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Cysteinyl leukotriene receptors, old and new; implications for asthma

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Summary

The cysteinyl leukotrienes (cys-LTs) are three structurally similar, but functionally distinct lipid mediators of inflammation. The parent cys-LT, LTC_4, is synthesized by and released from mast cells, eosinophils, basophils, and macrophages, and is converted to the potent constrictor LTD_4 and the stable metabolite, LTE_4. While only two cys-LT-selective receptors (CysLTRs) have been identified, cloned, and characterized, studies dating back three decades predicted the existence of at least three functional CysLTRs, each with a characteristic physiological function in airways and other tissues. The recent demonstration that mice lacking both known CysLTRs exhibit full (and in some instances, augmented) physiological responses to cys-LTs verifies the existence of unidentified CysLTRs. Moreover, the ability to manipulate receptor expression in both whole animal and cellular systems reveals that the functions of CysLTRs are controlled at multiple levels, including receptor-receptor interactions. Finally, studies in transgenic mice have uncovered a potentially major role for cys-LTs in controlling the induction of Th_2 responses to common allergens. This review focuses on these recent findings and their potential clinical implications.

Introduction

The cysteinyl leukotrienes (cys-LTs) are highly potent peptide-conjugated arachidonic acid-derived mediators closely linked to the pathobiology of allergic inflammation [1, 2]. Cys-LTs are also now thought to play roles in the pathophysiology of cardiovascular disease [3], cancer [4], fibrosis [5], and immune host defence[6]. Thus, a comprehensive understanding of the cellular targets and mechanisms of action of cys-LTs is potentially important to all of these processes. Cys-LTs were the first specific mediators successfully targeted for drug development in asthma [7–9], and cys-LT targeted drugs are now widely prescribed. While efficacious and useful, clinical responses to existing antagonists of the type 1 cysteinyl leukotriene receptor ($CysLT_1$ receptor) are heterogeneous [10]. Furthermore, increasingly precise molecular tools and transgenic animal studies have revealed that cys-LTs and their receptors comprise of a much more complex network than originally thought. This complexity is likely due to cross-regulation [11, 12] and physical complexes among the receptors [13, 14], close interactions with other receptors, and novel receptors [15, 16]. Each of these factors carries substantial implications for the modulation of the cys-LT system for

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therapy. The purpose of this review is to contextualize this complexity in terms of its potential therapeutic implications.

Production of cysteinyl leukotrienes

Cysteinyl leukotrienes are generated by eosinophils, basophils, mast cells, macrophages, and myeloid dendritic cells in response to activation [17]. In each cell type, arachidonic acid is oxidized by 5-lipoxygenase (5-LO) to generate the unstable precursor leukotriene A_4 (LTA_4) [18], and subsequently leukotriene C_4 synthase (LTC_4S) converts LTA_4 to the parent cys-LT, LTC_4 [19]. After energy-dependent export from the cell [20], LTC_4 is sequentially converted to LTD_4 [21], and then to the final and most stable cys-LT, LTE_4 [22]. Thus, three different ligands (LTC_4, LTD_4, and LTE_4) arise from a single intracellular synthetic event by successive enzymatic conversions. In addition to this intracellular pathway, there is also a transcellular mechanism for cys-LT generation that can be carried out by cells that express LTC_4S but not the proximal enzyme 5-LO in the pathway (e.g. platelets, endothelial cells). In the latter mechanism, the LTC_4S-expressing cells can convert extracellular LTA_4 (released by neutrophils or other cells with an active 5-LO enzyme) [23], and may serve as an additional source of cys-LTs in certain inflammatory states. Indeed, a recent study demonstrated that adherent platelets accounted for ~60% of the LTC_4S activity and cys-LT generation by granulocytes from individuals with aspirin exacerbated respiratory disease (AERD), a disorder consistently associated with high levels of cys-LT production (see below) [24].

