Prostaglandins as regulators of allergic inflammation

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Lipid mediators were first recognized as important products of allergic reactions in the 1940 report by Kellaway and Trethewie, describing contraction of airway smooth muscle in the lungs of egg protein-sensitized guinea pigs with re-exposure to the egg protein. These investigators did not know at the time that the “slow reacting substance” responsible for the smooth muscle contraction they witnessed were cysteinyl leukotrienes generated by various cell types in response to both IgE-dependent and –independent stimuli, and that have profound immunologic and physiologic roles in allergic responses. Moreover, recent evidence suggests that lipid mediators are also important as regulators of the initial dendritic cell response to antigens during primary sensitization. Therefore, our understanding of the scope of the mechanisms by which lipid mediators modulate allergic inflammation is more far reaching than was previously appreciated. In this chapter, we will review the pathways of lipid mediator generation, examine studies that confirm the presence of these products in allergic inflammatory states, and discuss in vivo intervention studies in humans and recent murine studies which elucidate the activity of these mediators in the pathogenesis of allergic disease.

**Generation of lipid mediator precursors by phospholipase A₂**

The phospholipases A₂ (PLA₂) are enzymes which hydrolyze fatty acids at the sn-2 position of membrane phospholipids, forming free fatty acids including arachidonic acid and lysoglycero-phospholipids. Arachidonic acid serves as the precursor for the synthesis of all prostaglandins and leukotrienes, collectively known as eicosanoids because the Greek word for twenty is “eikosi”, the number of carbon atoms in arachidonic acid. The lysoglycero-
phospholipids are precursors for lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P). These both the generation and biologic function of the eicosanoids and the lysoglycerophospholipids metabolites will be discussed in detail later in this chapter. The PLA2s are categorized into six major classes, secretory PLA2s (sPLA2), cytosolic PLA2s (cPLA2), Ca2+ independent PLA2 (iPLA2), platelet-activating factor acetylhydrolases (PAF-AH), lysosomal PLA2s, and adipose-specific PLA2. The current classification scheme of the PLA2s is based upon the catalytic mechanism of the individual PLA2, as well as their functional and structural properties, and consists of 16 groups (Table 1). Although these 16 groups of PLA2 have now been identified, the groups that lead to lipid mediator generation are, at least to this point, limited to group IIA, group IVA, group VI and group X.

The sPLA2 are secreted from their cellular source, are small enzymes (14-18 kDa) that utilize an active site histidine and a His/Asp dyad, and require μM levels of Ca2+ for their catalytic activity. After cellular release, the sPLA2s can participate in either paracrine or autocrine generation of arachidonic acid from the outer leaflet of plasma membranes. Group IIA sPLA2 is important in the generation of lysophosphatidyl choline for synthesis of LPA. Group V sPLA2 is important for the development of allergic airway inflammation in mice. In the house dust mite model, group V sPLA2-deficient mice had markedly reduced pulmonary inflammation and goblet cell metaplasia compared to wild type mice, perhaps through reduced antigen processing and maturation of antigen presenting cells. Mice lacking group X sPLA2 had decreased bronchial inflammation, airway remodeling, lung Th2 cytokine levels, and levels of multiple lipid mediators in a model of chicken ovalbumin (OVA)-induced airway disease, but this asthma phenotype can be restored with knock-in of human group X sPLA2. Group X sPLA2 is released in large quantities by the asthmatic airway epithelium and plays a particularly
important role in asthma provoked by exercise and severe asthma in providing arachidonic acid for the rapid transformation of cysteinyl LTs.\textsuperscript{5-7}

The cPLA\textsubscript{2} are present in the cytosol and are larger than the sPLA\textsubscript{2} (61-114 kDa).\textsuperscript{2} There are six subgroups (denoted A-F) of cPLA\textsubscript{2} enzymes in group IV and these use a catalytic serine in a Ser/Asp dyad. The group IVA cPLA\textsubscript{2} does not require Ca\textsuperscript{2+} for its catalytic activity, but Ca\textsuperscript{2+} is important for this enzyme’s translocation to intracellular membranes after binding to a C2-domain. Group IVA cPLA\textsubscript{2} not only hydrolyzes glycerophospholipids at the sn-2 position to liberate arachidonic acid, but also has lysophospholipase and transacylase activities.\textsuperscript{2} A recent report suggests that this enzyme may have a role in asthma pathogenesis as group IVA cPLA\textsubscript{2} was overexpressed in patients with persistent asthma.\textsuperscript{8}

The Ca\textsuperscript{2+} independent PLA\textsubscript{2} are termed iPLA\textsubscript{2} and are in group VI.\textsuperscript{2} Similar to the cPLA\textsubscript{2}, the iPLA\textsubscript{2} use a catalytic serine and there are also six subgroups (denoted A-F) of iPLA\textsubscript{2} enzymes in group VI. Group VIA and group VIB iPLA\textsubscript{2} act to generate arachidonic acid release for eicosanoid production, while group VIA has roles in glycerophospholipid remodeling, protein expression, acetylcholine-modulated endothelium-dependent relaxation of vessels, apoptosis, and lymphocyte proliferation. The platelet-activating factor acetylhydrolases (PAF-AH) hydrolyze the acetyl group from the sn-2 position of PAF.\textsuperscript{2} There are two groups of PAF-AH, classified as groups VII and VIII. While PAF-AH is not involved in eicosanoid formation, inactivation of PAF by PAF-AH protects against anaphylaxis as persons with lower levels of PAH-AF have more severe manifestations of anaphylaxis than those with higher levels of PAF-AH.\textsuperscript{9}
Thus, the PLA₂ enzymes, while critical for the generation of arachidonic acid, LPA, and S₁P from membrane phospholipids, also have many other important far-reaching biologic functions.

**EICOSANOID FORMATION**

**CYCLOOXYGENASE PATHWAY**

Arachidonic acid is oxidatively metabolized by the cyclooxygenase and lipoxygenase pathways.¹⁰ Cyclooxygenase catalyzes two reactions, first a cyclooxygenase reaction that inserts two molecules of oxygen into arachidonic acid to produce prostaglandin (PG)G₂, followed by an endoperoxidase reaction that reduces PGG₂ to PGH₂ (Figure 1). PHG₂ is the precursor for the prostanoids PGD₂, PGE₂, PGF₂α, and PGI₂, and thromboxane A₂ (TXA₂). Each prostanoid is produced by tissue specific enzymes and isomerases which will be discussed below. There are two cyclooxygenase enzymes, COX-1 and COX2, which are products of separate genes and have different biologic functions based on their different temporal and tissue specific expression.¹⁰ The human COX-1 gene is located on chromosome 9, is constitutively expressed in most tissues, and although inducible in some contexts is presumed to be involved in homeostatic prostanoid synthesis.¹¹ On the other hand, COX-2 expression is inducible and usually transient. The human COX-2 gene is present on chromosome 1. COX-2 expression can be induced by lipopolysaccharide (LPS) produced by Gram-negative bacteria, in addition to interleukin (IL)-1, IL-2, and TNF.¹¹ COX-2 expression can be induced in macrophages, endothelial cells, airway
epithelial cells, airway smooth muscle cells, and airway fibroblasts. The multitude and diversity of stimuli that induce COX-2 expression, and the myriad of cells capable of expressing it, ensures that its function is a frequent concomitant of inflammatory diseases. COX-2 is also constitutively expressed in some contexts, such as in cultured human lung epithelial cells, cortical thick ascending limb of the kidney, pancreatic islet cells, human brain cortical cells in Rett syndrome, and in human gastric carcinoma. The capacity of nonsteroidal anti-inflammatory drugs to inhibit COX-2 activity may constitute their major therapeutic effect, while inhibition of COX-1 may result in some of their undesired side effects.

There have been contradictory reports about the expression of COX-2 in the airway epithelium from persons with allergic diseases. One study reported a fourfold amplification in bronchial epithelial COX-2 immunostaining in asthmatic subjects compared to healthy controls, however, another study discerned no difference in the level of immunostaining in asthmatics, chronic bronchitics, or controls who had no lung disease. COX-2 mRNA expression and immunoreactive protein was increased in the airway epithelium of asthmatics that have not been treated with corticosteroids compared with non-asthmatic controls, while corticosteroid-treated asthmatics had decreased COX-2 expression compared to their non-treated counterparts. The relationship between the cytokines implicated in the allergic response and COX-2 expression is complex. IL-4 and IL-13 suppressed PGE2 production by inhibiting both COX-2 and microsomal PGE synthase (mPGES) through JAK1 and STAT6 signaling. Thus, in asthmatic subjects, increased TNF expression might result in COX-2 induction, while IL-4 and IL-13 might inhibit this enzyme’s expression. In subjects with nasal polyps, prednisone increased COX-2 mRNA expression in polyp tissue two weeks after treatment was started, while there was no effect on COX-1 mRNA expression. It is possible corticosteroids modulate COX-
2 expression by indirectly reducing IL-4 and IL-13, in so doing permitting TNF induction of COX-2. This interpretation might help explain the apparent contradictory in vitro data whereby COX-2 immunoreactivity in airway epithelial cells is reduced by corticosteroid treatment. Corticosteroids decreased basal and bradykinin-induced levels of PGE\(_2\) in airway epithelial cells, implying that COX-2 is the primary producer of PGE\(_2\) in airway epithelium. COX-1 and COX-2 mRNA is present in resting human T lymphocytes. T cell activation does not affect COX-1 expression in T cells, while T cell stimulation upregulates COX-2 mRNA levels with increased COX-2 protein and cyclooxygenase activity. Therefore, COX expression exists in both resident airway cells and cells of the adaptive immune response.

**Human studies of the COX pathway in allergic inflammation**

There is abundant data that COX products are increased as a result of allergic inflammation. For instance, COX products in the bronchoalveolar (BAL) fluid of allergic asthmatics are significantly increased compared to healthy nonasthmatic controls, and allergic antigen challenge of the airways further augments prostanoid production. BAL fluid levels of PGD\(_2\) and PGF\(_{2\alpha}\) were 12- to 22-fold greater in asthmatics than in nonallergic subjects, and 10-times greater in allergic asthmatics than in nonasthmatic subjects who had allergic rhinitis. Segmental allergen challenge led to a 17- to 208-fold increase in the levels of PGD\(_2\), thromboxane (Tx) B\(_2\), and 6-keto-PGF\(_{1\alpha}\), a PGI\(_2\) metabolite in allergic asthmatics. When these subjects were treated with prednisone for three days prior to segmental allergen challenge, there was no alteration in the BAL fluid prostanoid concentrations, revealing that corticosteroids do not inhibit activation of the COX pathway that occurs with an allergic inflammatory stimulus.
corroborating the results in prednisone-treated patients with nasal polyps discussed in the preceding paragraph.

Intervention studies examining the importance of the COX enzymes in allergic airway disease have been performed by treating subjects with indomethacin, which blocks both COX-1 and COX-2, before allergen challenge. Indomethacin did not affect lung function prior to allergen challenge in either allergic asthmatics or subjects with allergic rhinitis who did not have asthma. However, indomethacin treatment reduced the forced expiratory volume in one second (FEV₁) and specific airway conductance in nonasthmatic subjects with allergic rhinitis in response to inhaled allergen challenge. Indomethacin administration prior to allergen challenge caused a small but significant decrease in specific airway conductance in the allergic asthmatic subjects compared to placebo treatment, yet indomethacin had no effect on allergen-induced alterations in FEV₁. Indomethacin treatment had no significant effect on airway responsiveness to histamine, nor did it change the immediate or late phase pulmonary response to allergen challenge in allergic asthmatics. In subjects with exercise-induced asthma, indomethacin did not alter bronchoconstriction after exercise, but did prevent refractoriness after exercise. The apparent complex effect of COX inhibition on lung function reflects the diversity of the individual prostanoids and the receptors with which they interact (see below), some of which counteract one another’s actions.

