Leukotriene receptors as potential therapeutic targets

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**Review Series**

Leukotrienes, a class of arachidonic acid–derived bioactive molecules, are known as mediators of allergic and inflammatory reactions and considered to be important drug targets. Although an inhibitor of leukotriene biosynthesis and antagonists of the cysteinyl leukotriene receptor are clinically used for bronchial asthma and allergic rhinitis, these medications were developed before the molecular identification of leukotriene receptors. Numerous studies using cloned leukotriene receptors and genetically engineered mice have unveiled new pathophysiological roles for leukotrienes. This Review covers the recent findings on leukotriene receptors to revisit them as new drug targets.

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Leukotrienes, a class of arachidonic acid–derived bioactive molecules, are known as mediators of allergic and inflammatory reactions and considered to be important drug targets. Although an inhibitor of leukotriene biosynthesis and antagonists of the cysteinyl leukotriene receptor are clinically used for bronchial asthma and allergic rhinitis, these medications were developed before the molecular identification of leukotriene receptors. Numerous studies using cloned leukotriene receptors and genetically engineered mice have unveiled new pathophysiological roles for leukotrienes. This Review covers the recent findings on leukotriene receptors to revisit them as new drug targets.

Leukotrienes (LTs) are a class of mediators derived from arachidonic acid by the initiating activity of 5-lipoxygenase and 5-lipoxygenase–activating protein (FLAP). They are involved in self-defense systems against foreign bodies or microorganisms, but overproduction causes a variety of immune and inflammatory diseases (1). Currently, while only 5-lipoxygenase inhibitors and cysteiny1 leukotriene receptor 1 (CysLT1) antagonists are marketed to treat bronchial asthma and allergic rhinitis, other targets for at least four distinct types of receptors or their combinations are under consideration. The 3D structure analysis followed by the determination of the catalytic sites of LTC4 synthase and LTA4 hydrolase provides new structural bases for the development of LT synthesis inhibitors (2–6). As described here, the 3D structure of BLT1 has been resolved, enhancing the rational design of potent antagonists and inverse agonists. We also refer readers to a more comprehensive review of leukotriene receptors including agonist and antagonist structures and their applications (7).

Characterization of BLT receptors

Two G protein–coupled receptors (GPCRs) have been cloned as receptors for leukotriene B4 (LTB4) (Table 1 and refs. 8, 9). The first, BLT1, known as a high-affinity LTB4 receptor, is expressed in various subsets of leukocytes and is responsible for LTB4-dependent leukocyte migration. The second, BLT2, was originally reported as a low-affinity LTB4 receptor and is now considered as a receptor for various oxidized fatty acids, including 12-hydroxyheptadecatrienoic acid (12-HHT) and hydroxyeicosatetraenoic acids (HETEs). BLT1 is expressed in epidermal keratinocytes and epithelial cells of intestine, cornea, and lung and is responsible for wound healing and epidermal barrier function. In addition to other Reviews in this series, the reader may also refer to a comprehensive series of 9 recent reviews on LTB4 (10–18).

BLT1. Human BLT1 consists of 352 amino acids and is mainly expressed in various subsets of leukocytes, including granulocytes (8), eosinophils (19, 20), and effector-type CD4+ and CD8+ T cells (21–23), as well as certain subsets of dendritic cells (24, 25) and macrophages (26). BLT1 is also expressed in murine (27) and human (28) vascular smooth muscle cells, and is involved in atherogenesis and vascular injury. It is a high-affinity and LTB4–specific receptor with a Kd value of 0.15 nM for LTB4 when expressed in Cos-7 cells (8). In BLT1–transfected CHO cells, BLT1 is able to couple with both G-i-like and G-q-like (G16) G proteins, and an extensive mutagenesis study showed that intracellular loop 3 is important for the G coupling of BLT1 (29). Human BLT1 has two N-glycosylation sites (N2 and N164), and mutagenesis of these asparagine residues does not affect localization, ligand binding, or intracellular signaling of BLT1. BLT1 does not contain the cysteine residue that is often palmitoylated in the C-tails of various GPCRs; instead, it has a so-called helix 8 structure immediately following transmembrane 7. Helix 8 of BLT1 is important in the conformational change to the low-affinity state after G protein activation (30, 31) and internalization (32–34) of BLT1.

