

# Leukotriene receptors as potential therapeutic 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 cysteinyl leukotriene receptor 1 (CysLT<sub>1</sub>) 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 LTC<sub>4</sub> synthase and LTA<sub>4</sub> hydrolase provides new structural bases for the development of LT synthesis inhibitors (2–6). As described here, the 3D structure of BLT<sub>1</sub> 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 B<sub>4</sub> (LTB<sub>4</sub>) (Table 1 and refs. 8, 9). The first, BLT<sub>1</sub>, known as a high-affinity LTB<sub>4</sub> receptor, is expressed in various subsets of leukocytes and is responsible for LTB<sub>4</sub>-dependent leukocyte migration. The second, BLT<sub>2</sub>, was originally reported as a low-affinity LTB<sub>4</sub> receptor and is now considered as a receptor for various oxidized fatty acids, including 12-hydroxyheptadecatrienoic acid (12-HHT) and hydroxyeicosatetraenoic acids (HETEs). BLT<sub>2</sub> 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 LTB<sub>4</sub> (10–18).

BLT<sub>1</sub>. Human BLT<sub>1</sub> consists of 352 amino acids and is mainly expressed in various subsets of leukocytes, including granulocytes (8), eosinophils (19, 20), and effector-type CD4<sup>+</sup> and CD8<sup>+</sup>

T cells (21–23), as well as certain subsets of dendritic cells (24, 25) and macrophages (26). BLT<sub>1</sub> 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 LTB<sub>4</sub>-specific receptor with a K<sub>D</sub> value of 0.15 nM for LTB<sub>4</sub> when expressed in Cos-7 cells (8). In BLT<sub>1</sub>-transfected CHO cells, BLT<sub>1</sub> is able to couple with both G<sub>i</sub>-like and G<sub>q</sub>-like (G16) G proteins, and an extensive mutagenesis study showed that intracellular loop 3 is important for the G<sub>i</sub> coupling of BLT<sub>1</sub> (29). Human BLT<sub>1</sub> has two N-glycosylation sites (N2 and N164), and mutagenesis of these asparagine residues does not affect localization, ligand binding, or intracellular signaling of BLT<sub>1</sub>. BLT<sub>1</sub> 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 BLT<sub>1</sub> is important in the conformational change to the low-affinity state after G protein activation (30, 31) and internalization (32–34) of BLT<sub>1</sub>.

Recently, the crystal structure analysis of BLT<sub>1</sub> with the antagonist BIIL260 was achieved (Figure 1, A and B, and ref. 35). Docking study with LTB<sub>4</sub> and BLT<sub>1</sub> indicates that LTB<sub>4</sub> would interact with the residues H96, R158, E187, and S243 that were predicted to be involved with LTB<sub>4</sub> 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 BLT<sub>1</sub> (Figure 1). This observation suggests the possible application of the benzamidine moiety as a common structural feature of inverse agonists for various GPCRs, including BLT<sub>1</sub>.

The most important characteristic of LTB<sub>4</sub> is its potent chemotactic effect on leukocytes. BLT<sub>1</sub>-deficient granulocytes and eosinophils do not migrate toward LTB<sub>4</sub> (19–23, 37). BLT<sub>1</sub> stimulation in leukocytes leads to degranulation through the production of phosphatidylinositol tris-phosphates (IP<sub>3</sub>) via activation of phosphatidylinositol-3-kinase (PI3 kinase) (38). LTB<sub>4</sub> also activates phagocytosis in macrophages through the activation of G<sub>i</sub>, PI3 kinase, Rac, and Syk (38). Recently, the receptor for advanced glycation end products (RAGE) was identified as a BLT<sub>1</sub>-binding protein

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**Table 1. Characteristics of leukotriene receptors**