Mouse studies of the COX pathway in allergic inflammation

Mice with targeted deletions of COX-1 and COX-2 genes have been subjected to models of sensitization and challenge with OVA. COX-1 deficient mice showed heightened lung
eosinophilia, serum IgE levels, augmented airway responsiveness, increased numbers of CD4+ and CD8+ T cells, and exaggerated levels of Th2 cytokines, and amplified concentrations of eotaxin and thymus- and activation-regulated chemokine (TARC) compared to both mice deficient in COX-2 and wild type control mice.\textsuperscript{26,27} Thus, COX-1-derived prostanoids may be homeostatic during allergen-induced pulmonary inflammation. One study reported that COX-2-deficient mice on a C57BL/6 genetic background had increased serum IgE levels, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) levels compared to control mice, but no difference in pulmonary eosinophilia or airway responsiveness.\textsuperscript{26,27} Another group reported that COX-2 deficient mice, also expressed on a C57BL/6 background had greater allergen-induced lung eosinophilia as compared to wild type mice.\textsuperscript{28} Based on these findings that COX-1 inhibition augments allergic airway inflammation and airway responsiveness, one would suspect that overexpression of COX-1 would have the opposite effect. However, COX-1 overexpression targeted to the airway epithelium decreased basal airway responsiveness and yet had no effect on the degree of allergic inflammation.\textsuperscript{29}

Pharmacologic inhibition of COX enzymes produces profound effects on the development of allergic inflammation. Mice treated with oral indomethacin during the induction of allergic airway disease had increased Type 2 cytokines in the lungs, increased pulmonary eosinophilia, and greater airway responsiveness to methacholine compared to vehicle-treated mice.\textsuperscript{30} Although BAL leukotriene levels were increased as a result of indomethacin treatment, 5-LO deficient mice also had increased allergen-induced inflammation with indomethacin treatment, effectively ruling out a causative role for enhanced leukotriene production in the exaggerated inflammatory response.\textsuperscript{31} The heightened allergic inflammation with indomethacin was dependent upon CD4+ cells, but independent of IL-4, IL-4 receptor alpha signaling, and
STAT6, elements critical in the Th2 signaling pathway.\textsuperscript{32} This augmented allergic phenotype is not specific to indomethacin as both COX-1 and COX-2 inhibitors independently augmented lung levels of IL-13 and airway responsiveness when compared to vehicle-treated mice.\textsuperscript{33} COX-2 inhibition during epicutaneous sensitization with OVA in a mouse model of atopic dermatitis increased eosinophil skin infiltration, elevated total and antigen specific IgE, and resulted in a systemic Th2 response to antigen.\textsuperscript{34} As several studies reveal that COX inhibition during the development of allergic disease resulted in increased allergen-induced inflammation and airway responsiveness, these results imply that a COX product may restrain allergic inflammation and might be a therapeutic target for the treatment of allergic diseases such as asthma and atopic dermatitis.

It is critical to recognize that in these mouse studies COX inhibition was present throughout the entire development of allergic disease, from the initial stage of antigen presentation and throughout all allergen challenges. In the human studies using indomethacin, COX inhibition occurred only at the time of an antigen challenge, long after the regulatory elements of allergic inflammation in the lung had been set in place. It is also important to note that prostanoids such as PGD\textsubscript{2} that cause bronchoconstriction in humans fail to constrict mouse airways.\textsuperscript{35} Thus animal models of allergic pulmonary disease, whether COX functions are ablated pharmacologically or by gene deletion, are better suited to identify immunologic functions of prostanoids, rather than the direct effects on end-organ physiology seen in human studies.

Individual Prostanoids
Prostaglandin D₂

PGD₂ is the major mast cell-derived prostanoid, being released in nanogram quantities by this cell type in response to IgE-mediated activation.¹⁰ Recent evidence suggests that eosinophils also synthesize PGD₂.³⁶ There are two distinct forms of PGD₂-synthesizing enzymes, hematopoietic- and lipocalin- PGD₂ synthases (H-PGDS and L-PGDS, respectively); only the former is involved in PGD₂ production by mast cells and other hematopoietic cell types. L-PGDS is present in the choroid plexus, leptomeninges, oligodendrocytes, organs of the male genital tract, and in the hearts of humans and monkeys. L-PGDS gene expression in the central nervous system can be modulated by glucocorticoid, thyroid, and estrogen hormones, while L-PGDS expression in the heart is modulated by estrogen. H-PGDS is expressed to the greatest degree in humans in placenta, lung, adipose tissue, and fetal liver, while it is expressed in lower levels in the heart, lymph nodes bone marrow, and appendix. H-PGDS is also expressed in mast cells, CD4⁺ Th2 (but not Th1) T lymphocytes, CD8⁺ Tc2 cells, histiocytes, megakaryocytes, dendritic cells, and Kupffer cells. PGD₂ can be metabolized to PGF₂α, 9α,11β-PGF₂α (the stereoisomer of PGF₂α), and the J series of prostanoids which includes PGJ₂, Δ¹²-PGJ₂, and 15d-PGJ₂.¹⁰

As is the case for all eicosanoids, PGD₂ signals through distinct seven transmembrane, G-protein coupled receptors (GPCRs), termed DP₁ and DP₂ (Table 2).¹⁰ DP₁ is expressed on mucus-secreting goblet cells in the nasal and colonic mucosa, nasal serous glands, vascular endothelium, Th2 cells, dendritic cells, and eosinophils. DP₁ stimulation activates adenyl cyclase, resulting in an intracellular increase in cAMP levels and PKA activity. Signaling through DP₁ has been reported to promote sleep, survival of eosinophils, secretion of mucus,
vasodilation, and vascular permeability, while decreasing cytokine secretion and chemotaxis.

DP₂ is also known as chemoattractant receptor-like molecule expressed on Th2 cells (CRTH2). In addition to PGD₂, other DP₂ agonists include Δ₁₂-PGJ₂; 15-deoxy-Δ₁₂,₁⁴PGJ₂ (15d-PGJ₂); 13,15-dihydro-15-keto-PGD₂; 11-dehydro-TXB₂; and the COX inhibitor indomethacin. DP₂ is expressed on immune cells such as CD4+ Th2 and CD8+ Tc2 cells, eosinophils, and basophils. These cells each respond chemotactically to PGD₂ in a DP₂-dependent manner. In the BAL fluid of asthmatic subjects DP₂ is preferentially expressed by IL-4⁺/IL-13⁺ T cells compared to IFN-γ⁺ T cells.³⁷ DP₂ signaling in eosinophils also induces their release of eosinophils from bone marrow, initiates their respiratory burst, increases the chemotactic response to other chemokines such as eotaxin, and primes them for degranulation. In addition, DP₂ signaling is reported to increase microvascular permeability, depletion of goblet cells, and constrict coronary arteries. In contrast to DP₁ signaling, activation of DP₂ results in decreased intracellular cAMP.¹⁰ Thus, PGD₂ signaling through DP₂, via suppression of cAMP, would be predicted to facilitate allergic inflammation through its effect on chemotaxis, and mediator release by effector cells. The smooth muscle contractile properties of PGD₂ and its immediate metabolite, 9α, 11β-PGF₂ are thought to be mainly mediated through the thromboxane TP receptor.³⁸,³⁹

**Human studies of PGD₂ in allergic inflammation**

Inhalation challenge of allergic human asthmatic subjects with specific allergen increases the levels of PGD₂ in the BAL fluid.⁴⁰ PGD₂ is increased in the nasal lavage from subjects with allergic rhinitis,⁴¹ in tears from persons suffering from allergic conjunctivitis,⁴² and in the fluid from experimentally produced skin blisters in patients with late phase reactions of the skin.⁴³ In
subjects with asthma, the stable urinary PGD2 metabolite was not decreased by treatment with the COX-2 specific inhibitor celecoxib for three days, suggesting that PGD2 is primarily synthesized via COX-1.

PGD2 is a potent bronchoconstrictor and vasodilator, and potentiates airway responsiveness. Intranasal administration of PGD2 administration increased nasal resistance 10-times more potently than histamine and 100-times greater than bradykinin. PGD2 induced vascular leakage in the conjunctiva and skin, and led to eosinophil influx in the conjunctiva and trachea, suggesting that it may have a direct pathogenic role in allergic disease. The vascular effects of PGD2 are thought to largely reflect dilation mediated by DP1, whereas recruitment of effector cells is more likely to reflect chemotaxis via DP2.

While these data strongly suggest that PGD2 is involved in the pathogenesis of allergic diseases, there are no published studies that show that a specific PGD2 antagonist had a beneficial effect in the treatment of these disorders, despite the development of multiple DP1 and DP2 antagonists. Therefore, whether PGD2 mechanistic has a mechanistic role in allergen-induced inflammation is not clear. In regard to smooth muscle contraction by PGD2 released upon allergen exposure, TP receptor antagonists such as GR32191, partially antagonized the early bronchoconstrictor response, with other constrictor mediators, histamine and LTC4/LTD4 contributing to make up the difference.

**Mouse studies of PGD2 in allergic inflammation**

Mouse studies reveal a complex role of PGD2 in experimental allergic disease. Transgenic mice that overexpress L-PGDS had greater BAL fluid levels of Th2 cytokines, eotaxin, eosinophils, and lymphocytes following allergen sensitization and challenge compared
to nontransgenic littermates. Aerosolized PGD2 administered one day prior to inhalational challenge with low-dose antigen amplified the numbers of eosinophils, lymphocytes, and macrophages, as well as IL-4 and IL-5, in BAL fluid of sensitized mice. These results suggest that PGD2 augments pulmonary Th2 responses.

Rodent studies of DP1 function in allergic inflammation have been somewhat contradictory. Allergen sensitized and challenged DP1-deficient mice had significantly reduced BAL concentrations of IL-4, IL-5, and IL-13 compared to control mice, and diminished AHR, without a difference in the levels of IFN-γ in BAL fluid. These DP1-deficient mice had decreased BAL cellular influx with less eosinophils and lymphocytes compared to control mice, suggesting that DP1 signaling was important in the full expression of allergic inflammation. However, the DP1 agonist BW245C suppressed the function of lung dendritic cells, including lung migration and the ability of dendritic cells to stimulate T cell proliferation. Mice treated with BW245C or mice receiving adoptively transferred DP1-treated dendritic cells had increased numbers of Foxp3+ CD4+ regulatory T cells that suppressed inflammation in an interleukin 10–dependent mechanism. The reduction in allergic inflammation caused by the DP1 agonist on dendritic cell function was mediated by cyclic AMP–dependent protein kinase A. In addition, chimeric mice that lacked DP1 expression on hematopoietic cells had strongly enhanced airway inflammation when challenged with allergen, indicating an important homeostatic role of DP1 and endogenous PGD2. Collectively, this data suggests that DP1 signaling facilitates effector responses through structural cells, but may dampen responses of dendritic cells so as to restrain the allergic inflammatory process at the sensitization phase.

