Mechanisms of allergic diseases
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The leukotriene E4 puzzle: Finding the missing pieces and revealing the pathobiologic implications

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The intracellular parent of the cysteinyl leukotrienes (cysLTs), leukotriene (LT) C4, is formed by conjugation of LTA4 and reduced glutathione by LTC4 synthase in mast cells, eosinophils, basophils, and macrophages. After extracellular export, LTC4 is converted to LTD4 and LTE4 through sequential enzymatic removal of glutamic acid and then glycine. Only LTE4 is sufficiently stable to be prominent in biologic fluids, such as urine or bronchoalveolar lavage fluid, of asthmatic individuals. Although widely used as a biomarker of cystLT pathway activity in clinical studies, LTE4 has received little attention because it binds poorly to the classical cysteinyl leukotriene receptor [CysLT1R] and is much less active on normal airways than LTC4 or LTD4. However, early studies indicated that LTE4 caused skin swelling in human subjects as potently as LTC4 and LTD4, that airways of asthmatic subjects observed functionally in the skin of mice lacking the type 1 and 2 cysteinyl leukotriene receptor [CysLT2R]). However, several earlier studies clearly demonstrated that LTE4 had biologic activity that differed from that of its precursors, predicting (correctly in retrospect) the existence of specific LTE4-reactive receptors. This review will highlight LTE4 from a historical

Of the 3 cysteinyl leukotrienes (cysLTs; leukotriene [LT] C4, LTD4, and LTE4), only LTE4 is sufficiently stable so as to be detectable in extracellular fluids. Although widely used as a biomarker of cystLT pathway activity in clinical studies, LTE4 has received little attention in recent literature as a mediator of inflammation because of its poor activity at the classical cysteinyl leukotriene receptors (type 1 cysteinyl leukotriene receptor [CysLT1R] and type 2 cysteinyl leukotriene receptor [CysLT2R]). However, recent studies have begun to uncover receptors selective for LTE4, P2Y12, an adenosine diphosphate receptor, and CysLT2R, which was observed functionally in the skin of mice lacking the type 1 and 2 cysteinyl leukotriene receptors. These findings prompt a renewed focus on LTE4 receptors as therapeutic targets that are not currently addressed by available receptor antagonists. (J Allergy Clin Immunol 2009;124:406-14.)

Key words: Leukotriene E4, G protein-coupled receptor, bronchial asthma, inflammation, knockout mouse
by in vitro DISCOVERY OF LTE₄ and mechanisms of action.

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**CysLT2R:** Type 2 cysteinyl leukotriene receptor

**CysLT1R:** Type 1 cysteinyl leukotriene receptor

**ERK:** Extracellular signal-regulated kinase

**GPCR:** G protein–coupled receptor

**LT:** Leukotriene

**LTC₄S:** Leukotriene C₄ synthase

**MIP-1β:** Macrophage inflammatory protein 1B

**PG:** Prostaglandin

**PPAR-γ:** Peroxisome proliferator-activated receptor γ

**SRS-A:** Slow-reacting substance of anaphylaxis

**WT:** Wild-type


discovery of the identification of its receptors and mechanisms of action.

**DISCOVERY OF LTE₄**

The slow-reacting substance of anaphylaxis (SRS-A), so named by Brocklehurst,¹ was identified as a substance generated by *in vitro* antigen/allergen challenge of perfused lungs of actively sensitized guinea pigs or human lung fragments of allergic patients requiring resection. Its potent constrictor activity on guinea pig or human bronchioles in the presence of an antihistamine provided compelling evidence for its potential role in asthma. The initial analyses into the physical characteristics and composition of SRS-A from the rat suggested possible sulfur content.² This led to the identification by Murphy et al³ of LTC₄, the first component of SRS-A, by loading a mastocytoma cell line with [³⁵S]cysteine and identifying the radiolabeled component released in response to activation with calcium ionophore. LTC₄ was composed of a metabolite of *arachidonic acid* (eicosatetraenoic acid) with 3 conjugated double bonds and a peptide added through a sulfur bridge. The exact stereochremistry of the lipid and the amino acid sequence of the S-linked peptide of LTC₄ was obtained by comparing purified natural SRS-A with candidate synthetic cysLTs prepared by E. J. Corey.⁴ These synthetic cysLTs showed bioactivity consistent with the functional definition of SRS-A offered by Brocklehurst,¹ who noted that a range of activities could contract the guinea pig ileum in the presence of an antihistamine.

