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The leukotriene E₄ puzzle: finding the missing pieces and revealing the pathobiologic implications

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Abstract

The intracellular parent of the cysteinyl leukotrienes (cys-LTs), 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 by 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 and at sites of inflammation in animal models. LTE₄ has received little attention because it binds poorly to the classical receptors, CysLT₁R and CysLT₂R, and was much less active on normal airways than LTC₄ or LTD₄. However, early studies indicated that LTE₄ caused skin swelling in humans as potently as LTC₄ and LTD₄, that airways of asthmatic subjects (particularly those that were aspirin-sensitive) were selectively hyperresponsive to LTE₄, and that a potential distinct LTE₄ receptor was present in guinea pig trachea. Recent studies have begun to uncover receptors selective for LTE₄; P2Y₁₂, an ADP receptor, and CysLT_ER, observed functionally in skin of mice lacking CysLT₁R and CysLT₂R. These findings prompt a renewed focus on LTE₄ receptors as therapeutic targets that are not currently addressed by available receptor antagonists.

Keywords

Leukotriene E₄; G protein-coupled receptor; bronchial asthma; inflammation; knockout mouse

Of the three cysteinyl leukotrienes (cys-LTs) (LTC₄, LTD₄, LTE₄), only LTE₄ is sufficiently stable so as to be detectable in extracellular fluids. Though widely used as a biomarker of cys-LT pathway activity in clinical studies, LTE₄ has received little attention in recent literature as a mediator of inflammation due to its poor activity at the classical cys-LT receptors (termed type 1 (CysLT₁R) and type 2 (CysLT₂R) receptors for cys-LTs). However, several earlier studies clearly demonstrated that LTE₄ had biological activity that differed from its precursors, predicting (correctly, in retrospect) the existence of specific LTE₄-reactive receptors. This review will highlight LTE₄ from a historical perspective, from its discovery to the identification of its receptors and mechanisms of action.

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DISCOVERY OF LTE₄

The slow reacting substance of anaphylaxis (SRS-A), named by Brocklehurst, was identified as a substance generated by in vitro antigen/allergen challenge of perfused lung of actively sensitized guinea pigs or human lung fragments of allergic patients needing resection (1). 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 al of leukotriene C₄ (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 (3). LTC₄ was composed of a metabolite of arachidonic acid (eicosatetraenoic acid) with 3 conjugated double bonds, and a peptide adduct through a sulfur bridge. The exact stereochemistry of the lipid and the amino acid sequence of the S-linked peptide of LTC₄ were obtained by comparing purified natural SRS-A with candidate synthetic cys-LTs prepared by E. J. Corey (reviewed in 4). These synthetic cys-LTs showed bioactivity consistent with the functional definition of SRS-A offered by Brocklehurst, who noted that a range of activities could contract the guinea pig ileum in the presence of an antihistamine (1).

Since 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 undergoing IgG-dependent anaphylaxis was comprised of three products, all with three conjugated double bonds, and each having contractile activity for the guinea pig ileum. By comparison to active and inactive standards with different peptide adducts, we recognized the three components of authentic SRS-A to elute with the retention times of the previously defined LTC₄ (4,5) and two additional theoretical structures, LTD₄ and LTE₄ (6). These two 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 (Figure 1). Since LTC₄ is the only intracellular cys-LT generated by LTC₄ synthase (LTC₄S) (7), it seemed likely that LTD₄ was formed extracellularly from LTC₄ by deletion of glutamic acid (by a γ -glutamyl transpeptidase or γ -glutamyl leukotrienase) following 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 (6,8). 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 three components were detected in biologic fluids. These early studies not only showed natural SRS-A to be composed of three cys-LTs but also demonstrated that each cys-LT had contractile activity for ileal smooth muscle in vivo (Figure 2) and permeability enhancing activity by intradermal 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 cys-LTs, and to identify potential antagonists. This approach permitted the development of the prototypes of the orally available CysLT₁R-selective antagonists (“lukasts”) more than a decade before any receptor was defined in molecular terms. The 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 ten-fold lower affinity than did CysLT₁R. It was surprising that LTE₄ failed to 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 felt that the LTE₄ agonist

activity that had been demonstrated in pharmacologic studies in guinea pigs and humans was impressive and that the greater stability of LTE_4 relative to the other cys-LTs might favor a distinct pathobiologic role. The subsections that follow will consider some of the early findings for LTE_4 favoring the existence of a distinct receptor, and revealing its relative biologic stability. We will also consider the noteworthy potency of LTE_4 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 humans with asthma and in allergen sensitized mice. These studies have been key to the recognition of two functional receptors with a preference for LTE_4 by two different laboratories with different experimental approaches.