Recently, the crystal structure analysis of BLT1, with the antagonist BIIL260 was achieved (Figure 1, A and B, and ref. 35). Docking study with LTB4 and BLT1 indicates that LTB4 would interact with the residues H96, R158, E187, and S243 that were predicted to be involved with LTB4 binding by the mutation study (Figure 1C and ref. 36). The benzamidine moiety of BIIL260 interacted with the side chains of D66, V69, S106, W236, and S276, which are shared among most GPCRs (Figure 1B). These amino acid residues bind water molecules as the sodium ion–centered water cluster, which stabilizes the inactive form of BLT1 (Figure 1). This observation suggests the possible application of the benzamidine moiety as a common structural feature of inverse agonists for various GPCRs, including BLT1.

The most important characteristic of LTB4 is its potent chemo- tactic effect on leukocytes. BLT1–deficient granulocytes and eosinophils do not migrate toward LTB4 (19–23, 37). BLT1 stimulation in leukocytes leads to degranulation through the production of phosphatidylinositol tris-phosphates (IP3) via activation of phosphatidylinositol-3-kinase (PI3 kinase) (38). LTB4 also activates phagocytosis in macrophages through the activation of G16 PI3 kinase, Rac, and S6k (38). Recently, the receptor for advanced glycation end products (RAGE) was identified as a BLT1-binding protein.
Table 1. Characteristics of leukotriene receptors

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<th>Ligand</th>
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<th>CysLT&lt;sub&gt;1&lt;/sub&gt;</th>
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<tr>
<td>12-HHT &gt; LTB&lt;sub&gt;4&lt;/sub&gt; &gt; 12(R)-HETE &gt; 15(S)-HETE</td>
<td>12-HHT &gt; LTB&lt;sub&gt;4&lt;/sub&gt; &gt; 12(S)-HETE &gt; 15(S)-HETE</td>
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| Antagonist | BIL260, LY255283, ZK158252, CP99543, U75302 (weak agonist) | LY255283, ZK158252, CP99543 | Montelukast, zafirlukast, pranlukast, MK-571, pobilukast | Zafirlukast, pranlukast, BAY-u9773, gemilukast |

| Expression (human) | Leukocytes > spleen, smooth muscle, lung, intestine | Intestine, skin > endothelial cells | Leukocytes, spleen, smooth muscle > lung, intestine | Leukocytes, spleen, adrenal medulla, lung, heart, brain |

Table 1. Characteristics of leukotriene receptors

that regulates BLT<sub>1</sub> signaling (39). RAGE functions as a molecular switch for BLT<sub>1</sub>, inhibits BLT<sub>1</sub>-dependent NF-kB activation, and stimulates BLT<sub>2</sub>-dependent chemotaxis. RAGE was also shown to bind to GPCRs other than BLT, and is a new class of GPCR modulator and a new target of GPCR study (40).

BLT<sub>2</sub>. During the analysis of leukocyte-specific transcription of BLT<sub>1</sub> (41), we and others identified a putative open reading frame for a GPCR with similarity to BLT<sub>1</sub> (9, 42–44). As the membrane fraction of cells overexpressing this receptor exhibited a low-affinity LTB<sub>4</sub> binding with K<sub>d</sub> values of 10–20 nM, this receptor was named BLT<sub>2</sub>. BLT<sub>2</sub> shares amino acid identity of 45% with BLT<sub>1</sub>, and high interspecies homology. In contrast to BLT<sub>1</sub>, BLT<sub>2</sub> is a promiscuous receptor that can be activated by 12(S)-HETE, 12(S)-HPETE, and 15(S)-HETE at micromolar concentrations (45). In 2008, we identified BLT<sub>2</sub>-specific agonistic activity in lipid extract of rat small intestine, then partially purified and determined the structure of this BLT<sub>2</sub> agonist as 12(S)-hydroxyheptadecatrienoic acid (12-HHT) (46). Prior to our work, 12-HHT had been known as a nonenzymatic degradation product of prostaglandin endoperoxides or an equimolar byproduct of thromboxane biosynthesis from prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), a process that includes removal of three carbons to produce malondialdehyde (47–49). No biological activity of 12-HHT had been reported.

