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Cysteinyl Leukotrienes and Their Receptors; Emerging Concepts

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Cysteinyl leukotrienes (cys-LTs) are potent mediators of inflammation derived from arachidonic acid through the 5-lipoxygenase/leukotriene C₄ synthase pathway. The derivation of their chemical structures and identification of their pharmacologic properties predated the cloning of their classical receptors and the development of drugs that modify their synthesis and actions. Recent studies have revealed unanticipated insights into the regulation of cys-LT synthesis, the function of the cys-LTs in innate and adaptive immunity and human disease, and the identification of a new receptor for the cys-LTs. This review highlights these studies and summarizes their potential pathobiologic and therapeutic implications.

Key Words: Leukotrienes; 5-lipoxygenase; asthma; AERD

INTRODUCTION

Leukotrienes are lipid mediators generated from arachidonic acid through the 5-lipoxygenase (5-LO) pathway. They are named for their cells of origin (leukocytes) and the presence of three positionally conserved double bonds (trienes). The 2 classes of leukotrienes, cysteinyl leukotrienes (cys-LTs) and leukotriene B₄ (LTB₄), have broad array of bioactivities and cellular targets. Both 5-LO inhibitors and cys-LT receptor antagonists are useful for the treatment of asthma and rhinitis.¹⁻³ Recently studies using molecular approaches have demonstrated that cys-LTs possess multiple cell targets and immunologic functions, and act through a receptor system far more complex than previously anticipated. This review highlights these recent studies, and will consider their potential pathobiologic and therapeutic implications.

Regulation of leukotriene synthesis

Leukotriene synthesis is initiated during the activation of leukocytes, when arachidonic acid is liberated from the membrane phospholipids by a cytosolic phospholipase A₂.⁴ 5-LO activating protein presents arachidonic acid to 5-LO, which catalyzes the formation of 5-hydroperoxyeicosatetraenoic acid and then the unstable epoxide LTA₄.⁵ In mast cells, macrophages, eosinophils, and basophils, LTC₄ synthase (LTC₄S) conjugates LTA₄ to reduced glutathione, forming LTC₄, the parent of the cys-LTs.⁶ Once formed, LTC₄ is transported to extracellular space via the ATP-binding cassette (ABC) transporters-1 and-4

and then metabolized to LTD₄ and LTE₄ by γ -glutamyl transpeptidases and dipeptidases, respectively. The rapid extracellular metabolism of LTC₄ and LTD₄ results in short biologic half-lives relative to the stable mediator LTE₄, which is abundant and readily detected in biologic fluids. In neutrophils, LTA₄ is hydrolyzed by a cytosolic LTA₄ hydrolase enzyme to form LTB₄, a dihydroxy leukotriene that is a potent chemoattractant for neutrophils and monocytes.⁷

5-LO activity is substantially upregulated when granulocytes are exposed *ex vivo* to hematopoietic cytokines such as GM-CSF or (in the case of eosinophils) IL-5.⁸⁻¹¹ In cord blood-derived human mast cells, IL-3 and IL-5 enhance the function of 5-LO by inducing its import from the cytosol to the nucleoplasm, whereas IL-4 potently induces expression and function of LTC₄S.¹² LTC₄S enzymatic function can be inhibited by protein kinase C (PKC)-dependent phosphorylation, which can limit the generation of cys-LTs *ex vivo*.¹³ 5-LO activity is suppressed by stimuli that induce cyclic adenosine monophosphate (cAMP) accumulation, leading to serine phosphorylation of 5-LO by cAMP-dependent protein kinase A (PKA).¹¹ These *in vitro* studies suggest that LT production is tightly regulated by

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the microenvironment and intracellular phosphorylation events, with mechanisms that can respectively enhance and limit the expression and function of the critical metabolic enzymes dependent on context.

Cysteinyl leukotriene receptors

Early pharmacologic profiling studies predicted the existence of at least 2 cys-LT receptors in mammalian tissues.¹⁴ The molecular characterization of the classical G protein-coupled receptors (GPCRs) partially reconciled this pharmacology. The type 1 cys-LT receptor, CysLT₁R, is a high-affinity receptor for LTD₄ and the target of antagonists (Montelukast, Zafirlukast, and Pranlukast) that are used for the management of asthma. The cloned human CysLT₁R gene encodes a GPCR of 339 amino acids.¹⁵ Human CysLT₁R mRNA is expressed in bronchial smooth muscle and substantially in myeloid cells, such as macrophages and mast cells. The human CysLT₂R is 38% identical to CysLT₁R in amino acid sequence.¹⁶ CysLT₂R binds LTC₄ and LTD₄ with equal affinity, and binds LTD₄ with affinity one-log less than CysLT₁R. CysLT₂R is resistant to Montelukast, and is expressed both on cells that also express CysLT₁R (e.g., myeloid cells, smooth muscle), as well as endothelial cells, cardiac Purkinje cells, adrenal medulla, and brain.¹⁶ The incompletely overlapping distribution of the 2 classical receptors for cys-LTs suggests that they have both complementary and distinct functions.

In contrast to their affinities for LTC₄ and LTD₄, the cloned CysLT₁R and CysLT₂R receptors display trivial binding affinity for the stable metabolite LTE₄. Nonetheless, studies of human tracheal explants and guinea pig tracheal rings had predicted the existence of a third cys-LT receptor with a preference for LTE₄.^{14,17} LTE₄ also was equipotent to its precursors for inducing wheal and flare responses when injected intradermally into humans.¹⁸ Recently GPR99, previously reported as an oxylglutarate receptor,¹⁹ was identified as a potential LTE₄ receptor.²⁰ LTE₄ binds and activates GPR99 at low nM range concentrations in transfected cells, and resists blockade by MK571, a prototype CysLT₁R antagonist. The ability of LTE₄ to induce cutaneous vascular permeability in mice depends largely on the presence of GPR99. GPR99 mRNA is expressed strongly by kidney and smooth muscle. Precise definition of its cellular distribution awaits the development of suitable antibody reagents, and its role in allergic inflammation is to be determined.

