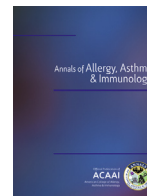


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Basic science for the clinician: lipid mediators

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ABSTRACT

Objective: To review the basic science and translational relevance of lipid mediators in the pathobiology of allergic diseases.

Data Sources: PubMed was searched for articles using the key terms *lipid mediator*, *prostaglandin*, *prostanoid*, *leukotriene*, *thromboxane*, *asthma*, and *allergic inflammation*.

Study Selections: Articles were selected based on their relevance to the goals of this review. Articles with a particular focus on clinical and translational aspects of basic science discoveries were emphasized.

Results: Lipid mediators are bioactive molecules generated from cell membrane phospholipids. They play important roles in many disease states, particularly in inflammatory and immune responses. Lipid mediators and their receptors are potentially useful as diagnostic markers of disease and therapeutic targets.

Conclusions: Several useful therapeutic agents have been developed based on a growing understanding of the lipid mediator pathways in allergic disease, notably the cysteinyl leukotriene receptor type 1 antagonists and the 5-lipoxygenase inhibitor, zileuton. Additional receptor agonists and antagonists relevant to these pathways are in development, and it is likely that future pharmacologic treatments for allergic disease will become available as our understanding of these molecules continues to evolve.

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Introduction

Lipids are a primary nutrient energy source and the major components of the cell membranes of all organisms. Lipid molecules also can function in intercellular signaling and have important roles in inflammatory and immune responses and in the regulation of many normal cellular functions and homeostasis. Prostaglandins (PGs), leukotrienes (LTs), and a variety of other lipid molecules with such signaling roles are known as lipid mediators or bioactive lipids. These molecules are synthesized on demand from precursor membrane lipids and act primarily by binding to G protein-coupled receptors (GPCRs).¹

The importance of lipid mediators as diagnostic markers and therapeutic targets in allergic diseases has been long recognized and continues to evolve. This is particularly true of asthma, for which there is the largest and broadest body of published literature among the allergic diseases. This review provides an overview of the pathways of lipid mediator generation and examines the relevance of specific lipid mediators to the pathobiology of allergic inflammation and implications for current and future clinical management of allergic diseases. Although lysophospholipids,

lipoxins, and other lipids are relevant to the mechanisms of allergic inflammation, this review focuses on PGs and LTs because of the abundance of existing literature, including clinical data, regarding these mediators. A summary of the mediators discussed in this review is presented in [Table 1](#).

Generation of Lipid Mediator Precursors

Enzymes known as phospholipases A₂ release arachidonic acid from the sn-2 position (ie, the second carbon of the glycerol molecule) of membrane phospholipids, also resulting in the formation of lysoglycerophospholipids.² Arachidonic acid is oxidatively metabolized by the cyclooxygenase and lipoxygenase pathways and is thereby the precursor for all PGs and LTs.³ These mediators are known as eicosanoids and are named for *eikosi*, the Greek word for 20, because arachidonic acid has 20 carbon atoms.⁴ Lysoglycerophospholipids are the precursors for lysophosphatidic acid and sphingosine-1-phosphate.²

Overview of the Cyclooxygenase Pathway

There are 2 cyclooxygenase (COX) enzyme isoforms: COX-1 and COX-2. These enzymes originate from different genes and have distinct tissue-specific expressions and biologic functions. Expression of COX-1 is constitutive in most tissues and believed to have a role in homeostasis. COX-2 expression is inducibly expressed by hematopoietic and nonhematopoietic cells in response to a variety of stimuli and is associated with inflammation and disease states.^{3,5,6}

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Table 1
Summary of selected lipid mediators

