

REVIEW

A review on leukotrienes and their receptors with reference to asthma

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Objective and methods: Leukotrienes (LTs) including cysteinyl leukotrienes (CysLTs) and LTB₄ are the most potent inflammatory lipid mediators and play a central role in the pathophysiology of asthma and other inflammatory diseases. These biological molecules mediate a plethora of contractile and inflammatory responses through specific interaction with distinct G protein-coupled receptors (GPCRs). The main objective of this review is to present an overview of the biological effects of CysLTs and their receptors, along with the current knowledge of mechanisms and role of LTs in the pathogenesis of asthma. **Results:** CysLTs including LTC₄, LTD₄ and LTE₄ are ligands for CysLT₁ and CysLT₂ receptors, and LTB₄ is the agonist for BLT₁ and BLT₂ receptors. The role of CysLT₁ receptor is well established, and most of the pathophysiological effects of CysLTs in asthma are mediated by CysLT₁ receptor. Several CysLT₁ antagonists have been developed to date and are currently in clinical practice. Most common among them are classical CysLT₁ receptor antagonists such as montelukast, zafirlukast, pranlukast, pobilukast, iralukast, cinalukast and MK571. The pharmacological role of CysLT₂ receptor, however, is less defined and there is no specific antagonist available so far. The recent demonstration that mice lacking both known CysLT receptors exhibit full/augmented response to CysLT points to the existence of additional subtypes of CysLT receptors. LTB₄, on the other hand, is another potent inflammatory leukotriene, which acts as a strong chemoattractant for neutrophils, but weaker for eosinophils. LTB₄ is known to play an important role in the development of airway hyper-responsiveness in severe asthma. However there is no LTB₄ antagonist available in clinic to date. **Conclusion:** This review gives a recent update on the LTs including their biosynthesis, biological effects and the role of anti-LTs in the treatment of asthma. It also discusses about the possible existence of additional subtypes of CysLT receptors.

KeywordsAsthma, anti-LTs, CysLT, CysLT receptors, CysLT₃R, CysLT_ER**History**

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Introduction

The leukotrienes (LTs) are the main inflammatory lipid mediators derived from the lipoxygenase pathway of the arachidonic acid metabolism and were so named, because of the salient feature that they can be produced by leukocytes and they have a common conjugated triene in their structure. The LTs consist of cysteinyl leukotrienes (CysLTs) and leukotriene B₄ (LTB₄) [1–4]. The CysLTs are characterized by the presence of a cysteine ring whereas LTB₄ is a non-cysteine containing dihydroxy-leukotriene. The various subtypes known for CysLTs are leukotriene C₄ (LTC₄), leukotriene D₄ (LTD₄) and leukotriene E₄ (LTE₄) [5,6]. The biological properties of LTs suggest that CysLTs, in

particular, play an important role in the pathogenesis of asthma. Three CysLT₁ antagonists are already in the market, that is, Pranlukast, Zafirlukast, Montelukast [7–9]. The specific LTB₄ modulators, BLT antagonists on the other hand are still in the preliminary stages of clinical development [10–14].

Methods

We carried out a series of searches, last updated March 2013, using the database PubMed. The strategy was intended to be broad in order to maximize the capture of citations for peer-reviewed publications relevant to the role of CysLTs in asthma and treatment of asthma with anti-LTs. The PubMed searches were carried out using the following algorithm of MeSH terms: asthma or CysLTs review or CysLT receptor, anti-leukotrienes or bronchoconstriction. The searches were repeated with these terms in combination with pathogenesis, treatment and prevention. The citation pool was further supplemented from manual assessment of the reference lists accompanying other systematic reviews of aspects related to role of CysLT in asthma and from other publications identified as being relevant for further review.

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Results

Biosynthesis of leukotrienes

The LTs are derived from the ubiquitous membrane constituent, arachidonic acid (AA) which is the member of a large family of molecules known as eicosanoids. These biologically active lipids are rapidly generated at the sites of inflammation by a series of reactions, initiated by cytosolic PLA₂ (cPLA₂) which in turn release the AA from the phospholipids present at the nuclear envelope (Figure 1). The AA binds to the 5-lipoxygenase (5-LO)-activating protein (FLAP) and is further available to the enzyme, 5-LO. The 5-LO enzyme contains a non-heme iron at its active center which undergoes transition from a divalent to a trivalent state during catalysis. The 5-LO enzyme acts on AA bound to FLAP along with molecular oxygen, resulting in the formation of 5-hydroperoxy-eicosatetraenoic acid (5-HPETE) with subsequent formation of unstable, short-lived intermediate LTA₄. LTA₄ is converted to LTB₄ by the enzyme LTA₄ hydrolase, a cytosolic enzyme [15–17]. The inflammatory cells such as eosinophils, basophils, mast cells and alveolar macrophages possessing the integral membrane protein LTC₄ synthase synthesize CysLTs in response to biological and non-biological stimuli. LTC₄ synthase conjugates with reduced glutathione at position C6 of LTA₄ to form LTC₄. LTC₄ synthase also appears to be associated with FLAP in a multimolecular complex. Both LTB₄ and LTC₄ are exported to extracellular space by transporters. After its export, LTC₄ is rapidly cleaved by transpeptidase to produce LTD₄ and finally LTD₄ is converted to LTE₄ by dipeptidase removal of glycine [12,18–21].