Platelets produce a large amount of 12-HHT in thromboxane A<sub>2</sub> synthase–dependent and –independent pathways, and aspirin and other NSAIDs inhibit 12-HHT production (50). We found that 12-HHT activates BLT<sub>2</sub> at lower concentrations than LTB<sub>4</sub>, leading to the activation of G<sub>α</sub> and G<sub>r</sub>-type G proteins. In some cancer cells, BLT<sub>2</sub> was shown to activate the generation of reactive oxygen species (51). Most classical BLT<sub>1</sub> antagonists inhibit both BLT<sub>1</sub> and BLT<sub>2</sub>, and a synthetic BLT<sub>2</sub>-specific agonist (CAY10583) is available (52–54). In contrast to BLT<sub>2</sub> expression in leukocytes, BLT<sub>2</sub> is expressed in keratinocytes (53), epithelial cells of intestine (55) and cornea (54), lung alveolar type 2 cells, and vascular endothelial cells (56).

BLT<sub>2</sub> in disease

Even before its molecular identification, BLT<sub>2</sub> had been considered as an important drug target, especially for inflammatory diseases (7, 57). Here we describe representative reports on animal disease models using BLT<sub>1</sub>-knockout (BLT<sub>1</sub>-KO) mice and BLT<sub>2</sub> antagonists.

Chemotaxis of leukocytes. Two groups independently reported attenuated leukocyte chemotaxis in the BLT<sub>1</sub>-KO mouse using peritonitis models (19, 20). The effect of BLT<sub>1</sub> deficiency was more prominent in eosinophils than in granulocytes. The importance of BLT<sub>2</sub> in eosinophils was later confirmed by nematode infection experiments in which BLT<sub>2</sub>-deficient eosinophils did not accumulate and kill nematodes (58). Although classical studies showed the restricted expression of BLT<sub>1</sub> in granulocytes, eosinophils, and macrophages, recent studies showed that BLT<sub>2</sub> expression is observed more widely in a variety of subsets of leukocytes, as follows. BLT<sub>2</sub> is not expressed in naïve T cells but is strongly induced by T cell differentiation: both CD4<sup>+</sup> and CD8<sup>+</sup> effector-type T cells abundantly express BLT<sub>2</sub>, and migrate to inflamed tissues (21–23). Interestingly, LTB<sub>4</sub> was shown to inhibit the differentiation of regulatory T cells and to stimulate naïve T cell differentiation into Th17 cells in vitro (59). Monoclonal antibodies against human (60) and mouse (61) BLT<sub>2</sub> have been established and will help to analyze the detailed expression pattern of BLT<sub>2</sub> in these species.

Allergic airway inflammation. The importance of BLT<sub>2</sub> in allergic airway inflammation was confirmed by murine models of bronchial asthma in which ovalbumin (OVA) was used for sensitization and challenge. Infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> T cells into the airway after OVA challenge was greatly reduced in BLT<sub>2</sub>-KO mice (23). In addition to T cells, eosinophil infiltration was attenuated in BLT<sub>1</sub>-KO mice with reduced production of Th2 cytokines and attenuated airway hyperresponsiveness (AHR) to methacholine (37). Miyahara et al. showed that IL-13–producing CD4<sup>+</sup> and CD8<sup>+</sup> T cells were reduced in allergic BLT<sub>1</sub>-KO lung, and transfer of OVA-sensitized BLT<sub>1</sub><sup>−/−</sup> T cells fully restored the reduced AHR in BLT<sub>1</sub>-KO mice, showing the importance of IL-13–producing T cells in AHR (62). They also showed that effector-type CD8<sup>+</sup> T cells express a higher level of BLT<sub>2</sub>, and LTB<sub>4</sub>-dependent trafficking of CD8<sup>+</sup> effector T cells is important in establishing AHR in mice (63). Dendritic cells also express BLT<sub>2</sub> (24, 25), and adoptive transfer experiments using OVA-loaded dendritic cells showed that BLT<sub>2</sub> expression in antigen-loaded dendritic cells is crucial in inducing asthmatic response (24).