	BLT <sub>1</sub>	BLT <sub>2</sub>	CysLT <sub>1</sub>	CysLT <sub>2</sub>
Ligand	LTB <sub>4</sub> > 20-OH-LTB <sub>4</sub>	12-HHT > LTB <sub>4</sub> > 12(S)-HETE > 12(R)-HETE > 15(S)-HETE	LTD <sub>4</sub> > LTC <sub>4</sub> >> LTE <sub>4</sub>	LTD <sub>4</sub> = LTC <sub>4</sub> >> LTE <sub>4</sub>
Antagonist	BIIL260, LY255283, ZK158252, CP195543, U75302 (weak agonist)	LY255283, ZK158252, CP195543	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

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-κB activation, and stimulates BLT<sub>1</sub>-dependent chemotaxis. RAGE was also shown to bind to GPCRs other than BLT<sub>1</sub> 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  $K_D$  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>i</sub>- and G<sub>q</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 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>1</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>1</sub> in disease

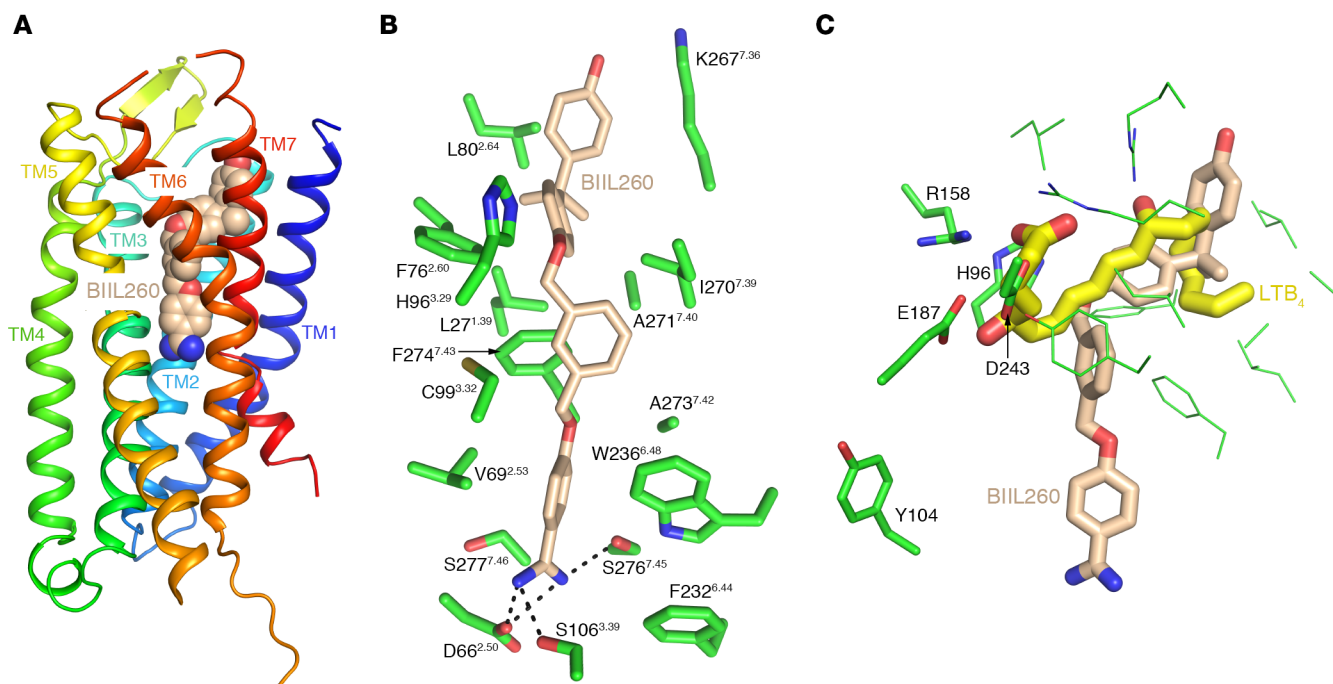
Even before its molecular identification, BLT<sub>1</sub> had been considered as an important drug target, especially for inflammatory diseases (7, 57). Here we describe representative reports on animal disease models using BLT<sub>1</sub>-knockout (BLT<sub>1</sub>-KO) mice and BLT<sub>1</sub> antagonists.