Several studies in different species examining DP2 support the notion that signaling through this receptor amplifies allergic inflammation. The DP2 receptor antagonist AM211
inhibited ovalbumin-induced airway eosinophil influx in a guinea pig model of allergic airway inflammation, while reducing the number of sneezes mice experienced following intranasal allergen challenge. Similarily, the DP2 antagonist MK-7246 inhibited antigen-induced late phase bronchoconstriction and airway responsiveness in sheep, and antigen-induced eosinophilia in both sheep and monkeys. In addition, an oral, potently selective alkynylphenoxyacetic acid DP2 antagonist decreased ovalbumin-induced airway eosinophilia in mice. Thus, PGD2 signaling through DP2 increases allergic inflammation, and interference with this receptor attenuates such inflammatory responses in animals. Whether these encouraging results in animal models of allergic inflammation translates to human disease remains to be seen.

Prostaglandin E2

There are three distinct enzymes that can metabolize PGH2 to PGE2. These are microsomal PGE synthase-1 (mPGES-1), mPGES-2, and cytosolic PGE synthase (cPGES). mPGES-1 is membrane-associated, localized to the perinuclear area, glutathione-dependent, and has a trimeric structure. PGE2 production was substantially greater in cells co-transfected with both mPGES-1 and COX-2, suggesting that mPGE-2 preferentially couples with COX-2 to immediately generate PGE2 when COX-2 is active in cells. mPGES-1 can metabolize PGH2 produced from COX-1, but required exogenous administration of arachidonic acid. For instance, mouse studies revealed that arachidonic acid generated by mast cell group IVA cPLA2 led to PGE2 production by fibroblast mPGES-1. The expression of cPGES is mostly constitutive and not induced by inflammatory stimuli. Compared to mPGES-1, cPGES coupled more efficiently with COX-1 than with COX-2 for PGE2 generation. Although this suggests that cPGE2 may
provide PGE₂ necessary for cellular homeostasis, mice lacking mPGES-1 show strikingly diminished levels of basal PGE₂ production in most organs. In contrast, genetic deletion studies in mice have been unable to support a role of either cPGES or mPGES-2 as PGESs in vivo. cPGES is localized to the cytosol, there was evidence that it translocates from the cytosol to the nuclear membrane to assemble with COX-1 in PGE₂ production, although it has a slight preference to interact with COX-2. Heat shock protein 90, casein kinase II, and bradykinin upregulated cPGE₂ activity, while dexamethasone decreased cPGES activation. In contrast to mPGES-1, mPGES-2 is not dependent on glutathione. mPGES-2 is expressed constitutively in many cells and tissues, but can be induced in colorectal adenocarcinoma cells to high levels. In transfected cells, mPGES-2 uses PGH₂ derived from COX-1 and COX-2 with equal efficiency. Local PGE₂ concentrations are modulated by COX-2 driven synthesis and PGE₂ degradation by 15-hydroxyprostaglandin dehydrogenase (15-PGDH).

The effects of PGE₂ both in vivo and in vitro are complex, relating to the fact that this prostanoid signals through four distinct GPCRs, termed EP receptors 1 through 4 (Table 3). Each EP receptor has a distinct G protein coupling preference and downstream signal activation, and some of these signals counteract with one another. All four receptor subtypes are present in the lung and other organs associated with allergic responses. Signaling through the EP₁ receptor increased inositol triphosphate and diacylglycerol, resulting in increased cell Ca²⁺ and smooth muscle contraction. Activation of the EP₂ and EP₄ receptors increased intracellular cAMP concentrations and relaxed smooth muscle. Stimulation of the EP₂ receptor inhibited mast cell and basophil mediator release. EP₂ is expressed most abundantly in the uterus, lung and spleen. EP₄ receptor expression is greatest in the kidney and peripheral blood leukocytes, but there is high level of expression in the thymus, lung and a number of other tissues.
receptors caused smooth muscle contraction by decreasing the rate of cAMP synthesis.\textsuperscript{67} EP\textsubscript{3} receptors are unique because of the diversity created by multiple splice variants that produce alternate sequences in the C-terminal tail of this receptor subtype; however, the functional importance of these alternative splice variants is not well understood.\textsuperscript{65} Usually these splice variants of EP\textsubscript{3} decrease cAMP generation, in contrast to signaling through EP\textsubscript{2} and EP\textsubscript{4} which increase cAMP.\textsuperscript{65} Thus, PGE\textsubscript{2} activity can be diverse and possibly competing, dependent upon the relative contributions of the receptors that are stimulated in a given context.

**Human studies of PGE\textsubscript{2} in allergic inflammation**

PGE\textsubscript{2} is a predominant COX product of the airway epithelium and smooth muscle.\textsuperscript{68;69} There is abundant evidence to support the proposition that endogenous PGE\textsubscript{2} may be bronchoprotective in human asthma.\textsuperscript{70} PGE\textsubscript{2} produced by epithelial cells inhibited vagal cholinergic contraction of airway smooth muscle.\textsuperscript{71} Bronchial epithelial cell-derived PGE\textsubscript{2} also dampened dendritic cell migration and pro-inflammatory cytokine secretion.\textsuperscript{72} There was a negative correlation between the sputum levels of PGE\textsubscript{2} from asthmatics and their sputum eosinophil count, suggesting that PGE\textsubscript{2} may have anti-inflammatory properties.\textsuperscript{73;74} PGE\textsubscript{2} inhalation also inhibited the pulmonary immediate and late phase responses to inhaled allergen.\textsuperscript{75;76} Inhaled PGE\textsubscript{2} decreased the change in methacholine airway reactivity and reduced the number of eosinophils after inhaled allergen challenge.\textsuperscript{75} In addition, PGE\textsubscript{2} inhibited both exercise induced and aspirin induced bronchoconstriction in patients sensitive to these challenges.\textsuperscript{77;78} Interestingly, although PGE\textsubscript{2} significantly protects against decrements in pulmonary function in challenge models, it does not alter baseline FEV\textsubscript{1} or methacholine
reactivity. The results from these studies suggest that PGE2 has greater immunomodulatory properties than directly affecting airway caliber. This is supported by the observation that PGE2 inhalation prior to segmental allergen challenge significantly reduced the BAL levels of PGD2, an important product of mast cell activation, and the concentrations of cys-LTs. Recent evidence suggests that the EP4 receptor in human, guinea pig, and rat airways mediates smooth muscle relaxation, whereas it is the EP3 receptor that mediates the cough properties of PGE2. PGE2 in combination with the β2-adrenergic receptor agonist albuterol also inhibited human airway smooth muscle migration and mitogenesis, revealing that PGE has pleiotropic effects on airway function.

PGE2 is rapidly metabolized and this has prompted investigators to utilize the more stable orally active PGE1 analogue, misoprostol, in studies of allergen-induced airway inflammation and lung function in humans, but the results have largely been negative. Misoprostol did not have an impact on pulmonary function, β2 agonist use, or asthma severity score in aspirin-sensitive asthmatics. In mild asthmatics, misoprostol had no effect on either baseline lung function or histamine reactivity, and but did elicit significant gastrointestinal side effects in one-third of the subjects enrolled in the study.

In vitro studies have not supported human in vivo studies that propose PGE2 decreases allergic inflammation. PGE2 in vitro inhibited lymphocyte production of the Type 1 cytokines IL-2 and interferon-γ, thus promoting T cell differentiation toward a Type 2 cytokine profile. These in vitro results suggesting PGE2 promoted Type 2 cytokine production may be regulated at the antigen presentation. Myeloid dendritic cells matured in the presence of IFN-γ produced Type 1 CD4+ T lymphocyte responses, while dendritic cells matured in PGE2 elicited Type 2 T cell responses. PGE2 induction of Type 2 cytokine production mostly through its activity at the
time of antigen presentation would not necessarily contradict *in vivo* human studies that have suggested PGE<sub>2</sub> is anti-inflammatory. More recently, in combination with IL-23, PGE<sub>2</sub> induced differentiation and expansion of CD4<sup>+</sup> Th17, along with secretion of the signature cytokines by this lineage.\textsuperscript{91} Acute antigen challenge models probably more precisely reflect effector cell function, since allergic sensitization to an antigen occurs much earlier in life.

Besides PGE<sub>2</sub>’s activity in the development of CD4<sup>+</sup> Th1 and Th2 cell development, this prostanoid has important immunomodulatory effects on other inflammatory cells presumed to be pathogenic in asthma. In a cell culture system, both PGE<sub>2</sub> and cAMP inhibited spontaneous eosinophil apoptosis, as did an EP2 agonist.\textsuperscript{92} Thus, by prolonging eosinophil survival, PGE<sub>2</sub> could potentially increase the inflammatory potential of these cells in asthma. However, PGE<sub>2</sub> was also reported to decrease eosinophil chemotaxis, aggregation, degranulation, and IL-5-mediated survival.\textsuperscript{93;94} PGE<sub>2</sub> inhibition of eosinophil trafficking was modulated through EP2 signaling.\textsuperscript{95} Therefore, the relevance of these *in vitro* findings to *in vivo* disease states is still to be determined.

PGE<sub>2</sub> also modulated the expression of a very important growth factor, granulocyte macrophage-colony stimulating factor (GM-CSF), from human airway smooth muscle cells.\textsuperscript{96} The COX inhibitor indomethacin upregulated GM-CSF production by cultured human airway smooth muscle cells; however, exogenous PGE<sub>2</sub> decreased this indomethacin induced GM-CSF production, suggesting that PGE<sub>2</sub> restrained GM-CSF expression and the inflammation that is associated with this cytokine.\textsuperscript{96} In contrast, PGE<sub>2</sub> increased IL-6 and GM-CSF production as a result of IgE-mediated mast cell degranulation by signaling through EP1 and EP3 receptors.\textsuperscript{97} PGE<sub>2</sub> has also been reported to have contrasting activities on the mast cell production of differing mediators. PGE<sub>2</sub> has been reported to either reduce\textsuperscript{98-100} or enhance\textsuperscript{101;102} the release of
histamine and other inflammatory mediators. These differences may relate to the relative dominance of EP3 (activating) versus EP2 (inhibitory) signaling in a given mast cell population. For instance PGE2 can activate human mast cells through EP3, but inhibit activation through the EP2-PKA signaling pathway.\textsuperscript{103}

PGE2 is also thought to play a key role in aspirin-intolerant asthma with inhibition of COX-1, but not COX-2, being closely aligned with the ability of NSAIDs such as aspirin for precipitating this form of asthma (see Chapter 81).\textsuperscript{104}