Inflammatory arthritis. The roles of the LT<sub>B</sub>/BLT<sub>1</sub> axis in inflammatory arthritis have been studied using several animal models. In the K/BxN serum transfer arthritis model, in which serum transferred from arthritic transgenic mice produces robust arthritis in WT strains, genetic or pharmacological ablation of BLT<sub>1</sub>, abrogated the development of arthritis, showing the requirement for BLT<sub>1</sub> in recruiting neutrophils into the joints (64). Although BLT<sub>1</sub> was initially shown to be important in the early phase of the disease, it is required for the continuous extravasation of neutrophils throughout the course of an arthritis model (65). Mice deficient in 5-lipoxygenase were also tested using the same model: in that study, 5-lipoxygenase but not LTC<sub>4</sub> synthase
and produced a larger amount of LTB₄, leading to the recruitment of tumor-associated macrophages (75). A 5-LOX inhibitor, zileuton, was shown to inhibit polyp formation in the APC Δ468 mouse colon (76) and the growth of ovarian cancer xenografts (75), possibly by inhibiting local inflammation. Recently, Jala et al. reported that BLT1 deficiency in ApcMin/+ mice resulted in increased size and number of intestinal tumors due to altered gut microbiota and increased chronic inflammation (77). Thus, depending on the context and experimental conditions, the LTB₄/BLT1 axis acts as either a tumor-promoting or a tumor-suppressing factor.

Clinical studies on BLT1-targeted therapy. Several BLT1 antagonists were tested in a few inflammatory diseases. Oral administration of a BLT1 antagonist, LY293111, attenuated LTB₄-dependent activation of Mac-1 in human neutrophils (78) and skin (79), but failed to decrease allergic inflammation induced by histamine and allergen challenges (80). Psoriasis was also a target of BLT1 antagonists, as recently shown in a mouse experiment (81); however, LY293111 was not effective on stable plaques (82), nor on relapse in human psoriasis (83). LY293111 was also tested in cystic fibrosis (84) and chondrosarcoma and melanoma (85, 86) without significant effectiveness.

BLT₂ in health and disease

Identification of BLT₂ was reported in 2000 (9), and the first report on BLT₂-KO mice appeared in 2010 (55). In some cases, it is difficult to distinguish the roles of BLT₁ and BLT₂, because both receptors are activated by LTB₄, transduce similar intracellular signaling, and are antagonized by most BLT antagonists; however, different tissue distribution gives a clue to distinguish biological
roles of these two receptors. Currently, BLT agonists are attracting interest as new drugs for skin, corneal, and intestinal ulcers.

Small intestine. The first phenotype of BLT-deficient mice was susceptibility to drug-induced inflammatory colitis. Because of the lack of BLT-specific antibody at that time, in situ hybridization was used to show BLT expression in epithelial and cryptic cells of mouse intestine. BLT-KO mice exhibited bloody stool and severe body weight loss following administration of 1% dextran sodium sulfate in the drinking water under conditions in which WT mice did not show any clinical manifestations. Histological examination showed severe intestinal inflammation in the BLT-KO intestine that may be linked to observations of increased STAT3 phosphorylation (55). In vitro study using BLT-overexpressing Madin-Darby canine kidney II (MDCKII) cells showed that BLT expression increased transepithelial electrical resistance and decreased FITC-dextran leakage through MDCKII monolayers, suggesting the barrier-enhancing activity of BLT (54). BLT-KO mice also showed enhanced transepidermal water loss and antigen uptake, suggesting the barrier-enhancing activity of BLT (55). The first phenotype of BLT-deficient mice was observed in mouse lung, and BLT-KO mice were evaluated in the pneumolysin-dependent (PLY-dependent) acute lung injury model. BLT-KO mice and NSAID-treated mice were sensitive to intratracheal infusion of PLY and died immediately as a result of the increased vascular permeability and subsequent pulmonary edema. PLY treatment induced the production of CysLTs in the lung, and the CysLT antagonist montelukast prevented the death of BLT-KO and NSAID-treated mice, suggesting the possible drug repositioning of CysLT antagonists for acute lung injury (56). BLT-KO mice showed a severe eosinophilic lung inflammation in an OVA-induced allergic airway disease model. This was explained by the enhanced production of IL-13 from BLT-deficient CD4+ T cells (89).