EARLY PHARMACOLOGY OF LTE_4 IN ANIMALS

The potency of LTE_4 for contraction of guinea pig tracheal spirals in vitro was ten-fold greater than that of either LTC_4 or LTD_4 , whereas for guinea pig parenchymal strips the potency of LTD_4 was six-fold that of LTE_4 and 20-fold that of LTC_4 . Further, the concentration-effect for LTD_4 and LTE_4 on parenchymal strips observed by Drazen and colleagues was biphasic, with the initial low concentration effect (studied only for LTD_4) being competitively antagonized by FPL55712. In contrast, LTC_4 was the least potent ligand and gave only a linear response (11,12). When these ligands were given intravenously to the intact anaesthetized or unanaesthetized guinea pig, LTD_4 and LTC_4 elicited a small increase in pulmonary resistance compared to the magnitude of the fall in dynamic compliance, while LTE_4 decreased compliance together with a robust increase in resistance indicating both peripheral and central airway effects (12,13). Another distinctive effect of LTE_4 was that it enhanced the contractile responses of the guinea pig tracheal smooth muscle to histamine, a property not shared with LTC_4 or LTD_4 . The latter effect of LTE_4 could be prevented by treatment of the tracheal tissue with indomethacin, indicating a key role for a cyclooxygenase (COX) product (14). Together, these in vitro and in vivo functional findings suggested the presence of three receptors for cys-LTs; a high affinity receptor for LTD_4 , a lower affinity receptor for LTC_4 , and a separate receptor for LTE_4 , with the latter potentially capable of eliciting the secondary production of a prostanoid (Table I) (12).

The observed ratio of potency for the three cys-LTs in different tissues could reflect not only the profile of receptor expression in the target tissue, but also the rate of conversion of one cys-LT to another of greater or lesser activity. This is readily demonstrated when LTC_4 and LTD_4 are separately applied to the guinea pig ileum at concentrations sufficient to give their maximum isotonic responses. $[^3\text{H}]\text{LTC}_4$ has a 60 second latent period before initiating a linear contractile response to 80% maximum over 2 min, which is followed by a further contraction associated with slow metabolism to the more potent $[^3\text{H}]\text{LTD}_4$. There is negligible conversion of $[^3\text{H}]\text{LTC}_4$ to $[^3\text{H}]\text{LTD}_4/\text{LTE}_4$ during the linear phase. Furthermore, the inclusion of serine borate to block bioconversion of LTC_4 to LTD_4 by membrane γ -glutamyl transpeptidase does not change the response of the tissue to LTC_4 , thus confirming that this response is mediated by a specific LTC_4 receptor (15). In contrast, $[^3\text{H}]\text{LTD}_4$ initiated an immediate linear contraction that reached maximum at 1 min and then declined sharply with linear conversion to $[^3\text{H}]\text{LTE}_4$ which has only one quarter the potency of LTD_4 in this assay. When the mucosa containing the dipeptidase activity had been removed from the ileal muscle, the linear contractile response to $[^3\text{H}]\text{LTD}_4$ was maintained, reflecting the loss of metabolism to $[^3\text{H}]\text{LTE}_4$ by removal of the glycine. These findings nicely reflect the exquisite receptor specificity of the cys-LT system that is conferred by modifications of the peptide adduct. (Fig. 3A, B).

EARLY STUDIES OF LTE₄ METABOLISM

The products of the granulocyte respiratory burst, which are abundant with inflammation, can alter the stability of each cys-LT in vitro and in vivo. Phorbol myristate acetate-activated human neutrophils converted each cys-LT to their subclass specific S-diastereoisomeric sulfoxides which retained their ability to be detected by cys-LT-specific antibodies but lost greater than 95% of function. Each sulfoxide was further processed to identical diastereoisomers of 6-trans LTB₄ which were non functional 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 (HOCl). Dose dependent attack on the sulfur bridge during HOCl formation showed that LTE₄ was substantially more resistant than the other cys-LTs (16,17). Systemic metabolism of the cys-LTs 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 humans indicate that ~5% is recovered in the urine, 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 beta oxidation with shortening of the carbon chain to 18-COOH-dinor-LTE₄ and beyond (18, 19 & reviewed in 20).