**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>1</sub> in eosinophils was later confirmed by nematode infection experiments in which BLT<sub>1</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>1</sub> expression is observed more widely in a variety of subsets of leukocytes, as follows. BLT<sub>1</sub> is not expressed in naive 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>1</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 naive T cell differentiation into Th17 cells in vitro (59). Monoclonal antibodies against human (60) and mouse (61) BLT<sub>1</sub> have been established and will help to analyze the detailed expression pattern of BLT<sub>1</sub> in these species.

**Allergic airway inflammation.** The importance of BLT<sub>1</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>1</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>1</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>1</sub> (24, 25), and adoptive transfer experiments using OVA-loaded dendritic cells showed that BLT<sub>1</sub> expression in antigen-loaded dendritic cells is crucial in inducing asthmatic response (24).

**Inflammatory arthritis.** The roles of the LTB<sub>4</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



**Figure 1. Structure of guinea pig BLT<sub>1</sub> bound with a BLT antagonist, BIIL260.** (A) Overall structure of BLT<sub>1</sub> and BIIL260 complex (35). BLT<sub>1</sub> is presented as a rainbow-colored cartoon model, and BIIL260 is presented as pink (carbon), blue (nitrogen), and red (oxygen) spheres. (B) Mode of BIIL260 binding by BLT<sub>1</sub>. BLT<sub>1</sub> side chains within 4 Å from BIIL260 are presented by stick models. Carbon and sulfur atoms of BLT<sub>1</sub> are colored green and gold, respectively. The salt bridge and hydrogen bond interactions are indicated by black dashed lines. (C) Docking of LTB<sub>4</sub> in the orthosteric binding site of BLT<sub>1</sub>. The docking study was performed with the program AutoDock 4 (184). Carbon atoms of LTB<sub>4</sub> are colored yellow. The residues involved in the LTB<sub>4</sub> binding shown by the mutation study (36) are presented by stick models.

proved requisite for the development of arthritis (66). BLT<sub>1</sub>-KO mice were also tested in a collagen-induced arthritis (CIA) model. BLT<sub>1</sub>-KO and BLT<sub>1</sub>/BLT<sub>2</sub> double-KO mice were completely protected from CIA, despite normal accumulation of serum anti-collagen antibody (67). BLT<sub>1</sub> antagonists (66, 68) were also effective for the attenuation of arthritis.

**Atherosclerosis.** The relationship between LTB<sub>4</sub> and atherosclerosis has been documented in several reports. LTB<sub>4</sub> triggered adhesion of human monocytes to endothelial cells in a β<sub>1</sub> and β<sub>2</sub> integrin-dependent fashion (69), and strongly increased monocyte chemoattractant protein-1 (MCP-1) (70). In LDLr<sup>-/-</sup> and apoE<sup>-/-</sup> mice, the BLT antagonist CP-105,696 reduced atherosclerosis without affecting the plasma lipid concentrations (71). Atherogenesis was attenuated in BLT<sub>1</sub>-KO mice crossed with apoE<sup>-/-</sup> mice, which was explained by the lack of LTB<sub>4</sub>-dependent expression of CD36 (a fatty acid translocase, B-type scavenger receptor) and CCL2 chemokine (72), and by the reduced recruitment of smooth muscle cells to the atherosclerotic lesions (27).