Because we had anticipated that the activity of SRS-A could be attributed to a single product, we were surprised to find that partially purified SRS-A from the peritoneal cavity of rats,


diagrammatically. The Editors wish to acknowledge Seema Aceves, MD, PhD, for preparing this glossary.

**GLOSSARY**

**ARACHIDONIC ACID:** Arachidonic acid is the precursor for both LTs and prostaglandins and is found on the nuclear membrane. Together, LTs and prostaglandins are called eicosanoids.

**ASPIRIN-EXACERBATED RESPIRATORY DISEASE (AERD):** AERD consists of a clinical constellation of nasal polyposis with eosinophilic sinusitis, asthma, and idiosyncratic sensitivity to nonstereoidal anti-inflammatory agents that inhibit COX-1. Treatment of patients with AERD includes LT inhibitors.

**COX-2:** COX-2 is induced by LPS, IL-1, and IL-2 to produce prostaglandin intermediates from arachidonic acid.

**CYSTEINYL LEUKOTRIENE** (cysLT): LTs, so named because of their origin from "leuko"cytes and conserved 3-double-bond "trienes," are generated from arachidonic acid by S-lipoxygenase/S-lipoxygenase–activating protein. Conversion of LT₄ to LTC₄ by means of addition of activating protein. Conversion of LTA₄ to LTC₄ by means of addition of glutathione is the first step in generating cysLTs. LTC₄ hydrolyase converts LT₄ to LTB₄. The major sources of LTC₄S (and thus cysLTs) are eosinophils, basophils, and macrophages. Mast cells make both LTC₄S and, along with neutrophils and macrophages, LTA₄ hydrolyase.

**CYSTEINYL LEUKOTRIENE RECEPTOR** (CysLTR): Both cysLT receptors and LTB₄ receptors are G protein-coupled receptors. CysLTR receptors can be upregulated by IL-4. LTB₄ uses 2 receptors, BLT1 and BLT2, which are expressed on most tissues and upregulated by IFN-γ and promote neutrophil chemotaxis when activated by LT₄.

**DYNAMIC COMPLIANCE:** Airway compliance is a measure of volume change per unit of pressure. Lungs from patients with longstanding asthma have been reported to show decreased compliance, perhaps because of airway remodeling and associated fibrosis.

**INDOMETHACIN:** Indomethacin inhibits COX-1 and can trigger AERD.

**IL-6:** IL-6 primes for T₂ effector cells, inhibits the suppressive functions of CD4⁺CD25⁺ regulatory T cells, and protects mast cells from apoptosis.

**IL-10:** Generally associated with dampening immune responses, IL-10 decreases mast cell functions, such as IgE-mediated activation and anaphylaxis, in murine models. But blocks the production of both cysLTs and LTB₄.

**MIP-1α (MIP-1b):** MIP-1 is involved in the chemotaxis and activation of monocytes. CysLTs can induce MIP-1β and MIP-1α production from monocytes through the CysLT₁R.

**PERMЕABILITY-ENHANCING ACTIVITY:** Activated vascular endothelium allows leakage of dyes (eg, Evans blue and fluorescein isothiocyanate-albumin) into the extravascular space, making tissues appear colored (blue) or fluorescent (fluorescein isothiocyanate).

**PERTUSSIS TOXIN:** Pertussis toxin inhibits the function of GPCRs through adenosine diphosphate ribosylation of Gα.

**PULMONARY RESISTANCE:** Mechanical factors that limit alveolar access to air. Pulmonary resistance is calculated by using Ohm’s and Poiseille’s laws, which factor in the pressure difference at the mouth and alveoli, the rate of airflow, viscosity, and the length and radius of the airways.