EARLY PHARMACOLOGY OF LTE₄ in HUMANS

Although the early pharmacology of the three sequentially generated cys-LTs identified LTE₄ as the most stable in physiologic and pathobiologic models, clinical attention shifted to LTD₄ and LTC₄ which upon inhalation were up to 1000x as potent as histamine (21,22). LTE₄ was only 39x as potent as histamine in reducing maximum expiratory flow at 30% of vital capacity in normal humans (21–23). Although each cys-LT was a potent bronchoconstrictor in patients with bronchial asthma, there was little difference between asthmatics and normal controls in sensitivity to cys-LTs, in contrast to the hyperresponsiveness to histamine or methacholine that is characteristic of asthma (24). An exception to that rule is in aspirin-exacerbated respiratory disease, an asthma variant associated with marked over-production of the cys-LTs. In these individuals, Christie and colleagues showed selective hyperresponsiveness to LTE₄, but not to LTC₄, relative to aspirin-tolerant asthmatic individuals (25).

Because we had recognized that LTE₄ induced permeability in guinea pig skin over the same dose range (5.0 to 50 ng), as for LTC₄ and LTD₄ (6), we compared their action at 1.0 nmol per site by intradermal injection in human 3 volunteers. Each cys-LT elicited a wheal and flare by 10 minutes which peaked at 1–2 hrs with a 10–20 mm wheal and 20–25 mm flare. The wheal resolved by 4 hrs (Figure 4) while the flare was still evident at 6 hrs (26). The 3 cys-LTs gave equiactive responses in each subject. Biopsies at 2 hrs 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 two 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 the microvasculature of the mouse to seek a functional receptor for LTE₄.

FUNCTIONAL and PHARMACOLOGIC CHARACTERIZATION OF CysLT_ER, A CUTANEOUS RECEPTOR PREFERENTIAL FOR LTE₄

In addition to addressing the pharmacology of the cys-LTs during the 1980s, we began to characterize LTC₄S, the integral protein of the outer nuclear membrane responsible for biosynthesis of LTC₄ by conjugation of glutathione to LTA₄ (7). After expression cloning of

human LTC₄S and then homology cloning of mouse LTC₄S (27,28), we turned to targeted disruption of mouse LTC₄S to explore for phenotypic characteristics that might depend on the functions of the cys-LTs (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*^{-/-}) as compared to wild-type 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 cys-LTs was at least as important as the preformed amines in this model. To analyze the contributions from individual cys-LT receptors, we next generated strains deficient in CysLT₁R (*Cyslt1r*^{-/-}) and CysLT₂R (*Cyslt2r*^{-/-}), respectively (30,31) based on the prior cloning of these 7 transmembrane, G protein-coupled human receptors (GPCRs) (9,10). In response to an intraperitoneal injection of zymosan, a yeast cell wall material which elicits cys-LT generation from macrophages, both the *Ltc4s*^{-/-} and *Cyslt1r*^{-/-} but not the *Cyslt2r*^{-/-} strains showed ~50% reductions in vascular leak, implying a key role for cys-LTs acting at CysLT₁R for this innate immune response.

The responsiveness of the mouse vasculature to the cys-LTs suggested that the existence of a distinct LTE₄-reactive receptor could be proven by studying cys-LT-dependent swelling responses in mice deficient in both receptors. This strain (*Cyslt1r/Cyslt2r*^{-/-}) was created by intercrossing the *Cyslt1r*^{-/-} and *Cyslt2r*^{-/-} strains (32). The resulting double receptor deficiency of the *Cyslt1r/Cyslt2r*^{-/-} strain was confirmed by the absence of both receptor transcripts. We then examined the dose-dependent ear edema elicited by each cys-LT in the respective *Cyslt1r*^{-/-}, *Cyslt2r*^{-/-}, and *Cyslt1r/Cyslt2r*^{-/-} strains (32). The dose-dependent ear edema elicited by injection of LTD₄ and LTC₄ in the *Cyslt1r/Cyslt2r*^{-/-} strain was equivalent to that in the wild-type (WT) controls, indicating the presence of a previously unrecognized receptor. The *Cyslt1r/Cyslt2r*^{-/-} 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 biopsies at 30 and 240 minutes showed an exaggerated magnitude and duration of ear edema without cellular infiltration in response to LTE₄ in the *Cyslt1r/Cyslt2r*^{-/-} strain. The LTE₄-mediated vascular leak in the *Cyslt1r/Cyslt2r*^{-/-} 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 G_{αi} proteins and Rho kinase (32). Additionally, the response to LTE₄ was blocked by ~30% by treatment of the mice with indomethacin, 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 CysLT_ER rather than a number until it is cloned (Figure 5).