Alternative route for leukotriene biosynthesis

CysLTs can also be produced through transcellular metabolism from neutrophils-derived LTA₄ by the cells which do not express 5-LO, including platelets, erythrocytes and vascular endothelial cells, and they utilize a different member of the FLAP/LTC₄ synthase gene family to synthesize LTB₄ and/or

LTC₄ (Figure 2). Such transcellular biosynthesis of LTC₄ has also been reported for mast cells [22], blood peripheral monocytes [23], human airway epithelial cells, alveolar macrophages [23], kidney-derived endothelial cells [24], keratinocytes and chondrocytes [25,26]. After their intracellular synthesis, these LTs are released to the extracellular space through specific carrier proteins that are potential targets for the new generation anti-LTs.

Gender variation in the synthesis of leukotrienes

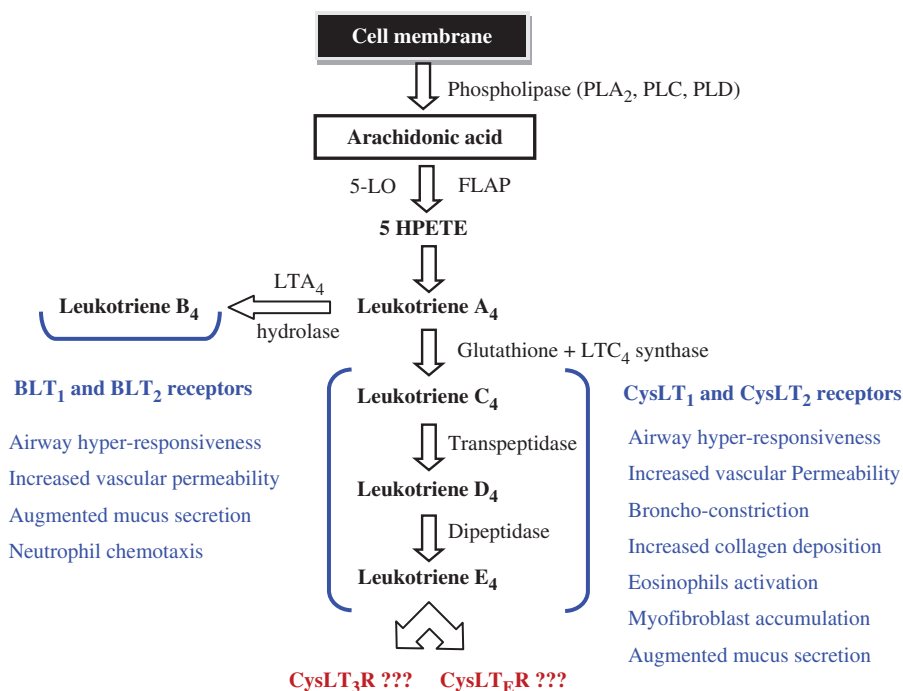
Pergola et al. have reported remarkable gender differences in the generation of the LTs [27]. Formation of LTs and its products on stimulation with lipopolysaccharide (LPS) plus *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) or with Ca²⁺ ionophore A23187 was significantly higher in female blood as compared to male. This gender difference in the capacities of LPS/fMLP-induced generation of LTs and other 5-LO products may also reflect pathophysiological conditions in the body. However, 5-LO protein levels and 5-LO activity in homogenates of blood or isolated neutrophils were not different between genders. In female neutrophils, 5-LO resided in the cytoplasm of resting cells and redistributed to nucleus upon stimulation, whereas in male neutrophils, 5-LO was detected in both cytosol and nuclear compartment of resting cells and the compartmentalization of 5-LO was not significantly altered on stimulation. The study by Pergola et al. suggests that male neutrophils should have higher levels of extracellular signal-regulated kinase (ERK) activity versus female cells. Thus, biosynthesis of LTs and its products and distribution and localization of 5-LO in human neutrophils depend on gender, connected to a differential activation of ERK [27].

Pharmacology of leukotriene receptors

Pharmacology of CysLT receptors

CysLTs exert their effect through cell surface receptors belonging to superfamily of GPCRs [28]. According to

Figure 1. Biosynthesis of leukotrienes. Arachidonic acid is catalyzed by the 5-LO enzyme. This reaction requires FLAP and results in the formation of LTA₄. The unstable epoxide LTA₄ is either acted upon by epoxide hydrolase to form LTB₄ or conjugated with glutathione by LTC₄ synthase and yields LTC₄. LTC₄ is metabolized by γ -glutamyltranspeptidase to LTD₄, which is, in turn, metabolized by dipeptidase to LTE₄.



International Union of Pharmacology (IUPHAR), CysLT receptor nomenclature was originally based on the sensitivity to the so-called classical antagonists, including montelukast, zafirlukast, pranlukast, pobelukast, iralukast, cinalukast and MK571 [4,5,29–31]. Accordingly, CysLT receptors have been mainly divided into two classes: CysLT₁, which are sensitive to the classical antagonists, and CysLT₂, which mediate several effects which are not inhibited by the classical antagonists. The only “dual antagonist” is an LTE₄ analogue, BAY u9773, that has been reported to exhibit antagonistic activity at both CysLT₁ and CysLT₂ receptors. BAY u9773, however, is neither very potent nor selective for the CysLT receptor classes, especially in human tissues [5,32–36]. BAYu9773 has also been reported as a partial agonist for CysLT₂ receptor. Recently, Wunder et al. have reported the identification of first potent and selective CysLT₂ receptor antagonist HAMI3379, which inhibits the cardiovascular effects mainly mediated through CysLT₂ receptors in rodents [37,38].