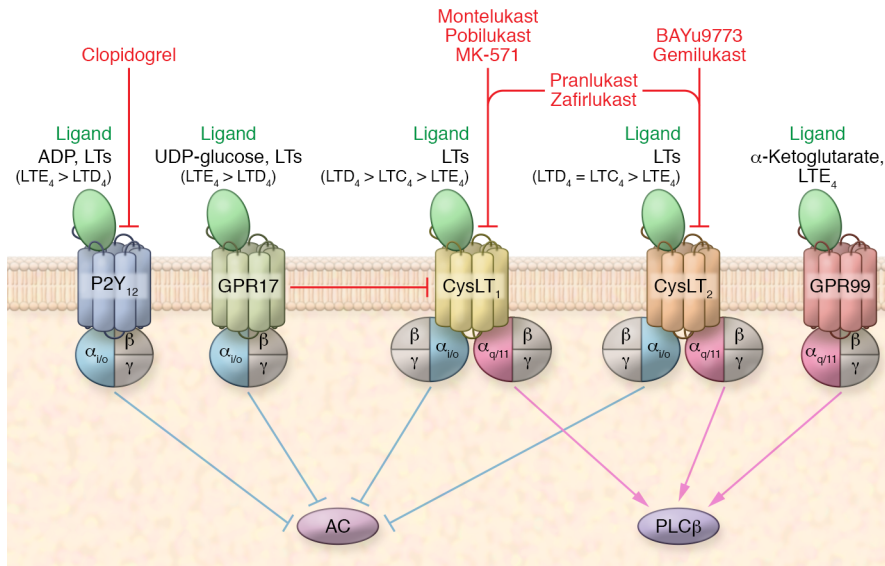
**Cancer.** Although the involvement of eicosanoids, especially PGE<sub>2</sub>, in carcinogenesis has been extensively studied (73), the contribution of LTB<sub>4</sub> to cancer has not been well demonstrated. Recently, inflammation has been considered as an important factor in the initiation and progression of certain cancers, and some research has focused on the roles of LTB<sub>4</sub> and BLT<sub>1</sub> in cancer (17). Infection of endothelial cells with Kaposi's sarcoma-associated herpesvirus induced higher expression of 5-LOX, FLAP, and LTA4H, and increased LTB<sub>4</sub> production (74). Hypoxic ovarian cancer cells express higher levels of LTB<sub>4</sub>-biosynthetic enzymes,

and produced a larger amount of LTB<sub>4</sub>, leading to the recruitment of tumor-associated macrophages (75). A 5-LOX inhibitor, zileuton, was shown to inhibit polyp formation in the APC<sup>d468</sup> mouse colon (76) and the growth of ovarian cancer xenografts (75), possibly by inhibiting local inflammation. Recently, Jala et al. reported that BLT<sub>1</sub> deficiency in ApC<sup>Min/+</sup> 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 LTB<sub>4</sub>/BLT<sub>1</sub> axis acts as either a tumor-promoting or a tumor-suppressing factor.

**Clinical studies on BLT<sub>1</sub>-targeted therapy.** Several BLT<sub>1</sub> antagonists were tested in a few inflammatory diseases. Oral administration of a BLT<sub>1</sub> antagonist, LY293111, attenuated LTB<sub>4</sub>-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 BLT<sub>1</sub> 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.

## BLT<sub>2</sub> in health and disease

Identification of BLT<sub>2</sub> was reported in 2000 (9), and the first report on BLT<sub>2</sub>-KO mice appeared in 2010 (55). In some cases, it is difficult to distinguish the roles of BLT<sub>1</sub> and BLT<sub>2</sub>, because both receptors are activated by LTB<sub>4</sub>, transduce similar intracellular signaling, and are antagonized by most BLT antagonists; however, different tissue distribution gives a clue to distinguish biological



**Figure 2. Signaling via CysLT<sub>1</sub>, CysLT<sub>2</sub>, GPR17, GPR99, and P2Y<sub>12</sub>.** Synthetic antagonists for each receptor are given in red text. Ligands and relative affinities are described adjacent to their respective receptor. GPR17 is a negative regulator for CysLT<sub>1</sub>. Shown below each receptor is its downstream coupling to G<sub>i/o</sub> and adenylyl cyclase (AC) and/or G<sub>q/11</sub> and phospholipase Cβ (PLCβ).

roles of these two receptors. Currently, BLT<sub>2</sub> agonists are attracting interest as new drugs for skin, corneal, and intestinal ulcers.