Mediator	Major Cellular Sources	Receptors	Receptor Locations	Pharmacologic Action (Human)	Agonists	Antagonists	Clinical Applications
PGD ₂	Mast cells, T _H 2 cells, DCs	DP ₁ , DP ₂	DP ₁ : goblet cells, vascular endothelium, immune cells; DP ₂ : immune cells	Bronchoconstriction, vasodilation	N/A	OC000459 and other DP ₂ antagonists in clinical trials	Under study for various allergic diseases
PGE ₂	Various cells, including airway epithelium and smooth muscle	EP ₁ , EP ₂ , EP ₃ , EP ₄	Ubiquitous	Bronchoprotection/airway relaxant	Various EP ₂ and EP ₄ agonists	N/A	Potential utility in asthma
PGI ₂	Many tissues, including heart, lung, smooth muscle	IP	Immune cells	Inhibition of platelet activation, vasodilation	Iloprost, cicaprost, treprostinil	N/A	Used in treatment of pulmonary hypertension, under study for asthma
TXA ₂	Activated platelets, granulocytes, mast cells	TP	Lung, heart, kidney, platelets	Bronchoconstriction, platelet activation	N/A	TP receptor antagonists and TXA synthesis inhibitors	Under study for asthma, with mixed results
Cysteinyl leukotrienes (LTC ₄ , LTD ₄ , LTE ₄)	Eosinophils, basophils, mast cells, macrophages, myeloid DCs	CysLT ₁ R: (LTD ₄ > LTC ₄ = LTE ₄); CysLT ₂ R: (LTC ₄ = LTD ₄ > LTE ₄); CysLT ₃ R: (LTE ₄ > LTD ₄ = LTC ₄)	CysLT ₁ R and CysLT ₂ R broadly expressed on structural and hematopoietic cells; CysLT ₃ R expression not yet known	Bronchoconstriction	N/A	CysLT ₁ R antagonists (montelukast, zafirlukast) and 5-LO inhibitors (zileuton)	Treatment of asthma and/or allergic rhinitis
LTB ₄	Mast cells, macrophages, neutrophils	BLT ₁ , BLT ₂	BLT ₁ : activated leukocytes; BLT ₂ : most tissues	Leukocyte chemotaxis	N/A	N/A	No published human studies

Abbreviations: 5-LO, 5-lipoxygenase; DC, dendritic cell; IP, prostaglandin I₂ inhibitor; LTB₄, leukotriene B₄; LTC₄, leukotriene C₄; LTD₄, leukotriene D₄; LTE₄, leukotriene E₄; N/A, not available; PGD₂, prostaglandin D₂; PGE₂, prostaglandin E₂; PGI₂, prostaglandin I₂; T_H2, T-helper cell type 2; TP, thromboxane A₂ receptor; TXA₂, thromboxane A₂; CysLT₁₋₃R, cysteinyl leukotriene receptors 1 to 3.

COX-1 and COX-2 catalyze 2 sequential reactions to initiate the COX pathway. First, 2 molecules of oxygen are inserted into arachidonic acid, cyclizing it to produce PGG₂. Next, an endoperoxidase reaction reduces PGG₂ to PGH₂.⁷ Terminal PG synthases then convert PGH₂ into 1 of 5 bioactive prostanoids: PGD₂ by hematopoietic PGD synthase or lipocalin-type PGDS; PGE₂ by microsomal PGE synthase (mPGES)-1, mPGES-2, or cytosolic PGES; PGF₂α by PGF synthase; PGI₂ by PGI synthase; and thromboxane A₂ (TXA₂) by thromboxane synthase⁷ (Fig 1). Selected individual PGs are discussed in detail below.

Studies in mice and humans have highlighted the role of the COX pathway in allergic inflammation. Mice deficient in COX-1 or COX-2 had increased airways hyperresponsiveness (AHR) compared with wild-type (WT) mice after lipopolysaccharide exposure, with similar levels of airway inflammation.⁸ In a model of sensitization and challenge with ovalbumin (OVA), mice lacking COX-1 or COX-2 had increased bronchoalveolar lavage (BAL) eosinophilia. COX-1-deficient mice had increased airway responsiveness, more CD4+ and CD8+ lymphocytes, and increased levels of T-helper cell type 2 (T_H2) cytokines, eotaxin, and thymus- and activation-regulated chemokine in the BAL fluid compared with mice deficient in COX-2 and WT mice.⁹ Another study described increased OVA-induced BAL eosinophilia in mice lacking COX-2 compared with WT mice.¹⁰ In contrast, overexpression of COX-1 in mouse airway epithelium decreased airway responsiveness but did not decrease OVA-induced airway inflammation.¹¹

Pharmacologic inhibition of COX also affects allergic inflammation in mice. OVA-sensitized mice treated with indomethacin (a nonselective COX-1/COX-2 inhibitor) had increased pulmonary eosinophilia, T_H2 cytokines, and AHR.¹² In another study, selective COX-1 and COX-2 inhibitors each increased airway responsiveness and lung interleukin (IL)-13 levels when given with OVA sensitization.¹³ Nonselective COX inhibition with diclofenac during

OVA challenge and before methacholine challenge resulted in increased AHR and decreased BAL inflammation.¹⁴ Collectively, these studies suggest the potential for COX inhibition to influence the magnitude of lung inflammatory responses and their physiologic consequences.