The relevance of cysteinyl leukotrienes to asthma

The bioactivities of the cys-LTs in the pre-clinical setting, particularly their potency as smooth muscle constrictors, spurred interest in these mediators as potential therapeutic targets in asthma. The ability to monitor urinary levels of LTE_4 as a reflection of systemic cys-LT generation *in vivo* allowed for the proof that cys-LTs are generated by subjects with acute asthma exacerbations [2]. Individuals with AERD, a variant of asthma characterized by nasal polyposis, chronic eosinophilic sinusitis, and idiosyncratic respiratory reactions induced upon ingestion of aspirin or other drugs that block COX-1, have especially high baseline levels of urinary LTE_4 (3–4-fold higher than aspirin-tolerant asthmatic controls), and a marked (approximately tenfold) further increment in these levels in response to oral challenge with aspirin [25]. The role of cys-LTs in asthma has been well validated by clinical trials using the available drugs. The 5-LO inhibitor zileuton, which reduces urinary cys-LT excretion by ~50%, and selective antagonists of $CysLT_1$ receptor both improve lung function, reduce the frequency of asthma exacerbations [7, 26], and reduce the severity of reactions to aspirin challenge in individuals with AERD [27]. Collectively, these findings establish a role for the cys-LTs as pertinent mediators of asthma, particularly in AERD, due to the high levels of cys-LTs generated in this disease variant.

Physiological and pharmacological evidence for multiple cysteinyl leukotriene receptors

While the three cys-LTs share certain functions *in vivo*, including smooth muscle contraction and vascular leak [28–30], there were important differences identified in early studies that suggested additional and distinct functions for each. Pharmacological profiling of guinea-pig lung demonstrated that LTC_4 and LTD_4 were equipotent as constrictors, whereas LTE_4 was inactive. Remarkably, however, LTE_4 was 10-times more potent for inducing guinea-pig tracheal ring contractions *in vitro* than was LTC_4 or LTD_4 [31, 32]. Intravenously administered LTC_4 and LTD_4 elicited large changes in dynamic lung compliance without major effects on resistance (indicative of primarily peripheral airway

effects), whereas LTE_4 elicited a robust increase in resistance (indicative of effects on the central airways), and changes in compliance [31]. Furthermore, LTE_4, but not LTC_4 or LTD_4, enhanced contractile responses of guinea-pig tracheal smooth muscle to histamine. This priming effect of LTE_4 for histamine responsiveness was prevented by pre-treatment of the tracheal tissue with indomethacin, indicating a key collaborative role for a cyclooxygenase (COX) product [32], putatively identified as thromboxane A_2 [33]. Together, these *in vitro* and *in vivo* functional findings predicted the existence of at least 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.

Studies on human subjects also provided compelling evidence for the existence of at least three receptors for cys-LTs. In both non-asthmatic and asthmatic subjects, inhalation of LTC_4 and LTD_4 elicited airflow obstruction at doses several 1000-fold lower than histamine [30, 34, 35]. Although, LTE_4 was only ~40-fold more potent than histamine in reducing expiratory flow rates in non-asthmatic subjects [36], asthmatic subjects were much more sensitive to LTE_4, as they developed airflow obstruction after inhalation of LTE_4 at doses 10-fold lower than the doses required to elicit this response in non-asthmatic controls. However, asthmatic and non-asthmatic subjects had equivalent dose-responses to LTC_4 and LTD_4 [29]. Moreover, subjects with AERD demonstrated even greater (16-fold) sensitivity to LTE_4-induced bronchoconstriction than did aspirin-tolerant asthmatic controls [37], but their responses to LTC_4 and histamine were similar to those in aspirin-tolerant controls. In another study, inhalation of LTE_4, but not of LTD_4, provoked the accumulation of eosinophils and basophils into the bronchial mucosa and sputum of asthmatic subjects when the two cys-LTs were administered at doses titrated to produce an equivalent degree of bronchoconstriction [38]. Inhalation of LTE_4 also enhanced the sensitivity of asthmatic subjects to histamine-induced bronchoconstriction, an effect that was blocked by pre-treatment with oral indomethacin [39]. These studies support the concept that LTE_4 acts through a receptor(s) that is distinct from those responsible for the actions of LTC_4 and LTD_4, and suggest that expression and/or function of the LTE_4 receptor may be selectively up-regulated in asthma, specifically enhancing pulmonary responsiveness to it. Lastly, as was the case for guinea-pig trachea, LTE_4 can potentiate hyperresponsiveness to histamine by the induction of or collaboration with COX products.