**Mouse studies of PGE2 in allergic inflammation**

In the OVA-sensitized and challenged model, mice that are deficient in the EP3 receptor had enhanced allergic inflammation compared to WT mice, while there were no differences in the pulmonary allergic inflammation between WT, EP1-deficient, EP2-deficient, and EP4-deficient mice.\textsuperscript{105} Compared to WT mice, EP3-deficient mice had greater airway numbers of eosinophils, neutrophils, and lymphocytes in BAL fluid, as well as augmented BAL concentrations of IL-4, IL-5, and IL-13.\textsuperscript{105} Administration of the EP3 agonist AE-248 to OVA-sensitized and challenged WT mice significantly suppressed allergic airway cellularity and tended to decrease airway mucus expression and airways responsiveness to methacholine.\textsuperscript{105} In *ex vivo* experiments, lungs from OVA-sensitized and challenged EP3-deficient or WT mice were harvested and then challenged with OVA.\textsuperscript{105} In these studies, lungs from WT mice treated with an EP3 agonist produced significantly decreased histamine and cys-LT, suggesting that PGE2 may signal through EP3 on mast cells *in vivo* to inhibit mediator release.\textsuperscript{105} It is important to note that the results of these studies could not have been predicted from *in vivo* analyses, since EP3
receptor signaling causes mast cell activation \textit{in vitro}, whereas EP$_2$ receptor signaling is inhibitory.\textsuperscript{103} Other studies also support the concept that PGE$_2$ restrains allergic airway inflammation in mice. Using the house dust mite model, subcutaneous PGE$_2$ inhibited lung eosinophilia and Th2 cytokine protein expression.\textsuperscript{106} The effect of PGE$_2$ on mouse mast cell function seems to be opposite that of other cells involved in the allergic inflammatory response. For instance, PGE$_2$ induced mast cell chemotaxis and cytokine production through mTORC2 activation.\textsuperscript{107} The chemotactic activity of PGE$_2$ on mouse mast cells occurs via EP$_3$ activation.\textsuperscript{108} There is evidence that PGE$_2$ expression is reduced in chronic allergen exposure, possibly a result of allergic inflammation, with a consequence being airway remodeling. In this model, there was an inverse relationship between the number of allergen challenges and both COX-2 and mPGES-1 expression in lung fibroblasts, resulting in decreased cytokine-induced PGE$_2$ production.\textsuperscript{109} Interestingly, mPGES-1 derived PGE$_2$ expression had no effect on allergen sensitization or effector T cell responses in a house dust mite model comparing the phenotype of mPGES-1-deficient and wild type mice.\textsuperscript{110} However, mPGES-1-deficient mice had significantly increased numbers of vascular smooth muscle cells and thickness of intrapulmonary vessels following allergen challenge.\textsuperscript{110} These results revealed that PGE$_2$ produced by mPGES-1 protected the pulmonary vasculature from remodeling during allergen-induced lung inflammation. PGE$_2$ also regulates airway tone in mice. Immunologically naïve mice that lack 15-hydroxyprostaglandin dehydrogenase, the major catabolic enzyme of PGE$_2$, and therefore have elevated levels of PGE$_2$, had a decreased bronchoconstrictor response to methacholine.\textsuperscript{111} Similarly, mice that had elevated PGE$_2$ levels as a result of overexpression of PGE$_2$ synthase in the lung had decreased methacholine-induced airway constriction.\textsuperscript{111} Thus PGE$_2$ protects against lower airway bronchoconstriction, and other studies suggest that this effect is mediated through EP$_2$. 
Pretreatment with aerosolized PGE$_2$ blunted methacholine-induced bronchoconstriction in WT mice, but not in EP$_2$-deficient animals.$^{112}$ In addition, methacholine-induced bronchoconstriction was reversed by aerosolized PGE$_2$ in WT, but not EP$_2$-deficient, mice.$^{112}$ These findings were confirmed by another group that reported that PGE$_2$-induced bronchodilation was a consequence of direct activation of EP$_2$ receptors on airway smooth muscle, while PGE$_2$ signaling through EP$_1$ and EP$_3$ led to bronchoconstriction.$^{113}$ Collectively, these studies suggest that PGE$_2$ regulates homeostasis of bronchomotor tone and pulmonary immune responses through different respective receptors.

Prostaglandin F$_{2\alpha}$

PGF$_{2\alpha}$ is produced by PGF synthase (PGFS).$^{114}$ PGFS has two activities: 1) catalyzing the formation of PGF$_{2\alpha}$ from PGH$_2$ by PGH$_2$ 9,11-endoperoxide reductase in the presence of NADPH, and 2) forming PGF$_{2\alpha}$ from PGD$_2$ by PGD$_2$ 11-ketoreductase.$^{114}$ The PGFS binding sites for PGH$_2$ and PGD$_2$ are proposed to be distinct.$^{114}$ PGFS is expressed in lung and peripheral blood lymphocytes, suggesting a possible role in allergic diseases such as asthma.$^{115}$ PGFS may also have a role in the nervous system, since it has been identified in neurons and vascular endothelial cells in the rat spinal cord.$^{116}$ PGFS is inhibited by non-steroidal anti-inflammatory drugs (NSAIDS) such as indomethacin and this may partially explain the protective effect of this class of drugs in some gastrointestinal tumors in which PGFS activity is high.$^{114}$ PGF$_{2\alpha}$ binds a sole receptor, termed FP (Table 4) which is the most promiscuous of the
prostanoid GPCRs in binding the principal prostaglandins, with PGD₂ and PGE₂ both binding to FP at nanomolar concentrations. Selective FP agonists such as fluprostenol and latanoprost have been produced that are used in clinical settings because of these agents’ ocular hypotensive properties. PGF₂α plays a critical function in reproduction, renal physiology, and modulation of intraocular pressure. Tissue distribution of FP receptor mRNA expression is highest in the ovarian corpus luteum, followed by the kidney, with lower-level expression in the lung, stomach, and heart. FP expression has not been reported in the spleen, thymus, or on immune cells. As a result, in contrast to the other prostaglandins, there is very little evidence to suggest an important contribution of PGF₂α-FP receptor signaling in inflammatory and immunological processes.

**Human studies of PGF₂α**

PGF₂α has not been studied to nearly the same extent as PGD₂ or PGE₂ in allergic disease and asthma. PGF₂α inhalation leads to a dose-related decrease in specific airway conductance in both control and asthmatic subjects. There has been a wide variation in the pulmonary function response to PGF₂α reported in asthmatics, in contrast to the relatively small inter-individual variation in healthy control subjects. Subjects who inhaled PGF₂α, experienced wheezing, coughing and chest irritation within 3 to 4 minutes, while watery sputum also occurred shortly thereafter. Maximal decrease in specific airway conductance after PGF₂α occurred 6 minutes after inhalation and recovery took place within 30 minutes. Asthmatic subjects experienced an approximate 150 times greater sensitivity to PGF₂α than did healthy controls, yes asthmatics were only 8.5 times more sensitive to histamine than nonasthmatic
there was decreased variation in individual responses to histamine compared to inhaled PGF$_{2\alpha}$ challenge, but sensitivity to both drugs correlated with each other. In general, women had a diminished bronchoconstrictor response to PGF$_{2\alpha}$ compared to men. Both PGE$_2$ and isoprenaline shortened recovery from the decrease in pulmonary function caused by inhalation of PGF$_{2\alpha}$; however, neither atropine, disodium cromoglycate, nor flufenamic acid prevented PGF$_{2\alpha}$-induced bronchoconstriction. PGF$_{2\alpha}$ (and PGE$_2$ as well) decreased exhaled nitric oxide (NO) concentrations in both health controls and asthmatic subjects; however, the meaning of this outcome is unknown. Although FP is not expressed on immune cells, there is some evidence that PGF$_{2\alpha}$ may have a role on airway inflammation. In subjects with asthma, the magnitude of sputum eosinophilia correlated with the log sputum PGF$_{2\alpha}$ concentrations, while there was a negative correlation between sputum eosinophilia and PGE$_2$ levels and no correlation between the number of sputum eosinophils and sputum levels of cys-LTs, thromboxane, and PGD$_2$.

**Mouse studies of PGF$_{2\alpha}$ in allergic inflammation**

To our knowledge, there are no published studies examining the effect of PGF$_{2\alpha}$ administration or signaling through the FP receptor in the mouse allergen challenge model. An FP-deficient mouse has been created and these mice had attenuated bleomycin-induced pulmonary fibrosis independent of TGF-β expression. Therefore, one might expect that FP-deficient mice might have increased collagen deposition and airway wall remodeling in a chronic allergen challenge model.
**Prostaglandin I₂ (prostacyclin)**

PGI₂ is converted from PGH₂ by PGI synthase (PGIS). The gene encoding PGIS is located on chromosome 20q13.11-13. PGIS is strongly expressed in the heart, lung, smooth muscle, kidney, and ovary and expressed at moderate levels in the brain, pancreas, and prostate. There is low level PGIS expression in the placenta, spleen, and leukocytes. PGI₂ signals through its receptor, IP, a GPCR (Table 4). Binding of PGI₂ to its receptor activates adenylate cyclase via G₄ in a dose-dependent manner, increasing the production of cAMP. This increase in intracellular cAMP mediates PGI₂’s effect of inhibiting platelet aggregation, and dispersing existing platelet aggregates both *in vitro* and in human circulation. Northern blot analysis reveals that IP mRNA is expressed to the greatest degree in the thymus, while high level of IP mRNA expression is also found in spleen, heart, lung, and neurons in the dorsal root ganglia. IP is also expressed on mouse bone marrow-derived dendritic cells (BMDC). The PGI₂ analogs iloprost and cicaprost decreased BMDC production of proinflammatory cytokines (IL-12, TNF-alpha, IL-1alpha, IL-6) and chemokines (MIP-1alpha, MCP-1), while these analogs increased the production of the anti-inflammatory cytokine IL-10 by BMDCs. The modulatory effect was associated with IP-dependent up-regulation of intracellular cAMP and down-regulation of NF-kappaB activity. Iloprost and cicaprost also suppressed LPS-induced expression of CD86, CD40, and MHC class II molecules by BMDCs and inhibited the ability of BMDCs to stimulate antigen-specific CD4 T cell proliferation and production of IL-5 and IL-13. Iloprost also enhanced human dendritic cell production of IL-10 and in co-culture experiments of iloprost-treated dendritic cells and naïve T cells, there was induction of T
regulatory cells.\textsuperscript{127} IP is also expressed in T cells of mice, along with the PGE\textsubscript{2} receptor (EP) subtypes and the thromboxane receptor (TP).\textsuperscript{128} IP has also been found in kidney smooth muscle and epithelial cells.\textsuperscript{129} Messenger RNA for IP is expressed in both CD4\textsuperscript{+} Th1 and Th2 cells.\textsuperscript{130} Thus, IP has been located on several different cell types, including those critical to the adaptive immune response.

**Human studies of PGI\textsubscript{2} in allergic inflammation**

PGI\textsubscript{2} and PGD\textsubscript{2} were the predominant COX products produced in antigen-induced anaphylactic reactions of human lung parenchyma, on the order of 3- to 7-times greater concentrations than that of the other prostanoids.\textsuperscript{131} The PGI\textsubscript{2} metabolite 6-keto-PGF\textsubscript{1\alpha} was produced in concentrations two-to-three-fold higher than all the other prostanoids in both airway and subpleural lung fragments in an \textit{in vitro} anaphylaxis assay of passively sensitized human lung.\textsuperscript{132} Plasma 6-keto-PGF\textsubscript{1\alpha} was also increased following antigen challenge in which asthmatic subjects were pretreated with indomethacin.\textsuperscript{133} Thus, PGI\textsubscript{2} is produced in abundance in allergic inflammatory responses in the lung, presumably a reflection of activated endothelial cells that express almost all the PGIS in the human airway.

Most of the published intervention studies examining the modulatory effect of PGI\textsubscript{2} in human asthma were performed over 15 years ago and the limitations of these older reports is that PGI\textsubscript{2} (half-life 3-5 minutes) was used, rather than the more recently developed stable analogs. Therefore, these studies may not accurately reflect the therapeutic capability of the currently available class of PGI\textsubscript{2} agents. Pretreatment with PGI\textsubscript{2} had no effect on allergen-induced immediate phase bronchoconstriction.\textsuperscript{134} In another study, PGI\textsubscript{2} protected against both exercise
and ultrasonic water-induced bronchospasm, yet again had no effect on allergen-induced airway reactivity.\textsuperscript{135} Inhaled PGI\textsubscript{2} also had no effect on specific airway conductance, but did result in consistent bronchodilation in two of the asthmatic subjects. In this study, there was a significant effect of PGI\textsubscript{2} on the cardiovascular system. Inhaled PGI\textsubscript{2} resulted in a fall in both diastolic (20±3 mmHg) and systolic (8±2 mmHg) blood pressure, as well as an increased pulse rate (29±3 beats per minute).\textsuperscript{136} Intravenous PGI\textsubscript{2} administration had no effect on the fall in airflow induced by aspirin in subjects with aspirin-induced asthma.\textsuperscript{137} Somewhat contradictory results of the effect of inhaled PGI\textsubscript{2} in subjects with mild asthma have been reported.\textsuperscript{138} In these studies PGI\textsubscript{2} did not alter specific airway conductance, but resulted in a concentration-dependent decrease in FEV\textsubscript{1}. In contrast, these same investigators found that PGI\textsubscript{2} protected against bronchoconstriction induced by either PGD\textsubscript{2} or methacholine. The authors proposed that these disparate findings might be explained by PGI\textsubscript{2}’s marked vasodilator effect, resulting in airway narrowing through mucosal blood engorgement, while this same phenomenon possibly reduced the spasmogenic properties of other inhaled mediators by increasing their clearance from the airways. An oral PGI\textsubscript{2} analog (OP-41483) had no effect on FEV\textsubscript{1} or airways responsiveness to methacholine in stable asthmatics.\textsuperscript{139} Since this last report which was published in 1991, to our knowledge, there have been no other published manuscripts examining PGI\textsubscript{2} in human allergic inflammation in the lung or asthma. The therapeutic potential of newer, more stable PGI\textsubscript{2} analogs in asthma remains unexplored.

