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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 C<sub>4</sub> 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  $B_4$  (LTB<sub>4</sub>), 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.<sup>1-3</sup> 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 A<sub>2</sub>.<sup>4</sup> 5-LO activating protein presents arachidonic acid to 5-LO, which catalyzes the formation of 5-hydroperoxyeicosatetraenoic acid and then the unstable epoxide LTA<sub>4</sub>.<sup>5</sup> In mast cells, macrophages, eosinophils, and basophils, LTC<sub>4</sub> synthase (LTC<sub>4</sub>S) conjugates LTA<sub>4</sub> to reduced glutathione, forming LTC<sub>4</sub>, the parent of the cys-LTS.<sup>6</sup> Once formed, LTC<sub>4</sub> is transported to extracellular space via the ATP-binding cassette (ABC) transporters-1 and-4 and then metabolized to LTD<sub>4</sub> and LTE<sub>4</sub> by  $\gamma$ -glutamyl transpeptidases and dipeptidases, respectively. The rapid extracellular metabolism of LTC<sub>4</sub> and LTD<sub>4</sub> results in short biologic half-lives relative to the stable mediator LTE<sub>4</sub>, which is abundant and readily detected in biologic fluids. In neutrophils, LTA<sub>4</sub> is hydrolyzed by a cytosolic LTA<sub>4</sub> hydrolase enzyme to form LTB<sub>4</sub>, a dihydroxy leukotriene that is a potent chemoattractant for neutrophils and monocytes.<sup>7</sup>

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.<sup>8-11</sup> 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 LTC<sub>4</sub>S.<sup>12</sup> LTC<sub>4</sub>S enzymatic function can be inhibited by protein kinase C (PKC)-dependent phosphorylation, which can limit the generation of cys-LTs *ex vivo*.<sup>13</sup> 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).<sup>11</sup> 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.<sup>14</sup> The molecular characterization of the classical G protein-coupled receptors (GPCRs) partially reconciled this pharmacology. The type 1 cys-LT receptor, CysLT<sub>1</sub>R, is a high-affinity receptor for LTD<sub>4</sub> and the target of antagonists (Montelukast, Zafirlukast, and Pranlukast) that are used for the management of asthma. The cloned human CysLT<sub>1</sub>R 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 CysLT<sub>2</sub>R is 38% identical to CysLT<sub>1</sub>R in amino acid sequence.<sup>16</sup> CysLT<sub>2</sub>R binds LTC<sub>4</sub> and LTD<sub>4</sub> with equal affinity, and binds LTD<sub>4</sub> with affinity one-log less than CysLT1R. CysLT2R is resistant to Montelukast, and is expressed both on cells that also express CysLT<sub>1</sub>R (e.g., myeloid cells, smooth muscle), as well as endothelial cells, cardiac Purkinje cells, adrenal medulla, and brain.<sup>16</sup> 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 LTC<sub>4</sub> and LTD<sub>4</sub>, the cloned CysLT<sub>1</sub>R and CysLT<sub>2</sub>R receptors display trivial binding affinity for the stable metabolite LTE<sub>4</sub>. 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 LTE<sub>4</sub>.<sup>14,17</sup> LTE<sub>4</sub> also was equipotent to its precursors for inducing wheal and flare responses when injected intradermally into humans.<sup>18</sup> Recently GPR99, previously reported as an oxyglutarate receptor,<sup>19</sup> was identified as a potential LTE<sub>4</sub> receptor.<sup>20</sup> LTE<sub>4</sub> binds and activates GPR99 at low nM range concentrations in transfected cells, and resists blockade by MK571, a prototype CysLT<sub>1</sub>R antagonist. The ability of LTE<sub>4</sub> 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 CysLT<sub>1</sub>R by human peripheral blood monocytes and monocyte-derived macrophages.<sup>21</sup> IL-13, but not IL-4, upregulates CysLT<sub>2</sub>R expression as well in human monocytes.<sup>22</sup> IL-13 and transforming growth factor beta induce CysLT<sub>1</sub>R expression by human bronchial smooth muscle cells.<sup>23</sup> CysLT<sub>1</sub>R can be inducibly expressed by mouse T cells stimulated through the T cell receptor.<sup>24</sup> CvsLT<sub>1</sub>R signaling is also controlled by PKA<sup>25</sup> - or PKC<sup>26</sup> -dependent phosphorylation and desensitization. PKC mediates ligand-induced internalization of CysLT<sub>1</sub>R following stimulation with LTD<sub>4</sub>.<sup>27</sup> 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 CysLT<sub>1</sub>R without causing its internalization.<sup>26</sup> Since nucleotides, the natural ligands for P2Y receptors, are released in large quantities during acute inflammatory responses,<sup>28</sup> signaling through the cognate P2Y receptors may limit potentially deleterious effects of CysLT<sub>1</sub>R 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.