COX pathway products also are important in allergic inflammation in humans. In the BAL fluid of allergic asthmatics, levels of PGD₂ and PGF₂α were 12- to 22-fold higher than in nonallergic individuals and 10-fold higher than in nonasthmatic individuals with allergic rhinitis.¹⁵ Segmental allergen challenge resulted in 5- to 14-fold increases in PGD₂, 9α, 11β-PGF₂ (the stereoisomer of PGF₂α), and thromboxane B₂ in the BAL fluid, and although pretreatment with prednisone inhibited the challenge-induced decrease in forced expiratory volume in 1 second (FEV₁), it did not affect the levels of these mediators.¹⁶

Nonsteroidal anti-inflammatory drugs inhibit COX-1 and COX-2, whereas drugs such as celecoxib are specific COX-2 inhibitors. COX inhibition with the nonsteroidal anti-inflammatory drug, indomethacin, did not affect lung function before the challenge in allergic asthmatics or nonasthmatic subjects with allergic rhinitis. However, indomethacin decreased FEV₁ in nonasthmatic subjects, but not in asthmatic subjects, after allergen challenge.¹⁷ In another study, indomethacin did not alter airway histamine responsiveness or the immediate or late phase pulmonary responses to allergen challenge in allergic asthmatics.¹⁸ Although the effects of COX inhibition on lung function in humans are complex, the combined human and mouse data indicate that COX inhibition generally results in increased allergic inflammation, suggesting that at least 1 COX product (likely PGE₂ and/or PGI₂) may conversely decrease allergic inflammation and thus might be a useful therapeutic target in allergic disease. Specific COX pathway products that have pro- or anti-inflammatory roles are discussed in more depth in the following sections.

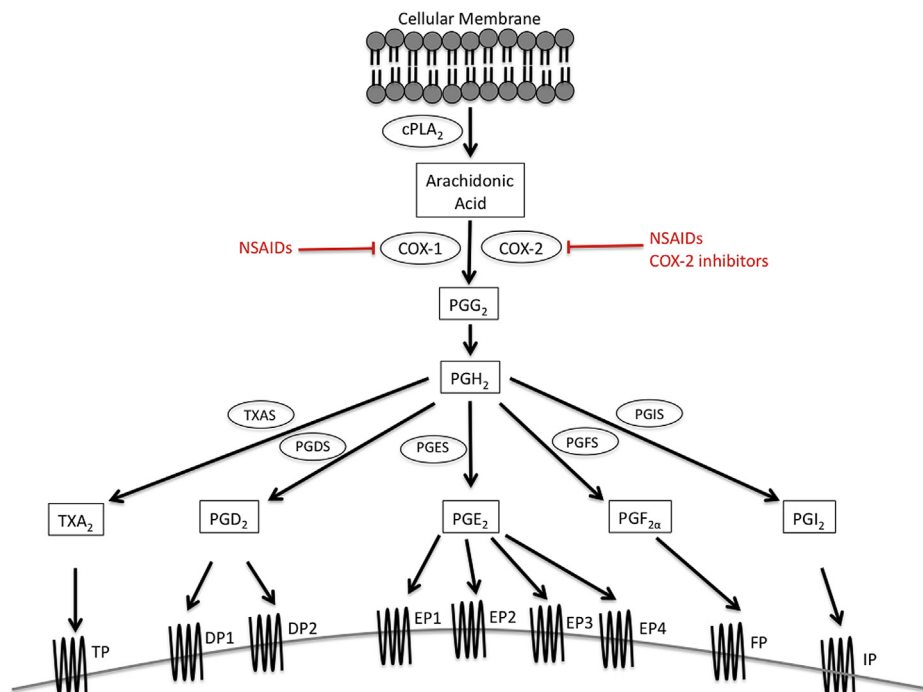


Figure 1. Prostaglandin biosynthesis. Cyclooxygenase (COX)-1 or COX-2 converts arachidonic acid to prostaglandin (PG) H₂. Thromboxane (TX) A₂, PGD₂, PGE₂, PGF_{2α}, and PGI₂ are then generated by specific PG synthase enzymes and signal by the indicated G protein-coupled receptors. cPLA₂, cytosolic phospholipase A₂; NSAIDs, nonsteroidal anti-inflammatory drugs.

Selected Prostanoids

PGD₂ and Its Receptors, DP₁ and DP₂/Chemoattractant Homologous Molecule Expressed on T_H2 Cells