\textbf{Mouse studies of PGI\textsubscript{2} in allergic inflammation}
Four studies using mouse models suggest that endogenous PGI₂ signaling through IP is involved in homeostatic control of airway inflammation. In a model of short-term OVA challenge, IP-deficient mice had increased lung production of IL-4 and IL-5, serum antigen-specific and total IgE levels, and airway leukocyte accumulation compared to wild type mice.¹⁴⁰ In another study, the period of allergen challenge was extended to generate signatures of “chronic” allergen exposure. In this study, IP-deficient mice had greater airway eosinophils and lymphocytes, Th2 cytokine levels, and hydroxyproline concentrations compared to wild type mice.¹⁴¹ Mice that lacked the ability to signal through IP had augmented inflammatory and physiologic changes compared to WT mice in the model of bleomycin-induced fibrosis.¹⁴² In another bleomycin model of lung injury, mice that overexpressed PGIS in airway epithelial cells were protected against lung injury and had decreased production of F₂-isoprostanes, marker of oxidant injury. In these experiments, PGI₂ induced the expression of Nqo1, an enzyme that prevents generation of reactive oxidant species.¹⁴³ Supporting the concept that PGI₂ restrains airway inflammation, inhaled iloprost inhibited the maturation and migration of lung DCs to the mediastinal LNs following intranasal antigen administration, resulting in decreased induction of an allergen-specific Th2 response in these nodes.¹⁴⁴ In this in vivo model, iloprost-treated DCs also downregulated Th2 differentiation from naive T cells and were unable to boost effector cytokine production in primed Th2 cells.¹⁴⁴ While these results in animal models of allergic inflammation are encouraging for the use of PGI₂ in the treatment of allergic airway inflammation, cost and difficulty in drug delivery are currently obstacles.¹⁴⁵;¹⁴⁶ However, the development of less expensive and longer acting agonists may make PGI2 a viable therapeutic option.
Thromboxane A\textsubscript{2} (TXA\textsubscript{2}) is the principal product of arachidonic acid metabolism formed by platelets and is a potent platelet aggregating agent.\textsuperscript{147} Thromboxane synthase (TXAS) is an endoplasmic reticulum membrane protein that catalyzes the conversion of prostaglandin H\textsubscript{2} to thromboxane A\textsubscript{2}.\textsuperscript{148} TXAS is a member of the cytochrome P450 superfamily and is localized to band q33-q34 of the long arm of chromosome 7 in humans.\textsuperscript{148} TXAS is expressed abundantly in lung, liver, kidney, and blood cells, including megakaryocytes and monocytes.\textsuperscript{148} Lower, but significant, levels of TXAS mRNA are observed in kidney, placenta and thymus.\textsuperscript{148} TXA\textsubscript{2} is principally produced by platelets, monocytes, macrophages, neutrophils and lung parenchyma.\textsuperscript{149} After it is formed, TXA\textsubscript{2} is nonezymatically hydrolyzed to thromboxane B\textsubscript{2}, which is further metabolized to the principle urinary metabolites 2,3-dinor-thromboxane B\textsubscript{2} and 11-dehydro-thromboxane B\textsubscript{2}.\textsuperscript{150} The TXA\textsubscript{2} receptor is termed TP (Table 4) and there are two isoforms, TP\(\alpha\) and TP\(\beta\), which are produced by alternative splicing occurring in the carboxy-terminal region after the seventh transmembrane domain.\textsuperscript{151} Both of these isoforms functionally couple to a Gq protein resulting in phospholipase C activation, calcium release, and activation of protein kinase C.\textsuperscript{152} However, these receptor isoforms couple oppositely to adenylate cyclase as TP\(\alpha\) activates adenylate cyclase while TP\(\beta\) inhibits this enzyme.\textsuperscript{153} The TP receptors are localized to both plasma membrane and cytosolic compartments and are mainly distributed in tissues rich in vasculature such as lung, heart and kidney.\textsuperscript{117} These GPCRs are involved in a multitude of physiological and pathological processes such as vasoconstriction implicated in vascular diseases such as hypertension, stroke, atherosclerosis, and myocardial infarction.\textsuperscript{154}
Human studies of TXA₂ in allergic inflammation

TXA₂ has a half-life of approximately 30 seconds,¹⁵⁵ and because of this lability there is a paucity of in vivo studies examining the effect of TXA₂ in the human airway. Although TXB₂ did not cause bronchoconstriction of human airway in vivo.¹⁵⁶ However, TXA₂ was a potent stimulant of in vitro smooth muscle constriction.¹⁴⁷ There is data that suggests that TXA₂ might have a role in the physiology associated with acute asthma exacerbations. Levels of TXA₂ metabolites were increased 4-6 fold in the urine of patients admitted to the hospital with asthma compared to non-smoking controls admitted for other diagnoses.¹⁵⁶ Allergic asthmatics subjected to inhaled allergen challenge had a significant increase in urinary excretion of TXA₂ products,²⁴,¹⁵⁷ yet others have not found similar results.¹⁵⁶ Inhibition of platelet COX by low dose aspirin inhibited the increase in urinary 2,3 dimer thromboxane, supporting that allergen inhalation causes platelet activation. Allergic asthmatics pre-treated with indomethacin before inhaled allergen challenge resulted in a significant decline in urinary TXA₂ metabolites; however, no change in pulmonary function was noted.²⁴ Subjects that experience ozone-induced airway hyperresponsiveness had significant increases in BAL concentrations of TXA₂ and airway neutrophilia.¹⁵⁸ Similarly, LTB₄ inhalation also resulted in increased levels of TXA₂ and neutrophils in BAL fluid.¹⁵⁹

Thromboxane A₂ antagonists have been used in challenge models to determine thromboxane’s effect on allergen-induced pulmonary function. In a non-randomized,
uncontrolled study the TP antagonist seratrodast (AA-2414) significantly reduced bronchial reactivity in asthmatic subjects after 4 weeks of once daily therapy compared to a pre-treatment baseline. In this study, seratrodast had no effect on either exhaled nitric oxide or on the percentage of eosinophils in sputum. In a follow-up double blind, placebo-controlled study of asthmatic treated for four weeks, seratrodast treatment resulted in significant improvements in symptom score, peak expiratory flow (PEF) rates, diurnal variation of PEF, and bronchial responsiveness compared with the placebo group. These improvements were associated with a significant reduction in the number of submucosal eosinophils on bronchial biopsy. Seratrodast treatment resulted in a significant decrease in the number of cells in the epithelium expressing RANTES and macrophage inflammatory protein (MIP)-1α, as well as a diminished number of cells in the submucosa expressing monocyte chemotactic protein-3, RANTES, MIP-1α, and eotaxin. These findings suggest that thromboxane antagonism may reduce allergic inflammation in the lung, although the mechanisms are not well defined.

**Mouse studies of TXA2 in allergic inflammation**

Both the TXA2 synthase inhibitor OKY-046 and the TP receptor antagonist S-1452 significantly decreased the number of total cells and eosinophils in BAL fluid in a dose responsive relationship in OVA-sensitized and challenged mice. Treatment with either the TXA2 synthase inhibitor or the TP receptor antagonist significantly inhibited antigen-specific activation of splenic mononuclear cells from sensitized mice in ex vivo experiments as defined by pro-inflammatory cytokine production.
LIPOXYGENASE PATHWAY

As is the case for prostanoids, arachidonic acid liberated by group IV cPLA2 is the precursor for lipoxygenase (LO) pathway products. Two major enzymes, 5-LO and 15-LO metabolize arachidonate in the initial steps that form distinctive respective mediator classes (Figure 2). The latter enzyme catalyzes the hydroperoxidation of arachidonic acid by the insertion of one molecule of oxygen at position 15 to form 15-HPETE, as well as the insertion of molecular oxygen into other polyunsaturated fatty acids and phospholipids. The 15-LO pathway is responsible for forming 15-hydroxyeicosatetraenoic acid (15-HETE) and the dihydroxy acids 8,15-diHETE and 14,15-diHETE. 5-LO translocates in a Ca2+-dependent manner from either the cytoplasm or the nucleus to the perinuclear membrane and catalyzes the insertion of molecular oxygen into arachidonic acid to produce 5-hydroperoxyeicosatetraenoic acid (5-HPETE). 5-HPETE can then either be dehydrated to leukotriene (LT) A4 by 5-LO, or reduced to 5-hydroxyeicosatetraenoic acid (5-HETE) and further converted to the 5-oxo-ETEs. Both of these catalytic functions require 5-LO activating protein (FLAP), an integral perinuclear membrane protein that transfers free arachidonic acid to 5-LO and is essential for the 5-LO function of generating LTA4. FLAP is a member of the structurally homologous microsome-associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) family, which includes mPGES-1, and LTC4 synthase (LTC4S).