The discovery of a CysLT_ER 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 *Cyslt1r*^{-/-} mice, and normal in magnitude but delayed in *Cyslt2r*^{-/-} mice, suggesting that CysLT₁R is the major signaling receptor for LTC₄ and LTD₄, whereas CysLT₂R is a negative regulator of CysLT₁R. LTE₄-elicited vascular leak was not attenuated in the *Cyslt1r*^{-/-} mice, but delayed and sustained in the *Cyslt2r*^{-/-} strain, suggesting that CysLT_ER is the dominant receptor for this ligand and that CysLT₂R is again a negative regulator. That the enhanced sensitivity to LTE₄-induced ear edema observed in the *Cyslt1r/Cyslt2r*^{-/-} strain was not seen with either single receptor null strain, *Cyslt1r*^{-/-} or *Cyslt2r*^{-/-}, implies that both CysLT₂R and CysLT₁R negatively regulate CysLT_ER. Indeed, administration of the selective CysLT₁R antagonist MK571 to *Cyslt2r*^{-/-} mice mimicked the phenotype of the *Cyslt1r/Cyslt2r*^{-/-} 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 CysLT_ER. Curiously, pretreatment with MK571 had opposing effects in WT and the *Cyslt1r/Cyslt2r*^{-/-} strain (32). Specifically, MK571 suppressed swelling of the skin in WT mice challenged intradermally with 0.5 nmol LTD₄, LTC₄, or LTE₄. In contrast, the same MK571 pretreatment and dose of

ligand produced an *enhanced* response to cys-LTs in *Cyslt1r/Cyslt2r*^{-/-} mice. Thus, MK571, which is a prototype of the lukast drugs, potentiates responses apparently mediated through CysLT_ER, in a setting where 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), may block a yet-to-be-defined receptor with negative regulatory properties for CysLT_ER.

DISCOVERY THAT THE P2Y₁₂ RECEPTOR IS A FUNCTIONAL PULMONARY AND MAST CELL-ASSOCIATED RECEPTOR FOR LTE₄

As is the case for many effector cells of bone marrow origin, mast cells express both CysLT₁R and CysLT₂R (35,36). 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 (35–37). 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 using RNA interference in primary human mast cells (38). 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, interleukin (IL)-6, and IL-10 (37). 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 (39). 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, as LTE₄ failed to activate a PPAR-γ driven reporter in bovine endothelial cells (39). 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 one previously unrecognized LTE₄ receptor that was MK571-resistant (and perhaps another that was sensitive).

Based on sequence homology between CysLT₁R, CysLT₂R, and the P2Y receptor family, it seemed likely that a putative “CysLT₃R” might be among the orphan “P2Y-like” GPCRs, or even a known member. Human mast cells express several such receptors (40), including the P2Y₁₂ receptor, a G_{ai}-linked receptor for adenosine diphosphate and the target of thienopyridine anti-thrombotic drugs. Because a computer modeling study had predicted that LTE₄ might be a surrogate ligand for this receptor (41), 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 (42). 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. Since LTE₄ (but not LTD₄) was previously shown to induce bronchial eosinophilia when administered by inhalation to asthmatic humans (43), 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 mouse skin microvasculature to LTE₄, indicating that P2Y₁₂ receptors (hereafter referred to as P2Y₁₂/CysLT₃R to reflect their dual specificity) are separate and distinct from the CysLT_ER in the skin (32).

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 cys-LT-mediated permeability effects in guinea pigs and humans 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 CysLT_ER function in mice was certainly unexpected but is supported by literature showing CysLT₂R to be a negative regulator of CysLT₁R for mouse and human mast cell proliferation (38) and by the potentiation of LTE₄-mediated skin swelling of *Cyslt2r*^{-/-} mice occurring in the presence of a CysLT₁R antagonist (32). In an inflammatory process, it seems possible that the generation of LTC₄/LTD₄, and occupancy of classical receptors followed by receptor internalization (44) could allow increased CysLT_ER function. The identification of at least two LTE₄-reactive GPCRs provides potential mechanistic explanations for the potency of LTE₄ as an inducer of vascular permeability and potentiator of mucosal inflammation that were identified by previous pharmacologic profiling studies in human and guinea pig tissues (13–15,25,26,43). Moreover, the fact that P2Y₁₂/CysLT₃R (and not CysLT_ER) 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 (Figure 6).