Molecular pharmacology of the CysLTRs and cross-regulation of the $CysLT_1$ receptor

The $CysLT_1$ and $CysLT_2$ receptors are both G-protein coupled receptors (GPCRs) and were cloned and characterized several years after the original descriptive pharmacology predicted their properties. These two GPCRs arise from different chromosomes and share only 38% sequence homology [40, 41]. Human and mouse $CysLT_1$ receptors are 87% identical [42] and the $CysLT_2$ receptors are 74% identical [43], suggesting a high level of functional conservation through evolution. Both receptors are structural homologues of the purinergic (P2Y) receptors that are specialized to recognize extracellular nucleotides [44], with 25–34% identity at the amino acid level. $CysLT_1$ receptor binds LTD_4 with high affinity (10^{-9} M) and LTC_4 with lesser affinity (10^{-8} M), whereas $CysLT_2$ receptor binds both LTC_4 and LTD_4 with equal affinity (10^{-8} M). Neither receptor exhibits substantial affinity for LTE_4 in radioligand binding assays nor does LTE_4 elicit strong signalling responses in cells expressing $CysLT_1$ receptor or $CysLT_2$ receptor in isolation [16, 40, 41]. It is therefore unlikely that the pharmacology of LTE_4 *in vivo* is attributable to $CysLT_1$ receptor and $CysLT_2$ receptor alone.

The $CysLT_1$ and $CysLT_2$ receptors are broadly expressed by structural and hematopoietic cells. Some cell types (vascular smooth muscle) express mostly Cys-LT_1 receptors [40], whereas others (endothelial cells) dominantly express $CysLT_2$ receptors [43]. Both receptors are expressed by cells of the innate (macrophages, monocytes, eosinophils, basophils, mast cells, dendritic cells) and adaptive (T cells, B cells) immune system, implying potentially cooperative functions in immunity and inflammation [17]. Studies of human mast cells *ex vivo* showed cys-LTs promoted proliferation of the cells through transactivation of the c-kit tyrosine kinase [45] and downstream phosphorylation of extracellular signal regulated kinase (ERK), and also induced cytokine generation [46]. LTD_4-induced mast cell proliferation and cytokine production were completely eliminated by knockdown of $CysLT_1$ receptors [12, 13] or by blockade of $CysLT_1$ receptors with MK571 [45, 46], an early prototype of the clinically available $CysLT_1$ receptor-selective antagonists. These studies suggested that the functions of cys-LTs included a direct role as agonists for activation of immune effector cells.

As the $CysLT_1$ and $CysLT_2$ receptors both mediate calcium flux and activate signalling cascades when expressed in isolation [47], it was surprising that blockade or knockdown of $CysLT_1$ receptors in mast cells eliminated most LTD_4-mediated signalling [13, 44, 48] despite the presence of $CysLT_2$ receptors on these cells. In fact, the knockdown of $CysLT_2$ receptors substantially potentiated LTD_4-induced signalling and cytokine generation [13]. The absence of $CysLT_2$ receptors resulted in approximately twofold higher levels of $CysLT_1$ receptor protein expressed on the cell surface, without changing the total cellular content of $CysLT_1$ receptors. $CysLT_1$ receptors and $CysLT_2$ receptors were found to heterodimerize in primary mast cells, a relatively common feature of GPCRs that recognizes similar ligands [49]. Thus $CysLT_2$ receptors, by interacting with $CysLT_1$ receptors, limit the surface expression levels and signalling ability of the latter receptors, at least on mast cells. This cross-regulation is likely to be important *in vivo*, since mice lacking $CysLT_2$ receptors (*cysltr2*−/− mice) show exaggerated ear swelling responses to direct intracutaneous challenges to LTD_4 relative to wild-type controls [15]. The absence of $CysLT_2$ receptors may also facilitate the formation of $CysLT_1$ receptor homodimers [41] that are strong signalling units for LTD_4 (Fig. 1).