Leukotrienes
Leukotrienes, named for their cells of origin (leukocytes) and three positionally conserved double bonds (triens) are potent inflammatory mediators generated from the unstable precursor LTA₄.¹⁶⁵ There are two distinct classes of leukotrienes; LTB₄ is a dihydroxyl compound formed by a cytosolic LTA₄ hydrolase (LTA₄H). LTA₄H is expressed by mast cells, macrophages, and neutrophils, the major cellular sources of LTB₄ in vivo. The human gene encoding LTA₄H maps to chromosome 12q22.¹⁶⁶ LTA₄ can also be conjugated to reduced glutathione to form LTC₄, the parent molecule of the cys-LTs by leukotriene C₄ synthase (LTC₄S), an integral perinuclear protein and member of the MAPEG family.¹⁰³;¹⁶⁷ The major cellular sources of cys-LTs are eosinophils, basophils, mast cells and macrophages, each of which express LTC₄S. LTC₄S expression is sharply upregulated in human mast cells by IL-4 STAT6-dependent transcription, potentially reflecting a mechanism for upregulating cys-LT production in allergic inflammation.¹⁶⁸ The gene encoding LTC₄S in humans maps to chromosome 5q35, distal to the Th2 cytokine gene cluster.¹⁶⁹ Both LTB₄ and LTC₄ are exported by specific respective transporter proteins. LTC₄ is converted to LTD₄ extracellularly by cleavage of glutamic acid is cleaved from the glutathione moiety by γ-glutamyl-transpeptidase, or by a more specific γ-glutamyl-leukotrienease.¹⁷⁰ A dipeptidase then cleaves glycine from LTD₄ to form LTE₄.¹⁷⁰

Leukotrienes in human studies of allergic inflammation and asthma

Leukotriene B₄ and the cys-LTs were increased in exhaled breath condensate from asthmatic subjects compared to healthy controls.¹⁷¹ After allergen challenge, there was a
significant increase in leukotriene levels in the BAL fluid of allergic subjects and this was associated with increased eosinophilic inflammation and bronchial responsiveness.\textsuperscript{172}

Leukotrienes can be measured in induced sputum and the levels of these mediators were not only increased in asthmatic subjects compared to nonasthmatic control subjects, but also correlated with severity of disease.\textsuperscript{74} Urinary LTE\textsubscript{4} is also increased in asthmatic subjects compared to controls and in proportion to disease severity.\textsuperscript{173} Therefore, leukotrienes can be measured in a wide range of biologic fluids and reflect ongoing inflammation in the lung and the physiologic changes associated with asthma. Corticosteroid treatment had no impact of leukotriene levels in asthmatics, suggesting that leukotriene generation is relatively resistant to this class of anti-inflammatory medication.\textsuperscript{171;174}

5-LO inhibitors block the formation of both LTB\textsubscript{4} and the cys-LTs, and as such provide insight into the role of both of these groups of mediators in the pathogenesis of both allergic disease and asthma. As mentioned above, both 5-LO and FLAP are critical to the generation of leukotrienes. While there is abundant clinical experience with 5-LO inhibitors, no FLAP inhibitors have been approved for human use. Zileuton is a 5-LO inhibitor and its activity is presumed to its ability to chelate iron at the active site of 5-LO, thus preventing its redox potential.\textsuperscript{175} In a study of asthmatics with mild-to-moderate disease, zileuton administration produced a 350 ml (15\% from pretreatment baseline) increase in FEV\textsubscript{1} within one hour and was statistically increased compared to placebo. After a four week study period, there was also a significant improvement in peak expiratory flow rate in the zileuton-treated subjects (600 mg four times daily) compared to placebo. Of note, after the four weeks of treatment, zileuton reduced urinary LTE\textsubscript{4} levels by only approximately 40\% reduction compared to the placebo group, indicating that even the highest clinically recommended dose of the 5-LO inhibitor did
not fully block leukotriene generation. In another 13 week study of asthmatic subjects who had FEV₁ between 40 and 80% predicted, zileuton significantly decreased the need for rescue β-agonists, reduced daytime and night time symptoms, increased symptom free days and nights compared to placebo. In this trial, zileuton also significantly reduced the number of subjects requiring corticosteroid therapy for an asthma exacerbation. In a trial to investigate the role of 5-LO inhibition on inflammation following segmental allergen challenge, zileuton inhibited urinary LTE₄ production by 86% and prevented the increase in BAL eosinophils that was noted in the placebo-treated subjects. The fact that 5-LO inhibition blunts allergen-induced inflammation while improving lung function and symptoms in asthmatic subjects validates the role of leukotrienes in asthma pathogenesis. What is somewhat disappointing is, beyond zileuton, no further 5-LO inhibitors have made it to clinical use for reasons that are unclear, although zileuton disturbed liver function has been a problem. In general, FLAP inhibitors have looked more promising.

**Murine studies of leukotriene inhibition**

Unlike human airways, mouse airways are resistant to the bronchoconstrictive effects of the cys-LTs. Nonetheless, mouse models of allergen-induced pulmonary inflammation have uncovered key roles for these mediators in the induction and amplification of this process. Mice that lack 5-LO as a result of targeted gene disruption have decreased allergen-induced BAL eosinophilia, serum IgE, and airway responsiveness compared to wild type mice. A similar phenotype was described for mice lacking group IVA PLA₂, which generates neither leukotrienes nor prostaglandins. Similarly, zileuton reduced allergen-induced leukotriene
release in the BAL and eosinophil recruitment to the lungs, while dose dependently reducing AHR, mucus accumulation, and remodeling.\textsuperscript{182} Specific inhibitors of 5-LO and FLAP independently blocked airway mucus release and airway infiltration by eosinophils indicating a key role for leukotrienes in these features of allergic pulmonary inflammation; however, they had no effect on allergen-induced airway responsiveness.\textsuperscript{183} Zileuton treatment blocked airway responsiveness and inflammation that occurred as a result of intratracheal instillation of IL-13, monocyte chemoattractant protein (MCP)-1, MCP-5, and KC, the mouse homolog of IL-8.\textsuperscript{184} It is also interesting that zileuton suppressed PGE\(_2\) and 6-keto-PGF\(_{1\alpha}\) production by mouse macrophages, presumably by interfering with arachidonic acid release.\textsuperscript{185} Thus, the effects of pharmacologic and genetic deletion of 5-LO pathway products in mice are profound and reflect the composite loss of both cys-LT and LTB\(_4\)-mediated actions (see below).

**LTB\(_4\)**

LTB\(_4\) exerts its biologic effects by signaling through two distinct GPCRs, BLT\(_1\) and BLT\(_2\).\textsuperscript{186} LTB\(_4\) binds with much greater affinity to BLT\(_1\) compared to BLT\(_2\).\textsuperscript{186} Other eicosanoids, specifically 12(s)-HETE, 12(S)-HPETE, and 15(S)-HETE can bind BLT\(_2\), but do not bind BLT\(_1\).\textsuperscript{186} BLT\(_1\) is predominantly expressed on activated leukocytes, with decreased expression in spleen, thymus, bone marrow, lymph nodes, heart, skeletal muscle, brain, and liver.\textsuperscript{186} BLT\(_2\) is expressed in most human tissues with the greatest degree in spleen, liver, ovary, and peripheral blood white cells.\textsuperscript{186} Among the leukocytes, there are some significant differences in LTB\(_4\) receptor expression.\textsuperscript{186} For instance, neutrophils and eosinophils both express high levels of both BLT\(_1\) and BLT\(_2\), while mononuclear cells express high levels of
BLT₂, but very low levels of BLT₁.¹⁸⁶ BLT₁ expression is upregulated on activated cells and expression is increased by both interferon-γ (IFN-γ) and glucocorticoids.¹⁸⁶ The primary known function of LTB₄ receptor signaling is leukocyte recruitment.¹⁸⁶ LTB₄ is a leukocyte chemoattractant and also changes leukocyte rolling to firm attachment through its upregulation of the integrin CD11b/CD18 on neutrophils.¹⁸⁶ With these properties, exogenous LTB₄ administration in both the skin and airways caused neutrophils migration to those sites.

**Human studies of LTB₄ in allergic inflammation**

To date, there are no intervention studies using specific BLT₁ or BLT₂ antagonists or agonists to examine the role of signaling through these receptors in modulating allergic inflammation in humans. However, there are recent studies which suggest that signaling through these receptors may regulate the allergic phenotype. For instance, in healthy subjects, T cells that express BLT₁ are rare in peripheral blood, but do express the activation markers CD38 and HLA-DR.¹⁸⁷ When compared to T cells that do not express BLT₁, a larger proportion of peripheral blood expressing T cells also express the effector cytokines IFN-γ and IL-4, as well as inflammatory chemokine receptors, CCR1, CCR2, CCR6, and CXCR1.¹⁸⁷ T cell BLT₁ expression is tightly regulated by inflammation and only expressed transiently after naive T lymphocytes are activated by dendritic cells. The number of peripheral blood T cells expressing BLT₁ was increased in frequency in the airways of asymptomatic allergic asthmatics.¹⁸⁷ LTB₄, via BLT₁, is strongly chemotactic for mast cell progenitors *in vitro*.¹⁸⁸ Activated mature mast cells produced LTB₄ which was highly chemotactic for 2-week-old mast cells that expressed high levels of mRNA for BLT₁ while expression of this receptor was not present on mature mast
There was also accumulation of immature cells \textit{in vivo} in response to intradermally injected LTB_4, and LTB_4 was extremely potent in recruiting mast cell progenitors from freshly isolated bone marrow cell suspensions.\textsuperscript{188} Additionally, LTB_4 was a potent chemoattractant for human cord blood-derived immature, but not mature, mast cells.\textsuperscript{188} To date, there are no published studies in humans or human cells examining the effect of signaling through BLT_2 on the regulation of allergic inflammation.


\textbf{Mouse studies of LTB_4 in allergic inflammation}

BLT_1 expression was low in naïve murine CD4+ T cells, but strong in activated Th0, Th1, and Th2 cells, while BLT_2 was not expressed in these cell populations.\textsuperscript{189} LTB_4 induced CD4+ T cell chemotaxis in wild type mice, but not in BLT_1-deficient mice, signifying the receptor specificity for this chemotaxis.\textsuperscript{189} BLT_1-deficient mice had decreased number of both CD4+ and CD8+ T cells in BAL fluid after one and two days, but not after three days of allergen challenge in which mice had been sensitized first with an intraperitoneal injection of OVA and the adjuvant aluminum hydroxide, while there was no difference in the numbers of CD4+ and CD8+ cells in the lung parenchyma. BLT_1-deficient and wild type mice did not differ in the expression of chemokines responsible for T cell recruitment, suggesting that reduced effector T cell trafficking into the airway in BLT_1-deficient mice was a direct consequence of the absence of LTB_4-BLT_1 signaling.\textsuperscript{189} In this model, there were no differences in serum IgE levels between the BLT_1-deficient and wild type mice, and neither airway eosinophilia nor mucus expression was reported. However, adoptively transferred antigen-specific transgenic T cells did not require BLT_1 for antigen-induced recruitment to the lungs of naïve mice.\textsuperscript{189} Thus, the role of
BLT<sub>1</sub> signaling in the setting of allergen challenge is dependent on the model used. Another group using OVA/aluminum hydroxide sensitization model found, that BLT<sub>1</sub>-deficient mice were protected from AHR, eosinophilic inflammation, and hyperplasia of goblet cells. These BLT<sub>1</sub>-deficient mice also had reduced IgE production, and levels of IL-5 and IL-13 in bronchoalveolar lavage fluid, suggesting BLT<sub>1</sub> signaling was critical for the generation of a Th2-type immune response. Another group found similar results in allergen-sensitized and challenged BLT<sub>1</sub>-deficient mice and was able to increase allergic inflammation by transfer of allergen-primed wild type T cells into the BLT<sub>1</sub>-deficient mice that were subsequently allergen challenged. Signaling through BLT<sub>1</sub> has also been proposed to mediate CD8-dependent allergic airway inflammation and AHR. CD8-deficient mice that were adoptively transferred CD8 cells from allergen-sensitized wild type, but not BLT<sub>1</sub>-deficient mice developed allergen inflammation and AHR. There have been no reports of murine studies examining the BLT<sub>2</sub> receptor in regulating allergic inflammation, although dendritic cells from mice deficient in both BLT<sub>1</sub> and BLT<sub>2</sub> had a marked defect in the ability to migrate to draining lymph nodes compared to dendritic cells from wild type mice.