CysLT<sub>1</sub>R functions can also be regulated by direct physical interactions with other GPCRs. CysLT<sub>1</sub>R and CysLT<sub>2</sub>R heterodimerize in cultured human mast cells.<sup>29</sup> The presence of CysL-T<sub>2</sub>R limits the levels of membrane expression of CysLT<sub>1</sub>R, and dampens the capacity of CysLT<sub>1</sub>R to induce phosphorylation of extracellular signal regulated kinase and proliferation in this cell type. GPR17, a GPCR homologous to CysLT<sub>1</sub>R and CysL-T<sub>2</sub>R,<sup>30</sup> was originally "deorphanized" as a dual-specific receptor for cys-LTs and uracil nucleotides.<sup>31</sup> However, we and others could not reproduce GPR17 activation by either ligand type in various assay systems.<sup>30,32,33</sup> Instead, GPR17 functions as a negative regulator of LTD<sub>4</sub>-mediated CysLT<sub>1</sub>R activation, and markedly reduces binding of LTD<sub>4</sub> when the two receptors are co-expressed in cell lines.<sup>30</sup> Accordingly, mice lacking GPR17 (*Gpr17* -/- mice) showed markedly enhanced CysLT<sub>1</sub>R-dependent tis-





**Figure.** Cross-regulation of the cysteinyl leukotriene receptors. CysLT<sub>1</sub>R function is inhibited both by direct physical interactions with CysLT<sub>2</sub>R or GPR17, and by heterologous, PKC-dependent phosphorylation by P2Y receptors. The lack of both CysLT<sub>1</sub>R and CysLT<sub>2</sub>R amplifies cutaneous responses to LTE<sub>4</sub>, suggesting that both classical receptors cross-regulate GPR99. The requirement for P2Y<sub>12</sub> receptors for the ability of LTE<sub>4</sub> to amplify pulmonary eosinophilia could reflect an interaction with GPR99.

sue edema induced by IgE-dependent passive cutaneous anaphylaxis.<sup>30</sup> Thus, at least two GPCRs (CysLT<sub>2</sub>R and GPR17) dampen CysLT<sub>1</sub>R function by direct physical interactions. The fact that both direct and indirect mechanisms can limit signaling through CysLT<sub>1</sub>R (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.<sup>34-36</sup> LTE<sub>4</sub> was a weaker bronchoconstrictor than LTC4 and LTD4, but was ~1-logfold more potent in inducing bronchoconstriction in asthmatic subjects relative to nonasthmatic controls.<sup>37</sup> Additionally, when delivered by inhalation, LTE4 caused the accumulation of eosinophils and basophils in the bronchial submucosa of mild asthmatic subjects, whereas LTD<sub>4</sub> did not.<sup>38</sup> In retrospect, these findings not only implied that end-organ reactivity to LTE<sub>4</sub> is specifically enhanced in asthma, but also suggested the existence of distinct receptors with a preference for binding and activation by LTE<sub>4</sub>.