**PROSTAGLANDIN D₂ (PGD₂):** A mast cell eicosanoid made in large quantities after IgE-mediated mast cell activation. PGD₂ levels are increased after allergen challenge, and it functions as a bronchconstrictor and vasodilator and is associated with eosinophil influx.

**PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ (PPAR-γ):** Transcription factors in the nuclear hormone receptor superfamily that binds retinoic acid. PPAR-γ1 and PPAR-γ2 are expressed in adipocytes and decrease proinflammatory cytokine production from macrophages, B and T cells, eosinophils, dendritic cells, and airway epithelium.

**RESPIRATORY BURST:** The neutrophil oxidative burst generates superoxide anions and reactive oxygen intermediates important for microbial killing through the reduced nicotinamide adenine dinucleotide phosphate oxidase complex. Mutations in this complex (*gp91, gp67phox, gp22, and gp47phox*) cause chronic granulomatous disease and recurrent infections with organisms such as *Staphylococcus aureus*, *Aspergillus* species, *Serratia* species, and *Burkholderia cepacia*.

"LUKASTS"s: Montelukast, pranlukast, and zafirlukast all block the CysLT₁R, whereas biosynthetic pathway inhibitors, such as zileuton, block the production of both cysLTs and LTB₄.

The Editors wish to acknowledge Seema Aceves, MD, PhD, for preparing this glossary.
undergoing IgGa-dependent anaphylaxis was comprised of 3 products, all with 3 conjugated double bonds and each having contractile activity for the guinea pig ileum. By comparison with active and inactive standards with different peptide adducts, we recognized the 3 components of authentic SRS-A to be detected in single-cell systems, whereas all extracellular space accounted for the fact that it was the only component of SRS-A to be detected in biologic fluids. These early studies demonstrated that the export of the former compound to the extracellular space. Further, CysLT1R exhibited a marked preference for binding of LTD4, whereas LTD4 and LTE4.6 These 2 additional structures differed from LTC4 in that the former possessed the sulfur-linked glutathione tripeptide adduct composed of glutamic acid, glycine, and cysteine, whereas LTD4 lacked the glutamic acid residue and LTE4 lacked both the glutamic acid and glycine residues (Fig 1). Because LTC4 is the only intracellular cysLT generated by LTD4 synthase (LTC4S), it seemed likely that LTC4 was formed extracellularly from LTC4 by means of deletion of glutamic acid (by γ-glutamyl transpeptidase or γ-glutamyl leukotriene) after the export of the former compound to the extracellular space. Further removal of glycine from the remaining dipeptide adduct of LTD4 by dipeptidases accounted for LTE4 with a remaining cysteine adduct.5 That LTC4 underwent enzymatic modification in the extracellular space accounted for the fact that it was the only component of SRS-A to be detected in single-cell systems, whereas all 3 components were detected in biologic fluids. These early studies not only showed natural SRS-A to be comprised of 3 cysLTSs but also demonstrated that each cysLT had contractile activity for ideal smooth muscle in vitro and in vivo (Fig 2) and permeability-enhancing activity by means of intradural injection into the guinea pig prepared with Evans blue dye.6

Members of the pharmaceutical industry then used contractile assays to characterize the putative “receptors” for cysLTSs and to identify potential antagonists. This approach permitted the development of the prototypes of the orally available CysLT1R-selective antagonists (“lukasts”)9,10 more than a decade before any receptor was defined in molecular terms. Human CysLT1R and CysLT2R were cloned by Evans and colleagues.9,10 CysLT1R exhibited a marked preference for binding of LTD4 over LTC4 and was the only receptor that was competitively blocked by the lukasts. CysLT2R had equal affinity for LTD4 and LTC4 and bound LTD4 at 10-fold lower affinity than did CysLT1R. It was surprising that LTE4 did not register as an appreciable binding ligand for these “classical” receptors expressed individually in cloned cells. The poor affinity of LTE4 for these cloned receptors prompted some to suggest that LTE4 was a relatively impotent extracellular metabolite and perhaps discouraged others from seeking a third receptor. In contrast, we believed that the LTE4 agonist activity that had been demonstrated in pharmacologic studies in guinea pigs and human subjects was impressive and that the greater stability of LTE4 relative to the other cysLTSs might favor a distinct pathobiologic role. The sections that follow will consider some of the early findings for LTE4, favoring the existence of a distinct receptor and revealing its relative biologic stability. We will also consider the noteworthy potency of LTE4 as a contractile agonist in guinea pig airways and in the human microvasculature, as well as a proinflammatory function based on studies using aerosolization challenge in human subjects with asthma and in allergen-sensitized mice. These studies have been key to the recognition of 2 functional receptors activated by LTE4 by 2 different laboratories with different experimental approaches.