**Cysteinyi leukotrienes**

Cys-LTs signal through at least two GPCRs, CysLT<sub>1</sub> and CysLT<sub>2</sub> (Table 5). While CysLT<sub>1</sub> and CysLT<sub>2</sub> appear to be the major CysLT receptors, LTE<sub>4</sub> was recently also found to signal through two other distinct receptors. P2Y<sub>12</sub> was identified as an LTE<sub>4</sub> receptor and was required for LTE<sub>4</sub>-induced pulmonary inflammation. In addition, PPARγ cooperated with an MK571-sensitive GPCR to mediate human mast cell LTE<sub>4</sub>-activated COX-2 induction, PGD<sub>2</sub>
generation, and ERK phosphorylation.\textsuperscript{196} In humans, CysLT\textsubscript{1} maps to the X chromosome, while CysLT\textsubscript{2} maps to chromosome 13q14.\textsuperscript{165} CysLT\textsubscript{1} binds LTD\textsubscript{4} with high affinity and LTC\textsubscript{4} and LTE\textsubscript{4} equally with lower affinity.\textsuperscript{165} In contrast, CysLT\textsubscript{2} binds LTC\textsubscript{4} and LTD\textsubscript{4} equally and with greater affinity than LTE\textsubscript{4}.\textsuperscript{165} In humans, CysLT\textsubscript{1} is expressed in the spleen, lung, placenta, and small intestine.\textsuperscript{165} Specific cell types on which CysLT\textsubscript{1} is expressed include bronchial smooth muscle, glandular epithelium, monocyte/macrophages, mast cells, basophils, eosinophils, dendritic cells, B and T lymphocytes, CD34\textsuperscript{+} hematopoietic progenitors, neutrophils, and human umbilical vein endothelial cells.\textsuperscript{165;197;198} CysLT\textsubscript{1} expression can be upregulated on peripheral blood mononuclear cells by IL-4 and IL-13, or on an eosinophilic leukemic cell line by IL-5, with a resulting increase in expression of CysLT\textsubscript{1} leading to enhanced chemotaxis to LTD\textsubscript{4}.\textsuperscript{165} CysLT\textsubscript{2} is expressed in the lung, spleen, heart, lymph nodes, and brain. Cells expressing CysLT\textsubscript{2} include monocyte/macrophages, mast cells, eosinophils, cardiac, Purkinje cells, bronchial smooth muscle, coronary smooth muscle, and human umbilical vein endothelial cells. CysLT\textsubscript{1} and CysLT\textsubscript{2} have been shown to interact at least on human mast cells, and possibly on other cells as well. CysLT\textsubscript{2} inhibited CysLT\textsubscript{1} mast cell surface expression and CysLT\textsubscript{1}-dependent proliferation of cord blood-derived mast cells.\textsuperscript{199} The specific CysLT\textsubscript{1} receptor antagonists such as montelukast, zafirlukast, and pranlukast have been critical in determining the effects of signaling through this receptor in biological systems and in humans \textit{in vivo}. Signaling through CysLT\textsubscript{1} dilates blood vessels with a resultant increase in vascular permeability, amplified mucus expression, bronchial smooth muscle constriction, and inflammatory cell recruitment. Specifically, CysLT\textsubscript{1} signaling augments transendothelial migration of CD34\textsuperscript{+} hematopoietic cells and eosinophil chemotaxis \textit{in vitro}. In addition, cys-LTs increase IgE-mediated mast cell production of IL-5 and TNF via the CysLT\textsubscript{1} receptor and regulate mast cell proliferation by
inducing transactivation of the c-Kit receptor tyrosine kinase.\textsuperscript{200} The consequences of CysLT\textsubscript{2}-mediated signaling are far less well understood than that of CysLT\textsubscript{1} signaling because there are no specific CysLT\textsubscript{2} receptor antagonists. Therefore, CysLT\textsubscript{2} receptor mediated properties in humans are inferred from studies in which the effects of the cys-LTs are not abrogated by CysLT\textsubscript{1} receptor antagonists. A primary role of CysLT\textsubscript{2} signaling may be activation of endothelial cells in vascular responses, as indicated by the CysLT\textsubscript{2}-dominant signaling reported in human umbilical vein endothelial cells.\textsuperscript{165}

**Human studies of cysteinyi leukotrienes in allergic inflammation**

Site-directed allergen challenge increases the concentrations of cys-LTs in the skin, eye, nose, and lung and these levels are strongly correlated with allergic symptoms.\textsuperscript{171,201,202} Direct administration of cys-LTs into the human airways confirms that these lipids are the most potent known bronchoconstrictors, are pro-inflammatory, and that their effects are receptor mediated. In asthmatic subjects, inhaled LTE\textsubscript{4} increased airway eosinophils and this eosinophils and this is blocked by treatment with the cysLT\textsubscript{1} receptor antagonist zafirlukast.\textsuperscript{203} CysLT\textsubscript{1} antagonists have been used in clinical trials of allergic conditions such as asthma, rhinitis, and urticaria and these studies reveal that signaling through this receptor is involved in the pathogenesis of these diseases. In a 12-week study of asthmatics with FEV\textsubscript{1} 50-85\% predicted, montelukast significantly improved pulmonary function and symptoms compared to placebo, but was not as effective as inhaled corticosteroids for the same endpoints.\textsuperscript{204} CysLT\textsubscript{1} receptor antagonists have also been shown to inhibit both early and late phase pulmonary reactions to allergen challenge as well as the airway obstruction medicated by exercise, SO\textsubscript{2}, hyperpnea, adenosine 5’-
monophosphate, and mannitol. In clinical studies of rhinitis, CysLT1 receptor antagonists have reduced rhinorrhea, nasal congestion, and sneezing, while improving daytime and nighttime symptoms, although there is variability between studies in the effectiveness of this medication class on these individual endpoints. CysLT1 antagonists have also been shown to reduce the symptoms of chronic urticaria compared to placebo. Several of these clinical trials identified significant suppression of blood and/or tissue eosinophil counts by the administration of CysLT1 antagonists, implying a role for cys-LTs in regulating eosinophil homeostasis.

**Mouse studies of cys-LTs in allergic inflammation**

In a “mast cell-dependent” model of OVA-induced pulmonary inflammation, BALB/c mice lacking LTC4S showed strikingly diminished AHR, goblet cell metaplasia, OVA-specific IgE, and cytokine production by restimulated lymph node cells when compared with wild-type, allergen-treated control mice. These mice also showed strikingly decreased mast cell numbers in the bronchial epithelium compared to wild-type controls. In another model of OVA-induced allergic pulmonary inflammation, wild-type BALB/c mice were treated with a long (76 day) period of allergen challenge to induce changes of airway remodeling. In this study, the administration of the CysLT1 receptor-selective antagonist montelukast during the challenge phase decreased lung expression of IL-4 and IL-5, an decreased bronchial eosinophil numbers, while inhibiting smooth muscle hypertrophy and collagen deposition. In this model, inflammation subsided after cessation of the allergen challenge, but the smooth muscle hypertrophy and collagen deposition persisted out to three months. Interestingly, montelukast reversed the remodeling signatures when administered from days 73-163 (after the challenge
phase of the experiment), whereas dexamethasone did not.\textsuperscript{215} Thus cys-LTs orchestrate both sensitization and remodeling events in models of allergic pulmonary inflammation in mice, consistent with their abundant generation by mast cells and eosinophils, as well as the broad distribution of their receptors on both hematopoietic and structural cells in the lung.

When pulsed \textit{ex vivo} with dust mite antigen, mouse myeloid dendritic cells exposed to exogenous LTD\(_4\) show augmented production of IL-10 and attenuated IL-12 generation compared with antigen pulsing alone.\textsuperscript{216} Treatment of these DCs with CysLT\(_1\) receptor-selective antagonists during antigen pulsing attenuates IL-10 generation and augments IL-12 production.\textsuperscript{216} Myeloid dendritic cells treated with CysLT\(_1\) antagonists during stimulation with dust mite allergen \textit{in vitro} were substantially less able to support an eosinophil-dominated inflammatory response to inhaled allergen following when adoptively transferred into the tracheas of naïve recipient mice.\textsuperscript{216} Thus as is the case for PGD\(_2\), cys-LTs participate in the regulation of dendritic cell maturation responses. Whether cys-LTs, like LTB\(_4\), also act on T cells remains to be determined.

\textbf{Lipoxins}

Lipoxins (LXs) are produced at sites of either vascular or tissue injury and abundant data suggest that these are involved in resolution of inflammation (reviewed in \textsuperscript{217}). LX can be formed by several pathways. In the vasculature, LTA\(_4\) produced by activated leukocytes can be converted to LX by platelet 12-LO. In the lung parenchyma, LTA\(_4\) can be converted to LX by epithelial cell 15-LO. LXs can also be formed by transformation of 15-LO–derived 15-hydroperoxy-eicosatetraenoic acid (15-H(p)ETE) by 5-LO. 15-epimer-LXs are produced by 5-
LO-mediated conversion of 15(R)-hydroxy-eicosatetraenoic acid (15(R)-HETE) to 15-epi-LXA₄ and 15-epi-LXB₄. Interestingly, statins also demonstrate the capability of inducing 15-epi-LXA₄ formation. In addition, cell-cell interactions between neutrophils and airway epithelial cells in the presence of statins leads to 15-epi-LXA₄ biosynthesis.

LXA₄ and 15-epi-LXA₄ are both agonists for a LXA₄ receptor termed ALX/FPR2, a GPCR that binds these lipid products with high affinity. ALX is expressed on both human airway epithelial cells and leukocytes, and can be induced by specific inflammatory mediators. In addition to LXs signaling through ALX, LXs can also act as antagonists at CysLT₁ receptors and can also signal via the aryl hydrocarbon receptor. LXs can inhibit granulocyte locomotion, shape change, transmigration, and degranulation. Through these actions, LXs are both anti-inflammatory for neutrophils and eosinophils, and appear to have an important role in the clearance of inflamed tissue by monocytes and macrophages. In addition to these leukocyte-specific actions, LXs promote restoration of injured airway epithelium by stimulating bronchial basal epithelial cell proliferation, blocking the release of the proinflammatory cytokines IL-6 and IL-8, and inhibiting neutrophil transmigration across differentiated human bronchial cells. Injury upregulates ALX receptor expression in both the proximal and distal airway. In line with promoting resolution of injury, LXs inhibit inflammation-induced angiogenesis and endothelial cell migration in response to proinflammatory mediators.

Animal models reveal that LXs block inflammation and its consequences in several different models of lung injury. Allergic airway inflammation is significantly reduced by LXs in mouse models and resolution occurs more quickly compared to vehicle-treatment. Similar findings have been published for a rat model of allergic pleurisy, bleomycin-induced fibrosis in mice, acid-induced acute lung injury, and pneumonia. LXs are decreased in the whole blood,
sputum, and BAL fluids of persons with severe asthma, suggesting that decreased generation of
LXs, and therefore inability to resolved inflammation, may be responsible for the severe
phenotype. There is also a decrease in LXs in subjects with aspirin-intolerant asthma, exercise-
induced asthma, scleroderma lung disease, and cystic fibrosis compared to healthy persons.
Further research in the LX field is necessary to determine if these lipids may serve a therapeutic
purpose.