Prostaglandin D₂ is released in large quantities by mast cells in response to IgE-mediated activation.¹⁹ Other leukocytes, including T_H2 cells²⁰ and dendritic cells (DCs),²¹ produce PGD₂ at lower levels. PGD₂ signals through receptors DP₁ and DP₂ (also known as chemoattractant homologous molecule expressed on T_H2 cells [CRT_H2]).²² Aerosolized PGD₂ administered to sensitized mice before antigen challenge increased the numbers of eosinophils and the levels of T_H2 cytokines found in BAL fluid.²³ Mice with lung-targeted overexpression of lipocalin-type PGDS had more eosinophils, lymphocytes, T_H2 cytokines, and eotaxin in the BAL fluid after allergen sensitization and challenge compared with WT mice.²⁴ However, treatment of mice with the DP₁ agonist BW245C suppressed the ability of DCs to migrate to the lung and to stimulate T-cell proliferation.²⁵ In another study, mice treated with BW245C or with adoptively transferred BW245C-treated DCs had more Foxp3+ CD4+ regulatory T cells that suppressed inflammation through an IL-10–dependent mechanism.²⁶ PGD₂ signaling through DP₂ is chemotactic for mouse eosinophils. A specific DP₂ agonist also increased eosinophil recruitment at sites of inflammation in mouse models of atopic dermatitis and asthma.²⁷ Conversely, the DP₂ antagonists TM30089 and ramatroban decreased pulmonary inflammation in a mouse model of allergic airway disease by decreasing peribronchial eosinophilia and mucus cell hyperplasia.²⁸ Thus, mouse studies suggest that PGD₂ can facilitate tolerance or promote eosinophilia in the lung depending on the receptor stimulated.

Allergen challenge of atopic asthmatic humans increased the levels of PGD₂ in the BAL fluid.²⁹ Similar observations have been reported in response to provocative nasal³⁰ and ocular³¹ challenges. These findings are thought to reflect the generation of PGD₂ by mast cells activated in response to IgE receptor crosslinking. Of note, PGD₂ is a potent bronchoconstrictor in humans,³² but not in

mice.³³ PGD₂ likely mediates bronchoconstriction through the actions of a major metabolite at thromboxane receptors (TP), rather than DP₁ or DP₂.³² Several specific inhibitors of DP₂ are being studied currently in human clinical trials. The DP₂ antagonist with the largest body of published human data is OC000459. A randomized, double-blinded, placebo-controlled trial of OC000459 in 132 patients with moderate persistent asthma showed a significant improvement in the primary end point of FEV₁ in patients who received OC000459 compared with placebo, but only when noncompliant patients were excluded from the analysis.³⁴ In a randomized, double-blinded, placebo-controlled, 2-way crossover study, 35 male patients with grass pollen allergy had a significant decrease in nasal and ocular symptoms after allergen challenge after OC000459 treatment compared with placebo.³⁵ A randomized, double-blinded, placebo-controlled, 2-way crossover study of 16 patients with allergic asthma showed a 25.4% decrease in the late asthmatic response to allergen challenge with OC000459 compared with placebo, but no change in the early response. Sputum eosinophil counts 1 day after allergen challenge also were significantly lower after OC000459 treatment.³⁶ Most recently, a randomized, double-blinded, placebo-controlled trial of OC000459 in 38 adults with eosinophilic esophagitis showed a significant decrease in esophageal eosinophil load after 8 weeks of treatment, with no change in the placebo group. Disease activity and extracellular deposits of eosinophil peroxidase and tenascin C also were decreased.³⁷ Another DP₂ antagonist that also blocks DP₁, AMG 853, did not alleviate asthma symptoms or improve lung function compared with placebo in patients with moderate to severe asthma.³⁸ Thus, although PGD₂ is clearly associated with allergic inflammation, its potential as a therapeutic target remains unclear.

PGE₂ and Its Receptors

Prostaglandin E₂ signals through 4 distinct GPCRs (EP receptors 1 through 4), resulting in complex downstream effects. These 4

receptors are expressed in various combinations by essentially all cell types and tissues.³⁹ Naive mice that had elevated levels of PGE₂ owing to a lack of 15-hydroxyprostaglandin dehydrogenase, a major catabolic enzyme of PGE₂, or to overexpression of mPGES-1 showed decreased methacholine responsiveness.⁴⁰ In addition, pretreatment with PGE₂ before methacholine challenge decreased bronchoconstriction in WT mice, but not in mice lacking the EP₂ receptor. PGE₂ given after methacholine also reversed bronchoconstriction in WT mice but not in EP₂-deficient mice, suggesting that these effects of PGE₂ are mediated through EP₂.⁴¹ In a model of OVA sensitization and challenge, mice lacking EP₃ had increased BAL cellular inflammation and T_H2 cytokines compared with WT mice, with no differences between WT mice and mice lacking EP₁, EP₂, or EP₄. In addition, treatment of WT mice with the EP₃ agonist decreased allergic airway inflammation and methacholine responsiveness.⁴² The effect of the EP₃ agonist in this study was attributed to the suppression of chemokine generation by epithelial cells. Mice lacking mPGES-1 (which accounts for ~80% of PGE₂ generated in the lung) developed profoundly increased bronchovascular inflammation and AHR compared with WT controls when treated with a house dust mite extract.⁴³ Thus, exogenous and endogenous PGE₂ in mice have effects on smooth muscle and the inflammatory response.