The genes for human CysLT₁ and CysLT₂ receptors are located on the long arms of chromosomes 10 and 13 and share 38% amino acid identity. Both CysLT₁ and CysLT₂ receptors are reported to be coupled to G α_q -protein. Their activation involves an increase in intracellular calcium through PLC pathway [34,39–44]. Recently, a third receptor was shown to respond to both CysLTs and uracil nucleotides. Most of the effects of CysLTs that are relevant to the pathophysiology of asthma are mediated by activation of the CysLT₁ receptor, which is expressed in monocytes and macrophages, eosinophils, basophils, mast cells, neutrophils, T cells, B lymphocytes, pluripotent hematopoietic stem cells (CD³⁴⁺), interstitial cells of the nasal mucosa, airway smooth muscle cells, bronchial fibroblasts and vascular endothelial cells [30,41,43,45–48]. CysLT₂ receptors on the other hand are strongly expressed throughout the entire human heart, including ventricles, atrium, septum, apex and Purkinje fiber cells, as well as in vascular endothelial and smooth muscle cells. As CysLT₂ receptors are highly expressed in heart and blood vessels, a role for this receptor in the pathogenesis of various cardiovascular diseases might be anticipated. They are also expressed in human peripheral basophils, endothelial cells, cultured mast cells, and in nasal eosinophils and mast cells in patients with active seasonal allergic rhinitis. However, at present, the role of the CysLT₂ receptor in allergic inflammation is poorly known [34,38,40,44,49,50].

Pharmacology of LTB₄ receptors

BLT₁ and BLT₂ are the two receptor subtypes identified for LTB₄. Both of them are the GPCR, and the coding genes for both the receptors are located on chromosome 14. These receptors differ in their affinity and specificity for LTB₄ and their expression pattern. The cDNA for BLT₁ encodes a cell-surface protein of 352 amino acids. BLT₁ is a specific high-affinity receptor for LTB₄ and is expressed predominantly on leukocytes including granulocytes, monocytes, macrophages, mast cells, dendritic cells and effector T cells. BLT₂, on the other hand, is a low-affinity receptor which can also bind to other eicosanoids. BLT₂ is expressed ubiquitously; however, their biological role in humans is unknown. Ligation of

BLT₁ and/or BLT₂ by LTB₄ triggers various intracellular signal transduction and cellular events in inflammatory cells, which include intracellular Ca²⁺ mobilization, activation of ERK, phosphoinositide-3 kinase and Akt, chemotaxis, degranulation and the production of inflammatory proteins [2,10–12,14,31,51,52].

The major signal transduction pathway for BLT₁ receptor in native systems is through activation of Phospholipase C (PLC) coupled to phosphoinositide (PI) turnover and mobilization of intracellular calcium. A pertussis toxin-sensitive G-protein has been implicated in signal transduction. BLT₂ receptor shows a high homology with BLT₁ (36–45% amino acid identity). It mediates LTB₄-induced chemotaxis, Ca²⁺ flow and inhibition of adenylate cyclase. It is insensitive to several BLT₁ antagonists and thus it is a pharmacologically distinct receptor subtype [8,9,37,52,53].

Evidences for additional subtypes of receptors

There are several reports suggesting the presence of additional CysLT receptor subtypes in human tissues. It has been observed that activation of the CysLT receptor with LTE₄ alone or inhibition using the dual antagonist BAYu9773 was not sufficient to alter all the CysLT functional responses [21,54–57]. Contractions due to LTC₄ were found to be more potent than LTD₄ but the CysLT₁ receptor antagonist or the dual CysLT₁/CysLT₂ receptor antagonist could only slightly inhibit the contractions caused by both the agonists in endothelium intact porcine pulmonary arteries. After denuding the endothelium, however, LTC₄ and LTD₄ are found to be equipotent, and the LTC₄-induced contractions in this situation are resistant to both CysLT₁ receptor antagonist and the dual CysLT₁/CysLT₂ receptor antagonist. Further, the contractile response of human pulmonary artery to CysLTs was resistant to the CysLT₁R antagonist MK-571 and to the dual CysLT₁/CysLT₂R antagonistic activity [1,58,59]. This suggests the occurrence of another receptor in the human pulmonary artery, which is different from the existing CysLT₁ and CysLT₂ receptors. These functional observations were further supported by the results obtained from ligand-binding studies in human tissues [58,60–63].