Isoprostanes

Isoprostanes (IsoPs) are prostaglandin-like molecules that are not formed by the action of
the COX enzymes, but instead are formed by the free radical-catalyzed peroxidation of
arachidonic acid. IsoP formation is not only a dependable marker of oxidant injury both in
vivo and in vitro, but these mediators also are biologically active and may regulate oxidant
injury. There are several classes of IsoPs that differ based on the functional groups present on
the prostane ring. The classes include the F2-IsoPs, the D2/E2-IsoPs, the A2/J2-IsoPs, the
Isothromboxanes, and the Isoketals (IsoKs). These classes are distinguished by the type of
isoprostane ring that each contains. A2/J2-IsoPs are formed from the dehydration of E2/D2-
IsoPs, respectively. F2-IsoP can be detected in all normal biologic fluids in both humans and
animals, while the D2/E2-IsoPs, the A2/J2-IsoPs, and the isothromboxanes cannot. However,
levels of all of these classes are substantially increased in vivo following oxidant injury such as
carbon tetrachloride administrational, with the exception of the A2/J2-IsoPs and the IsoKs which
still cannot be detected. The neuroprostanes are similar to the IsoPs in that they contain
various prostane ring functional groups. However, the neuroprostanes are formed from docosahexaenoic acid that is present in high levels of neural tissue.\textsuperscript{218}

IsoPs have been shown to have potent biologic activity and perhaps regulate adverse effects of oxidant injury. The biologic activities of 15-F\textsubscript{2t}-IsoP (also known as 8-isoprostane or 8-iso-PGF\textsubscript{2a}) and 15-E\textsubscript{2t}-IsoP have been particularly well studied and these were potent vasoconstrictors in the kidney, lung, heart, retina, portal vein, and lymphatics.\textsuperscript{219-225} At least part of the vasoconstrictive properties of 15-F\textsubscript{2t}-IsoP resulted from its interaction with the thromboxane receptor TP.\textsuperscript{226} 15-F\textsubscript{2t}-IsoP also promoted the release of endothelin and vascular smooth muscle cell proliferation.\textsuperscript{227,228} Other activities ascribed to 15-F\textsubscript{2t}-IsoP include osteoclastic differentiation resulting in bone resorption and augmented resistance to aspirin-induced blockade of platelet aggregation.\textsuperscript{229-231}

As the F\textsubscript{2}-IsoPs can be easily detected in human biologic fluid at baseline, changes in the levels of these compounds have been used as an \textit{in vivo} index of endogenous lipid peroxidation or oxidant stress. The fact that the F\textsubscript{2}-IsoPs can be measured in urine further increases the usefulness of these products as a noninvasive marker of oxidant stress activity and the activity of antioxidant compounds \textit{in vivo}. Similar to the prostaglandins, measurement of F\textsubscript{2}-IsoPs in the urine is the most dependable method to assess total endogenous levels of these products as blood levels may reflect only recent trends because the biologic half-life of these mediators is very short, on the order of 16 minutes. The preferred method of measurement of the IsoPs is gas chromatography/negative ion chemical ionization mass spectroscopy. Immunoassays are commercially available; however, the presence of substances in biological fluids that interfere with these assays may confound results obtained with this technique. It is important to note that
biologic material in which IsoPs are to be measured should be stored at -70°C or with the addition of antioxidants to avoid production of IsoPs ex vivo.

**Human studies of isoprostanes in allergic inflammation**

Levels of IsoPs have been studied in asthma and other allergic diseases such as asthma. Compared to healthy non-asthmatic controls, exhaled breath condensates of the F₂-IsoP series member 15- F₂-IsoP were greater in children with asthma, whether they had either mild persistent asthma, stable mild-to-moderate persistent asthma that was treated with inhaled corticosteroids, or unstable asthma. Similarly, steroid-naive asthmatic children and children in stable condition with mild-to-moderate persistent asthma who were being treated with inhaled corticosteroids had greater levels of 15-F₂-IsoP compared to healthy non-asthmatic children. In children experiencing an asthma exacerbation, oral corticosteroids significantly reduced exhaled breath condensate levels of 15-F₂-IsoP, although not to the same concentrations as measured in non-asthmatics, suggesting that corticosteroids may not be fully effective in reducing oxidative stress in children with an exacerbation of asthma. IsoPs have also been measured in exhaled breath condensates in adults. In women with mild allergic asthma, the levels of 15-F₂-IsoP were inversely correlated with the percent predicted FEV₁. Exhaled breath condensate levels of 15-F₂-IsoP were also elevated in steroid-naïve subjects with aspirin-induced asthma compared to both subjects with aspirin-induced asthma treated with steroids and healthy control subjects. Allergen challenge increased 15-F₂-IsoP in exhaled breath condensate, indicating that allergen challenge increases airway oxidative stress in allergic asthma. Induced sputum has also been used as a biologic fluid in which IsoPs may be
measured. Adults who either had asthmatic or bronchiectasis had greater levels of sputum 15-F_{2\tau}-IsoP than in healthy control subjects. In addition, 15-F_{2\tau}-IsoP levels decreased in the induced sputum of in asthmatics as they recovered from an exacerbation. Urinary levels of IsoPs were also elevated in asthmatic subjects experiencing allergen challenge. 15-F_{2\tau}-IsoP was elevated from as early as two hours, and as long as eight hours, following inhaled allergen challenge in subjects with mild allergic asthma. In contrast, there was no increase in the urinary excretion of 8-isoprostane occurred after methacholine challenge. Therefore, IsoP levels are increased during ongoing allergic inflammatory responses and decrease with treatment.

**Mouse studies of isoprostanes in allergic inflammation**

IsoP measurements have also been performed in mouse models of allergic lung inflammation. In the OVA-model, F_{2}-isoprostanes in whole lung were increased on the ninth day of daily aerosol allergen challenge. Increased immunoreactivity to 15-F_{2\tau}-IsoP or to isoketal protein adducts was found in epithelial cells 24 hours after the first aerosol challenge and after 5 days of allergen exposure in macrophages. Collagen surrounding airways and blood vessels, and airway and vascular smooth muscle, also exhibited increased 15-F_{2\tau}-IsoP immunoreactivity after OVA challenge. Dietary vitamin E restriction in conjunction with allergic inflammation led to increased whole lung F_{2}-isoprostanes while supplemental vitamin E suppressed their formation. Similar changes in immunoreactivity to F_{2}-isoprostanes were seen. Airway responsiveness to methacholine was also increased by vitamin E depletion and decreased slightly by supplementation with the antioxidant. Therefore, IsoPs are also
increased in the mouse model of allergic inflammation and these are reduced with anti-oxidant treatment.

**Sphingosine-1-phosphate**

There are two primary members of lysosphingolipids (LPLs) that have immunomodulatory functions. These include the lysoglycero-phospholipids, such as lysophosphatidic acid (LPA), and the lysosphingolipids, of which sphingosine-1-phosphate (S1P) is a key member. S1P is synthesized intracellularly with the primary sources among immune cells being mast cells, platelets, and macrophages; however, a variety of nonimmune cells can also produce this mediator. The first step in S1P production is the sphingomyelinase conversion of endogenous membrane-derived sphingomyelin to ceramide, then ceramidase converts ceramide to sphingosine. Sphinogosine is then converted to S1P through phosphorylation by either of two Sph kinases, SphK1 or SphK2. In mouse bone marrow-derived mast cells SphK2 was the principal regulator of intracellular signaling events such as calcium flux and downstream calcium-dependent activation of protein kinase C, NF-κB, eicosanoid production, and cytokine secretion. SphK1 had no apparent role in these intracellular activities, but instead regulated the concentration of extracellular S1P and the sensitivity of mast cells to antigen-driven degranulation. S1P can be transformed back into sphingosine by S1P phosphatase or removed from the production pathway by a S1P lyase. S1P functions within cells as a modulator of calcium homeostasis and as a regulator of cellular survival and proliferation. The receptors responsible for these intracellular functions are not
clearly defined. S1P can be transported extracellularly and functions to activate cell motility.\textsuperscript{243} This function is mediated by S1P signaling through five GPCRs S1P\textsubscript{1-5}.\textsuperscript{243} The principal functional receptor for leukocyte chemotaxis to S1P is S1P\textsubscript{1} which is expressed on T and B lymphocytes, mononuclear phagocytes, dendritic cells, mast cells, and NK cells.\textsuperscript{245} S1P signaling through S1P\textsubscript{1} is a major regulator of T lymphocyte function in preventing apoptosis, promoting CD4\textsuperscript{+}CD25\textsuperscript{+} T regulatory cell activity, and enhancing chemotaxis.\textsuperscript{245} S1P\textsubscript{1} signaling promotes thymocyte emigration and movement of lymphocytes from lymph nodes, but not from the spleen, into efferent lymph and subsequently to blood.\textsuperscript{245} S1P\textsubscript{1} receptors also regulate the chemotaxis of mast cells toward antigen, while S1P\textsubscript{2} receptors facilitate IgE-dependent mast cell degranulation. It is thus likely that S1P generated by mast cells plays an important autocrine role in their function.\textsuperscript{246}

**Human studies of S1P in allergic inflammation**

Segmental allergen challenge in allergic asthmatics induced a two-fold increased in S1P levels in BAL fluid 24 hours after the challenge; however, there was no change in BAL S1P levels in nonallergic nonasthmatic controls after challenge.\textsuperscript{247} In *in vitro* experiments using human airway smooth muscle cells, S1P administration resulted in a dose dependent increase in both DNA synthesis and cell proliferation.\textsuperscript{247} S1P also induced IL-6 production by human smooth muscle airway cells that was further increased by treatment of the cells with both S1P and TNF. However, S1P inhibited TNF-induced RANTES expression. Therefore, further work will need to be performed to conclusively define the role of S1P in the human allergic inflammatory response.
Mouse studies of S1P in allergic inflammation

The pharmacologic agent FTY720 downregulates the activity of S1P1 in addition to S1P2 and S1P5, but not S1P3 or S1P4, and has been used in animal models to test the activity of signaling through the receptors in which it exerts inhibitory activity.245 In a model in which OVA-specific polarized Th2 cells were adoptively transferred into naïve mice, orally administered FTY720 inhibited the influx of T lymphocytes and eosinophils into the lungs when these mice were subsequently challenged with aerosolized OVA.248 In mice that were sensitized with an intraperitoneal injection of OVA formulated with aluminum hydroxide, oral FTY720 at the time of OVA challenge inhibited the airway accumulation of lymphocytes and eosinophils, prevented the induction of bronchial hyperresponsiveness, and reduced goblet cell hyperplasia.248 Intratracheal administration of FTY720 significantly reduced the number of BAL macrophages, neutrophils, lymphocytes, and eosinophils when it was given 30 minutes prior to OVA aerosolization in mice that had been previously sensitized to OVA.249 In addition, intratracheal FTY720 inhibited allergen-induced AHR and parenchymal lung inflammation.249 Although FTY720 had no effect on the number of blood circulating lymphocytes or lymphocytes in peripheral lymph nodes, the number of T cells in lung draining lymph nodes was significantly reduced.249 The effect of FTY720 seemed to be predominantly mediated by its inhibition of the migration of lung dendritic cells to the mediastinal lymph nodes, which in turn blunted the formation of allergen-specific Th2 cells in lymph nodes.249 In addition, FTY720-treated dendritic cells were intrinsically less effective in activating naive and effector Th2 cells due to a inhibited capacity to form stable interactions with T cells and thus to produce an immunological
Thus, inhibition of signaling through several S1P receptors downregulates allergic inflammation in the mouse. Very recently, FTY720 was reported to potently and selectively inhibit group IVA PLA2. It is thus possible that some of its immunosuppressive effects may be mediated by interference with eicosanoid generation.

**Summary**

The lipid mediators are a diverse array of potent molecules that can be rapidly generated by structural cells as well as leukocytes in response to environmental perturbations. The spectrum of homeostatic, immunologic, and inflammatory functions served by these mediators can now be better understood due to the identification of the GPCRs and enzyme systems responsible for the actions and synthesis of each, and the development of receptor-deficient mice and receptor-selective agonists. While the development of specific enzyme inhibitors and receptor antagonists for therapeutic use is still in its infancy, the success of the COX and 5-LO inhibitors and the CysLT1 antagonists strongly support the role of eicosanoids in human disease. It is likely that additional reagents under development, such as FTY720, will both provide efficacious treatment for human disease and new insights into the biologic importance of lipid mediators in both health and disease. Furthermore, the increasing recognition that lipid mediator receptors can form heterodimers and even trimers raises the level of complexity of eicosanoid signaling, but at the same time identifies new opportunities for selective interventions on causal pathways of allergic disease.
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