Regulation of cysteinyl leukotriene receptor function

As is the case for the cys-LT synthesis, cellular responsiveness to cys-LTs can be modulated both by exogenous stimuli and intracellular phosphorylation events. IL-4 and IL-13 upregulate the expression and function of CysLT₁R by human peripheral blood monocytes and monocyte-derived macrophages.²¹ IL-13, but not IL-4, upregulates CysLT₂R expression as well in human monocytes.²² IL-13 and transforming growth factor beta

induce CysLT₁R expression by human bronchial smooth muscle cells.²³ CysLT₁R can be inducibly expressed by mouse T cells stimulated through the T cell receptor.²⁴ CysLT₁R signaling is also controlled by PKA²⁵ - or PKC²⁶ -dependent phosphorylation and desensitization. PKC mediates ligand-induced internalization of CysLT₁R following stimulation with LTD₄.²⁷ PKC activation by members of the purinergic (P2Y) family of GPCRs, which are homologous to the cys-LT receptors, can induce heterologous, PKC-dependent phosphorylation and desensitization of CysLT₁R without causing its internalization.²⁶ Since nucleotides, the natural ligands for P2Y receptors, are released in large quantities during acute inflammatory responses,²⁸ signaling through the cognate P2Y receptors may limit potentially deleterious effects of CysLT₁R signaling in cells that express both classes of receptors (Figure). Moreover, the overlap in the cytokines (IL-4) and protein kinases (PKA, PKC) that respectively enhance and suppress the functions of the synthetic and receptor systems suggest that cys-LT production may be regulated in parallel with end-organ responsiveness.

CysLT₁R functions can also be regulated by direct physical interactions with other GPCRs. CysLT₁R and CysLT₂R heterodimerize in cultured human mast cells.²⁹ The presence of CysLT₂R limits the levels of membrane expression of CysLT₁R, and dampens the capacity of CysLT₁R to induce phosphorylation of extracellular signal regulated kinase and proliferation in this cell type. GPR17, a GPCR homologous to CysLT₁R and CysLT₂R,³⁰ was originally “deorphanized” as a dual-specific receptor for cys-LTs and uracil nucleotides.³¹ However, we and others could not reproduce GPR17 activation by either ligand type in various assay systems.^{30,32,33} Instead, GPR17 functions as a negative regulator of LTD₄-mediated CysLT₁R activation, and markedly reduces binding of LTD₄ when the two receptors are co-expressed in cell lines.³⁰ Accordingly, mice lacking GPR17 (*Gpr17*^{-/-} mice) showed markedly enhanced CysLT₁R-dependent tis-

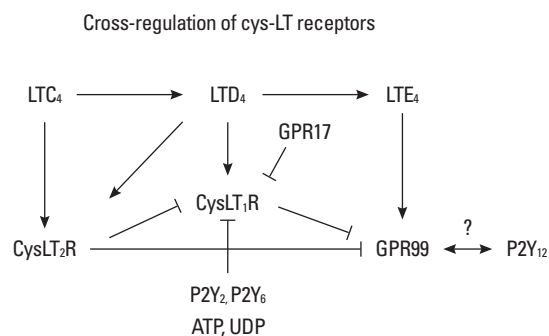


Figure. Cross-regulation of the cysteinyl leukotriene receptors. CysLT₁R function is inhibited both by direct physical interactions with CysLT₂R or GPR17, and by heterologous, PKC-dependent phosphorylation by P2Y receptors. The lack of both CysLT₁R and CysLT₂R amplifies cutaneous responses to LTE₄, suggesting that both classical receptors cross-regulate GPR99. The requirement for P2Y₁₂ receptors for the ability of LTE₄ to amplify pulmonary eosinophilia could reflect an interaction with GPR99.

sue edema induced by IgE-dependent passive cutaneous anaphylaxis.³⁰ Thus, at least two GPCRs (CysLT₂R and GPR17) dampen CysLT₁R function by direct physical interactions. The fact that both direct and indirect mechanisms can limit signaling through CysLT₁R (Figure) implies that such limitation is critical for homeostasis of immune and inflammatory responses.

Cys-LTs in human allergic disease

Asthma and rhinitis

Based on their potencies as airway smooth muscle spasmogens and inducers of vascular leak, cys-LTs were considered potential pathogenetic mediators of asthma and rhinitis decades before the cloning of the cys-LT receptors. When administered by inhalation to asthmatic and nonasthmatic human subjects, both LTC₄ and LTD₄ induced bronchoconstriction at doses several log-fold lower than histamine.³⁴⁻³⁶ LTE₄ was a weaker bronchoconstrictor than LTC₄ and LTD₄, but was ~1-log-fold more potent in inducing bronchoconstriction in asthmatic subjects relative to nonasthmatic controls.³⁷ Additionally, when delivered by inhalation, LTE₄ caused the accumulation of eosinophils and basophils in the bronchial submucosa of mild asthmatic subjects, whereas LTD₄ did not.³⁸ In retrospect, these findings not only implied that end-organ reactivity to LTE₄ is specifically enhanced in asthma, but also suggested the existence of distinct receptors with a preference for binding and activation by LTE₄.

Cys-LT production increases substantially in association with allergic inflammation and asthma, likely reflecting the activation of mast cells and eosinophils in the lesional tissues.³⁹ Unfractionated leukocytes from subjects with asthma generate several fold higher levels of both LTB₄ and LTC₄ than do leukocytes from the blood of nonasthmatic controls in response to stimulation with calcium ionophore.⁴⁰ Urinary levels of LTE₄ increase during spontaneous asthma exacerbations,⁴¹ and correlate with decline in FEV₁.⁴² Treatments with either zileuton, a 5-LO inhibitor,⁴³ or with antagonists of CysLT₁R⁴⁴ each reduce the frequency of asthma exacerbations. Intravenous Montelukast increases peak expiratory flow rates in adult asthmatic subjects presenting to the emergency department with airflow obstruction compared with placebo.⁴⁵ These findings suggest that cys-LTs contribute substantially to exacerbations of asthma. CysLT₁R antagonists also attenuate the magnitude of decline in FEV₁ in response to allergen challenge.⁴⁶ Cys-LT-generating enzymes are expressed by eosinophils, monocytes, and mast cells in nasal biopsies from subjects with allergic rhinitis,³⁹ and CysLT₁R and CysLT₂R localize to both hematopoietic and non-hematopoietic cell types in the nasal tissue.^{39,47} Additionally, CysLT₁R is expressed by human Th2 cells in peripheral blood from atopic subjects.⁴⁸ Montelukast, alone or in combination with an H₁ histamine receptor antagonist, is superior to placebo for reducing nasal congestion in the treatment of seasonal allergic rhinitis.³ The effects of CysLT₁R antagonists on rhinitis may re-

flect the actions of the cys-LTs on the vasculature as well as resident inflammatory cells.