Prostaglandin E₂ is also bronchoprotective in humans. Inhalation of PGE₂ by atopic individuals inhibited the early and late phase responses to inhaled allergen challenge and decreased methacholine reactivity and sputum eosinophilia.^{44,45} Randomized controlled trials also have shown that inhaled PGE₂ decreases exercise-induced⁴⁶ and aspirin-induced⁴⁷ bronchoconstriction in patients with these disease triggers. In patients with aspirin-exacerbated respiratory disease (AERD), inhaled PGE₂ also blocked the aspirin-induced increase in urinary leukotriene E₄ (LTE₄),⁴⁷ likely because of the capacity of PGE₂ to suppress 5-lipoxygenase (5-LO) activation through protein kinase A.⁴⁸ The airway tissue relaxant properties of several EP receptor agonists were tested in a variety of mammalian tissues, in a recent study seeking new targets for bronchodilator therapy. These *in vitro* studies found, through the use of the EP₄ selective agonist ONO-AE1-329, that the EP₄ receptor plays a role in human airway relaxant responses. In addition, the EP₂ agonist AH13205 was found to have relaxant activity in the guinea pig but not in the human airway, which the investigators suggested may explain the reported failure of this agonist in clinical studies.⁴⁹ Another *in vitro* study found that selective EP₄ agonists (ONO-AE1-329 or L-902688), but not a selective EP₂ agonist (ONO-AE1-259), induced relaxation of bronchial tissues precontracted with histamine or anti-IgE.⁵⁰ EP₂ receptor agonists suppressed the release of inflammatory mediators by activated mast cells⁵¹ and inhibited T-cell proliferation.⁵² Thus, the bronchoprotective effects of PGE₂ likely reflect stimulation of different EP receptors on smooth muscle and hematopoietic effector cells.

PGI₂ and Its Receptor, IP

Prostaglandin I₂, also known as prostacyclin, inhibits platelet activation and is a potent vasodilator that plays an important role in vascular homeostasis.³⁹ PGI₂ signals through its receptor, IP, which is expressed on immune cells including mouse bone marrow-derived DCs (BMDCs)⁵³ and on CD4+ T_H1 and T_H2 cells.⁵⁴ The PGI₂ analogs iloprost, cicaprost, and treprostinil decreased the production of proinflammatory cytokines (IL-12, tumor necrosis factor- α , IL-1 α , and IL-6) and chemokines (macrophage inflammatory protein-1 α and monocyte chemotactic protein-1) and increased the production of an anti-inflammatory cytokine (IL-10) by BMDCs.⁵³ Iloprost and cicaprost also decreased the lipopolysaccharide-induced expression of CD86, CD40, and major

histocompatibility complex class II molecules on BMDCs and decreased their ability to stimulate antigen-specific CD4+ T-cell proliferation and production of IL-5 and IL-13.⁵³ Subsequently, cicaprost was shown to decrease antigen uptake by immature BMDCs and increase their expression of CCR7 and chemotactic migration toward CCL19 and CCL21.⁵⁵ Iloprost and cicaprost also inhibited the production of T_H1 and T_H2 cytokines by CD4+ T cells *in vitro*.⁵⁴

Like PGE₂, mouse studies indicate that PGI₂ may be anti-inflammatory in the lung *in vivo*. Mice lacking IP had increased lung production of IL-4 and IL-5, higher serum total and antigen-specific IgE levels, and greater airway leukocyte accumulation compared with WT mice in a short-term OVA model.⁵⁶ In a model of long-term allergen exposure, IP-deficient mice also had more airway eosinophils and lymphocytes and higher T_H2 lung cytokine levels compared with WT mice.⁵⁷ Mice treated with ONO-1301, a PGI₂ analog that also has thromboxane synthase inhibitory activity, had decreased AHR and decreased findings of airway remodeling compared with vehicle-treated mice in a long-term OVA model.⁵⁸ Together, these studies suggest that PGI₂ signaling through IP down-regulates allergic inflammation and may decrease lung fibrosis and remodeling.

Although PGI₂ analogs are used in humans for the treatment of pulmonary hypertension, there are not many interventional studies of these agents in human asthma. A recent study of mild asthmatics demonstrated the feasibility of administering inhaled iloprost for further study. No changes in lung function or methacholine responsiveness were found in this nonrandomized, noncontrolled observational study.⁵⁹ An oral PGI₂ analog (OP-41483) similarly had no effect on FEV₁ or bronchial responsiveness in stable asthmatics.⁶⁰ Several older studies showed variable effects of inhaled or intravenous PGI₂ but were limited by the short half-life of PGI₂ compared with the stable analogs currently available.