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.<sup>39</sup> 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.<sup>40</sup> Urinary levels of LTE<sub>4</sub> increase during spontaneous asthma exacerbations,<sup>41</sup> 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. CysLT<sub>1</sub>R antagonists also attenuate the magnitude of decline in FEV1 in response to allergen challenge.<sup>46</sup> Cys-LT-generating enzymes are expressed by eosinophils, monocytes, and mast cells in nasal biopsies from subjects with allergic rhinitis,<sup>39</sup> and CysL-T<sub>1</sub>R and CysLT<sub>2</sub>R localize to both hematopoietic and non-hematopoietic cell types in the nasal tissue.<sup>39,47</sup> Additionally, CysL-T<sub>1</sub>R is expressed by human Th2 cells in peripheral blood from atopic subjects.<sup>48</sup> 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 CysLT<sub>1</sub>R antagonists on rhinitis may reflect 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).<sup>49</sup> Baseline levels of urinary LTE<sub>4</sub> 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.<sup>50</sup> The administration of either Zileuton or CysLT<sub>1</sub>R antagonists attenuates the severity of aspirin-induced bronchoconstriction in AERD.<sup>51</sup> Both classes of drugs were also superior to placebo for improving sinonasal function.<sup>2,52</sup> 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 LTC<sub>4</sub>S protein relative to eosinophils in biopsies from aspirin tolerant controls.<sup>53,54</sup> Platelets, which lack 5-LO, also express LTC<sub>4</sub>S and can convert granulocyte-derived LTA<sub>4</sub> to LTC<sub>4</sub> through a transcellular mechanism.<sup>55</sup> 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.<sup>56</sup> These adherent platelets contribute as much as 60% of the LTC<sub>4</sub>S 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 LTE<sub>4</sub>.56 Mast cell activation accompanies the responses to aspirin challenge in AERD,<sup>57</sup> and the administration of mast cell stabilizing cromone drugs blocks the rise in urinary LTE<sub>4</sub> that accompanies reactions.<sup>58</sup> 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  $LTE_4^{59}$  and  $LTD_4^{60}$  at significantly lower doses. The numbers and percentages of CysLT<sub>1</sub>R-positive mast cells, eosinophils, and monocytes in nasal biopsies from patients with AERD exceed those observed in the tissues of aspirin-tolerant asthmatic controls.<sup>47,61</sup> CysLT<sub>1</sub>R expression on hematopoietic cells decreases following desensitization to aspirin,<sup>61</sup> a procedure that attenuates bronchial reactivity to  $LTE_4$ .<sup>62</sup> The numbers and distributions of CysLT<sub>2</sub>R-positive cells do not differ between aspirin tolerant asthmatics and subjects with AERD. Interestingly, bronchial reactivity to inhaled LTD<sub>4</sub> in AERD or aspirin tolerant

asthma does not correlate with the numbers of  $CysLT_1R$ - or  $CysLT_2R$ - expressing cells in bronchial biopsies.<sup>60</sup> 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 LTE<sub>4</sub>) observed in AERD.

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

The development of mice lacking LTC4S (*Ltc4s*-/-), CysLT<sub>1</sub>R (*Cysltr1*-/-), CysLT<sub>2</sub>R (*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 .<sup>63</sup> Intraperitoneal injections of zymosan, a yeast cell wall glycan that elicits LTC<sub>4</sub> generation from macrophages, induced vascular leak that was reduced in both the *Ltc4s*-/- and *Cysltr1*-/- strains by ~50% compared with WT controls.<sup>63,64</sup> The responses of *Cysltr2*-/- mice were equivalent to those of WT controls. Thus, *CysLT*.R 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 LTE<sub>4</sub> 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 LTE<sub>4</sub>.<sup>65</sup> Given that GPR99 bound LTE<sub>4</sub> 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 LTE<sub>4</sub>-mediated vascular permeability, but not to LTC4 or LTD4, suggesting a preference of GPR99 for LTE<sub>4</sub>.<sup>20</sup>

#### Th2 Immunity

Lung Th2 immunity to the house dust mite *Dermatophagoides farinae* (*Df*) requires stimulation of the myeloid C-type lectin receptor, Dectin-2.<sup>66,67</sup> Based on a protocol of sensitization of naive WT mice by means of adoptive transfer of *Df*-pulsed dendritic cells (DCs), Th2 responses to Df require the expressions of LTC<sub>4</sub>S and CysLT<sub>1</sub>R 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.<sup>18</sup> 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 *Cvsltr1-/*allele,68 whereas the introduction of the *Ltc4s*-/- allele eliminated the potentiation of Th2 immunity induced by transfer of Cysltr2-/- DCs.<sup>69</sup> Thus, constitutive downregulation of CysLT<sub>1</sub>R function by GPR17 and CysLT<sub>2</sub>R 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.<sup>70</sup> 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 CysLT<sub>1</sub>R, and responded to stimulation in vitro and in vivo with LTD<sub>4</sub> by proliferating and releasing cytokines. Interestingly, while both LTD<sub>4</sub> and IL-33 caused lung ILC2 to generate IL-5 and IL-13, only LTD<sub>4</sub> 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 LTD<sub>4</sub> could be blocked by Montelukast, LTE<sub>4</sub>-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.<sup>72,73</sup> 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 CysLT<sub>1</sub>R and CysL-T<sub>2</sub>R,<sup>74,75</sup> as well as the P2Y<sub>12</sub> receptor, a homologue of the cys-LT receptors that binds ADP. Stimulation of mouse platelets with LTC<sub>4</sub> strongly induces their expression of P-selectin in an entirely CysLT<sub>2</sub>R-dependent manner, while LTD<sub>4</sub> or LTE<sub>4</sub> are inactive. Intratracheal administration of LTC<sub>4</sub>, but not LTD<sub>4</sub>, markedly 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 LTC<sub>4</sub> on platelet-associated CysLT<sub>2</sub>R in the lung vasculature.