**Small intestine.** The first phenotype of BLT<sub>2</sub>-deficient mice was susceptibility to drug-induced inflammatory colitis. Because of the lack of BLT<sub>2</sub>-specific antibody at that time, *in situ* hybridization was used to show BLT<sub>2</sub> expression in epithelial and cryptic cells of mouse intestine. BLT<sub>2</sub>-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<sub>2</sub>-KO intestine that may be linked to observations of increased STAT3 phosphorylation (55). *In vitro* study using BLT<sub>2</sub>-overexpressing Madin-Darby canine kidney II (MDCKII) cells showed that BLT<sub>2</sub> expression increased transepithelial electrical resistance and decreased FITC-dextran leakage through MDCKII monolayers, suggesting the barrier-enhancing activity of BLT<sub>2</sub> (55). The intracellular mechanism of BLT<sub>2</sub>-dependent barrier function will be described in the next section.

**Skin and cornea.** In skin, BLT<sub>2</sub> expression is restricted to dermal keratinocytes. Wound-healing assays after skin punching were performed using BLT<sub>2</sub>-KO mice. Mass spectrometric analysis showed the accumulation of 12-HHT in the wound exudates, and pretreating the mice with high-dose aspirin completely abolished the accumulation of 12-HHT. Both BLT<sub>2</sub> deficiency and aspirin treatment delayed skin wound healing with the attenuated re-epithelialization, and the aspirin effect was not seen in BLT<sub>2</sub>-KO mice. Thus, BLT<sub>2</sub> stimulates keratinocyte migration after skin injury, and BLT<sub>2</sub> agonists might be a therapeutic tool for intractable skin ulcers (53, 87). It is of note that aspirin-dependent delays in wound healing occur as a consequence of decreased production of 12-HHT but not of prostaglandins. Similarly, BLT<sub>2</sub> is expressed in corneal epithelial cells and stimulates corneal wound healing (54). BLT<sub>2</sub>-KO mice also showed enhanced transepidermal water loss and antigen uptake, suggestive of attenuated skin barrier function in BLT<sub>2</sub>-KO mice. BLT<sub>2</sub>-dependent barrier function involves the enhanced expression of the tight junctional protein claudin-4 downstream of BLT<sub>2</sub> (88).

**Lung.** Small but significant BLT<sub>2</sub> expression was observed in mouse lung, and BLT<sub>2</sub>-KO mice were evaluated in the pneumolysin-dependent (PLY-dependent) acute lung injury model. BLT<sub>2</sub>-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<sub>1</sub> antagonist montelukast prevented the death of BLT<sub>2</sub>-KO and NSAID-treated mice, suggesting the possible drug repositioning of CysLT<sub>1</sub> antagonists for acute lung injury (56). BLT<sub>2</sub>-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<sub>2</sub>-deficient CD4<sup>+</sup> T cells (89).

## Characterization of CysLT receptors

So far, five CysLT receptors have been identified: CysLT<sub>1</sub>, CysLT<sub>2</sub>, P2Y<sub>12</sub>, GPR99, and GPR17 (Figure 2 and Table 1). CysLT<sub>1</sub> is widely expressed in spleen, leukocytes, lung, small intestine, colon, and skeletal muscle (90–92). CysLT<sub>2</sub> exhibits 37.3% amino acid identity with CysLT<sub>1</sub> (93), and is exclusively expressed in heart, adrenals, leukocytes, spleen, lymph nodes, and brain (93–96). CysLT<sub>1</sub> is preferentially activated by LTD<sub>4</sub>, whereas CysLT<sub>2</sub> binds both LTC<sub>4</sub> and LTD<sub>4</sub> with equal affinity. Recently, P2Y<sub>12</sub>, GPR99, and GPR17 were reported as receptors for LTE<sub>4</sub> (97–99). Moreover, GPR17 has been proposed as a putative negative regulator of CysLT<sub>1</sub>. This section serves to give information on these receptors.