**EARLY PHARMACOLOGY OF LTE4 IN ANIMALS**

The potency of LTE4 for contraction of guinea pig tracheal spirals in vitro was 10-fold greater than that of either LTC4 or LTD4, whereas for guinea pig parenchymal strips, the potency of LTD4 was 6-fold that of LTE4 and 20-fold that of LTC4. Furthermore, the concentration effect for LTD4 and LTE4 on parenchymal strips observed by Drazen et al12 was biphasic, with the initial low concentration effect (studied only for LTD4) being competitively antagonized by FPL55712. In contrast, LTC4 was the least potent ligand and produced only a linear response.11,12 When these ligands were administered intravenously to the intact anesthetized or unanesthetized guinea pig, LTD4 and LTC4 elicited a small increase in pulmonary resistance compared with the magnitude of the decrease in dynamic compliance, whereas LTE4 decreased compliance together with a robust increase in resistance, indicating both peripheral and central airway effects.12,13

Another distinctive effect of LTE4 was that it enhanced the contractile responses of the guinea pig tracheal smooth muscle to histamine, a property not shared with LTC4 or LTD4. The latter effect of LTE4 could be prevented by treatment of the tracheal tissue with indomethacin, indicating a key role for a COX product.14 Together, these in vitro and in vivo functional findings suggested the presence of 3 receptors for cysLTSs: a high-affinity receptor for LTD4, a low-affinity receptor for LTC4, and a separate receptor for LTE4, with the latter potentially capable of eliciting the secondary production of a prostanoi (Table I).12

The observed ratio of potency for the 3 cysLTSs in different tissues could reflect not only the profile of receptor expression in the target tissue but also the rate of conversion of 1 cysLT to another of greater or lesser activity. This is readily demonstrated when LTC4 and LTD4 are separately applied to the guinea pig ileum at concentrations sufficient to result in their maximum isometric responses. Tritiated LTC4 has a 60-second latent period before initiating a linear contractile response to 80% of maximum over 2 minutes, which is followed by a further contraction associated with slow metabolism to the more potent tritiated LTD4.
There is negligible conversion of tritiated LTC4 to tritiated LTD4/LTE4 during the linear phase. Furthermore, the inclusion of serine borate to block bioconversion of LTC4 to LTD4 by membrane γ-glutamyl transpeptidase does not change the response of the tissue to LTC4, thus confirming that this response is mediated by a specific LTC4 receptor.15 In contrast, tritiated LTD4 initiated an immediate linear contraction that reached maximum at 1 minute and then decreased sharply with linear conversion to tritiated LTE4, which has only one quarter the potency of LTD4 in this assay. When the mucosa containing the dipeptidase activity had been removed from the ileal muscle, the linear contractile response to tritiated LTD4 was maintained, reflecting the loss of metabolism to tritiated LTE4 by means of removal of the glycine. These findings nicely reflect the exquisite receptor specificity of the cysLT system that is conferred by modifications of the peptide adduct (Fig 3).

**TABLE I. Effects of various LTs on airways**

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*Cdyn*, Dynamic compliance; *RL*, lung resistance.

*In vitro activity is recorded as the ratio of the molar concentration of LTs required to achieve a half-maximal response to the concentration of histamine required to achieve an equivalent response.

*In vivo activity is recorded as the response to infusion of 3 μg/kg LT (1+, minimal response; 4+, maximal response).