Abbreviations

COX, cyclooxygenase
 cys-LT, cysteinyl leukotriene
 CysLT₁R and CysLT₂R, type 1 and type 2 cys-LT receptors
 ERK, extracellular signal-regulated kinase
 GPCR, G protein-coupled receptor
 IL, interleukin
 LT, leukotriene
 LTC₄S, LTC₄ synthase
 MIP-1β, macrophage inflammatory protein-1β
 PG, prostaglandin
 PPAR-γ, peroxisome proliferator-activated receptor-γ
 SRS-A, slow reacting substance of anaphylaxis
 WT, wild-type

References

1. Brocklehurst WE. The release of histamine and formation of a slow reacting substance (SRS-A) during anaphylactic shock. *J Physiol (London)* 1960;151:416–435. [PubMed: 13804592]

2. Orange RP, Murphy RC, Karnovsky ML, Austen KF. The physicochemical characteristics and purification of slow reacting substance of anaphylaxis. *J Immunol* 1973;110:760–770. [PubMed: 4347478]
3. Murphy RC, Hammarstrom S, Samuelsson B. Leukotriene C₄ a slow-reacting substance from murine mastocytoma cells. *Proc Natl Acad Sci USA* 1979;76:4275–4279. [PubMed: 41240]
4. Marfat, A.; Corey, EJ. Synthesis and structure elucidation of leukotrienes. In: Pike, JE.; Morton, DR., Jr, editors. *Advances in Prostaglandin, Thromboxane, and Leukotriene Research*. Vol. 14. New York: Raven; 1985. p. 155-228.
5. Morris HR, Taylor GW, Piper PJ, Samhoun MN, Tippins JR. Slow reacting substances (SRSs): the structure identification of SRSs from rat basophil leukemia (RBL-1) cells. *Prostaglandins* 1980;19:185–201. [PubMed: 6104346]
6. Lewis RA, Drazen JM, Austen KF, Clark DA, Corey EJ. Identification of the C(6)-S-conjugate of leukotriene A with cysteine as a naturally occurring slow reacting substance of anaphylaxis (SRS-A). Importance of the 11-*cis*-geometry for biological activity. *Biochem Biophys Res Commun* 1980;96:271–277. [PubMed: 6254506]
7. Yoshimoto T, Soberman RJ, Spur B, Austen KF. Properties of highly purified leukotriene C₄ synthase of guinea pig lung. *J Clin Invest* 1988;81:866–871. [PubMed: 3343345]
8. Parker CW, Falkenheim SF, Huber MM. Sequential conversion of the glutathionyl side chain of slow reacting substance (SRS) to cysteinyl-glycine and cysteinyl-glycine and cysteine in rat basophilic leukemia cells stimulated with A-23187. *Prostaglandins* 1980;20:863–886. [PubMed: 6110221]
9. Lynch KR, O'Neil GP, Liu Q, Im D-S, Sawyer N, Metters KM, et al. *Nature* 1999;399:789–793. [PubMed: 10391245]
10. Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im D-S, et al. *J Biol Chem* 2000;275:30531–30536. [PubMed: 10851239]
11. Drazen JM, Austen KF, Lewis RA, Clark DA, Goto G, Marfat A, et al. Comparative airway and vascular activities of leukotrienes C-1 and D *in vivo* and *in vitro*. *Proc Natl Acad Sci USA* 1980;77:4354. [PubMed: 6933488]
12. Drazen, JM.; Lewis, RA.; Austen, KF.; Corey, EJ. Pulmonary Pharmacology of the SRS-A Leukotrienes. In: Berti, F.; Folco, G.; Velo, GP., editors. *Leukotrienes and Prostacyclin*. New York: Plenum Publishing Corporation; 1983.
13. Drazen JM, Venugopalan CS, Austen KF, Brion F, Corey EJ. Effects of leukotriene E on pulmonary mechanics in the guinea pig. *Am Rev Respir Dis* 1982;125:290–294. [PubMed: 6279000]
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 USA* 1984;81:4922–4925. [PubMed: 6087352]
15. Krilis S, Lewis RA, Corey EJ, Austen KF. Bioconversion of C-6 sulfidopeptide leukotrienes by the responding guinea pig ileum determines the time course of its contraction. *J Clin Invest* 1983;71:909–915. [PubMed: 6300193]
16. Lee CW, Lewis RA, Corey EJ, Barton A, Oh H, Tauber AI, Austen KF. Oxidative inactivation of leukotriene C₄ by stimulated human polymorphonuclear leukocytes. *Proc Natl Acad Sci USA* 1982;79:4166–4170. [PubMed: 6955794]
17. Lee CW, Lewis RA, Tauber AI, Mehrotra M, Corey EJ, Austen KF. The myeloperoxidase-dependent metabolism of leukotrienes C₄, D₄ and E₄ to 6-*trans*-leukotriene B₄ diastereoisomers and the subclass-specific S-diastereoisomeric sulfoxides. *J Biol Chem* 1983;258:15004–15010. [PubMed: 6317683]
18. Sala A, Voelkel N, Maclof J, Murphy RC. Leukotriene E₄ elimination and metabolism in normal human subjects. *J Biol Chem* 1990;265:21771–21778. [PubMed: 2174886]
19. Jedlitschky G, Huber M, Volk A, Müller M, Leier I, Müller J, et al. Peroxisomal Degradation of leukotrienes by β -oxidation from the ω -end. *J Biol Chem* 1991;266:24763–24772. [PubMed: 1761571]
20. Murphy RC, Gijon MA. Review Article: Biosynthesis and metabolism of leukotrienes. *Biochem J* 2007;405:379–395. [PubMed: 17623009](Printed in Great Britain).
21. Weiss JW, Drazen JM, Coles N, McFadden ER Jr, Weller PF, Corey EJ, et al. Bronchoconstrictor effects of leukotriene C in humans. *Science* 1982;216:196–198. [PubMed: 7063880]