**CysLT<sub>1</sub>.** Consistent with the clinical effectiveness of CysLT antagonists in asthma, CysLT<sub>1</sub> is expressed in a variety of inflammatory cells, *i.e.*, neutrophils, mast cells, and monocytes/macrophages (100, 101). Human CysLT<sub>1</sub>-expressing cells respond selectively to CysLTs with rank order of potency LTD<sub>4</sub> > LTC<sub>4</sub> > LTE<sub>4</sub> (90, 91). Antagonist affinities are similar to those investigated in other functional assays for CysLT<sub>1</sub> as well as in binding experiments (90, 102). Activation of CysLT<sub>1</sub> by LTD<sub>4</sub> results in the production of several second intracellular messengers through phospholipase Cβ (103, 104). Several reports demonstrated that CysLTs elicit Ca<sup>2+</sup> responses via a pertussis toxin-sensitive

(PTX-sensitive) G protein ( $G_{i/o}$ ) in peripheral blood mononuclear cells (105, 106), or through two distinct G proteins, PTX-sensitive and -insensitive ( $G_{q/11}$ ), in monocyte/macrophage U937 cells (Figure 2 and ref. 107) as well as a human epithelial cell line, suggesting the promiscuity of CysLT<sub>1</sub> in G protein coupling (108).

**CysLT<sub>2</sub>.** Comparison of human CysLT<sub>1</sub> and CysLT<sub>2</sub> revealed negligible existence of CysLT<sub>1</sub> but high expression of CysLT<sub>2</sub> 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 CysLT<sub>2</sub> is expressed in interstitial macrophages and smooth muscle cells (93). Moreover, the presence of human CysLT<sub>2</sub> mRNA was determined in atrium, ventricle, and intermediate coronary arteries by in situ hybridization (110). The potency ranking for the competition with tritiated [<sup>3</sup>H]LTD<sub>4</sub> binding to human CysLT<sub>2</sub> is LTD<sub>4</sub> = LTC<sub>4</sub> >> LTE<sub>4</sub> (93). The CysLT<sub>1</sub> antagonists are either weak (zafirlukast and pranlukast) or inactive (montelukast and pobelukast) at competing for [<sup>3</sup>H]LTD<sub>4</sub> binding to human CysLT<sub>2</sub> (Figure 2 and refs. 93, 111, 112), whereas full competition was observed with the dual CysLT<sub>1</sub>/CysLT<sub>2</sub> antagonist BAY-u9773 (112). LTC<sub>4</sub> and LTD<sub>4</sub> evoke a dose-dependent activation of Ca<sup>2+</sup> flux through a PTX-insensitive G protein ( $G_{q/11}$ ) in human CysLT<sub>2</sub>-expressing oocytes (Figure 2 and ref. 93). On the other hand, in human mast cells, the CysLT<sub>2</sub> stimulation elicits IL-8 secretion, and the effect is completely blocked by PTX, suggesting it occurs via  $G_{i/o}$  protein (113). Recent reports demonstrated that CysLT<sub>2</sub> negatively regulates the development of Th2 pulmonary inflammation by inhibiting the CysLT<sub>1</sub> functions on dendritic cells (114). Furthermore, LTC<sub>4</sub>, but not LTD<sub>4</sub> and LTE<sub>4</sub>, activates mouse platelets via CysLT<sub>2</sub>, although these cells express both CysLT<sub>1</sub> and CysLT<sub>2</sub> (115).

**Other receptors related to CysLTs.** Some of LTE<sub>4</sub>-mediated responses are resistant in mice deficient in CysLT<sub>1</sub> and CysLT<sub>2</sub>, implying the existence of LTE<sub>4</sub> receptors. Based on the similarity among CysLT<sub>1</sub>, CysLT<sub>2</sub>, and the nucleotide P2Y receptors, LTE<sub>4</sub> receptors seemed to be P2Y-like GPCRs.