AERD

AERD is characterized by adult onset asthma, severe rhinosinusitis with nasal polyps, and idiosyncratic respiratory reactions to aspirin and other nonselective inhibitors of cyclooxygenase (COX).⁴⁹ Baseline levels of urinary LTE₄ in subjects with AERD exceed the levels seen in aspirin tolerant asthmatic controls by several fold, and increase further and markedly in response to provocative challenge with aspirin.⁵⁰ The administration of either Zileuton or CysLT₁R antagonists attenuates the severity of aspirin-induced bronchoconstriction in AERD.⁵¹ Both classes of drugs were also superior to placebo for improving sinonasal function.^{2,52} Thus, cys-LTs are involved in both the upper and lower respiratory tract pathology typical of AERD.

Eosinophils are the most abundant effector cell in bronchial and nasal biopsies from patients with AERD, and show over-expression of LTC₄S protein relative to eosinophils in biopsies from aspirin tolerant controls.^{53,54} Platelets, which lack 5-LO, also express LTC₄S and can convert granulocyte-derived LTA₄ to LTC₄ through a transcellular mechanism.⁵⁵ In the blood and nasal polyps from patients with AERD, eosinophils, monocytes, and neutrophils display markedly increased numbers of adherent platelets compared to samples from aspirin tolerant controls.⁵⁶ These adherent platelets contribute as much as 60% of the LTC₄S activity associated with peripheral blood granulocytes obtained from subjects with AERD, and the percentages of blood granulocytes that are platelet-adherent correlated strongly with the levels of urinary LTE₄.⁵⁶ Mast cell activation accompanies the responses to aspirin challenge in AERD,⁵⁷ and the administration of mast cell stabilizing cromone drugs blocks the rise in urinary LTE₄ that accompanies reactions.⁵⁸ Collectively, these studies suggest that the dysregulation of cys-LT production in AERD reflects several cell types. Recently developed models of AERD in mice (see below) may more precisely define the cellular and molecular mechanisms responsible for dysregulated cys-LT production in AERD.

In addition to dysregulated cys-LT generation, subjects with AERD show enhanced end-organ reactivity to cys-LTs. Compared with aspirin tolerant asthmatic controls, individuals with AERD demonstrate bronchoconstriction in response to inhaled LTE₄⁵⁹ and LTD₄⁶⁰ at significantly lower doses. The numbers and percentages of CysLT₁R-positive mast cells, eosinophils, and monocytes in nasal biopsies from patients with AERD exceed those observed in the tissues of aspirin-tolerant asthmatic controls.^{47,61} CysLT₁R expression on hematopoietic cells decreases following desensitization to aspirin,⁶¹ a procedure that attenuates bronchial reactivity to LTE₄.⁶² The numbers and distributions of CysLT₂R-positive cells do not differ between aspirin tolerant asthmatics and subjects with AERD. Interestingly, bronchial reactivity to inhaled LTD₄ in AERD or aspirin tolerant

asthma does not correlate with the numbers of CysLT₁R- or CysLT₂R- expressing cells in bronchial biopsies.⁶⁰ It is tempting to speculate that non-classical receptors, such as GPR99, may account for a component of the end organ responsiveness to cys-LTs (particularly to LTE₄) observed in AERD.

Understanding functions of the cys-LTs and their receptors in mice

The development of mice lacking LTC₄S (*Ltc4s*^{-/-}), CysLT₁R (*Cysltr1*^{-/-}), CysLT₂R (*Cysltr2*^{-/-}), and both receptors (*Cysltr1/Cysltr2*^{-/-}) has permitted in-depth studies of the role of cys-LTs in immune and inflammatory responses. These studies have revealed complex and, in some instances, unanticipated functions for cys-LTs and their receptors in a variety of biologic responses detailed below.

Vascular leak

In a mast cell and IgE-dependent model of passive cutaneous anaphylaxis, *Ltc4s*^{-/-} mice displayed reductions in ear skin swelling of ~50% compared to wild-type (WT) mice.⁶³ Intraperitoneal injections of zymosan, a yeast cell wall glycan that elicits LTC₄ generation from macrophages, induced vascular leak that was reduced in both the *Ltc4s*^{-/-} and *Cysltr1*^{-/-} strains by ~50% compared with WT controls.^{63,64} The responses of *Cysltr2*^{-/-} mice were equivalent to those of WT controls. Thus, CysLT₁R plays a key role in mediating vascular leak in models where cys-LTs are generated in response to antigen- or pathogen-dependent stimuli.

To determine whether additional cys-LT receptors participated in vascular leak, we subjected *Cysltr1/Cysltr2*^{-/-} mice to direct intracutaneous challenges with cys-LTs. Surprisingly, LTC₄ and LTD₄ induced tissue edema in *Cysltr1/Cysltr2*^{-/-} mice that was comparable to WT mice, and LTE₄ induced marked tissue edema in this strain, with 64-fold enhanced sensitivity to LTE₄ over the WT controls. This enhanced response to LTE₄ was inhibited by pretreatment of the mice with pertussis toxin and a Rho kinase inhibitor, suggesting that it was mediated by a previously unrecognized G protein-coupled cys-LT receptor with a preference to LTE₄.⁶⁵ Given that GPR99 bound LTE₄ in transfected cells, we generated *Gpr99*^{-/-} and *Gpr99/Cysltr1/Cysltr2*^{-/-} mice for comparison with WT and *Cysltr1/Cysltr2*^{-/-} mice. GPR99 deletion from the *Cysltr1/Cysltr2*^{-/-} mice eliminated the vascular leak in response to the cys-LT ligands, indicating that GPR99 is likely to be a true cys-LT receptor. Furthermore, the *Gpr99*^{-/-} mice showed a dose-dependent loss of LTE₄-mediated vascular permeability, but not to LTC₄ or LTD₄, suggesting a preference of GPR99 for LTE₄.²⁰