The $CysLT_2$ receptor is not the only GPCR likely to cross-regulate the functions of $CysLT_1$ receptors *in vivo*. A P2Y-like GPCR termed GPR17 shows sequence homology to the CysLTRs, and was reported to be a dual-responsive receptor for both cys-LTs and nucleotides when over-expressed in an astrocytoma cell line [50]. GPR17 heterodimerizes with $CysLT_1$ receptors in transfectants and in primary macrophages [14], but does not exhibit specific binding to LTD_4 in these contexts. Instead, co-transfection of $CysLT_1$ receptors with GPR17 markedly reduced the binding affinity of $CysLT_1$ receptors for LTD_4 (Fig. 1) Importantly, mice lacking GPR17 [51] show exaggerated Th_2 responses and eosinophilic pulmonary inflammation in the house dust mite-induced model of pulmonary disease, whereas mice lacking $CysLT_1$ receptors (*cysltr1*−/− mice) [14] or LTC_4 synthase (*ltc4s*−/− mice) [52] show dramatically attenuated responses in this model. In addition, *in vitro* studies indicate that $CysLT_1$ receptors can be heterologously desensitized by nucleotides that signal through its homologues, the $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors, through protein kinase C-induced phosphorylation [11]. Collectively, these studies argue that multiple mechanisms exist to limit the magnitude and duration of signalling events occurring through $CysLT_1$ receptors, reflecting a need to maintain homeostasis of the powerful biological effects induced *in vivo* by LTD_4.

Novel receptors for cysteinyl leukotrienes

As noted previously, both animal and human studies suggested that biological responses to LTE_4 were likely mediated by receptors that were different from those responsible for the effects of LTC_4 and LTD_4. As LTE_4, but not LTD_4, elicits eosinophil and basophil recruitment to the bronchial mucosa of asthmatic subjects [38], a mouse model was employed to determine the mechanism(s) responsible. Cys-LTs alone failed to cause eosinophil recruitment to the lungs of naïve BALB/c mice. However, when administered intranasally to mice that were sensitized and challenged with ovalbumin (OVA), LTE_4, but not LTD_4, significantly increased the numbers of eosinophils recovered from the BAL fluid, and also increased the extent of bronchovascular leucocyte infiltration and goblet cell metaplasia [53]. Remarkably, these effects of LTE_4 were completely intact in double-knockout mice lacking both $CysLT_1$ and $CysLT_2$ receptors (*cysltr1/cysltr2*−/− mice), providing further evidence for the presence of a distinct LTE_4 receptor [53].

A computer modelling study had predicted that the ADP-binding $P2Y_{12}$ receptor could potentially recognize LTE_4 as a surrogate ligand [54]. Thus, sensitized BALB/c mice were treated with clopidogrel, a thienopyridine drug that is an antagonist of $P2Y_{12}$ receptors [55]. Clopidogrel completely eliminated the increments in eosinophil recruitment, inflammation, and goblet cell metaplasia induced by LTE_4. The actions of LTE_4 in this model were also absent in mice lacking $P2Y_{12}$ receptors. Depletion of platelets, the major cell type expressing $P2Y_{12}$ receptors, from the sensitized mice before their challenges with OVA and LTE_4 also eliminated the ability of LTE_4 to amplify pulmonary eosinophilia [53]. This study established that the ability of LTE_4 to amplify bronchial eosinophilia depends not on classical CysLTRs, but rather on ADP-reactive $P2Y_{12}$ receptors and platelets, implying the existence of an effector pathway that is likely to resist blockade by conventional $CysLT_1$ receptor antagonists. As naïve mouse airways fail to contract directly to cys-LTs [56], it was not possible to assess whether $P2Y_{12}$ receptors mediate bronchoconstriction in response to LTE_4, or whether the involvement of platelets explains the indomethacin-sensitive aspects of airway responses to LTE_4 observed in previous studies of human and guinea-pig [32, 39].