22. Weiss JW, Drazen JM, McFadden ER Jr, Weller PF, Corey EJ, Lewis RA, et al. Airway constriction in normal humans produced by inhalation of leukotriene D: potency, time course, and effect of aspirin therapy. *JAMA* 1983;249:2814–2817. [PubMed: 6842792]
23. Davidson AB, Lee TH, Scanlon PD, Solway J, McFadden ER Jr, Ingram RH Jr, et al. Bronchoconstrictor effects of leukotriene E₄ in normal and asthmatic individuals. *Am Rev Respir Dis* 1987;135:333–337. [PubMed: 3028218]
24. Griffin M, Weiss JW, Leitch AG, McFadden ER Jr, Corey EJ, Austen KF, et al. Effects of leukotriene D on the airways in asthma. *N Engl J Med* 1983;308:436–439. [PubMed: 6823253]
25. Christie PE, Schmitz-Schumann M, Spur BW, Lee TH. Airway responsiveness to leukotriene C₄, leukotriene E₄, and histamine in aspirin-sensitive asthmatic subjects. *Eur Respir J* 1993;6:1468–1473. [PubMed: 8112440]
26. 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;80:115–119. [PubMed: 6296237]
27. Lam BK, Penrose JF, Freeman GJ, Austen KF. Expression cloning of a cDNA for human leukotriene C₄ synthase, a novel integral membrane protein conjugating reduced glutathione to leukotriene A₄. *Proc Natl Acad Sci USA* 1994;91:7663–7667. [PubMed: 8052639]
28. Lam BK, Penrose JF, Rokach J, Xu K, Baldasaro MH, Austen KF. Molecular cloning, expression, and characterization of mouse leukotriene C₄ synthase. *Eur J Biochem* 1996;238:606–612. [PubMed: 8706658]
29. 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;276:22608–22613. [PubMed: 11319240]
30. 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;277:20820–20824. [PubMed: 11932261]
31. Beller TC, Maekawa A, Friend DS, Austen KF, Kanaoka Y. Targeted gene disruption reveals the role of the cysteinyl leukotriene 2 receptor in increased vascular permeability and in bleomycin-induced pulmonary fibrosis in mice. *J Biol Chem* 2004;44:46129–46134. [PubMed: 15328359]
32. 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 USA* 2008;105:16695–16700. [PubMed: 18931305]
33. Mitra P, Oskertizian CA, Payne SG, Beaven MA, Milstien S, Spiegel S. Role of ABCC1 in export of sphingosine-1-phosphate from mast cells. *Proc Natl Acad Sci USA* 2006;103:16394–16399. [PubMed: 17050692]
34. Mamedova L, Capra V, Accomazzo MR, Gao ZG, Ferrario S, Fumagalli M, et al. CysLT₁ leukotriene receptor antagonists inhibit the effects of nucleotides acting at P2Y receptors. *Biochem Pharmacol* 2005;71:115–125. [PubMed: 16280122]
35. Mellor EA, Maekawa A, Austen KF, Boyce JA. Cysteinyl leukotriene receptor 1 is also a pyrimidinergic receptor and is expressed by human mast cells. *Proc Natl Acad Sci USA* 2001;98:7964–7969. [PubMed: 11438743]
36. Mellor EA, Frank N, Soler D, Jora JM, Hodge MR, Austen KF, et al. Expression of the type 2 cysteinyl leukotriene receptor by human mast cells; demonstration of function distinct from that of CysLTR1. *Proc Natl Acad Sci USA* 2003;100:11589–11593. [PubMed: 13679572]
37. Jiang Y, Kanaoka Y, Feng C, Nocka K, Rau S, Boyce JA. Interleukin 4-dependent mast cell proliferation requires intracrine/autocrine cysteinyl leukotriene-dependent signaling. *J Immunol* 2006;177:2755–2759. [PubMed: 16920908]
38. Jiang Y, Feng C, Backskai B, Boyce JA. CysLT₂ receptors interact with CysLT₁ receptors and down-modulate cysteinyl leukotriene-dependent mitogenic responses of mast cells. *Blood* 2007;100:3263–3270. [PubMed: 17693579]
39. Paruchuri S, Jiang Y, Feng C, Francis SA, Plutzky J, Boyce JA. Leukotriene E₄ activates peroxisome proliferator activated receptor gamma and induces prostaglandin D₂ generation by human mast cells. *J Biol Chem* 2008;283:16477–16487. [PubMed: 18411276]