LTE₄ binds poorly to the classical CysLT₁ and CysLT₂ receptors as compared to other CysLTs and is much less active on normal airways. However, earlier studies have reported that LTE₄ caused skin swelling in human subjects with similar potency as other CysLTs and the inhalation of LTE₄ caused an increase in the airway inflammatory cells and airway hyper-responsiveness of aspirin-sensitive asthmatics [21,54]. Austen et al. [21,54] have shown that the ear edema produced by injection of LTD₄ and LTC₄ in strain deficient in both CysLT₁ and CysLT₂ receptor was equivalent to that in the wild-type control animals, indicating the presence of a novel receptor. Further, they were sensitive to LTE₄, exhibiting the same extent of ear swelling in a much lower dose as compared to wild-type control animals. The LTE₄-mediated vascular leak was markedly inhibited by pretreatment with pertussis toxin or Rho kinase inhibitor in the mice deficient in both CysLT₁ and CysLT₂ receptor, suggesting involvement of a GPCR linked to G α_i proteins and Rho kinase. This particular sensitivity of an unknown receptor to LTE₄

raises the possibility of a novel receptor, designated as CysLT_ER [54,64].

GPR99, previously described as an oxoglutarate receptor (Oxgr1), has been shown to have both, a functional and a binding response to LTE₄ in cysLTR1/cysLTR2(-/-) mice. Additionally, GPR99 deficiency in the cysLTR1/cysLTR2(-/-) mice virtually eliminated the vascular leak in response to the LTE₄, indicating GPR99 as a potential receptor for LTE₄. Importantly, the Gpr99 knockout mice showed a dose-dependent loss of LTE₄-mediated vascular permeability, but not to LTC₄ or LTD₄, revealing a preference of GPR99 for LTE₄, even when CysLT₁R is present. As LTE₄ is the predominant cysLT species in inflamed tissues, GPR99 may provide a new therapeutic target [65]. The existence of a functional crosstalk between the nucleotide and the CysLT systems has been documented, and both types of mediators accumulate at the site of inflammation. The inflammatory cells often coexpress both P2Y and CysLT receptors. There are close structural and phylogenetic relationships between P2Y and CysLT receptors, which cluster together into the “purine receptor cluster” of the rhodopsin family of GPCRs [66,67]. Mellor et al. proposed [56] that both CysLT₁ and a yet unidentified LTC₄-preferring receptor (probably CysLT₃ receptor) were upregulated in human mast cells by treatment with the proinflammatory cytokine IL-4 and mediated dual responses to both CysLTs and UDP. GPR17, an orphan receptor present at an intermediate phylogenetic position between P2Y and CysLT receptor families, may represent the yet unidentified elusive receptor responding to both nucleotides and CysLTs. Ciana et al. have reported [67] that the GPR17 receptors overexpressed in various different cell lines responded to both nucleotides and CysLTs in a specific and concentration-dependent manner. These data suggested the role of GPR17 in the P2Y and CysLT receptor signaling systems. Moreover, GPR17 has been found to be highly expressed in organs that can typically undergo ischemic injury (brain, heart and kidney) and also *in vivo* knockdown of GPR17 by either CysLT/P2Y receptor antagonists or by antisense technology clearly prevented evolution of ischemic brain damage and also reduced tissue damage and related histological and motor deficits during spinal cord injury, suggesting GPR17 as a common molecular target mediating the inflammatory effects induced *in vivo* by nucleotides and CysLTs [67,68].

It has also been reported that purinergic (P2Y₁₂) receptor is required for LTE₄-mediated pulmonary inflammation [54,69]. Austen et al. have shown that [54] LTE₄ can induce the activation of ERK in the Chinese hamster ovary (CHO) cells stably transfected with human P2Y₁₂ receptors exceeding the potency of LTD₄, and this effect of LTE₄ is inhibited by clopidogrel, a P2Y₁₂ receptor-selective antagonist. This signaling event was sensitive to pertussis toxin but resistant to MK571. 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.

In sensitized mice, administration of LTE₄ potentiates eosinophilia and goblet cell metaplasia in response to low-dose aerosolized allergen. These responses persist in mice

lacking both CysLT₁ receptor and CysLT₂ receptor, but not in mice lacking P2Y₁₂ receptors, indicating that the P2Y₁₂ receptor is required for proinflammatory actions of the stable abundant mediator LTE₄ and is a novel potential therapeutic target for asthma [57].

Altogether these evidences support for the possibility for the existence of additional CysLT receptor subtypes which need further characterization with respect to pathophysiology of different inflammatory disorders including asthma.

Role of leukotrienes and their receptors in asthma

Asthma is a chronic respiratory disease characterized by airway inflammation and hyperresponsiveness to various stimuli. It includes the narrowing of the small airways of the lung upon exposure to certain “triggers” resulting in the difficulty in breathing and increased responsiveness. It is one of the common diseases with an increasing prevalence among children (~10%) as well as adult (~5%) population [2,5,29,70]. Although pathogenesis of asthma involves several different cells and mediators and varies from individual to individual depending on the stimulus, however CysLTs and LTB₄ are the most potent bronchoconstrictors found in human to date and are believed to play important role in the pathogenesis of asthma [11,34,70–73]. In last three decades, there are a lot of reports suggesting the pathophysiological role of LTs in several inflammatory conditions with an emphasis on asthma. The inherent tone of human airway is thought to be maintained by a balance between the effects of contractile mediators including CysLTs and LTB₄, histamine and the relaxing elements such as PGE₂ [52,74–76].