Characterization of CysLT receptors
So far, five CysLT receptors have been identified: CysLT, CysLT, P2Y, GPR99, and GPR17 (Figure 2 and Table 1). CysLT is widely expressed in spleen, leukocytes, lung, small intestine, colon, and skeletal muscle (90–92). CysLT exhibits 37.3% amino acid identity with CysLT (93), and is exclusively expressed in heart, adrenals, leukocytes, spleen, lymph nodes, and brain (93–96). CysLT is preferentially activated by LTD whereas CysLT binds both LTC and LTD with equal affinity. Recently, P2Y, GPR99, and GPR17 were reported as receptors for LTE (97–99). Moreover, GPR17 has been proposed as a putative negative regulator of CysLT. This section serves to give information on these receptors.

CysLT. Consistent with the clinical effectiveness of CysLT antagonists in asthma, CysLT is expressed in a variety of inflammatory cells, i.e., neutrophils, mast cells, and monocytes/macrophages (100, 101). Human CysLT-expressing cells respond selectively to CysLTs with rank order of potency LTD > LTC > LTE (90, 91). Antagonist affinities are similar to those investigated in other functional assays for CysLT, as well as in binding experiments (90, 102). Activation of CysLT by LTD results in the production of several second intracellular messengers through phospholipase Cβ (103, 104). Several reports demonstrated that CysLTs elicit Ca responses via a pertussis toxin-sensitive
(PTX-sensitive) G protein (Gq) in peripheral blood mononuclear cells (105, 106), or through two distinct G proteins, PTX-sensitive and -insensitive (Gq/11), in monocyte/macrophage U937 cells (Figure 2 and ref. 107) as well as a human epithelial cell line, suggesting the promiscuity of CysLT, in G protein coupling (108).

CysLT1. Comparison of human CysLT1 and CysLT2 revealed negligible existence of CysLT1, but high expression of CysLT2 in the heart and eosinophils (109). In contrast, both receptors are highly expressed in spleen (93–95). In situ hybridization analyses of human lung demonstrated that CysLT1, is expressed in interstitial macrophages and smooth muscle cells (93). Moreover, the presence of human CysLT1 mRNA was determined in atrium, ventricle, and intermediate coronary arteries by in situ hybridization (110). The potency ranking for the competition with tritiated [3H]LTD4 binding to human CysLT1 is LTD4 = LTC4 >> LTE4 (93). The CysLT1 antagonists are either weak (zafirlukast and pranlukast) or inactive binding to human CysLT2 (Figure 2 and refs. 93, 111, 112), whereas full competition was observed with the dual CysLT1/CysLT2 antagonist BAY-u9773 (112). LTC4 and LTD4 evoke a dose-dependent activation of Ca2+ flux through a PTX-insensitive G protein (Gq/11) in human CysLT2-expressing oocytes (Figure 2 and ref. 93). On the other hand, in human mast cells, the CysLT1 stimulation elicits IL-8 secretion, and the effect is completely blocked by PTX, suggesting it occurs via Gq protein (113). Recent reports demonstrated that CysLT1 negatively regulates the development of Th2 pulmonary inflammation by inhibiting the CysLT1 functions on dendritic cells (114). Furthermore, LTC4 but not LTD4 and LTE4 activates mouse platelets via CysLT2, although these cells express both CysLT1 and CysLT2, (115).

Other receptors related to CysLTs. Some of LTE4-mediated responses are resistant in mice deficient in CysLT1, and CysLT2, implying the existence of LTE4 receptors. Based on the similarity among CysLT1, CysLT2, and the nucleotide P2Y2 receptors, LTE4 receptors seemed to be P2Y-like GPCRs.

Human mast cells express P2Y12, an adenosine diphosphate receptor, and knockdown of this receptor impaired the LTE4-elicted production of MIP-1β and PGD2, in LAD2 human mast cells without altering their responses to LTD4 (116). LTE4 induces the activation of ERK1/2 in CHO cells expressing P2Y12, which is sensitive to PTX (Figure 2 and ref. 97). Furthermore, administration of LTE4 to the airways of sensitized BALB/c mice induces eosinophilia, goblet cell metaplasia, and IL-13 production in response to low-dose aerosolized OVA. These effects are intact in CysLT1/CysLT2-null mice but are completely blocked by administration of clopidogrel, a P2Y12-selective antagonist. A recent study showed that clopidogrel prevents airway hyperresponsiveness and eosinophilic inflammation in a mouse model of asthma (117), suggesting a possible link between platelet activation and inflammatory responses.