40. Feng CL, Mery AG, Beller EM, Favot CL, Boyce JA. Adenine nucleotides inhibit cytokine generation by human mast cells through a Gs-coupled receptor. *J Immunol* 2004;173:7539–7547. [PubMed: 15585881]
41. 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;337:281–288. [PubMed: 16185654]
42. Paruchuri S, Feng C, Tashimoto H, Jiang Y, Conley P, Boyce JA. Leukotriene E₄-mediated potentiation of airway inflammation requires the P2Y₁₂ receptor. submitted
43. Gauvreau GM, Parameswaran KN, Watson RM, O'Byrne PM. Inhaled leukotriene E₄, but not leukotriene D₄, increased airway inflammatory cells in subjects with atopic asthma. *Am J Respir Crit Care Med* 2001;164:1495–1500. [PubMed: 11704602]
44. Naik S, Billington CK, Pascual RM, Deshpande DA, Stefano FP, Kohout TA, et al. Regulation of cysteinyl leukotriene type 1 receptor internalization and signaling. *J Biol Chem* 2005;280:8722–8732. [PubMed: 15590629]

Figure 1.

Biosynthesis and molecular structures of cys-LTs. Cytosolic phospholipase A₂α (cPLA₂α) catalyzes the liberation of arachidonic acid from nuclear membranes. 5-lipoxygenase (5-LO) translocates to the nuclear envelope, requiring the integral membrane protein 5-LO-activating protein (FLAP) to convert arachidonic acid to the precursor LTA₄. LTA₄ is further conjugated to reduced glutathione (GSH) by LTC₄S, forming LTC₄, the first committed molecule of the cys-LTs. Following energy-dependent export, LTC₄ is converted by the extracellular enzymes γ-glutamyl transpeptidase (γ-GT) or γ-glutamyl leukotrienase (γ-GL) to LTD₄, and to LTE₄ by dipeptidases (DiPs).

**RP-HPLC
of 3
major
peaks of
rat SRS-A**

Figure 2.

Resolution by RP-HPLC of three major peaks of SRS-A produced in the peritoneal cavity of rats. (A) Retention times of synthetic cysteinyl leukotrienes, (B) retention time of the resolved of natural components of SRS-A, and (C) arbitrary units of biologic activity of these natural components for contraction of the guinea pig ileum. Published from reference #6 with permission from Elsevier Inc.

Figure 3.

LTD₄-elicited contraction of guinea pig ileum and associated metabolism to LTE₄. A) Time course of contractile response to 3.6 ng of [³H]LTD₄ from guinea ileum expressed as percentage of maximal response (closed circles) and of metabolism of [³H]LTD₄ (open squares) to [³H]LTE₄ (open triangles) expressed as percent of total labeled leukotrienes recovered. B) Time course of contractile response to 3.6 ng of [³H]LTD₄ from longitudinal muscle strips of guinea pig ileum expressed as percent of maximal response (closed circles) and of metabolism of [³H]LTD₄ (open squares) to [³H]LTE₄ (open triangles) expressed as percent of total leukotrienes recovered. Published from reference #15 with permission from the American Society for Clinical Investigation.

Wheal formation after intracutaneous injections

Figure 4.

Wheal formation occurring with intracutaneous injections of various eicosanoids into 3 human subjects. The agonists were LTC₄ (closed square, 1.0 nmole/site), LTD₄ (closed circle, 1.0 nmole/site), LTE₄ (closed triangle, 1.0 nmole/site), LTB₄ (open triangle, 1.6 nmole/site), PGD₂ (open circle, 3.0 nmole/site) and saline (open square). The greatest diameter of the wheal in mm is depicted vs. time from 10 minutes to 6 hrs. Published from reference #26 with permission from Nature Publishing Group.

Figure 5.