Potential role of CysLTs in asthma

CysLTs are potent bronchoconstrictors and one of the important inflammatory components of asthma. They are biosynthesized by eosinophils, basophils, mast cells and alveolar macrophages (Figure 2). The effects of CysLTs include increased microvascular permeability leading to pulmonary edema, increased mucus secretion and decreased clearance by impairing the ciliary activity and airway smooth muscle cell hyperplasia which further causes airway remodeling [77–79]. Many cell types including mast cells, eosinophils, macrophages, epithelial cells and endothelial cells coexpress CysLT₂ with CysLT₁ receptor. However, abundance of CysLT₂ appears to exceed over CysLT₁ in both eosinophils and nasal epithelium. CysLTs are produced mainly by the eosinophils and also

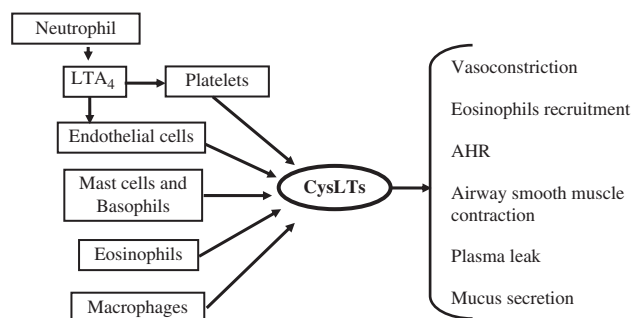


Figure 2. Cellular sources of cysteinyl leukotrienes and summary of their effects on airway and inflammatory cells.

by the mast cells at the rapid onset of allergic asthma. The number and activity of the eosinophils are increased resulting in the increased concentration of CysLTs in the bronchoalveolar lavage and urine [80]. The eosinophils are thought to play a pivotal role in the process of chronic inflammation associated with asthma, and CysLTs increase eosinophils survival in response to paracrine signals from mast cells and lymphocytes. CysLTs promote leukocyte maturation and migration from the bone marrow into the circulatory system and they are chemoattractant for eosinophils, increasing their cellular adhesion and transendothelial migration across the vessel wall into the airways. Some of the cytokines and peptides, such as GM-CSF, IL-5, ICAM-1 and VLA-4, stimulate CysLT production, which further causes the release of more cytokines and other adhesion molecules from eosinophils [81].

The study by Zhu and colleagues [48] further demonstrates that the numbers of CysLT₁ receptor mRNA and protein-positive inflammatory cells are significantly high in stable asthmatic subjects and patients hospitalized for exacerbation of their asthma as compared to controls. There also exists a strong positive correlation between this observation and the increased numbers of CD45⁺ progenitors. In human nasal mucosa, CysLT₁ receptor has been identified at both gene as well as protein levels in blood vessels and in the interstitial cells such as vascular endothelial cells, eosinophils, mast cells, macrophages and neutrophils. Because subjects with aspirin-induced asthma have greater airway hyperresponsiveness to the effects of inhaled CysLTs than aspirin-tolerant asthmatics (ATA), Sousa and colleagues hypothesized that this could be because of the elevated expression of CysLT₁ receptor on inflammatory cells [82].

Subjects with more severe asthma were found to have higher concentration of CysLTs in sputum than in non-asthmatic control subjects. Bronchoalveolar lavage (BAL) fluids from asthmatic subjects were found to show increased concentrations of LTC₄ after allergen challenge and provide a direct measurement of inflammation [8–11,34,48,52,70,76,77,83]. These reports suggest that CysLT levels are increased in patients with asthma, increase after allergen challenge, exercise challenge and with severity of asthma, and decrease on treatment with CysLT₁ antagonist. Evidence of a proinflammatory role for CysLTs has been provided by means of direct administration of CysLTs to the human airway.

Effects on airway smooth muscle tone

Contraction of smooth muscle of bronchi was the first biological property recognized for the CysLTs, even before their structure was elucidated. LTC₄ and LTD₄ were potent inducers of bronchoconstriction in guinea pig airways *in vitro* and *in vivo* and caused contractions of isolated human bronchi. *In vivo* experiments have clearly shown that CysLTs are able to induce bronchoconstriction, when inhaled, both in healthy and in asthmatic individuals. In chronic asthma, it appears that human bronchi possess an increased contractile tone, due to constitutive release of CysLTs, and the administration of a CysLT₁ receptor antagonist induces an apparent bronchodilation, despite the fact that these compounds are devoid of direct effects on bronchial tone [59,71,84–86].

The biological effects of LTE₄ have been studied much less, possibly because this LT was found to be less potent agonist than LTC₄ and LTD₄ in some smooth muscle preparations such as guinea pig ileum [21]. There are reports for the existence of a separate receptor for LTE₄. Lee et al. have reported that LTE₄, the most stable of the leukotrienes, comprising slow reacting substance of anaphylaxis, enhances the contractile response of guinea pig tracheal spirals but not of parenchymal strips to histamine in a time- and dose-dependent fashion. The ability of LTE₄ to increase histamine responsiveness was not produced by LTC₄ and LTD₄, which elicited the same magnitude of contraction of tracheal smooth muscle as LTE₄. These findings suggest that LTE₄-induced airway hyperirritability is not mediated by the contractile response *per se* and may be mediated through a receptor distinct from those for LTC₄ and LTD₄ [87].