GPR99, which belongs to the P2Y receptor subfamily, was initially identified as a receptor for α-ketoglutarate (118). Because the α-ketoglutarate–dependent inositol phosphate formation in GPR99-expressing cells is insensitive to PTX, GPR99 seems to act via a Gq/11 pathway (Figure 2 and ref. 118). Kanaoka et al. reported that GPR99 is a high-affinity receptor for LTE4 (99). The binding study revealed a specificity of GPR99 to [3H]LTE4 with a KD value of 2.5 nM. GPR99 is highly expressed in kidney, placenta, trachea, salivary glands, lung, and smooth muscle (118–120), and GPR99 deficiency eliminated vascular leaks in response to CysLTs in the CysLT1/CysLT2-KO mice (99). GPR99, which is also expressed in respiratory epithelial cells, mediates mucin release and submucosal swelling in response to LTE4 induced by Alternaria fungi (121). GPR99-KO mice are protected from epithelial cell mucin release and swelling by Alternaria or intranasal administration of LTE4. Moreover, GPR99 regulates a baseline number of mucin-containing goblet cells. Because LTE4 elicits airflow obstruction and lung inflammation in asthmatics, inhibition of LTE4/GPR99 signaling may have therapeutic benefit in asthma.

GPR17, which also belongs to the P2Y receptor family, responds to two unrelated ligands: uracil nucleotides and CysLTs (122). Activation of GPR17 leads to intracellular Ca2+ increase and inhibition of cAMP synthesis, suggesting a coupling with Gαi/proteins (Figure 2 and refs. 98, 122). Recent studies demonstrate that the administration of montelukast, a CysLT1 antagonist, leads to reduced neuroinflammation, elevation of hippocampal neurogenesis, and improved learning and memory in old rats (123, 124). These effects are abolished by GPR17 deficiency, suggesting the involvement of this receptor in the rejuvenation of the aged brain. Maekawa et al. demonstrated that GPR17 suppresses CysLT1-mediated signaling on the cell surface through heterodimerization, proposing GPR17 as a negative regulator for CysLT1 (125). In vivo, they demonstrated that in IgE-dependent passive cutaneous anaphylaxis, vascular permeability is increased in GPR17-KO mice and that this response is blocked by administration of a CysLT1 antagonist (125). Furthermore, they recently reported the negative regulation of CysLT1 by GPR17 in both the antigen-presentation and downstream phases of allergic pulmonary inflammation, suggesting physiological evidence for its negative regulatory role (126). Further studies are necessary on the mechanism and biological output of negative regulations.

CysLTs and cognate receptors in health and diseases

CysLTs are inflammatory lipid mediators implicated in multiple diseases, including asthma, allergic rhinitis, cardiovascular disease, atopic dermatitis, and experimental autoimmune encephalitis (a model of multiple sclerosis). The identification of CysLT receptors, generation of CysLT receptor–deficient mice, and development of specific antagonists have expanded the scope of functions of these mediators in disease. In particular, signaling via these receptors is implicated in many components of these diseases, such as bronchoconstriction, increased microvascular permeability, recruitment of effector cells, mucus and cytokine secretion, and fibrosis (127–133). In this section, we discuss the functional relevance of CysLT receptors to various diseases as determined by animal experiments.