Human mast cells express P2Y<sub>12</sub>, an adenosine diphosphate receptor, and knockdown of this receptor impaired the LTE<sub>4</sub>-elicited production of MIP-1 $\beta$  and PGD<sub>2</sub> in LAD2 human mast cells without altering their responses to LTD<sub>4</sub> (116). LTE<sub>4</sub> induces the activation of ERK1/2 in CHO cells expressing P2Y<sub>12</sub>, which is sensitive to PTX (Figure 2 and ref. 97). Furthermore, administration of LTE<sub>4</sub> 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 CysLT<sub>1</sub>/CysLT<sub>2</sub>-null mice but are completely blocked by administration of clopidogrel, a P2Y<sub>12</sub>-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  $\alpha$ -ketoglutarate (118). Because the  $\alpha$ -ketoglutarate-dependent inositol phosphate formation in GPR99-expressing cells is insensitive to PTX, GPR99 seems to act via a  $G_{q/11}$  pathway (Figure 2 and ref. 118). Kanaoka et al. reported that GPR99 is a high-affinity receptor for LTE<sub>4</sub> (99). The binding study revealed a specificity of GPR99 to [<sup>3</sup>H]LTE<sub>4</sub> with a  $K_D$  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 CysLT<sub>1</sub>/CysLT<sub>2</sub>-KO mice (99). GPR99, which is also expressed in respiratory epithelial cells, mediates mucin release and submucosal swelling in response to LTE<sub>4</sub> induced by *Alternaria* fungi (121). GPR99-KO mice are protected from epithelial cell mucin release and swelling by *Alternaria* or intranasal administration of LTE<sub>4</sub>. Moreover, GPR99 regulates a baseline number of mucin-containing goblet cells. Because LTE<sub>4</sub> elicits airflow obstruction and lung inflammation in asthmatics, inhibition of LTE<sub>4</sub>/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 Ca<sup>2+</sup> increase and inhibition of cAMP synthesis, suggesting a coupling with  $G_{i/o}$  proteins (Figure 2 and refs. 98, 122). Recent studies demonstrate that the administration of montelukast, a CysLT<sub>1</sub> 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 CysLT<sub>1</sub>-mediated signaling on the cell surface through heterodimerization, proposing GPR17 as a negative regulator for CysLT<sub>1</sub> (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 CysLT<sub>1</sub> antagonist (125). Furthermore, they recently reported the negative regulation of CysLT<sub>1</sub> 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.** LTC<sub>4</sub> and LTD<sub>4</sub> are equipotent in guinea pig tracheal smooth muscle, while LTD<sub>4</sub> is more effective in peripheral airways (134). For example, the potency of LTD<sub>4</sub> 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. LTE<sub>4</sub> elicits smooth muscle constriction in isolated guinea pig trachea in preference to LTC<sub>4</sub> and LTD<sub>4</sub>, which required an intact epithelium (136). Moreover, patients with bronchial asthma show an increased sensitivity to LTE<sub>4</sub> leading to airflow obstruction (137–139). Similarly, LTE<sub>4</sub> 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  $\gamma$ -glutamyl transpeptidase (141, 142). Using this model, they demonstrated that both CysLT<sub>1</sub> and CysLT<sub>2</sub> promote LTC<sub>4</sub>- or antigen-induced bronchoconstriction. In humans, both CysLT<sub>1</sub> and CysLT<sub>2</sub> 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 CysLT<sub>1</sub> antagonist, effectively ameliorates regional air trapping due to small airway obstruction in asthma, although contribution of CysLT<sub>2</sub> in this disease has not been fully clarified (144). Intriguingly, Sekioka et al. recently suggested that inhalation of LTC<sub>4</sub> causes CysLT<sub>2</sub>-mediated bronchoconstriction and lung air trapping in an *S*-hexyl GSH-treated guinea pig model (145).