Airway smooth muscle proliferation

Airway smooth muscle cell hyperplasia is another feature of chronic severe asthma. It has been suggested that LTD₄ is involved in modulating the proliferation of airway smooth muscle cells and airway remodeling in rats. The CysLT₁ antagonist inhibits the increase of the airway smooth muscle mass induced by ovalbumin in sensitized rats. LTD₄ has been found to be inactive *per se* in human airway smooth muscle cells culture; however, it markedly potentiated the proliferation induced by epidermal growth factor. Interestingly, such effect was antagonized by pobelukast or pranlukast, but not by zafirlukast, indicating that the receptor involved might be different from that of CysLT₁ receptors. These data suggest that the CysLTs play a role in the regulation of airway smooth muscle proliferation [29,52,85,86]. However, LTB₄ neither affects bronchial tone nor induces smooth muscle cell proliferation.

Vascular permeability

It has long been known that LTC₄ and LTD₄ cause exudation of plasma proteins in postcapillary venules in the hamster cheek pouch, and their intradermal application produce a flare and wheal reaction in humans. Local injection of these two CysLTs also increased accumulation of intravenous injected Evans blue in the skin, suggesting an increase in microvascular permeability. In the guinea pig, it was shown that each of LTC₄, LTD₄ and LTE₄ was capable of causing accumulation of the plasma protein tracer Evans blue in the airways. This is very likely to be due to the retraction of endothelial cells at the level of postcapillary venules, as shown for LTE₄. It is interesting to note that increased veno-permeability and plasma extravasation *in vivo* might be a result of the cooperation between LTB₄ and CysLTs. The plasma exudation occurred in all airway segments, ranging from the most peripheral small bronchi to trachea, and there was evidence of Evans blue accumulation in superficial as well as deep layers of the airway mucosa. In fact, LTB₄, being a potent chemoattractant, can participate in migration, rolling and adhesion of leukocytes. The proximity of leukocytes to the vascular wall facilitates the transfer of LTA₄ from the former to endothelial cells, thus triggering the biosynthesis of LTC₄ precisely, where this and/or the other CysLTs can induce

the opening of gaps between adjacent endothelial cells [61,62,88,89].

Bronchial hyperresponsiveness

The hyperresponsiveness of asthmatics to LTC₄ and LTD₄, relative to methacholine, is lower than that in normal individuals. This may be because the chronically elevated production of LTs in asthmatics induces adaptive changes in the airway effector cells or at the receptor level. However, it has been reported that asthmatics were especially hyperresponsive to LTE₄. LTE₄ induces significant hyperresponsiveness to histamine both in guinea pig trachea *in vitro* and in humans *in vivo*. Furthermore, the airways of asthmatic subjects are hyperresponsive to LTE₄, but not to the other CysLTs, although the degree of hyperresponsiveness to CysLTs is lower than histamine or methacholine. Inhibition of methacholine-induced hyperresponsiveness by CysLT antagonists *in vivo* suggests the role of endogenous CysLTs in this phenomenon [1,54,83,84,90–93].

Potential role of LTB₄ in asthma

The leukocytes are found to be the primary targets for the biological activity of LTB₄. LTB₄ is important for the activation and recruitment of inflammatory cells including leukocytes, mast cells, dendritic cells and, more recently, effector T cells to inflamed tissues in various inflammatory diseases. LTB₄ is a potent stimulus for various leukocyte functions, eliciting aggregation, chemotactic and chemokinetic responses, release of lysosomal enzymes, increased rolling and adhesion to endothelium followed by their emigration into the extravascular space. It is also a chemokinetic factor and a weak chemoattractant for eosinophils [7–11,14,52,76,79,88,94].

It has been shown that LTB₄ is a chemoattractant for neutrophils and may stimulate production of IL-5 in T lymphocytes. In addition to the effects on leukocyte adhesion and migration, LTB₄ like other secretagogues stimulates the secretion of superoxide anion and release of different granulae constituents from leukocytes [52,95–97]. With regard to the effects of LTB₄ on the lung, it has been established that LTB₄ causes contractile activity in the guinea pig lung parenchyma. This response is indirect, involving the release of TXA₂ and histamine, possibly from pulmonary mast cells. It has been shown that the effect in the lung parenchyma is due to activation (vasoconstriction) of pulmonary blood vessels rather than the airway elements. Although inhalation of LTB₄ by healthy human volunteers was followed by distinct cellular changes in the airways, and also some plasma exudation, the lack of immediate bronchoconstrictive properties was confirmed in healthy volunteers as well as in subjects with asthma [7,14,29,88,98,99].

LTB₄ is associated with the development of AHR during an asthma attack. Inhaled methacholine stimulates LTB₄ release in patients with asthma, but not in healthy subjects, without affecting the number of inflammatory cells in BAL fluid. The LTB₄ antagonist, LY293111, prevented the development of airway hyperresponsiveness and neutrophil accumulation but failed to inhibit accumulation of eosinophils in sensitized and challenged animals. However, recent studies

using another BLT₁ antagonist (CP-105,696) and BLT₁-deficient mice have confirmed the role of LTB₄/BLT₁ pathway in the recruitment of not only neutrophils but also effector T cells including effector memory CD⁸⁺ T cells into the lungs of allergen-induced allergic airway inflammatory responses in mice. Inhibition of the LTB₄/BLT₁ pathway resulted in decrease in airway hyperresponsiveness and allergic airway inflammation including accumulation of neutrophils and lymphocytes in the airway. This suggests LTB₄/BLT₁ pathway appears to play an important role in the pathophysiology of asthma along with other mediators including CysLTs, cytokines and chemokines [11,12,79,100–102].