Th2 Immunity

Lung Th2 immunity to the house dust mite *Dermatophagoides farinae* (*Df*) requires stimulation of the myeloid C-type lectin receptor, Dectin-2.^{66,67} Based on a protocol of sensitization of naive WT mice by means of adoptive transfer of *Df*-pulsed den-

dritic cells (DCs), Th2 responses to *Df* require the expressions of LTC₄S and CysLT₁R by DCs. Interestingly, both *Cysltr2*^{-/-} mice and *Gpr17*^{-/-} mice showed markedly augmented eosinophilic pulmonary inflammation, serum IgE, and Th2 cytokine generation in response to *Df* sensitization and challenge compared to WT controls.¹⁸ *Df*-pulsed DCs derived from *Cysltr2*^{-/-} mice and *Gpr17*^{-/-} mice induce an enhanced pulmonary eosinophilic and Th2 immune response in WT mice when compared with WT DCs. The enhanced response induced by *Gpr17*^{-/-} DCs was eliminated by introduction of the *Cysltr1*^{-/-} allele,⁶⁸ whereas the introduction of the *Ltc4s*^{-/-} allele eliminated the potentiation of Th2 immunity induced by transfer of *Cysltr2*^{-/-} DCs.⁶⁹ Thus, constitutive downregulation of CysLT₁R function by GPR17 and CysLT₂R may be critical to maintain homeostasis during the induction of Th2 immunity, at least to allergens (and potentially microbes) that bear ligands for Dectin-2.

Activation of innate lymphoid cells

Type 2 innate lymphoid cells (ILC2) are innate lymphocytes that release large quantities of IL-5 and IL-13 when activated by cytokines, such as IL-33, IL-25, or thymic stromal lymphopoietin (TSLP), derived from epithelial cells.⁷⁰ A recent study implicated the cys-LTs in the activation of ILC2 cells. Intrapulmonary challenge of mice with an extract from the mold *Alternaria alternata* strongly induced the generation of cys-LTs in the lung, and the recruitment and activation of ILC2.⁷¹ ILC2 expressed CysLT₁R, and responded to stimulation in vitro and in vivo with LTD₄ by proliferating and releasing cytokines. Interestingly, while both LTD₄ and IL-33 caused lung ILC2 to generate IL-5 and IL-13, only LTD₄ caused them to generate IL-4. Ex vivo stimulation of lung ILC2 with either LTD₄ or LTE₄ caused the production of IL-5. While the IL-5 production in response to LTD₄ could be blocked by Montelukast, LTE₄-induced IL-5 production was resistant to Montelukast. This study suggests that cys-LTs can contribute to Th2 immunity through direct actions at ILC2. These effects reflect cys-LT actions both classical and nonclassical receptors that can induce effector cytokine production.

Platelet-dependent pulmonary eosinophilia

Platelets are essential for the development of pulmonary eosinophilia and airway remodeling in mouse models of ovalbumin (OVA) sensitization and challenge.^{72,73} Activated platelets express P-selectin, which permits their adherence to leukocytes and primes leukocytes for directed migration via integrins. Mouse and human platelets express both CysLT₁R and CysLT₂R,^{74,75} as well as the P2Y₁₂ receptor, a homologue of the cys-LT receptors that binds ADP. Stimulation of mouse platelets with LTC₄ strongly induces their expression of P-selectin in an entirely CysLT₂R-dependent manner, while LTD₄ or LTE₄ are inactive. Intratracheal administration of LTC₄, but not LTD₄, mark-

edly amplifies the recruitment of eosinophils to the airways of sensitized mice challenged with low-dose OVA. This amplification requires platelets, and is attenuated in *Cysltr2*^{-/-} mice, suggesting a direct stimulatory effect of LTC₄ on platelet-associated CysLT₂R in the lung vasculature.

Although LTE₄ fails to directly activate mouse or human platelets *in vitro*,^{75,76} intratracheal administration of LTE₄, like that of LTC₄, potentiates OVA-induced eosinophilia in a platelet-dependent manner in WT mice.⁷⁶ In this model, LTE₄ is fully active in *Cysltr1/Cysltr2*^{-/-} mice, suggesting that it acts at a non-classical cys-LT receptor. Both the effects of LTE₄ (*in vivo*) and of LTC₄ (*in vivo* and *in vitro*) depend exquisitely on the P2Y₁₂ receptor.^{75,76} A computer modeling study predicted that P2Y₁₂ receptors might recognize LTE₄ as a surrogate ligand,⁷⁷ and LTE₄ elicits calcium flux⁷⁷ and phosphorylation of extracellular signal regulated kinase⁷⁶ in transfected cells over-expressing human P2Y₁₂ receptors. Nonetheless, radiolabeled LTE₄ does not directly bind to microsomal membranes from P2Y₁₂ receptor-expressing transfectants. It is presently unknown whether the involvement of P2Y₁₂ in LTE₄-dependent signaling responses and airway inflammation reflects a direct interaction between P2Y₁₂ receptors and a bona fide LTE₄ receptor, such as GPR99. The therapeutic potential of drugs that block P2Y₁₂ receptors in asthma or AERD is unexplored.

AERD-like models

Although several cellular abnormalities in eicosanoid synthesis and receptor function have been described in AERD,⁴⁹ the lack of a valid model of the disease has restrained progress in defining the key pathogenetic steps. Hirata *et al.* generated a transgenic mouse strain over expressing LTC₄S and examined the phenotype in OVA-induced pulmonary inflammation with or without treatment with a COX inhibitor, sulpyrine.⁷⁸ OVA-challenged LTC₄S-transgenic mice, but not similarly treated WT mice, demonstrated a significant increase in airway resistance by sulpyrine treatment. This is associated with increases in LTC₄ and LTB₄ in bronchoalveolar lavage (BAL) fluid in sulpyrine-treated OVA-challenged transgenic mice. Importantly, the increase in airway resistance was inhibited by Pranlukast, a CysLT₁R antagonist. This study demonstrates that the pathognomic feature of aspirin-induced bronchoconstriction can be reproduced in a mouse model, and suggests that the overexpression of LTC₄S described in tissues from patients with AERD⁵³ has a potentially causal role.