**Recruitment of effector leukocytes.** In humans, peripheral blood cells, e.g., monocytes (93, 100), eosinophils (146), and lung macrophages (90, 93), all express both CysLT<sub>1</sub> and CysLT<sub>2</sub>. 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 LTC<sub>4</sub>, LTD<sub>4</sub>, or LTE<sub>4</sub> 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 CysLT<sub>1</sub> pathway, initiate features of chronic inflammation (150). Furthermore, several CysLT<sub>1</sub> antagonists decreased LTD<sub>4</sub>-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 LTC<sub>4</sub> 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 CysLT<sub>2</sub> in OVA-induced airway inflammation. A recent study further demonstrated that intranasal administration of LTC<sub>4</sub> to OVA-sensitized mice induces airway eosinophilia via a platelet- and CysLT<sub>2</sub>-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 CysLT<sub>1</sub>. Zymosan A-induced plasma protein leakage and IgE-dependent passive cutaneous anaphylaxis are reduced in both CysLT<sub>1</sub>-KO mice and LTC<sub>4</sub> synthase-KO mice (160). These results indicate the pivotal role of CysLT<sub>1</sub> 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 LTC<sub>4</sub> synthase-KO or CysLT<sub>1</sub>-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 CysLT<sub>2</sub>-KO mice, although the zymosan A-

induced peritoneal inflammation is not altered (162). CysLT<sub>2</sub>-mediated vascular permeability via transendothelial vesicle transport was further investigated in a CysLT<sub>2</sub>-KO LacZ mouse model (163). In this model, CysLT<sub>2</sub> mediated inflammatory reactions in a vascular bed-specific manner by altering transendothelial vesicle transport-based vascular permeability. Further reports corroborate CysLT/CysLT<sub>2</sub>-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 LTC<sub>4</sub> synthase-KO mice (166). Although these injuries are not prevented by CysLT<sub>1</sub> deficiency in mice, CysLT<sub>1</sub>-KO mice do show exaggerated alveolar septal thickening with reticular fiber deposition when compared with WT or LTC<sub>4</sub> synthase-KO mice (166). Additionally, CysLT levels in the BALF recovered from CysLT<sub>1</sub>-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 CysLT<sub>2</sub>. Intriguingly, alveolar septal thickening after intratracheal injection of bleomycin is significantly reduced in CysLT<sub>2</sub>-KO mice (166). Because the amount of CysLTs in BALF is similar in CysLT<sub>2</sub>-KO mice and WT mice, CysLT<sub>2</sub> 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, LTC<sub>4</sub> and LTD<sub>4</sub> 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 LTD<sub>4</sub> > LTC<sub>4</sub> > LTE<sub>4</sub> (172).

Although CysLT<sub>2</sub> is predominantly expressed in vascular smooth muscle cells, the expression of CysLT<sub>1</sub> is also induced by stimulation with lipopolysaccharide in human coronary artery vascular smooth muscle cells (173). Intriguingly, in these cells, CysLT<sub>1</sub> is localized in the perinuclear region of human aortic valve myofibroblasts, and its activation is coupled to a predominantly nuclear Ca<sup>2+</sup> signaling (173, 174). Furthermore, a recent report suggested that CysLTs elicit inflammation and proliferation of endothelial cells through CysLT<sub>2</sub> and CysLT<sub>1</sub>, respectively (175). In this study, the authors further demonstrated that CysLT<sub>2</sub> 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<sub>1</sub>, 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<sub>1</sub> antagonists and inhibitors of LT biosynthesis are clinically useful to ameliorate the symptoms of bronchial asthma and allergic rhinitis. Although the past clinical studies on BLT<sub>1</sub> antagonists

failed to attenuate arthritis and psoriasis, studies using BLT<sub>1</sub>-KO mice and BLT<sub>1</sub> antagonists are expanding the BLT<sub>1</sub>-related inflammatory diseases, suggesting the usefulness of BLT<sub>1</sub> antagonists in the future. BLT<sub>2</sub> agonists will target intractable skin and corneal ulcers, including bedsores. Thus, leukotriene receptors are still important drug targets.

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