;55:Suppl 2: S28-S31.
  82. Asano K, Shiomi T, Hasegawa N, et al. Leukotriene C<sub>4</sub> synthase gene A(-444)C polymorphism and clinical response to a Cys-LT<sub>1</sub> antagonist, pranlukast, in Japanese patients with moderate asthma. *Pharmacogenetics* 2002;12:565-70.
  83. Currie GP, Lima JJ, Sylvester JE, Lee DK, Cockburn WJ, Lipworth BJ. Leukotriene C<sub>4</sub> synthase polymorphisms and responsiveness to leukotriene antagonists in asthma. *Br J Clin Pharmacol* 2003;56:422-6.
  84. Klotzman M, York TP, Pillai SG, et al. Pharmacogenetics of the 5-lipoxygenase biosynthetic pathway and variable clinical response to montelukast. *Pharmacogenet Genomics* 2007;17:189-96.
  85. Barnes N, Thomas M, Price D, Tate H. The national montelukast survey. *J Allergy Clin Immunol* 2005;115:47-54.
  86. Szefler SJ, Phillips BR, Martinez FD, et al. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. *J Allergy Clin Immunol* 2005;115:233-42.
  87. Peters-Golden M, Swern A, Bird SS, Hustad CM, Grant E, Edelman JM. Influence of body mass index on the response to asthma controller agents. *Eur Respir J* 2006;27:495-503.
  88. Lazarus SC, Chinchilli VM, Rollings NJ, et al. Smoking affects response to inhaled corticosteroids or leukotriene receptor antagonists in asthma. *Am J Respir Crit Care Med* 2007;175:783-90.
  89. Reiss TF, Chervinsky P, Dockhorn RJ, Shingo S, Seidenberg B, Edwards TB. Montelukast, a once-daily leukotriene receptor antagonist, in the treatment of chronic asthma: a multicenter, randomized, double-blind trial. *Arch Intern Med* 1998;158:1213-20.
  90. Sandrini A, Ferreira IM, Gutierrez C, Jardim JR, Zamel N, Chapman KR. Effect of montelukast on exhaled nitric oxide and nonvolatile markers of inflammation in mild asthma. *Chest* 2003;124:1334-40.
  91. Dempsey OJ, Kennedy G, Lipworth BJ. Comparative efficacy and anti-inflammatory profile of once-daily therapy with leukotriene antagonist or low-dose inhaled corticosteroid in patients with mild persistent asthma. *J Allergy Clin Immunol* 2002;109:68-74.
  92. Berger W, De Chandt MT, Cairns CB. Zileuton: clinical implications of 5-lipoxygenase inhibition in severe airway disease. *Int J Clin Pract* 2007;61:663-76.
  93. Spanbroek R, Grabner R, Lotzer K, et al. Expanding expression of the 5-lipoxygenase pathway within the arterial wall during human atherogenesis. *Proc Natl Acad Sci U S A* 2003;100:1238-43.
  94. Qiu H, Gabrielsen A, Agardh HE, et al. Expression of 5-lipoxygenase and leukotriene A<sub>4</sub> hydrolase in human atherosclerotic lesions correlates with symptoms of plaque instability. *Proc Natl Acad Sci U S A* 2006;103:8161-6.
  95. Aiello RJ, Bourassa PA, Lindsey S, Weng W, Freeman A, Showell HJ. Leukotriene B<sub>4</sub> receptor antagonism reduces monocytic foam cells in mice. *Arterioscler Thromb Vasc Biol* 2002;22:443-9.
  96. Bäck M, Bu DX, Brännström R, Sheikine Y, Yan ZQ, Hansson GK. Leukotriene B<sub>4</sub> signaling through NF-κB-dependent BLT1 receptors on vascular smooth muscle cells in atherosclerosis and intimal hyperplasia. *Proc Natl Acad Sci U S A* 2005;102:17501-6.
  97. Zhao L, Moos MP, Gräbner R, et al. The 5-lipoxygenase pathway promotes pathogenesis of hyperlipidemia-dependent aortic aneurysm. *Nat Med* 2004;10:966-73.
  98. Uzonyi B, Lötzer K, Jahn S, et al. Cysteinyl leukotriene 2 receptor and protease-activated receptor 1 activate strongly correlated early genes in human endothelial cells. *Proc Natl Acad Sci U S A* 2006;103:6326-31.
  99. Kajimoto K, Shioji K, Ishida C, et al. Validation of the association between the gene encoding 5-lipoxygenase-activating protein and myocardial infarction in a Japanese population. *Circ J* 2005;69:1029-34.
  100. Hakonarson H, Thorvaldsson S, Helgadottir A, et al. Effects of a 5-lipoxygenase-activating protein inhibitor on biomarkers associated with risk of myocardial infarction: a randomized trial. *JAMA* 2005;293:2245-56.
  101. Meschia JF, Brott TG, Brown RD Jr, et al. Phosphodiesterase 4D and 5-lipoxygenase activating protein in ischemic stroke. *Ann Neurol* 2005;58:351-61.
  102. Zee RY, Cheng S, Hegener HH, Erlich HA, Ridker PM. Genetic variants of arachidonate 5-lipoxygenase-activating protein, and risk of incident myocardial infarction and ischemic stroke: a nested case-control approach. *Stroke* 2006;37:2007-11.
  103. Kaushal R, Pal P, Alwell K, et al. Association of ALOX5AP with ischemic stroke: a population-based case-control study. *Hum Genet* 2007;121:601-7.
  104. Iovannisci DM, Lammer EJ, Steiner L, et al. Association between a leukotriene C<sub>4</sub> synthase gene promoter polymorphism and coronary artery calcium in young women: the Muscatine Study. *Arterioscler Thromb Vasc Biol* 2007;27:394-9.
  105. Dwyer JH, Allayee H, Dwyer KM, et al. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. *N Engl J Med* 2004;350:29-37.
  106. González P, Reguero JR, Lozano I, Moris C, Coto E. A functional Sp1/Egr1-tandem repeat polymorphism in the 5-lipoxygenase gene is not associated with myocardial infarction. *Int J Immunogenet* 2007;34:127-30.
  107. Mezhybovska M, Wikström K, Ohl JF, Sjölander A. The inflammatory mediator leukotriene D<sub>4</sub> induces beta-catenin signaling and its association with antiapoptotic Bcl-2 in intestinal epithelial cells. *J Biol Chem* 2006;281:6776-84.