Bronchoconstriction. LTC4 and LTD4 are equipotent in guinea pig tracheal smooth muscle, while LTD4 is more effective in peripheral airways (134). For example, the potency of LTD4 in the guinea pig lung parenchymal tissues is significantly different from that observed in the tracheal preparations (135), implying the existence of distinct CysLT receptors. LTE4 elicits smooth muscle constriction in isolated guinea pig trachea in preference to LTC4 and LTD4 which required an intact epithelium (136). Moreover, patients with bronchial asthma show an increased sensitivity to LTE4 leading to airflow obstruction (137–139). Similarly, LTE4 elicits eosinophil...
influx in asthmatic subjects (140). Recently, Yonetomi et al. established a novel guinea pig model of asthma induced by treatment with S-hexyl glutathione (S-hexyl GSH), an inhibitor of γ-glutamyl transpeptidase (141, 142). Using this model, they demonstrated that both CysLT1 and CysLT2 promote LTC4, or antigen-induced bronchoconstriction. In humans, both CysLT1 and CysLT2 are expressed in lung specimens isolated from asthmatic patients, suggesting the involvement of these receptors in antigen-induced bronchoconstriction (143). Previous study showed that montelukast, a CysLT1 antagonist, effectively ameliorates regional air trapping due to small airway obstruction in asthma, although contribution of CysLT2 in this disease has not been fully clarified (144). Intriguingly, Sekioka et al. recently suggested that inhalation of LTC4 causes CysLT2-mediated bronchoconstriction and lung air trapping in an S-hexyl GSH–treated guinea pig model (145).

**Reproof of effector leukocytes.** In humans, peripheral blood cells, e.g., monocytes (93, 100), eosinophils (146), and lung macrophages (90, 93), all express both CysLT1 and CysLT2. The expressions of these receptors are further confirmed in eosinophils, mononuclear cells, and resident mast cells in nasal biopsy tissue from humans with seasonal allergic rhinitis (100). Moreover, inhaled LTC4, LTD4, or LTE4 increases airway eosinophil numbers in bronchoalveolar lavage fluid (BALF) prepared from humans and guinea pigs (129, 147–149). Together, these results indicate that CysLTs may serve as chemotactic ligands and activating mediators for human effector leukocytes. In the chronic asthma model, treatment with montelukast significantly reduced eosinophil infiltration, mucus plugging, and smooth muscle hyperplasia, demonstrating that CysLTs, particularly the CysLT2 pathway, initiate features of chronic inflammation (150). Furthermore, several CysLT1 antagonists decreased LTD4-induced chemotaxis of peripheral blood eosinophils from humans, rats, guinea pigs, and cynomolgus monkeys (148, 151–153). In contrast, in the OVA-induced asthma model, the level of LTC4 in the BALF of challenged mice increased compared with those of the saline controls (154). These increases are correlated with an influx of predominantly eosinophils in airway tissues and BALF, suggesting the contribution of CysLT1 in OVA-induced airway inflammation. A recent study further demonstrated that intranasal administration of LTC4 to OVA-sensitized mice induces airway eosinophilia via a platelet- and CysLT2-dependent pathway (155).

**Microvascular permeability.** CysLTs increase microvascular permeability in hamster cheek pouches (127) and guinea pig airways by promoting the contraction of endothelial cells, leading to gaps in the endothelium of venules (156–159). The latter effect is inhibited by pranlukast (156, 158), indicating the involvement of CysLT2. Zymosan A–induced plasma protein leakage and IgE–dependent passive cutaneous anaphylaxis are reduced in both CysLT2–KO mice and LTC4 synthase–KO mice (160). These results indicate the pivotal role of CysLT2 in mediating increased vascular permeability in the models of both innate and adaptive immunity. However, several controversial data have been reported. For example, neutrophil recruitment is not impaired in either LTC4 synthase–KO or CysLT2–KO mice in the zymosan A–induced peritonitis model (160, 161). Moreover, the enhanced vascular permeability associated with the IgE–dependent passive cutaneous anaphylaxis is decreased in CysLT2–KO mice, although the zymosan A–induced peritoneal inflammation is not altered (162). CysLT2–mediated vascular permeability via transendothelial vesicle transport was further investigated in a CysLT2–KO LacZ model mouse (163). In this model, CysLT2 mediated inflammatory reactions in a vascular bed–specific manner by altering transendothelial vesicle transport–based vascular permeability. Further reports corroborate CysLT2/CysLT2–induced permeability of human vascular endothelial cells (164).