Prostaglandin E₂ (PGE₂) controls cys-LT generation by activating PKA and inducing phosphorylation of 5-LO.⁷⁹ Tissue inflammation is typically associated with increased PGE₂ production, reflecting the co-expression of 2 inducible enzymes; COX-2 (a largely aspirin-resistant enzyme) and microsomal PGE₂ synthase-1 (mPGES-1), which isomerizes COX-2-derived PGH₂ to PGE₂.⁸⁰ Nasal polyps from subjects with AERD contain less PGE₂ than nasal polyps from aspirin tolerant controls,⁸¹ possi-

bly relating to epigenetic modifications of COX-2⁸² and/or mPGES-1 expression.⁸³ Mice lacking mPGES-1 (*Ptges*^{-/-}) cannot upregulate PGE₂ production with inflammation, and display a remarkably AERD-like phenotype when subjected to a model of *Df*-induced pulmonary disease. Compared with WT controls, *Ptges*^{-/-} mice show increased eosinophilic inflammation and levels of cys-LTs in the BAL fluid. Challenge with inhaled lysine aspirin causes marked increases in airway resistance, robust release of cys-LTs, and pulmonary mast cell activation in the *Ptges*^{-/-} strain, but not in WT controls.⁸⁴ Aspirin challenge profoundly depletes lung PGE₂ in the *Ptges*^{-/-} mice, but not in the WT controls, suggesting that the mPGES-1 is needed to maintain PGE₂ levels when COX-1 is inhibited. *Ptges*^{-/-} mice also show increased numbers of platelet-adherent granulocytes in both the peripheral blood and lungs compared with WT controls. Importantly, cys-LT production, mast cell activation, and the changes in airway resistance were blocked by depletion of platelets or blockade of the TP receptor for thromboxane A₂. This model may be useful in defining the potential pathogenetic role of GPR99, CysLT₂R, and P2Y₁₂ receptors in AERD, as well as unraveling the complex interplay between cys-LTs, platelets, and mast cells that lead to the physiologic response to aspirin challenges.

CONCLUSIONS

While the drugs capable of inhibiting cys-LT formation and blocking CysLT₁R are useful, it is clear that the cys-LT system is far more complex than initially appreciated. The involvement of the cys-LTs in the induction of Th2 immunity and the effector phase of the immune response suggests additional potential applications for currently available pharmacologic agents. However, the recognition that cys-LTs act through at least three receptors and the resistance of 2 of these (CysLT₂R and GPR99) to the blockade by currently available drugs presents both challenges and opportunities for further therapeutic development. The availability of a broad array of valid animal models should facilitate progress in this area, while continuing to reveal unanticipated biological functions for the cys-LTs and their receptors.

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REFERENCES

1. Israel E, Rubin P, Kemp JP, Grossman J, Pierson W, Siegel SC, Tinkelman D, Murray JJ, Busse W, Segal AT, Fish J, Kaiser HB, Ledford D, Wenzel S, Rosenthal R, Cohn J, Lanni C, Pearlman H, Karahalios