**EARLY STUDIES OF LTE4 METABOLISM**

The products of the granulocyte respiratory burst, which are abundant with inflammation, can alter the stability of each cysLT in vitro and in vivo. Phorbol 12-myristate 13-acetate–activated human neutrophils converted each cysLT to their subclass-specific S-diastereoisomeric sulfoxides, which retained their ability to be detected by cysLT-specific antibodies but lost greater than 95% of function. Each sulfoxide was further processed to identical diastereoisomers of 6-trans LTB4, which were nonfunctional and no longer immunoreactive with the original antibodies. This neutrophil-mediated inactivation involved interaction of released myeloperoxidase, newly generated H2O2, and extracellular chloride ion to form hypochlorous acid. Dose-dependent attack on the sulfur bridge during hypochlorous acid formation showed that LTE4 was substantially more resistant than the other cysLTs.16,17 Systemic metabolism of the cysLTs begins after export of intracellular LTC4 and its rapid extracellular physiologic sequential conversion through LTD4 to LTE4. Studies using intravascular administration of labeled LTE4 or LTC4 to human subjects indicate that approximately 5% is recovered in the urine and is composed of LTE4 and N-acetyl LTE4. At the level of tissue peroxisomes, omega oxidation at the C-terminus yields 20-COOH-LTE4 and formation of the 20-CoA ester, which allows for sequential β oxidation with shortening of the carbon chain to 18-COOH-dinor-LTE4 and beyond.18-20

**EARLY PHARMACOLOGY OF LTE4 IN HUMAN SUBJECTS**

Although the early pharmacology of the 3 sequentially generated cysLTs identified LTE4 as the most stable in physiologic and pathobiologic models, clinical attention shifted to LTD4 and LTC4, which on inhalation were up to 1000 times as potent as histamine.21,22 LTE4 was only 39 times as potent as histamine in reducing maximum expiratory flow at 30% of vital capacity.
in healthy human subjects. Although each cysLT was a potent bronchoconstrictor in patients with bronchial asthma, there was little difference between asthmatic and healthy control subjects in sensitivity to cysLTs, which is in contrast to the hyperresponsiveness to histamine or methacholine that is characteristic of asthma. An exception to that rule is in aspirin-exacerbated respiratory disease, an asthma variant associated with marked overproduction of the cysLTs. In these subjects Christie et al showed selective hyperresponsiveness to LTE₄, but not to LTC₄, relative to that seen in aspirin-tolerant asthmatic subjects.

Because we had recognized that LTE₄ induced permeability in guinea pig skin over the same dose range (5.0-50 ng) as for LTC₄ and LTD₄, we compared their action at 1.0 nmol per site by means of intradermal injection in 3 human volunteers. Each cysLT elicited a wheal-and-flare response by 10 minutes, which peaked at 1 to 2 hours with a 10- to 20-mm wheal and a 20- to 25-mm flare. The wheal resolved by 4 hours (Fig 4), whereas the flare was still evident at 6 hours. The 3 cysLTs produced equiactive responses in each subject. Biopsy specimens at 2 hours showed dermal edema, marked and uniform dilation of the microvasculature,
and deep venules with activation of endothelial cells and some dilation of arterioles. That LTE4 was apparently as potent a permeability factor as LTC4 and LTD4 in 2 species clearly indicated that it was not a disposal product. The advent of molecular biology and the development of gene-deleted mice later permitted the use of murine microvasculature to seek a functional receptor for LTE4.

3.0 nmol per site), and saline (open squares). The greatest diameter of the wheal in millimeters is depicted versus time from 10 minutes to 6 hours. Reprinted from Soter et al26 with permission from Nature Publishing Group.

FIG 4. Wheal formation occurring with intracutaneous injections of various eicosanoids into 3 human subjects. The agonists were LTC4 (solid squares; 1.0 nmol per site), LTD4 (solid circles; 1.0 nmol per site), LTE4 (solid triangles; 1.0 nmol per site), LTB4 (open triangles; 1.6 nmol per site), PGD2 (open circles; 3.0 nmol per site), and saline (open squares). The greatest diameter of the wheal in millimeters is depicted versus time from 10 minutes to 6 hours. Reproduced from Soter et al26 with permission from Nature Publishing Group.