**Pulmonary fibrosis.** Bleomycin (165), an anticancer agent, causes chronic pulmonary inflammation and fibrosis by intratracheal or systemic administration in mice. The induced injuries, e.g., pulmonary macrophage and neutrophil recruitment, fibroblast accumulation, and collagen deposition, are significantly reduced in LTC4 synthase–KO mice (166). Although these injuries are not prevented by CysLT1 deficiency in mice, CysLT2–KO mice do show exaggerated alveolar septal thickening with reticular fiber deposition when compared with WT or LTC4 synthase–KO mice (166). Additionally, CysLT levels in the BALF recovered from CysLT2–KO mice are greater than those of WT mice. These findings suggest that the CysLTs are crucial for bleomycin–induced chronic inflammatory and fibrotic insult, presumably working via other types of receptors, including CysLT2. Intriguingly, alveolar septal thickening after intratracheal injection of bleomycin is significantly reduced in CysLT2–KO mice (166). Because the amount of CysLTs in BALF is similar in CysLT2–KO mice and WT mice, CysLT2 promotes chronic pulmonary inflammation with fibrosis in response to a particular pathological stimulus.

**Cardiovascular effects.** After reports of the reduced coronary blood flow induced by slow-reacting substance of anaphylaxis (SRS-A) (167), several groups have investigated the cardiovascular effects of CysLTs in several animal models. In sheep and pigs, CysLTs cause induction of coronary vasoconstriction and ischemia and impairment of left ventricular function (168, 169). Moreover, in isolated perfused guinea pig heart preparations, LTC4 and LTD4 reduce myocardial contractility concomitant with the vasoconstriction (170, 171). In human heart, the negative inotropic effect of CysLTs is similar to that in guinea pigs, with rank order of potency LTD4 > LTC4 > LTE4 (172).

Although CysLT2 is predominantly expressed in vascular smooth muscle cells, the expression of CysLT1 is also induced by stimulation with lipopolysaccharide in human coronary artery vascular smooth muscle cells (173). Intriguingly, in these cells, CysLT1 is localized in the perinuclear region of human aortic valve myofibroblasts, and its activation is coupled to a predominantly nuclear Ca2+ signaling (173, 174). Furthermore, a recent report suggested that CysLTs elicit inflammation and proliferation of endothelial cells through CysLT1 and CysLT2, respectively (175). In this study, the authors further demonstrated that CysLT2 activation leads to endothelial cell contraction and barrier disruption via the Rho kinase pathway, suggesting the critical roles of CysLT receptors in the pathology of cardiovascular diseases such as atherosclerosis.

**Clinical studies on CysLT receptor–targeted therapy**

CysLT receptors play an important role in the pathogenesis of bronchial asthma and allergic rhinitis. The effects of blockers of these receptors indicate that interventions in the signaling path-
way via CysLT receptors may be of therapeutic use in the treatment of these diseases. Blockers of CysLT, including montelukast (marketed as Singulair and Kipres), pranlukast (Onon), and zafirlukast (Accolate), are used in asthma and rhinitis. Montelukast, which is administered orally once daily, is the most prescribed antagonist for asthmatic patients (176). This drug is effective in allergic rhinitis and several types of asthma, e.g., exercise-induced asthma, asthma in obese patients, asthma in smokers, and aspirin-induced asthma (176, 177). The beneficial effect of montelukast in cardiovascular disease has been under clinical trial (178-180). Pranlukast is an orally administered, selective, and competitive antagonist. Clinical studies demonstrated that prophylactic treatment with this drug is effective for chronic bronchial asthma in pediatric and adult patients. Moreover, recent studies suggested that pretreatment with this drug significantly inhibits pollinosis (181) and decreases nasal eosinophil cationic protein and obstruction (182). Zafirlukast is approved for treatment of asthma in patients 7 years or older. The most common adverse effects are pharyngitis, headache, rhinitis, and gastritis. Transient increases in liver enzymes and rare but significant liver dysfunction have prompted recommendations against prescribing this drug to patients with hepatic dysfunction (183).

Conclusion
CysLT antagonists and inhibitors of LT biosynthesis are clinically useful to ameliorate the symptoms of bronchial asthma and allerg ic rhinitis. Although the past clinical studies on BLT antagonists failed to attenuate arthritis and psoriasis, studies using BLT-KO mice and BLT antagonists are expanding the BLT-related inflammatory diseases, suggesting the usefulness of BLT antagonists in the future. BLT agonists will target intractable skin and corneal ulcers, including bedsores. Thus, leukotriene receptors are still important drug targets.

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