- P, Drazen JM. The effect of inhibition of 5-lipoxygenase by zileuton in mild-to-moderate asthma. *Ann Intern Med* 1993;119:1059-66.
2. Dahlén B, Nizankowska E, Szczeklik A, Zetterström O, Bochenek G, Kumlin M, Mastalerz L, Pinis G, Swanson LJ, Boodhoo TI, Wright S, Dubé LM, Dahlén SE. Benefits from adding the 5-lipoxygenase inhibitor zileuton to conventional therapy in aspirin-intolerant asthmatics. *Am J Respir Crit Care Med* 1998;157:1187-94.
 3. Meltzer EO, Malmstrom K, Lu S, Prenner BM, Wei LX, Weinstein SE, Wolfe JD, Reiss TF. Concomitant montelukast and loratadine as treatment for seasonal allergic rhinitis: a randomized, placebo-controlled clinical trial. *J Allergy Clin Immunol* 2000;105:917-22.
 4. Clark JD, Milona N, Knopf JL. Purification of a 110-kilodalton cytosolic phospholipase A2 from the human monocytic cell line U937. *Proc Natl Acad Sci U S A* 1990;87:7708-12.
 5. Reid GK, Kargman S, Vickers PJ, Mancini JA, Léveillé C, Ethier D, Miller DK, Gillard JW, Dixon RA, Evans JF. Correlation between expression of 5-lipoxygenase-activating protein, 5-lipoxygenase, and cellular leukotriene synthesis. *J Biol Chem* 1990;265:19818-23.
 6. Lam BK, Penrose JF, Freeman GJ, Austen KF. Expression cloning of a cDNA for human leukotriene C4 synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A4. *Proc Natl Acad Sci U S A* 1994;91:7663-7.
 7. Palmantier R, Rocheleau H, Laviolette M, Mancini J, Borgeat P. Characteristics of leukotriene biosynthesis by human granulocytes in presence of plasma. *Biochim Biophys Acta* 1998;1389:187-96.
 8. Cowburn AS, Holgate ST, Sampson AP. IL-5 increases expression of 5-lipoxygenase-activating protein and translocates 5-lipoxygenase to the nucleus in human blood eosinophils. *J Immunol* 1999;163:456-65.
 9. Boyce JA, Lam BK, Penrose JF, Friend DS, Parsons S, Owen WF, Austen KF. Expression of LTC4 synthase during the development of eosinophils in vitro from cord blood progenitors. *Blood* 1996;88:4338-47.
 10. Owen WF Jr, Rothenberg ME, Silberstein DS, Gasson JC, Stevens RL, Austen KF, Soberman RJ. Regulation of human eosinophil viability, density, and function by granulocyte/macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. *J Exp Med* 1987;166:129-41.
 11. Flamand N, Surette ME, Picard S, Bourgoin S, Borgeat P. Cyclic AMP-mediated inhibition of 5-lipoxygenase translocation and leukotriene biosynthesis in human neutrophils. *Mol Pharmacol* 2002;62:250-6.
 12. Hsieh FH, Lam BK, Penrose JF, Austen KF, Boyce JA. T helper cell type 2 cytokines coordinately regulate immunoglobulin E-dependent cysteinyl leukotriene production by human cord blood-derived mast cells: profound induction of leukotriene C(4) synthase expression by interleukin 4. *J Exp Med* 2001;193:123-33.
 13. Ali A, Ford-Hutchinson AW, Nicholson DW. Activation of protein kinase C down-regulates leukotriene C4 synthase activity and attenuates cysteinyl leukotriene production in an eosinophilic sub-strain of HL-60 cells. *J Immunol* 1994;153:776-88.
 14. Lee TH, Austen KF, Corey EJ, Drazen JM. Leukotriene E4-induced airway hyperresponsiveness of guinea pig tracheal smooth muscle to histamine and evidence for three separate sulfidopeptide leukotriene receptors. *Proc Natl Acad Sci U S A* 1984;81:4922-5.
 15. Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM, Coulombe N, Abramovitz M, Figueroa DJ, Zeng Z, Connolly BM, Bai C, Austin CP, Chateaufneuf A, Stocco R, Greig GM, Kargman S, Hooks SB, Hosfield E, Williams DL Jr, Ford-Hutchinson AW, Caskey CT, Evans JF. Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 1999;399:789-93.
 16. Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS, Stocco R, Bellefeuille JN, Abramovitz M, Cheng R, Williams DL Jr, Zeng Z, Liu Q, Ma L, Clements MK, Coulombe N, Liu Y, Austin CP, George SR, O'Neill GP, Metters KM, Lynch KR, Evans JF. Characterization of the human cysteinyl leukotriene 2 receptor. *J Biol Chem* 2000;275:30531-6.
 17. Jacques CA, Spur BW, Johnson M, Lee TH. The mechanism of LTE4-induced histamine hyperresponsiveness in guinea-pig tracheal and human bronchial smooth muscle, in vitro. *Br J Pharmacol* 1991;104:859-66.
 18. Soter NA, Lewis RA, Corey EJ, Austen KF. Local effects of synthetic leukotrienes (LTC4, LTD4, LTE4, and LTB4) in human skin. *J Invest Dermatol* 1983;80:115-9.
 19. He W, Miao FJ, Lin DC, Schwandner RT, Wang Z, Gao J, Chen JL, Tian H, Ling L. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* 2004;429:188-93.
 20. Kanaoka Y, Maekawa A, Austen KF. Identification of GPR99 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4 ligand. *J Biol Chem* 2013;288:10967-72.
 21. Thivierge M, Stanková J, Rola-Pleszczynski M. IL-13 and IL-4 up-regulate cysteinyl leukotriene 1 receptor expression in human monocytes and macrophages. *J Immunol* 2001;167:2855-60.
 22. Shirasaki H, Seki N, Fujita M, Kikuchi M, Kanaizumi E, Watanabe K, Himi T. Agonist- and T(H)2 cytokine-induced up-regulation of cysteinyl leukotriene receptor messenger RNA in human monocytes. *Ann Allergy Asthma Immunol* 2007;99:340-7.
 23. Espinosa K, Bossé Y, Stankova J, Rola-Pleszczynski M. CysLT1 receptor upregulation by TGF-beta and IL-13 is associated with bronchial smooth muscle cell proliferation in response to LTD4. *J Allergy Clin Immunol* 2003;111:1032-40.
 24. Prinz I, Gregoire C, Mollenkopf H, Aguado E, Wang Y, Malissen M, Kaufmann SH, Malissen B. The type 1 cysteinyl leukotriene receptor triggers calcium influx and chemotaxis in mouse alpha beta and gamma delta effector T cells. *J Immunol* 2005;175:713-9.
 25. Capra V, Accomazzo MR, Gardoni F, Barbieri S, Rovati GE. A role for inflammatory mediators in heterologous desensitization of CysLT1 receptor in human monocytes. *J Lipid Res* 2010;51:1075-84.
 26. Capra V, Ravasi S, Accomazzo MR, Citro S, Grimoldi M, Abbracchio MP, Rovati GE. CysLT1 receptor is a target for extracellular nucleotide-induced heterologous desensitization: a possible feedback mechanism in inflammation. *J Cell Sci* 2005;118:5625-36.
 27. Naik S, Billington CK, Pascual RM, Deshpande DA, Stefano FP, Kohout TA, Eckman DM, Benovic JL, Penn RB. Regulation of cysteinyl leukotriene type 1 receptor internalization and signaling. *J Biol Chem* 2005;280:8722-32.
 28. Idzko M, Hammad H, van Nimwegen M, Kool M, Willart MA, Muskens F, Hoogsteden HC, Luttmann W, Ferrari D, Di Virgilio F, Virchow JC Jr, Lambrecht BN. Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat Med* 2007;13:913-9.
 29. Jiang Y, Borrelli LA, Kanaoka Y, Bacskai BJ, Boyce JA. CysLT2 receptors interact with CysLT1 receptors and down-modulate cysteinyl leukotriene dependent mitogenic responses of mast cells. *Blood* 2007;110:3263-70.
 30. Maekawa A, Balestrieri B, Austen KF, Kanaoka Y. GPR17 is a negative regulator of the cysteinyl leukotriene 1 receptor response to