108. Nielsen CK, Campbell JI, Ohd JF, et al. A novel localization of the G-protein-coupled CysLT<sub>1</sub> receptor in the nucleus of colorectal adenocarcinoma cells. *Cancer Res* 2005;65:732-42.
109. Ihara A, Wada K, Yoneda M, Fujisawa N, Takahashi H, Nakajima A. Blockade of leukotriene B<sub>4</sub> signaling pathway induces apoptosis and suppresses cell proliferation in colon cancer. *J Pharmacol Sci* 2007;103:24-32.
110. Avis IM, Jett M, Boyle T, et al. Growth control of lung cancer by interruption of 5-lipoxygenase-mediated growth factor signaling. *J Clin Invest* 1996;97:806-13.
111. Romano M, Catalano A, Nutini M, et al. 5-Lipoxygenase regulates malignant mesothelial cell survival: involvement of vascular endothelial growth factor. *FASEB J* 2001;15:2326-36.
112. Hoque A, Lippman SM, Wu TT, et al. Increased 5-lipoxygenase expression and induction of apoptosis by its inhibitors in esophageal cancer: a potential target for prevention. *Carcinogenesis* 2005;26:785-91.
113. Runarsson G, Liu A, Mahshid Y, et al. Leukotriene B<sub>4</sub> plays a pivotal role in CD40-dependent activation of chronic B lymphocytic leukemia cells. *Blood* 2005;105:1274-9.
114. Rioux N, Castonguay A. Inhibitors of lipoxygenase: a new class of cancer chemopreventive agents. *Carcinogenesis* 1998;19:1393-400.
115. Chen X, Li N, Wang S, et al. Leukotriene A<sub>4</sub> hydrolase in rat and human esophageal adenocarcinomas and inhibitory effects of bestatin. *J Natl Cancer Inst* 2003;95:1053-61.
116. Sun Z, Sood S, Li N, et al. Involvement of the 5-lipoxygenase/leukotriene A<sub>4</sub> hydrolase pathway in 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced oral carcinogenesis in hamster cheek pouch, and inhibition of carcinogenesis by its inhibitors. *Carcinogenesis* 2006;27:1902-8.
117. Peters-Golden M, Canetti C, Mancuso P, Coffey MJ. Leukotrienes: underappreciated mediators of innate immune responses. *J Immunol* 2005;174:589-94.
118. Coffey MJ, Phare MS, Kazanjian PH, Peters-Golden M. 5-Lipoxygenase metabolism in alveolar macrophages from subjects infected with the human immunodeficiency virus. *J Immunol* 1996;157:393-9.
119. Cederholm T, Lindgren JA, Palmblad J. Impaired leukotriene C<sub>4</sub> generation in granulocytes from protein-energy malnourished chronically ill elderly. *J Intern Med* 2000;247:715-22.
120. Konstan MW, Doring G, Lands LC. Results of a phase II clinical trial of BIII 284 BS (an LTB<sub>4</sub> receptor antagonist) for the treatment of CF lung disease. *Pediatr Pulmonol* 2005;40:125-6. abstract.

Copyright © 2007 Massachusetts Medical Society.

RECEIVE IMMEDIATE NOTIFICATION WHEN  
A JOURNAL ARTICLE IS RELEASED EARLY

To be notified when an article is released early  
on the Web and to receive the table of contents  
of the *Journal* by e-mail every Wednesday evening,  
sign up through our Web site at  
[www.nejm.org](http://www.nejm.org)