Dose-dependence of LTC₄-, LTD₄-, and LTE₄-induced ear edema in WT and *Cysltr1/Cysltr2*^{-/-} mice. WT (A, C, and E) and *Cysltr1/Cysltr2*^{-/-} (B, D, and F) mice received intradermal injections of LTC₄ (A and B), LTD₄ (C and D), or LTE₄ (E 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 S.D. Published from reference #32 from the Proceedings of the National Academy of Sciences.

Receptor specificity and potency of the cys-LTs

Figure 6.

Schematic presentation of the diversity of the cys-LT receptor system.

Table 1

Effects of Various Leukotrienes on Airways

	Leukotriene		
	C	D	E
<u>In vitro</u> ^a			
Parenchymal strip	1/300	1/6000	1/1000
Tracheal spiral	1/30	1/30	1/300
<u>In vivo</u> ^b			
C _{dyn}	3+	4+	3+
R _L	1+	1+	3+

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

^b In vivo activity recorded as the response to infusion of 3 µg/kg of LT. 1+ minimal response to 4+, maximal response.

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TERM	DEFINITION
ARACHIDONIC ACID	Arachidonic acid is the precursor for both leukotrienes and prostaglandins and is found on the nuclear membrane. Together, leukotrienes 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 non-steroidal anti-inflammatory agents that inhibit cyclooxygenase-1. Treatment of patients with AERD includes leukotriene inhibitors.
COX-2	Cyclooxygenase-2 is induced by lipopolysaccharide, IL-1, and IL-2 to produce prostagland intermediates from arachidonic acid.
CYSTEINYL LEUKOTRIENE (cys-LT)	Leukotrienes, so named due to their generation from "leuko"cytes and conserved 3 double bonds "trienes", are generated from arachidonic acid by 5-lipoxygenase/5-lipoxygenase activating protein. Conversion of LTA ₄ to LTC ₄ by the addition of glutathione is the first step to generating cysteinyl leukotrienes. LTA ₄ hydrolase converts LTA ₄ to LTB ₄ . The major sources of LTC ₄ synthase (and thus cys-LTs) are eosinophils, basophils, and macrophages. Mast cells make both LTC ₄ synthase and, along with neutrophils and macrophages, LTA ₄ hydrolase.
CYSTEINYL LEUKOTRIENE RECEPTOR (CysLTR)	Both CysLT receptors and LTB ₄ receptors are 7 transmembrane G protein-coupled receptors. CysLT receptors can be upregulated by IL-4. LTB ₄ uses 2 receptors, BLT1 and 2 which are expressed on most tissues, upregulated by IFN γ , and promote neutrophil chemotaxis when activated by LTB ₄ .
DYNAMIC COMPLIANCE	Airway compliance is a measure of volume change per unit of pressure. Lungs from patients with long standing asthma have been reported to have decreased compliance, perhaps due to airway remodeling and associated fibrosis.
INDOMETHACIN	Indomethacin inhibits cyclooxygenase-1 and can trigger aspirin exacerbated respiratory disease.
IL-6	IL-6 primes for Th2 effector cells, inhibits the suppressive functions of CD4+CD25+ T regulatory 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.
IL-13	IL-13 and IL-6 can be produced by mast cells in response to activating signals such as IL-33.
"LUKASTS"	Montelukast, pranlukast, and zafirlukast all block the CysLT1 receptor while biosynthetic pathway inhibitors, such as zileuton, block the production of both cysteinyl leukotrienes and LTB ₄ .
MIP-1 β	Macrophage inflammatory protein-1 is involved in the chemotaxis and activation of monocytes. Cysteinyl leukotrienes can induce MIP-1 β and MIP-1 α production from monocytes through the CysLT1 receptor.
PERMEABILITY ASSAY	Activated vascular endothelium allows leakage of dyes, e.g. Evan's blue, FITC-albumin, into the extravascular space, making tissues appear colored (blue) or fluorescent (FITC).
PERTUSSIS TOXIN	Inhibits the function of G protein coupled receptors through ADP-ribosylation of G α .
PULMONARY RESISTANCE	Mechanical factors that limit alveolar access to air. Pulmonary resistance is calculated using Ohm's and Poiseuille'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.
PGD ₂	A mast cell eicosanoid made in large quantities following IgE-mediated mast cell activation. PGD ₂ is increased following allergen challenge, functions as a bronchconstrictor, 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 γ 1 and γ 2 are expressed in adipocytes and decrease pro-inflammatory 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 via the NADPH oxidase complex. Mutations in this complex (gp91, gp67 ^{phox} , gp22, gp47 ^{phox}) cause chronic granulomatous disease and recurrent infections with organisms such as <i>Staphylococcus aureus</i> , <i>Aspergillus species</i> , <i>Serratia</i> , and <i>Burkholderia cepacia</i> .