Three lines of evidence supported the concept that $P2Y_{12}$ receptors were bona fide receptors for LTE_4. First, computer modelling predicted the ability of $P2Y_{12}$ receptors to bind LTE_4 [54]. Second, heterologous expression by transfection of human $P2Y_{12}$ receptors in Chinese hamster ovary (CHO) cells permitted both calcium flux [54] (when the cells were co-transfected with the Gα16 G protein subunit) and phosphorylation of ERK [53] in response to stimulation with LTE_4. Third, targeted knockdown of $P2Y_{12}$ receptor expression in the human mast cell line LAD2 markedly reduced the ability of the cells to generate chemokines and cytokines when stimulated with LTE_4, whereas reactivity to LTD_4 was unaffected; conversely, knockdown of $CysLT_1$ receptors eliminated the cellular responses to LTD_4 without altering responses to LTE_4 [16, 53]. Although these findings indicate that $P2Y_{12}$ receptors mediate certain functional and signalling responses to LTE_4, heterologously expressed $P2Y_{12}$ receptors failed to exhibit specific binding of radiolabelled LTE_4 [53]. Curiously, however, LTE_4 competitively inhibited the binding of ADP (the natural ligand for $P2Y_{12}$ receptors) to the membranes of LAD2 cells, and this competition was eliminated by knockdown of $P2Y_{12}$ receptors from the parent cell line [53]. Collectively, these observations imply that $P2Y_{12}$ may function as a component of a complex with another GPCR that specifically recognizes LTE_4 (Fig. 2, top) This would be consistent with the previously recognized complexes formed by $CysLT_1$ receptors with both $CysLT_2$ receptors and GPR17 [13, 14] (Fig. 1).

Additional studies using c*ysltr1/cysltr2*−/− mice led to the recognition of another potential LTE_4-reactive receptor in the cutaneous microvasculature. Cys-LTs caused swelling of the

ear skin when injected intradermally into wild-type BALB/c mice. The dose-dependent ear oedema elicited by injection of LTD_4 and LTC_4 in the c*ysltr1/cysltr2*−/− strain was equivalent to that in the wild-type controls [15], indicating that vascular responses to the cys-LTs, surprisingly, did not require the presence of either classical receptor. Remarkably, and in sharp contrast to the pharmacology of the cloned receptors, LTE_4 was the most potent agonist, eliciting the same extent of ear swelling in c*ysltr1/cysltr2*−/− mice at a dose of 0.008 nmol as the response of the WT mice to 0.5 nmol (a 64-fold increase in sensitivity to LTE_4). The LTE_4-mediated vascular leak in the c*ysltr1/cysltr2*−/− strain was inhibited by pre-treatment of the mice with pertussis toxin or a Rho kinase inhibitor, supporting that the mechanism involved a GPCR linked to Gαi proteins and Rho kinase [15], and was blocked by ~30% by treatment of the mice with indomethacin, reminiscent of the indomethacin sensitivity of the LTE_4 response of guinea-pig tracheal rings [32] and human airway smooth muscle [39]. However, the ear swelling response to LTE_4 was resistant to clopidogrel, indicating that it did not depend on $P2Y_{12}$ receptors. Curiously, treatment of the *cysltr1/cysltr2*−/− mice with MK571 dramatically *increased* the extent of their swelling response to LTE_4, implying the ability of the drug to block targets other than $CysLT_1$ receptors when the latter receptor is deleted, and that the LTE_4 receptor(s) responsible for the effects of LTE_4 in the cutaneous vasculature are insensitive to $CysLT_1$ receptor antagonists. This receptor has been designated a '$CysLT_E$ receptor', pending molecular identification (Fig. 2, bottom). Given the potentiation of the putative $CysLT_E$ receptor function by MK571, it seems likely that a lukast-susceptible GPCR restrains the former receptor, analogous to the arrangement for GPR17 and $CysLT_2$ receptors in the case of the $CysLT_1$ receptor (Fig. 1).

Summary and potential clinical implications

Since the cloning of the two classical CysLTRs, the incremental availability of antibody reagents and the application of molecular technology and genetics to the cys-LT system have not only provided mechanistic explanations for observations made in classical pharmacological and physiological studies, but have revealed much greater complexity and functional diversification of the cys-LT system than could possibly have been anticipated. While a comprehensive list of potential clinical implications is beyond the scope of this article, we present three areas of cys-LT biology that could greatly impact the field of asthma and allergy in the near future.