FUNCTIONAL AND PHARMACOLOGIC CHARACTERIZATION OF CysLT2R, A CUTANEOUS RECEPTOR PREFERENTIAL FOR LTE4

In addition to addressing the pharmacology of the cysLTs during the 1980s, we began to characterize LTC4S, the integral protein of the outer nuclear membrane responsible for biosynthesis of LTC4, by means of conjugation of glutathione to LTA4. After expression cloning of human LTC4S and then homology cloning of murine LTC4S,27,28 we turned to targeted disruption of murine LTC4S to explore for phenotypic characteristics that might depend on the functions of the cysLTs.29 In a model of passive cutaneous anaphylaxis, there was more than 50% reduction in ear swelling (indicative of vascular leak) in LTC4S-deficient mice (Ltc4s−/−) compared with wild-type (WT) mice after local sensitization of mast cells with specific IgE and systemic challenge with hapten-specific antigen. Thus the permeability-enhancing function of mast cell–derived cysLTs was at least as important as the preformed amines in this model. To analyze the contributions from individual cysLT receptors, we next generated strains deficient in CysLT1R (Cyst1r−/−) and CysLT2R (Cyst2r−/−), respectively,30,32 based on the prior cloning of these 7-transmembrane, human G protein–coupled receptors (GPCRs).9,10 In response to an intraperitoneal injection of zymosan, a yeast cell-wall material that elicits cysLT generation from macrophages, both the Ltc4s−/− and Cyslt1r−/−, but not the Cyst2r−/−, strains showed approximately 50% reductions in vascular leak, implying a key role for cysLTs acting at CysLT1R for this innate immune response.

The responsiveness of the murine vasculature to the cysLTs suggested that the existence of a distinct LTE4−reactive receptor could be proved by studying cysLT-dependent swelling responses in mice deficient in both receptors. This strain (Cyst1r/Cyst2r−/−) was created by intercrossing the Cyst1r−/− and Cyst2r−/− strains.32 The resulting double-receptor deficiency of the Cyst1r/Cyst2r−/− strain was confirmed by the absence of both receptor transcripts. We then examined the dose-dependent ear edema elicited by each cysLT in the respective Cyst1r−/−, Cyst2r−/−, and Cyst1r/Cyst2r−/− strains.32 The dose-dependent ear edema elicited by means of injection of LTD4 and LTC4 in the Cyst1r/Cyst2r−/− strain was equivalent to that in the WT control animals, indicating the presence of a previously unrecognized receptor. The Cyst1r/Cyst2r−/− mice were especially sensitive to LTE4, exhibiting the same extent of ear swelling in response to an LTE4 dose of 0.008 nmol as the response of the WT mice to 0.5 nmol (a 64-fold increase in sensitivity to LTE4). Histologic analysis of biopsy specimens at 30 and 240 minutes showed an exaggerated magnitude and duration of ear edema without cellular infiltration in response to LTE4 in the Cyst1r/Cyst2r−/− strain. The LTE4-mediated vascular leak in the Cyst1r/Cyst2r−/− strain was markedly inhibited by pretreatment of the mice with pertussis toxin or a Rho kinase inhibitor, supporting that the mechanism involved a GPCR linked to Goi proteins and Rho kinase.33 Additionally, the response to LTE4 was blocked by approximately 30% by means of treatment of the mice with indomethacin, which is reminiscent of the indomethacin sensitivity of the LTE4 response of guinea pig tracheal rings.14 The particular sensitivity of this novel receptor to LTE4 prompts the designation of CysLT2R rather than a number until it is cloned (Fig 5).