- leukotriene D4. *Proc Natl Acad Sci U S A* 2009;106:11685-90.
31. Ciana P, Fumagalli M, Trincavelli ML, Verderio C, Rosa P, Lecca D, Ferrario S, Parravicini C, Capra V, Gelosa P, Guerrini U, Belcredito S, Cimino M, Sironi L, Tremoli E, Rovati GE, Martini C, Abbracchio MP. The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor. *EMBO J* 2006;25:4615-27.
 32. Qi AD, Harden TK, Nicholas RA. Is GPR17 a P2Y/leukotriene receptor? examination of uracil nucleotides, nucleotide sugars, and cysteinyl leukotrienes as agonists of GPR17. *J Pharmacol Exp Ther* 2013;347:38-46.
 33. Hennen S, Wang H, Peters L, Merten N, Simon K, Spinrath A, Blättermann S, Akkari R, Schrage R, Schröder R, Schulz D, Vermeiren C, Zimmermann K, Kehraus S, Drewke C, Pfeifer A, König GM, Mohr K, Gillard M, Müller CE, Lu QR, Gomeza J, Kostenis E. Decoding signaling and function of the orphan G protein-coupled receptor GPR17 with a small-molecule agonist. *Sci Signal* 2013;6:ra93.
 34. Drazen JM. Comparative contractile responses to sulfidopeptide leukotrienes in normal and asthmatic human subjects. *Ann N Y Acad Sci* 1988;524:289-97.
 35. Weiss JW, Drazen JM, Coles N, McFadden ER Jr, Weller PF, Corey EJ, Lewis RA, Austen KF. Bronchoconstrictor effects of leukotriene C in humans. *Science* 1982;216:196-8.
 36. Weiss JW, Drazen JM, McFadden ER Jr, Weller PF, Corey EJ, Lewis RA, Austen KF. Comparative bronchoconstrictor effects of histamine, leukotriene C, and leukotriene D in normal human volunteers. *Trans Assoc Am Physicians* 1982;95:30-5.
 37. Davidson AB, Lee TH, Scanlon PD, Solway J, McFadden ER Jr, Ingram RH Jr, Corey EJ, Austen KF, Drazen JM. Bronchoconstrictor effects of leukotriene E4 in normal and asthmatic subjects. *Am Rev Respir Dis* 1987;135:333-7.
 38. Gauvreau GM, Parameswaran KN, Watson RM, O'Byrne PM. Inhaled leukotriene E(4), but not leukotriene D(4), increased airway inflammatory cells in subjects with atopic asthma. *Am J Respir Crit Care Med* 2001;164:1495-500.
 39. Figueroa DJ, Borish L, Baramki D, Philip G, Austin CP, Evans JF. Expression of cysteinyl leukotriene synthetic and signalling proteins in inflammatory cells in active seasonal allergic rhinitis. *Clin Exp Allergy* 2003;33:1380-8.
 40. Sampson AP, Thomas RU, Costello JE, Piper PJ. Enhanced leukotriene synthesis in leukocytes of atopic and asthmatic subjects. *Br J Clin Pharmacol* 1992;33:423-30.
 41. Drazen JM, O'Brien J, Sparrow D, Weiss ST, Martins MA, Israel E, Fanta CH. Recovery of leukotriene E4 from the urine of patients with airway obstruction. *Am Rev Respir Dis* 1992;146:104-8.
 42. Green SA, Malice MP, Tanaka W, Tozzi CA, Reiss TF. Increase in urinary leukotriene LTE4 levels in acute asthma: correlation with airflow limitation. *Thorax* 2004;59:100-4.
 43. Liu MC, Dubé LM, Lancaster J. Acute and chronic effects of a 5-lipoxygenase inhibitor in asthma: a 6-month randomized multicenter trial. Zileuton Study Group. *J Allergy Clin Immunol* 1996;98:859-71.
 44. Israel E, Chervinsky PS, Friedman B, Van Bavel J, Skalky CS, Ghanam AF, Bird SR, Edelman JM. Effects of montelukast and beclomethasone on airway function and asthma control. *J Allergy Clin Immunol* 2002;110:847-54.
 45. Camargo CA Jr, Gurner DM, Smithline HA, Chapela R, Fabbri LM, Green SA, Malice MP, Legrand C, Dass SB, Knorr BA, Reiss TF. A randomized placebo-controlled study of intravenous montelukast for the treatment of acute asthma. *J Allergy Clin Immunol* 2010;125:374-80.
 46. Hamilton A, Faiferman I, Stober P, Watson RM, O'Byrne PM. Pranlukast, a cysteinyl leukotriene receptor antagonist, attenuates allergen-induced early- and late-phase bronchoconstriction and airway hyperresponsiveness in asthmatic subjects. *J Allergy Clin Immunol* 1998;102:177-83.
 47. Corrigan C, Mallett K, Ying S, Roberts D, Parikh A, Scadding G, Lee T. Expression of the cysteinyl leukotriene receptors cysLT(1) and cysLT(2) in aspirin-sensitive and aspirin-tolerant chronic rhinosinusitis. *J Allergy Clin Immunol* 2005;115:316-22.
 48. Parmentier CN, Fuerst E, McDonald J, Bowen H, Lee TH, Pease JE, Woszczek G, Cousins DJ. Human T(H)2 cells respond to cysteinyl leukotrienes through selective expression of cysteinyl leukotriene receptor 1. *J Allergy Clin Immunol* 2012;129:1136-42.
 49. Laidlaw TM, Boyce JA. Pathogenesis of aspirin-exacerbated respiratory disease and reactions. *Immunol Allergy Clin North Am* 2013;33:195-210.
 50. Christie PE, Tagari P, Ford-Hutchinson AW, Charlesson S, Chee P, Arm JP, Lee TH. Urinary leukotriene E4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am Rev Respir Dis* 1991;143:1025-9.
 51. White A, Ludington E, Mehra P, Stevenson DD, Simon RA. Effect of leukotriene modifier drugs on the safety of oral aspirin challenges. *Ann Allergy Asthma Immunol* 2006;97:688-93.
 52. Dahlén SE, Malmström K, Nizankowska E, Dahlén B, Kuna P, Kowalski M, Lumry WR, Picado C, Stevenson DD, Bousquet J, Pauwels R, Holgate ST, Shahane A, Zhang J, Reiss TF, Szczeklik A. Improvement of aspirin-intolerant asthma by montelukast, a leukotriene antagonist: a randomized, double-blind, placebo-controlled trial. *Am J Respir Crit Care Med* 2002;165:9-14.
 53. Cowburn AS, Sladek K, Soja J, Adamek L, Nizankowska E, Szczeklik A, Lam BK, Penrose JF, Austen FK, Holgate ST, Sampson AP. Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J Clin Invest* 1998;101:834-46.
 54. Adamjee J, Suh YJ, Park HS, Choi JH, Penrose JF, Lam BK, Austen KF, Cazaly AM, Wilson SJ, Sampson AP. Expression of 5-lipoxygenase and cyclooxygenase pathway enzymes in nasal polyps of patients with aspirin-intolerant asthma. *J Pathol* 2006;209:392-9.
 55. Maclouf J, Murphy RC, Henson PM. Transcellular biosynthesis of sulfidopeptide leukotrienes during receptor-mediated stimulation of human neutrophil/platelet mixtures. *Blood* 1990;76:1838-44.
 56. Laidlaw TM, Kidder MS, Bhattacharyya N, Xing W, Shen S, Milne GL, Castells MC, Chhay H, Boyce JA. Cysteinyl leukotriene overproduction in aspirin-exacerbated respiratory disease is driven by platelet-adherent leukocytes. *Blood* 2012;119:3790-8.
 57. Fischer AR, Rosenberg MA, Lilly CM, Callery JC, Rubin P, Cohn J, White MV, Igarashi Y, Kaliner MA, Drazen JM, Israel E. Direct evidence for a role of the mast cell in the nasal response to aspirin in aspirin-sensitive asthma. *J Allergy Clin Immunol* 1994;94:1046-56.
 58. Yoshida S, Amayasu H, Sakamoto H, Onuma K, Shoji T, Nakagawa H, Tajima T. Cromolyn sodium prevents bronchoconstriction and urinary LTE4 excretion in aspirin-induced asthma. *Ann Allergy Asthma Immunol* 1998;80:171-6.
 59. Christie PE, Schmitz-Schumann M, Spur BW, Lee TH. Airway responsiveness to leukotriene C4 (LTC4), leukotriene E4 (LTE4) and histamine in aspirin-sensitive asthmatic subjects. *Eur Respir J*