TXA₂ and Its Receptor, TP

Thromboxane A₂ is produced by activated platelets, granulocytes, and mast cells. It is a potent inducer of platelet aggregation.⁶¹ It is also a powerful bronchoconstrictor. TP (the TXA₂ receptor) is found in highly vascular tissues such as the lung, heart, and kidney and has 2 isoforms, TP α and TP β , which are formed by alternative splicing.⁶² TP receptor signaling on lung endothelial cells potentially up-regulates intercellular adhesion molecule-1, thus facilitating the recruitment of granulocytes to the lung.⁶³

In mice sensitized and challenged with OVA, treatment with a TXA₂ synthase inhibitor (OKY-046) or a TP receptor antagonist (S-1452) decreased BAL eosinophilic inflammation and decreased antigen-specific activation of splenic mononuclear cells.⁶⁴ In mice with selective deficiency of PGE₂, deletion of the gene encoding the TP receptor or treatment with a TP antagonist profoundly suppressed eosinophilic airway inflammation and airway reactivity to methacholine after a challenge with a dust mite extract.⁴³ Thus, effector functions of TXA₂ in pulmonary inflammation are cross-regulated by PGE₂ and EP receptors.

In humans, urinary TXA₂ levels were markedly increased in patients hospitalized with severe asthma compared with control patients admitted for other illnesses.⁶⁵ Inhaled allergen challenge also increased urinary excretion of TXA₂ products in patients with allergic asthma.^{66,67} TP receptor antagonists and thromboxane synthase inhibitors have been used to study the role of TXA₂ in pulmonary function and bronchial responsiveness. The TP receptor antagonist seratrodast significantly decreased AHR in asthmatics after 4 weeks of therapy in a nonrandomized, uncontrolled study.⁶⁸ In a subsequent randomized, double-blinded, placebo-controlled trial, seratrodast treatment for 4 weeks resulted in significant improvements in symptom score, peak expiratory flow, and

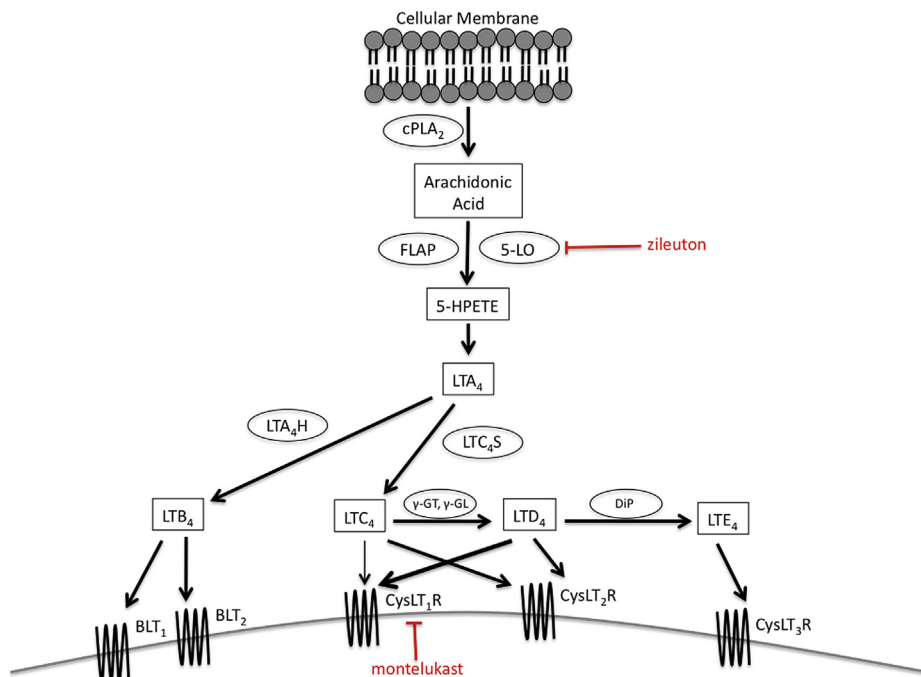


Figure 2. Leukotriene biosynthesis. 5-Lipoxygenase (5-LO) and 5-LO activating protein (FLAP) metabolize arachidonic acid to leukotriene (LT) A₄, which can be converted to LTB₄ (by LTA₄H) or the cysteinyl leukotrienes (cys-LTs; by leukotriene C₄ synthase [LTC₄S]). LTs then bind to the indicated G protein-coupled receptors. cPLA₂, cytosolic phospholipase A₂; BLT, leukotriene B receptor; DiP, dipeptidase; γ-GT, γ-glutamyl leukotrienase; γ-GT, γ-glutamyl transpeptidase; 5-HPETE, 5-hydroxyperoxyeicosatetraenoic acid.

bronchial responsiveness compared with placebo.⁶⁹ In contrast, the thromboxane synthase inhibitor OKY-046 (ozagrel) failed to improve FEV₁ or bronchial reactivity in a double-blinded, placebo-controlled, crossover trial.⁷⁰ TXA₂ levels in BAL fluid from patients with AERD were 4- to 5-fold higher than those found in BAL fluid from nonasthmatic controls.⁷¹ The pathogenetic contribution of TXA₂ in asthma may vary depending on the specific endophenotype.