Although LTE<sub>4</sub> fails to directly activate mouse or human platelets *in vitro*,<sup>75,76</sup> intratracheal administration of LTE<sub>4</sub>, like that of LTC<sub>4</sub>, potentiates OVA-induced eosinophilia in a platelet-dependent manner in WT mice.<sup>76</sup> In this model, LTE<sub>4</sub> is fully active in Cysltr1/Cysltr2-/- mice, suggesting that it acts at a nonclassical cys-LT receptor. Both the effects of LTE4 (in vivo) and of LTC<sub>4</sub> (in vivo and in vitro) depend exquisitely on the P2Y<sub>12</sub> receptor.<sup>75,76</sup> A computer modeling study predicted that P2Y<sub>12</sub> receptors might recognize LTE<sub>4</sub> as a surrogate ligand,<sup>77</sup> and LTE<sub>4</sub> elicits calcium flux<sup>77</sup> and phosphorylation of extracellular signal regulated kinase<sup>76</sup> in transfected cells over-expressing human P2Y<sub>12</sub> receptors. Nonetheless, radiolabeled LTE<sub>4</sub> does not directly bind to microsomal membranes from P2Y<sub>12</sub> receptor-expressing transfectants. It is presently unknown whether the involvement of P2Y<sub>12</sub> in LTE<sub>4</sub>-dependent signaling responses and airway inflammation reflects a direct interaction between P2Y<sub>12</sub> receptors and a bona fide LTE<sub>4</sub> receptor, such as GPR99. The therapeutic potential of drugs that block P2Y<sub>12</sub> 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,<sup>49</sup> 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 LTC<sub>4</sub>S and examined the phenotype in OVA-induced pulmonary inflammation with or without treatment with a COX inhibitor, sulpyrine.78 OVAchallenged LTC<sub>4</sub>S-transgenic mice, but not similarly treated WT mice, demonstrated a significant increase in airway resistance by sulpyrine treatment. This is associated with increases in LTC<sub>4</sub> and LTB4 in bronchoalveolar lavage (BAL) fluid in sulpyrinetreated OVA-challenged transgenic mice. Importantly, the increase in airway resistance was inhibited by Pranlukast, a CysL-T<sub>1</sub>R antagonist. This study demonstrates that the pathognomonic 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 AERD<sup>53</sup> has a potentially causal role.

Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) controls cys-LT generation by activating PKA and inducing phosphorylation of 5-LO.<sup>79</sup> Tissue inflammation is typically associated with increased PGE<sub>2</sub> production, reflecting the co-expression of 2 inducible enzymes; COX-2 (a largely aspirin-resistant enzyme) and microsomal PGE<sub>2</sub> synthase-1 (mPGES-1), which isomerizes COX-2-derived PGH<sub>2</sub> to PGE<sub>2</sub>.<sup>80</sup> Nasal polyps from subjects with AERD contain less PGE<sub>2</sub> than nasal polyps from aspirin tolerant controls,<sup>81</sup> possibly relating to epigenetic modifications of COX-2<sup>82</sup> and/or mP-GES-1 expression.83 Mice lacking mPGES-1 (Ptges-/-) cannot upregulate PGE<sub>2</sub> 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.<sup>84</sup> Aspirin challenge profoundly depletes lung PGE<sub>2</sub> in the Ptges-/- mice, but not in the WT controls, suggesting that the mPGES-1 is needed to maintain PGE<sub>2</sub> 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, cvs-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 A<sub>2</sub>. This model may be useful in defining the potential pathogenetic role of GPR99, CysLT<sub>2</sub>R, and P2Y<sub>12</sub> 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 CysLT<sub>1</sub>R 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 (CysLT<sub>2</sub>R 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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