- 1993;6:1468-73.
60. Corrigan CJ, Napoli RL, Meng Q, Fang C, Wu H, Tochiki K, Reay V, Lee TH, Ying S. Reduced expression of the prostaglandin E2 receptor E-prostanoid 2 on bronchial mucosal leukocytes in patients with aspirin-sensitive asthma. *J Allergy Clin Immunol* 2012;129:1636-46.
 61. Sousa AR, Parikh A, Scadding G, Corrigan CJ, Lee TH. Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. *N Engl J Med* 2002;347:1493-9.
 62. Arm JP, O'Hickey SP, Spur BW, Lee TH. Airway responsiveness to histamine and leukotriene E4 in subjects with aspirin-induced asthma. *Am Rev Respir Dis* 1989;140:148-53.
 63. Kanaoka Y, Maekawa A, Penrose JF, Austen KF, Lam BK. Attenuated zymosan-induced peritoneal vascular permeability and IgE-dependent passive cutaneous anaphylaxis in mice lacking leukotriene C4 synthase. *J Biol Chem* 2001;276:22608-13.
 64. Maekawa A, Austen KF, Kanaoka Y. Targeted gene disruption reveals the role of cysteinyl leukotriene 1 receptor in the enhanced vascular permeability of mice undergoing acute inflammatory responses. *J Biol Chem* 2002;277:20820-4.
 65. Maekawa A, Kanaoka Y, Xing W, Austen KF. Functional recognition of a distinct receptor preferential for leukotriene E4 in mice lacking the cysteinyl leukotriene 1 and 2 receptors. *Proc Natl Acad Sci U S A* 2008;105:16695-700.
 66. Barrett NA, Rahman OM, Fernandez JM, Parsons MW, Xing W, Austen KF, Kanaoka Y. Dectin-2 mediates Th2 immunity through the generation of cysteinyl leukotrienes. *J Exp Med* 2011;208:593-604.
 67. Barrett NA, Maekawa A, Rahman OM, Austen KF, Kanaoka Y. Dectin-2 recognition of house dust mite triggers cysteinyl leukotriene generation by dendritic cells. *J Immunol* 2009;182:1119-28.
 68. Maekawa A, Xing W, Austen KF, Kanaoka Y. GPR17 regulates immune pulmonary inflammation induced by house dust mites. *J Immunol* 2010;185:1846-54.
 69. Barrett NA, Fernandez JM, Maekawa A, Xing W, Li L, Parsons MW, Austen KF, Kanaoka Y. Cysteinyl leukotriene 2 receptor on dendritic cells negatively regulates ligand-dependent allergic pulmonary inflammation. *J Immunol* 2012;189:4556-65.
 70. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, McKenzie AN, Mebius RE, Powrie F, Vivier E. Innate lymphoid cells--a proposal for uniform nomenclature. *Nat Rev Immunol* 2013;13:145-9.
 71. Doherty TA, Khorram N, Lund S, Mehta AK, Croft M, Broide DH. Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production. *J Allergy Clin Immunol* 2013;132:205-13.
 72. Pitchford SC, Momi S, Giannini S, Casali L, Spina D, Page CP, Gresele P. Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. *Blood* 2005;105:2074-81.
 73. Pitchford SC, Riffo-Vasquez Y, Sousa A, Momi S, Gresele P, Spina D, Page CP. Platelets are necessary for airway wall remodeling in a murine model of chronic allergic inflammation. *Blood* 2004;103:639-47.
 74. Hasegawa S, Ichiyama T, Hashimoto K, Suzuki Y, Hirano R, Fukano R, Furukawa S. Functional expression of cysteinyl leukotriene receptors on human platelets. *Platelets* 2010;21:253-9.
 75. Cummings HE, Liu T, Feng C, Laidlaw TM, Conley PB, Kanaoka Y, Boyce JA. Cutting edge: Leukotriene C4 activates mouse platelets in plasma exclusively through the type 2 cysteinyl leukotriene receptor. *J Immunol* 2013;191:5807-10.
 76. Paruchuri S, Tashimo H, Feng C, Maekawa A, Xing W, Jiang Y, Kanaoka Y, Conley P, Boyce JA. Leukotriene E4-induced pulmonary inflammation is mediated by the P2Y12 receptor. *J Exp Med* 2009;206:2543-55.
 77. Nonaka Y, Hiramoto T, Fujita N. Identification of endogenous surrogate ligands for human P2Y12 receptors by in silico and in vitro methods. *Biochem Biophys Res Commun* 2005;337:281-8.
 78. Hirata H, Arima M, Fukushima Y, Honda K, Sugiyama K, Tokuhisa T, Fukuda T. Over-expression of the LTC4 synthase gene in mice reproduces human aspirin-induced asthma. *Clin Exp Allergy* 2011;41:1133-42.
 79. Luo M, Jones SM, Flamand N, Aronoff DM, Peters-Golden M, Brock TG. Phosphorylation by protein kinase A inhibits nuclear import of 5-lipoxygenase. *J Biol Chem* 2005;280:40609-16.
 80. Murakami M, Naraba H, Tanioka T, Semmyo N, Nakatani Y, Kojima F, Ikeda T, Fueki M, Ueno A, Oh S, Kudo I. Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. *J Biol Chem* 2000;275:32783-92.
 81. Yoshimura T, Yoshikawa M, Otori N, Haruna S, Moriyama H. Correlation between the prostaglandin D(2)/E(2) ratio in nasal polyps and the recalcitrant pathophysiology of chronic rhinosinusitis associated with bronchial asthma. *Allergol Int* 2008;57:429-36.
 82. Picado C, Fernandez-Morata JC, Juan M, Roca-Ferrer J, Fuentes M, Xaubet A, Mullol J. Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. *Am J Respir Crit Care Med* 1999;160:291-6.
 83. Cheong HS, Park SM, Kim MO, Park JS, Lee JY, Byun JY, Park BL, Shin HD, Park CS. Genome-wide methylation profile of nasal polyps: relation to aspirin hypersensitivity in asthmatics. *Allergy* 2011;66:637-44.
 84. Liu T, Laidlaw TM, Katz HR, Boyce JA. Prostaglandin E2 deficiency causes a phenotype of aspirin sensitivity that depends on platelets and cysteinyl leukotrienes. *Proc Natl Acad Sci U S A* 2013;110:16987-92.