Cross-regulation of $CysLT_1$ receptors

Given that at least two receptors ($CysLT_2$ receptor and GPR17) substantially dampen $CysLT_1$ receptor function *in vivo*, it is possible that functional variation in either receptor could influence clinical reactivity to $CysLT_1$ receptor antagonists, susceptibility to LTD_4-induced bronchoconstriction, or both. In support of this concept, polymorphic sequence variants of the human $CysLT_2$ receptor have been reported that predict response to $CysLT_1$ receptor antagonists. Other sequence variants of the $CysLT_2$ receptor predict the magnitude of bronchoconstriction induced in response to oral aspirin challenge in individuals with AERD [57], a feature of the reaction that is sensitive to $CysLT_1$ receptor antagonists. To date, no studies have addressed whether variants in GPR17 influence sensitivity to LTD_4 or clinical responses to $CysLT_1$ receptor antagonists. Interestingly, the P2Y receptors capable of inducing heterologous desensitization of $CysLT_1$ receptor ($P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$) [11] are all sufficiently structurally similar to the CysLTRs that they are susceptible to blockade by clinically active $CysLT_1$ receptor antagonists *in vitro* [58], and the same is true of GPR17 [50]. It is thus possible that the ability of $CysLT_1$ receptor antagonists to block these putative negative regulators could 'cancel out' the benefits of these drugs and attenuate the potential therapeutic benefit for some individuals. Perhaps this could account for some of the heterogeneity of response to these agents [59].

Receptors for LTE_4

As LTE_4 is abundant due to its long half-life, the existence of distinct receptors that are not blocked by currently available antagonists is potentially of great importance. This may be particularly true of AERD, in which high systemic levels of LTE_4 are accompanied by selectively increased sensitivity to bronchoconstriction in response to inhaled LTE_4 [37]. The dependence of LTE_4-mediated increases in eosinophil recruitment to the respiratory mucosa on $P2Y_{12}$ and platelets [53] suggest a potential application for thienopyridine drugs in the treatment of asthma and AERD. The molecular identification of $CysLT_E$ receptor could open new avenues towards the development of antagonists with a more complete blockade of cys-LT-dependent pathobiological effects, including antagonists of LTC_4S to prevent the formation of cys-LTs. Finally, the dependence of certain LTE_4-induced pulmonary effects on the presence of COX products [39] may explain why desensitization to aspirin produces a rapid loss of LTE_4-mediated bronchoconstriction in individuals with AERD [60], and why sequence variants in the gene encoding the receptor for thromboxane (*TPR*) are risk alleles for AERD [61].

The role of *cysteinyl leukotrienes* in sensitization to common allergens

Myeloid dendritic cells release LTC_4 via an innate, Dectin 2-dependent mechanism that can be activated by dust mite and *Aspergillus fumigatus* allergens [62]. The fact that dendritic cells from *ltc4s*−/− and *cysltr1*−/− mice fail to induce sensitization to these allergens in adoptive transfer experiments, and that mice lacking either $CysLT_1$ receptor or LTC_4S are resistant to the induction of Th_2 responses by dust mite allergens *in vivo* imply that cys-LTs play a critical role in the sensitization phase of this immune response [52]. Although there is no direct proof at this stage that this is the case in humans, variants of the genes encoding the $CysLT_1$ receptor [63], the $CysLT_2$ receptor [64], and LTC_4S [65] that associate with atopy in general or sensitization to dust mites in particular have all been reported in various human populations. As the $CysLT_1$ receptor is the key receptor in this process, early life intervention with the available antagonists could potentially alter or delay sensitization to Dectin 2-activating allergens in infants at high risk.

In summary, the full complexity of the cys-LT receptor system is still being determined. The development of mice lacking the classical CysLTRs, alone and in combination has proven invaluable. Studies using these mice verified older studies suggesting the existence of distinct receptors for LTE_4, demonstrated that the function of the $CysLT_1$ receptor is regulated by other GPCRs, and identified previously unanticipated functions for cys-LTs in fibrosis [5] and Th_2 immunity [52]. Each of these carries potential implications for the clinical setting.