LTB₄ levels were increased in the plasma, sputum and BAL fluid of asthmatic patients but not in the healthy subjects. Increased synthesis of LTB₄ was accompanied by increased transcriptional upregulation of 5-LO and LTA₄ hydrolase in peripheral blood leukocytes of asthmatics. Generation of LTB₄ by calcium ionophore-stimulated alveolar macrophages and peripheral blood neutrophils was increased in asthmatic patients. Taken together, these data suggest that an upregulation of the LTB₄ synthesis in the circulating leukocytes and lungs is associated with asthma [79,101,102].

Leukotriene receptor antagonists used in asthma

The clinical development of anti-leukotriene drugs started in the mid-1980s. There were two main approaches used in the discovery of anti-leukotriene drugs: (1) development of LT biosynthesis inhibitors (such as cPLA₂ inhibitors, 5-LO inhibitors, FLAP inhibitors) and (2) development of LT receptor antagonist (mainly cysLT₁ receptor antagonists). The LT biosynthesis inhibitors result in inhibition of both LTB₄ and CysLTs, whereas the LT receptor antagonists (LTRAs) selectively inhibit the action of CysLTs at CysLT₁ receptors within the airways. From 1990 and onwards, a number of studies were published, supporting the concept that CysLTs mediate significant components of bronchoconstriction evoked by common triggers of asthma through CysLT receptors and hence further clinical development were dominated mainly by the CysLT receptor antagonist. In the second half of the 1990s, the 5-LO inhibitor, zileuton, and the LTRAs such as pranlukast, zafirlukast and montelukast entered into clinical practice [73,100,103,104]. There are several CysLT₁ receptors antagonists in different clinical phases of development and are listed in Table 1.

With regard to CysLT₂ antagonists, there are only early preclinical candidates and it is not clear whether CysLT₂ receptor has an important role in asthma. CysLT₂ receptors are classically localized to human pulmonary veins, but they are also expressed in airway smooth muscle and inflammatory cells. It is not certain whether CysLT₂ receptors play any role in mediating the effects of CysLTs in asthma. There is some evidence suggesting that CysLT₂ receptors may mediate the proliferative response of airway smooth muscle cells to CysLTs [4,30].

LTRAs combine anti-inflammatory, mainly anti-eosinophilic activity with mild, bronchodilator properties, based on antagonism of CysLTs at the CysLT₁ receptor within the airways and on inflammatory cells. LTRAs have been shown

Table 1. List of CysLT receptors antagonists (alone or in combination with other drugs) in different phases of development.

Drug name	Originator	Route of administration	Status	Remarks
Zafirlukast	AstraZeneca	Per-oral	Launched (Japan, UK, USA)	–
Montelukast	Merck & Co	Intravenous, Per-oral	Launched (India, Japan, USA, UK, EU, Canada, New Zealand)	–
Pranlukast	Ono Pharmaceuticals	Per-oral	Launched (Japan, South America)	–
Acitazanolast	Wakamoto	Ophthalmic	Launched (Japan)	–
Levocetirizine/Monteleukast	Hanmi Pharmaceutical	Per-oral	Phase III	
Masilukast	AstraZeneca	Per-oral	Phase III	Since 2009
Loratidine/Montelukast	Schering-Plough/Merck	Per-oral	Phase III	Since 2009
Tipelukast	Kyorin Pharmaceuticals	Per-oral	Phase II/III	Since 2009
KP 496	Kaken Pharmaceuticals	Inhalation	Phase II	Since 2009
Montelukast/Mometasone	Merck & Co	Inhalation	Phase II	Initiated in 2010
YM 57158	AstellasPharma	Per-oral	Phase I	Since 2007
CR 3465	Rottapharm	Per-oral	Phase I	Initiated in 2010

Source: ADIS R&D Insight, 2013.

to improve symptoms of asthma and suppress the airway inflammation throughout the entire airways. They also have been shown to possess bronchoprotective properties and providing partial protections against airway narrowing stimuli and also reducing the AHR [7,72,100,105].

LT modifiers interfere with the pathway of leukotriene mediators, which are released from mast cells, eosinophils and basophils. These medications include LTRAs (montelukast and zafirlukast) and a 5-lipoxygenase inhibitor (zileuton). LTRAs are alternative, but not preferred, therapy for the treatment of patients with mild persistent asthma. LTRAs also can be used as adjunctive therapy with inhaled corticosteroids (ICS), but for youths (12 years of age) and adults, they are not preferred adjunctive therapy, compared to long-acting beta₂ adrenergic agonists (LABAs). Zileuton can be used as alternative, but not preferred, adjunctive therapy in adults; liver function monitoring is essential [51,106–108].