The discovery of a CysLT2R prompted us to re-evaluate the findings in WT mice and single-receptor-deficient strains. The permeability response to 0.5 nmol LTC4 or LTD4 was 50% reduced in Cyst1r−/− mice and normal in magnitude but delayed in Cyst2r−/− mice, suggesting that CysLT2R is the major signaling receptor for LTC4 and LTD4, whereas CysLT1R is a negative regulator of CysLT2R. LTE4-elicted vascular leak was not attenuated in the Cyst1r−/− mice but delayed and sustained in the Cyst2r−/− strain, suggesting that CysLT2R is the major receptor for this ligand and that CysLT1R is again a negative regulator. That the enhanced sensitivity to LTE4−induced ear edema observed in the Cyst1r/Cyst2r−/− strain was not seen with either single-receptor-null strain, Cyst1r−/− or Cyst2r−/−, implies that both CysLT3R and CysLT1R negatively regulate CysLT2R. Indeed, administration of the selective CysLT-R antagonist MK571 to Cyst2r−/− mice mimicked the phenotype of the Cyst1r/Cyst2r−/− strain in terms of the markedly increased vascular leak of the ear to intradermal LTE4. This, of course, meant that MK571 was not an inhibitor of CysLT1R. Curiously, pretreatment with MK571 had opposing effects in WT mice and the Cyst1r/Cyst2r−/− strain.32 Specifically, MK571 suppressed swelling of the skin in WT mice challenged intradermally with 0.5 nmol of LTD4, LTC4, or LTE4. In contrast, the same MK571 pretreatment and dose of ligand produced an enhanced
response to cysLTs in Cyslt1r/Cyslt2r−/− mice. Thus MK571, which is a prototype of the lukast drugs, potentiates responses apparently mediated through CysLT2R, in a setting in which neither CysLT1R nor CysLT2R is present to impart negative regulation. It is possible that MK571, which is now known to block certain transporter proteins and some purinergic (P2Y) receptors for nucleotides,[33,34] might block a yet-to-be-defined receptor with negative regulatory properties for CysLT2R.

FIG 5. Dose dependence of LTC4, LTD4, and LTE4-induced ear edema in WT and Cyslt1r/Cyslt2r−/− mice. WT (A, C, and E) and Cyslt1r/Cyslt2r−/− (B, D, and F) mice received intradermal injections of LTC4 (Fig 5A and B), LTD4 (Fig 5C and D), or LTE4 (Fig 5E and F) in the right ear and vehicle in the left ear (2 mice per group). Ear thickness was measured with calipers at the indicated times after the injection. Error bars indicate SDs. Reproduced from Maekawa et al[32] with permission from the Proceedings of the National Academy of Sciences.
**DISCOVERY THAT THE P2Y12 RECEPTOR MEDIATES MAST CELL ACTIVATION AND PULMONARY INFLAMMATION BY LTE4**

As is the case for many effector cells of bone marrow origin, mast cells express both CysLT1R and CysLT2R. LTC4 and LTD4 both induce calcium flux, cytokine and chemokine generation, phosphorylation of extracellular signal-regulated kinase (ERK), and proliferation of human mast cells in vitro. These responses, like those of the cutaneous microvasculature, are regulated positively by CysLT1R but negatively regulated by CysLT2R based on experiments in which each receptor is selectively knocked down by using RNA interference in primary human mast cells. During these studies, Jiang et al made the unanticipated finding that LTE4 exceeded the potency of LTC4 and LTD4 for increasing the numbers of human mast cells arising from cultures of cord blood–derived progenitor cells maintained in the presence of stem cell factor, IL-6, and IL-10. Subsequently, Paruchuri et al demonstrated that LTE4 not only exceeded the potency of LTC4 and LTD4 as a mitogen for a human mast cell line, LAD2, but far exceeded its potency for causing the production of the inflammatory chemokine macrophage inflammatory protein-1β (MIP-1β) and was also substantially more potent for causing the expression of inducible COX-2 and promoting delayed prostaglandin D2 (PGD2) generation. Curiously, the latter effects required the activation of peroxisome proliferator-activated receptor γ (PPAR-γ), a nuclear transcription factor that is activated by several dietary lipids and eicosanoids. However, the effect of LTE4 on PPAR-γ is indirect because LTE4 failed to activate a PPAR-γ-driven reporter in bovine endothelial cells. Indeed, the effects of LTE4 on PGD2 generation and MIP-1β production were sensitive to MK571 and pertussis toxin, whereas LTE4-mediated ERK activation was insensitive to MK571, and all LTE4 responses were completely resistant to knockdowns of CysLT1R and CysLT2R. It was thus clear that mast cells expressed at least 1 previously unrecognized LTE4 receptor that was MK571 resistant (and perhaps another that was sensitive).