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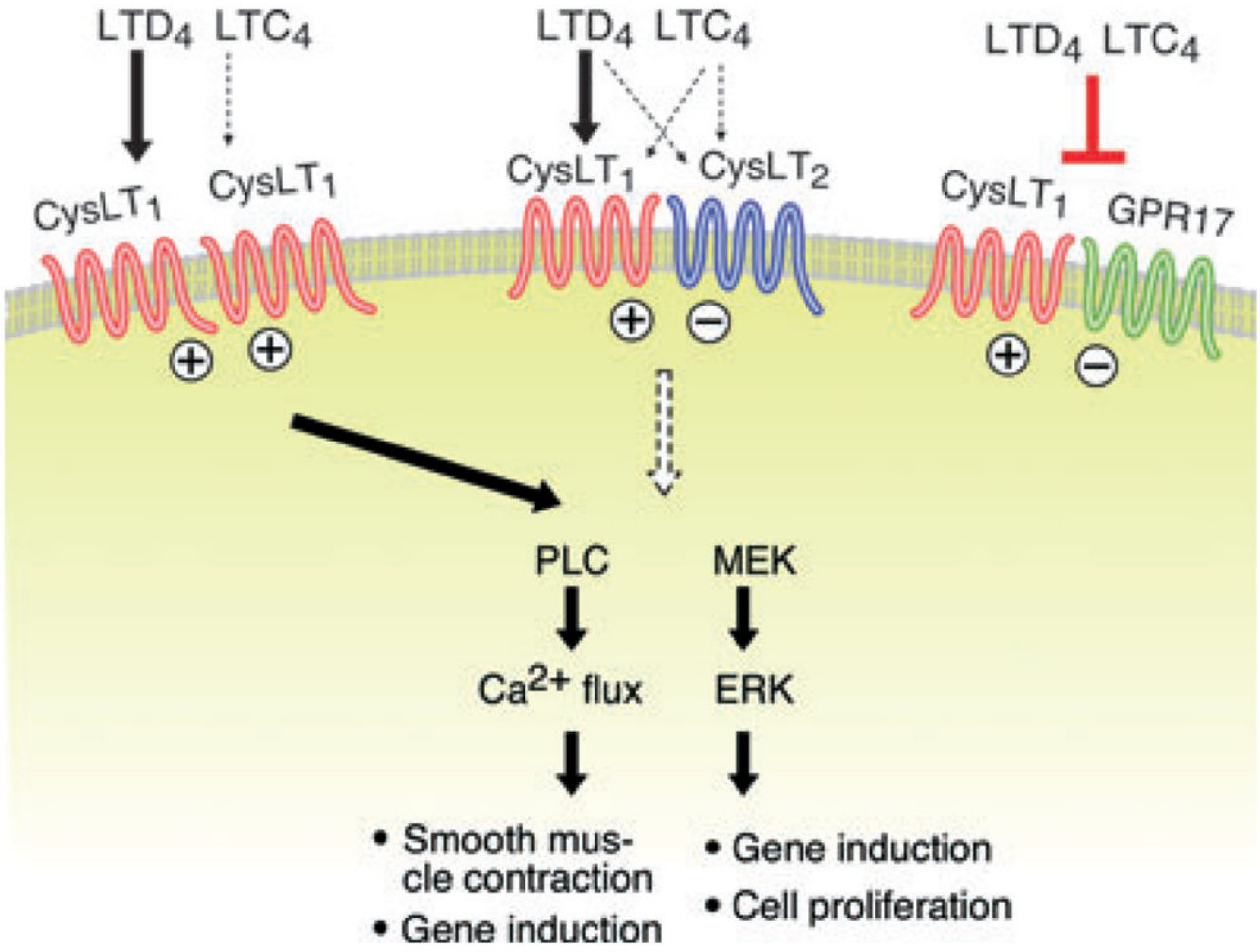

Fig. 1.