LTRAs can attenuate exercise-induced bronchoconstriction (EIB). EIB affects between 70% and 80% of asthmatic patients. Shortly after strenuous exercise, several inflammatory mediators, including CysLTs, induce bronchoconstriction. Evidence of this phenomenon includes the increase in urinary LTE₄ excretion, after exercise and the inhibition of EIB by the LT synthesis inhibitor, zileuton. Similarly, LTRAs, montelukast and zafirlukast have significantly reduced the decrease in pulmonary function after exercise and shortened the time to recovery. Because exercise is generally a less predictable event in children, EIB can be more difficult to manage in pediatric than in adult asthmatic patients. However, LTRA use may temper this problem. Montelukast administered once daily at bedtime protected pediatric patients against EIB throughout the entire day. Zafirlukast attenuated EIB within 4 hours of dosing in 6- to 17-year-old patients who had mild-to-moderate asthma. Currently, inhaled LABAs are widely prescribed for EIB, but when used long term, their efficacy may wane because of tolerance. In contrast, one of the advantages offered by LTRA therapy is the absence of tolerance [7,51,72,108,109].

LTRAs are well tolerated with few side effects, suggesting that the endogenous CysLTs are not important in regulating normal physiological functions. Headaches and gastrointestinal side effects are the most common, but these are rarely severe enough to cause discontinuation of the therapy. The major concern is Churg–Strauss syndrome, with circulating

eosinophilia, cardiac failure and associated eosinophilic vasculitis which have been reported in a few patients treated with LTRAs. There are several case reports of Churg–Strauss syndrome developing in patients, who have not been treated with inhaled or oral corticosteroids, suggesting that it may be a direct effect of the LTRAs. As these drugs are metabolized by the liver, the possibility for significant drug interactions with other drugs metabolized by the Cytochrome P450 enzyme system may exist. However, LTRAs have an excellent safety profile and have a good therapeutic index and limited toxicity [5,29,106–109]. Despite recent concerns, thorough analysis of existing data suggests anti-LTs are well-tolerated drugs. However, the possible link with Churg–Strauss syndrome requires further investigation.

Further to this, some selective and potent antagonists of LTB₄ have also been developed. A few compounds have entered into early clinical testing in man but most of them have been discontinued. The compound LY293111 (Eli Lilly) was found to inhibit LTB₄-induced neutrophil responses *in vivo* and allergen-induced neutrophil activation, but it had no effect on allergen-induced airway obstruction in asthmatics. CP105696 (Pfizer) has shown good efficacy *in vitro* and also *in vivo*, but it was discontinued because of long terminal elimination half-life. A prodrug, BIIL284 (Boehringer Ingelheim), was also developed, but discontinued for various reasons. To date, there is no LTB₄-specific antagonist in clinical use.

Discussion

CysLTs are important mediators of various asthmatic responses and they remain the most potent bronchoconstrictors known to date. CysLTs exert a range of proinflammatory effects and have proved to be important mediators in asthma, allergic rhinitis and several other inflammatory conditions such as cardiovascular diseases, cancer and certain CNS diseases such as multiple sclerosis. The CysLTs exert their effects through CysLT₁ and CysLT₂ receptors, which are the members of the GPCR superfamily. However, it has been well documented that the pathophysiological effect of CysLTs in asthma is mainly mediated through CysLT₁ receptor, and the role of CysLT₂ receptors in asthma is not well documented; there is no specific antagonist for CysLT₂ receptors developed yet. Moreover, the reason for the coexpression of the CysLT₁

and CysLT₂ receptors in eosinophils needs to be investigated. It is possible that in some disorders in which tissues and cells that coexpress both types of receptors, specific CysLT₁ receptor antagonists might be less efficacious than nonselective antagonists because of their inability to interfere with all the functions exerted by CysLTs.

In addition, some more studies are required to confirm the role of additional subtypes of receptors such as CysLT₃ receptor and CysLT_{ER} in human asthma. This is mainly important for the CysLT_{ER} as LTE₄ appears to be playing a role in the recruitment of eosinophils in human airways. It is worth noting that very recent evidence for the existence of additional receptor subtypes (such as CysLT₃R and CysLT_{ER}) and receptors crosstalk might in part be responsible for the known CysLT functions. It is therefore important to unravel the specific roles of these receptor subtypes in asthma and other airway diseases and also for the development of selective antagonists of these receptors.

With regard to LTB₄, recent research findings have revealed the important roles of LTB₄-BLT₁ pathway in the pathophysiology of various allergic diseases such as severe persistent asthma, aspirin- and exercise-induced asthma, allergic rhinitis and atopic dermatitis. LTB₄ production is in general resistant to corticosteroid treatment and corticosteroids, in fact, can upregulate BLT₁ expression on corticosteroid-resistant inflammatory cells such as neutrophils, monocytes and effector memory CD8⁺ T cells. As a result, this corticosteroid-resistant, LTB₄-BLT₁ pathway may contribute to the development of inflammation in allergic diseases that do not respond to the introduction of corticosteroids. Inhibition of this pathway has potential therapeutic benefit in various allergic diseases that have involvement of corticosteroid insensitivity [110]. LTB₄ receptor antagonists though do not have a major role in eosinophil recruitment and do not improve lung physiology in humans in response to allergen; they however reduce neutrophil influx to the airway following antigen challenge in atopic asthma. This evidence broadly supports the pathogenic role of LTB₄ in chronic neutrophil-mediated inflammation and LTB₄ receptor antagonists may prove to be beneficial in such disorders.

In conclusion, more research work is needed to define the precise roles of CysLTs and LTB₄ and their respective receptors in the normal and disease state for successful development of therapeutic agents which are safer than currently available therapies for asthma and other inflammatory disorders.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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