Overview of the 5-LO Pathway

As for the COX pathway, arachidonic acid is the precursor for the 5-LO pathway. Arachidonic acid is first oxidized to 5-hydroxyperoxyeicosatetraenoic acid and then dehydrated to leukotriene A₄ (LTA₄), an unstable precursor, by 5-LO.^{48,72} The generation of LTA₄ by 5-LO also depends on the function of 5-LO activating protein, a perinuclear membrane protein that transfers free arachidonic acid to 5-LO.⁴⁸ Two different classes of LTs can be produced from LTA₄. LTB₄ is formed by LTA₄ hydrolase, which is expressed by mast cells, macrophages, and neutrophils.⁷³ LTA₄ also can be conjugated to reduced glutathione by leukotriene C₄ synthase (LTC₄S), converting LTA₄ to LTC₄, the parent cysteinyl leukotriene (cys-LT). LTC₄ is then exported from the cell, where it is converted to LTD₄ and then to LTE₄, the most stable cys-LT⁷² (Fig 2). Cys-LT generation also can occur by a transcellular mechanism, in which cells that contain 5-LO but not LTC₄S (such as neutrophils) produce and release LTA₄, which can be used by other cells that express LTC₄S but not 5-LO (such as platelets or endothelial cells) as a precursor for cys-LT generation.⁷⁴ A recent study demonstrated the relevance of such a mechanism to AERD, in which cys-LT overproduction was shown to originate from platelet-adherent leukocytes.⁷⁵

Mice deficient in 5-LO have decreased allergen-induced AHR, BAL eosinophilia, and serum IgE compared with WT mice.⁷⁶ Zileuton, a 5-LO inhibitor, likewise decreased AHR and decreased eosinophil recruitment to the lungs and LT release in the BAL fluid.⁷⁷ Similar results were obtained in mice treated with a 5-LO

activating protein inhibitor.⁷⁸ Thus, global interference with 5-LO function in mice markedly impairs the development of allergic inflammation and its consequences.

In humans, levels of LTB₄ and cys-LTs were higher in exhaled breath condensate from asthmatic patients compared with nonasthmatic controls.⁷⁹ Cys-LTs also were higher in the induced sputum of asthmatic subjects compared with nonasthmatic controls, and levels of sputum cys-LTs correlated with disease severity.⁸⁰ LTE₄ also was increased in the urine of asthmatics compared with controls.⁸¹ Urinary LTE₄ was particularly high in patients with AERD (6-fold greater on average than in aspirin-tolerant asthmatics) and further increased by as much as 10-fold from baseline after oral aspirin challenge.⁸² Clinical experience with the 5-LO inhibitor zileuton has confirmed the importance of cys-LTs in asthma. In 1 study, zileuton significantly improved FEV₁ and decreased exacerbations requiring corticosteroid treatment compared with placebo over a 3-month treatment period.⁸³ Similar outcomes were found in a 6-month study of zileuton vs placebo.⁸⁴ Zileuton also prevented the decrease in FEV₁ in response to oral aspirin challenge in patients with AERD.⁸⁵

Several 5-LO activating protein inhibitors have been developed, and some (MK-886, BAY X1005, and MK-591) completed phase I and II clinical trials in the 1990s, with promising results.^{86,87} However, these drugs were never brought to market.

Specific LO Pathway Products

Cys-LTs and Their Receptors

The Cys-LTs function by signaling through at least 3 GPCRs: CysLT₁R, CysLT₂R,⁷² and the recently reported CysLT₃R.⁸⁸ CysLT₁R binds LTD₄ with highest affinity and LTC₄ and LTE₄ with lower affinity. CysLT₂R has equal affinity for LTC₄ and LTD₄ and lower affinity for LTE₄.⁸⁹ CysLT₃R exhibits a strong binding preference for LTE₄, the most stable and abundant cys-LT.⁸⁸ CysLT₁R and CysLT₂R are each expressed by bronchial smooth muscle and various cells of

the immune system.⁸⁹ Patients with AERD had elevated levels of CysLT₁R-expressing inflammatory leukocytes in nasal tissues and bronchial biopsies compared with aspirin-tolerant controls, and aspirin desensitization resulted in a decrease in the number of CysLT₁R-positive cells.⁹⁰ CysLT₂R expression was similar among patient groups. The localization of CysLT₃R expression in human tissues has yet to be determined.