Homotypic and heterotypic interactions of the $CysLT_1$ receptor. $CysLT_1$ receptors can form homodimers that bind LTD_4 with high affinity and LTC_4 with lesser affinity, causing both calcium flux and extracellular signal regulated kinase activation that lead to smooth muscle contraction, cytokine gene induction, and cell proliferation. Both $CysLT_2$ receptors and GPR17 can heterodimerize with $CysLT_1$ receptors, attenuating signalling through the latter receptor and, in the case of GPR17, markedly attenuating ligand binding. (+) signs indicate positive signalling, (−) signs denote inhibitory signalling, and ⊥ indicates blockade of ligand binding,

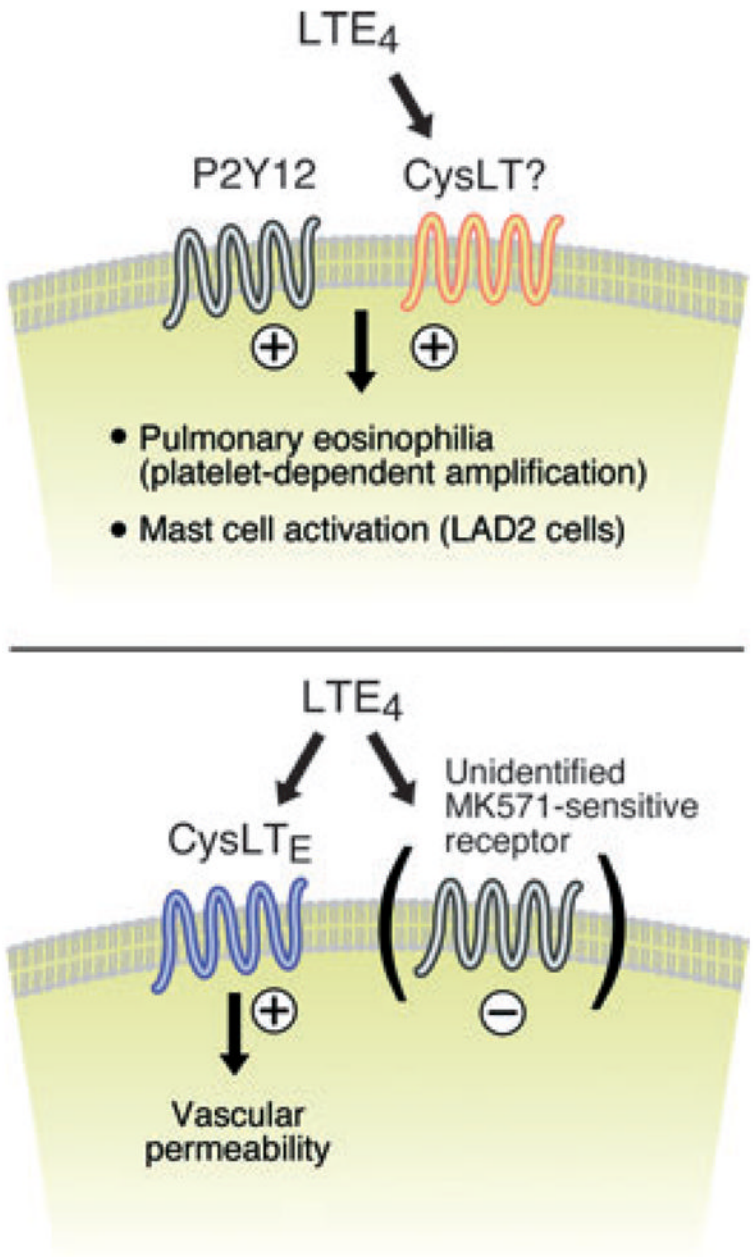

Fig. 2.

Hypothetical receptors for LTE_4 based on functional studies in mice lacking both $CysLT_1$ receptors and $CysLT_2$ receptors. An LTE_4-binding receptor combines functionally with $P2Y_{12}$ receptors (Top) to facilitate the recruitment of eosinophils to the bronchial mucosa in a platelet-dependent manner, while inducing chemokine generation by mast cells. In the cutaneous microvasculature, a putative G-protein coupled receptors ($CysLT_E$ receptor) directly mediate vascular leakage in response to LTE_4 that is potentiated by the presence of MK571 (and likely other $CysLT_1$ receptor antagonists), suggesting the presence of a lukast-sensitive negative regulator. (+) signs indicate positive signalling, (−) signs denote inhibitory signalling.