Based on sequence homology between CysLT1R, CysLT2R, and the P2Y12 receptor family, it seemed likely that a putative CysLT2R might be among the orphan P2Y-like GPCRs or even a known member. Human mast cells express several such receptors, including the P2Y12 receptor, a Gai-linked receptor for adenosine diphosphate and the target of thienopyridine antithrombotic drugs. Because a computer modeling study had predicted that LTE4 might be a surrogate ligand for this receptor, we sought to determine whether recombinant P2Y12 receptors reacted to LTE4 and mediated the LTE4-dependent signaling events recognized in mast cells. LTE4 induced the activation of ERK in Chinese hamster ovary cells stably transfected with human P2Y12 receptors exceeding the potency of LTD4. This signaling event was sensitive to pertussis toxin but resistant to MK571 (unpublished data). Although P2Y12 did not bind LTE4 directly, knockdown of P2Y12 receptors by RNA interference blocked LTE4-mediated MIP-1β generation and PGD2 production by LAD2 cells without significantly altering their responses to LTD4. Because LTE4 (but not LTD4) was previously shown to induce bronchial eosinophilia when administered by means of inhalation to asthmatic human subjects, we sought to determine whether pulmonary inflammation amplified by LTE4 in mice depended on P2Y12 receptors. Administration of LTE4, but not LTD4, to the airways of sensitized BALB/c mice potentiated eosinophilia, goblet cell metaplasia, and expression of IL-13 in response to low-dose aerosolized ovalbumin. These effects were completely intact in the Cyslt1r/Cyslt2r−/− mice but were completely blocked by oral administration of the P2Y12 receptor–selective antagonist clopidogrel. The effect of P2Y12 receptor blockade was similar to the effect of platelet depletion with an antibody, suggesting that LTE4 acted as an agonist for platelet activation in the pulmonary vasculature in this model. Importantly, clopidogrel had failed to block the response of the murine skin microvasculature to LTE4, indicating that P2Y12 receptors are separate and distinct from the CysLT2R in the skin.

**EPILOGUE**

At this early stage, clinical considerations must be circumspect and limited based on these findings for receptors in naive mice in model systems or with targeted disruption of classical receptors. Nonetheless, the history of cysLT-mediated permeability effects in guinea pigs and human subjects suggests that this important aspect of the inflammatory process is as responsive to LTE4 as to its precursors, LTC4 and LTD4, which are only transiently present during an inflammatory process. The finding that the classical CysLT1R and CysLT2R are negative regulators of CysLT1R function in mice was certainly unexpected but is supported by literature showing CysLT2R to be a negative regulator of CysLT1R for murine and human mast cell proliferation and by the potentiation of LTE4-mediated skin swelling of Cyslt2r−/− mice occurring in the presence of a CysLT1R antagonist. In an inflammatory process it seems possible that the generation of...
LTC₄ and LTD₄ and occupancy of classical receptors followed by receptor internalization could allow increased CysLT₁R function. The identification of at least 2 LTE₄-reactive GPCRs provides potential mechanistic explanations for the potency of LTE₄ as an inducer of vascular permeability and potentiator of mucosal inflammation, which were identified by previous pharmacologic profiling studies in human and guinea pig tissues. Moreover, the fact that P2Y₁₂ (and not CysLT₁R) is responsible for LTE₄-mediated activation and proliferation of mast cells, as well as amplification of allergic pulmonary inflammation, indicates that the receptors for LTE₄ evolved to serve functions that are anatomically and contextually distinct yet potentially complementary in inflammation (Fig 6).

REFERENCES