Mice lacking LTC₄S, and thus unable to make cys-LTs, had decreased eosinophilic lung inflammation and AHR compared with WT mice.⁹¹ Subsequently, LTC₄S-null mice were shown to have marked impairments in their capacity to develop T_H2-type immune response to dust mite challenge.⁹² This is attributable to a critical role for endogenous cys-LTs in DCs. CysLT₁R-null mice displayed a similar phenotype,⁹² whereas CysLT₂R-null mice showed exaggerated inflammatory responses in this model.⁹³ In vivo and in vitro studies have supported an inhibitory function for CysLT₂R in CysLT₁R expression and function.⁹⁴ In a model of long-term OVA exposure, treatment with the CysLT₁R antagonist montelukast decreased not only airway eosinophilia but also features of airway remodeling.⁹⁵ The mechanisms responsible are not clear.

In humans, cys-LTs are potent bronchoconstrictors. Inhalation of LTC₄ or LTD₄ resulted in airflow obstruction at doses several thousand-fold lower than histamine.^{96,97} Although LTE₄ is a less potent bronchoconstrictor than LTC₄ or LTD₄, asthmatic patients were more sensitive to LTE₄ than nonasthmatic controls.⁹⁸ Individuals with AERD were even more sensitive to bronchoconstriction induced by LTE₄ than were aspirin-tolerant asthmatics, although responses to LTC₄ and to histamine were similar between the 2 groups.⁹⁹ Given the ligand preference of the recently identified CysLT₃R, it is tempting to speculate that responses to LTE₄ may be mediated by this receptor. CysLT₁R antagonists, such as montelukast, have been shown to decrease the early and late phase pulmonary reactions to allergen challenge. The clinical response to these drugs in asthma has been variable, but in general, when used as monotherapy, they are not as effective as inhaled corticosteroids at improving FEV₁ and symptom measures.¹⁰⁰ Similarly, CysLT₁R antagonists have decreased symptoms in clinical studies of allergic rhinitis, but also have shown variable efficacy.¹⁰¹ Some studies have shown that CysLT₁R antagonists decrease symptoms of chronic urticaria compared with placebo, but negative studies also exist.¹⁰²

LTB₄ and Its Receptors, BLT₁ and BLT₂

Leukotriene B₄ signals through 2 GPCRs, BLT₁ and BLT₂, and its primary functions are in leukocyte chemotaxis.¹⁰³ In mice, antigen sensitization and challenge induced LTB₄ release into the airways, in addition to BAL eosinophilia and AHR.⁷⁸ Mice lacking BLT₁ were resistant to eosinophilic inflammation and AHR in an OVA model.¹⁰⁴ In a primate model of asthma, an LTB₄ receptor antagonist (CP-105,696) prevented the increase in AHR after multiple antigen challenges.¹⁰⁵ However, the very long half-life of this drug has prevented its translation into clinical use.¹⁰⁶ The dual BLT₁ and BLT₂ antagonist RO5101576 inhibited allergen and ozone-induced BAL neutrophilia in nonhuman primates.¹⁰⁷

In humans, increased levels of LTB₄ can be found in the BAL fluid, exhaled breath condensates, and blood of asthmatic patients.^{81,108} LTB₄ is strongly chemotactic for neutrophils and eosinophils in vitro.¹⁰⁸ These cells play an important role in asthma pathobiology, and LTB₄ may play a role in asthma pathogenesis through their recruitment to the airways. Thus far, there are no published studies of specific BLT₁ or BLT₂ agonists or antagonists in human asthma.

Discussion

Lipid mediators have important roles in the pathophysiology of disease and the maintenance of homeostasis. In addition to their

role in the development of acute inflammation, lipid mediators influence innate and adaptive immunities through complex signaling networks. The effectiveness of existing therapies, such as CysLT₁R antagonists and 5-LO inhibitors for asthma, demonstrates the importance of lipid mediators in human allergic disease. The identification of novel receptors not only helps to explain the pharmacology of the system but also presents potential new avenues for therapeutic and diagnostic advances.

Conclusions

Lipid mediators can have pro- and anti-inflammatory effects in allergic disease and asthma. These molecules, including PGs and LTs, are important as diagnostic markers and therapeutic targets. The development of new therapeutic agents for allergic disease will evolve from a rapidly expanding understanding of these mediators, their receptors, and the context within which they function.

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