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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.

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 ($CysLT_1$) 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_4 synthase and LTA4 hydrolase provides new structural bases for the development of LT synthesis inhibitors (2–6). As described here, the 3D structure of BLT_1 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_4 (LTB_4) (Table 1 and refs. 8, 9). The first, BLT_1, known as a high-affinity LTB_4 receptor, is expressed in various subsets of leukocytes and is responsible for LTB_4-dependent leukocyte migration. The second, BLT_2, was originally reported as a low-affinity LTB_4 receptor and is now considered as a receptor for various oxidized fatty acids, including 12-hydroxyheptadecatrienoic acid (12-HHT) and hydroxyeicosatetraenoic acids (HETEs). BLT_2 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_4 (10–18).

BLT_1. Human BLT_1 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). BLT_1 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_4-specific receptor with a K_D value of 0.15 nM for LTB_4 when expressed in Cos-7 cells (8). In BLT_1-transfected CHO cells, BLT_1 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_i coupling of BLT_1 (29). Human BLT_1 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_1. BLT_1 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_1 is important in the conformational change to the low-affinity state after G protein activation (30, 31) and internalization (32–34) of BLT_1.

Recently, the crystal structure analysis of BLT_1 with the antagonist BIIL260 was achieved (Figure 1, A and B, and ref. 35). Docking study with LTB_4 and BLT_1 indicates that LTB_4 would interact with the residues H96, R158, E187, and S243 that were predicted to be involved with LTB_4 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_1 (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_1.

The most important characteristic of LTB_4 is its potent chemotactic effect on leukocytes. BLT_1-deficient granulocytes and eosinophils do not migrate toward LTB_4 (19–23, 37). BLT_1 stimulation in leukocytes leads to degranulation through the production of phosphatidylinositol tris-phosphates (IP_3) via activation of phosphatidylinositol-3-kinase (PI3 kinase) (38). LTB_4 also activates phagocytosis in macrophages through the activation of G_i, PI3 kinase, Rac, and Syk (38). Recently, the receptor for advanced glycation end products (RAGE) was identified as a BLT_1-binding protein

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Table 1. Characteristics of leukotriene receptors

	BLT_1	BLT_2	$CysLT_1$	$CysLT_2$
Ligand	LTB_4 > 20-OH-LTB_4	12-HHT > LTB_4 > 12(S)-HETE > 12(R)-HETE > 15(S)-HETE	LTD_4 > LTC_4 >> LTE_4	LTD_4 = LTC_4 >> LTE_4
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_1 signaling (39). RAGE functions as a molecular switch for BLT_1, inhibits BLT_1-dependent NF-κB activation, and stimulates BLT_1-dependent chemotaxis. RAGE was also shown to bind to GPCRs other than BLT_1 and is a new class of GPCR modulator and a new target of GPCR study (40).

BLT_2. During the analysis of leukocyte-specific transcription of BLT_1 (41), we and others identified a putative open reading frame for a GPCR with similarity to BLT_1 (9, 42–44). As the membrane fraction of cells overexpressing this receptor exhibited a low-affinity LTB_4 binding with K_D values of 10–20 nM, this receptor was named BLT_2. BLT_2 shares amino acid identity of 45% with BLT_1 and high interspecies homology. In contrast to BLT_1, BLT_2 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_2-specific agonistic activity in lipid extract of rat small intestine, then partially purified and determined the structure of this BLT_2 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_2 (PGH_2), 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_2 synthase–dependent and –independent pathways, and aspirin and other NSAIDs inhibit 12-HHT production (50). We found that 12-HHT activates BLT_2 at lower concentrations than LTB_4, leading to the activation of G_i- and G_q-type G proteins. In some cancer cells, BLT_2 was shown to activate the generation of reactive oxygen species (51). Most classical BLT antagonists inhibit both BLT_1 and BLT_2, and a synthetic BLT_2-specific agonist (CAY10583) is available (52–54). In contrast to BLT_1 expression in leukocytes, BLT_2 is expressed in keratinocytes (53), epithelial cells of intestine (55) and cornea (54), lung alveolar type 2 cells, and vascular endothelial cells (56).

BLT_1 in disease

Even before its molecular identification, BLT_1 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_1-knockout (BLT_1-KO) mice and BLT_1 antagonists.

Chemotaxis of leukocytes. Two groups independently reported attenuated leukocyte chemotaxis in the BLT_1-KO mouse using peritonitis models (19, 20). The effect of BLT_1 deficiency was more prominent in eosinophils than in granulocytes. The importance of BLT_1 in eosinophils was later confirmed by nematode infection experiments in which BLT_1-deficient eosinophils did not accumulate and kill nematodes (58). Although classical studies showed the restricted expression of BLT_1 in granulocytes, eosinophils, and macrophages, recent studies showed that BLT_1 expression is observed more widely in a variety of subsets of leukocytes, as follows. BLT_1 is not expressed in naive T cells but is strongly induced by T cell differentiation: both $CD4^+$ and $CD8^+$ effector-type T cells abundantly express BLT_1 and migrate to inflamed tissues (21–23). Interestingly, LTB_4 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_1 have been established and will help to analyze the detailed expression pattern of BLT_1 in these species.

Allergic airway inflammation. The importance of BLT_1 in allergic airway inflammation was confirmed by murine models of bronchial asthma in which ovalbumin (OVA) was used for sensitization and challenge. Infiltration of $CD4^+$ and $CD8^+$ T cells into the airway after OVA challenge was greatly reduced in BLT_1-KO mice (23). In addition to T cells, eosinophil infiltration was attenuated in BLT_1-KO mice with reduced production of Th2 cytokines and attenuated airway hyperresponsiveness (AHR) to methacholine (37). Miyahara et al. showed that IL-13–producing $CD4^+$ and $CD8^+$ T cells were reduced in allergic BLT_1-KO lung, and transfer of OVA-sensitized $BLT_1^{+/+}$ T cells fully restored the reduced AHR in BLT_1-KO mice, showing the importance of IL-13–producing T cells in AHR (62). They also showed that effector-type $CD8^+$ T cells express a higher level of BLT_1, and LTB_4-dependent trafficking of $CD8^+$ effector T cells is important in establishing AHR in mice (63). Dendritic cells also express BLT_1 (24, 25), and adoptive transfer experiments using OVA-loaded dendritic cells showed that BLT_1 expression in antigen-loaded dendritic cells is crucial in inducing asthmatic response (24).

Inflammatory arthritis. The roles of the LTB_4/BLT_1 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_1 abrogated the development of arthritis, showing the requirement for BLT_1 in recruiting neutrophils into the joints (64). Although BLT_1 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_4 synthase

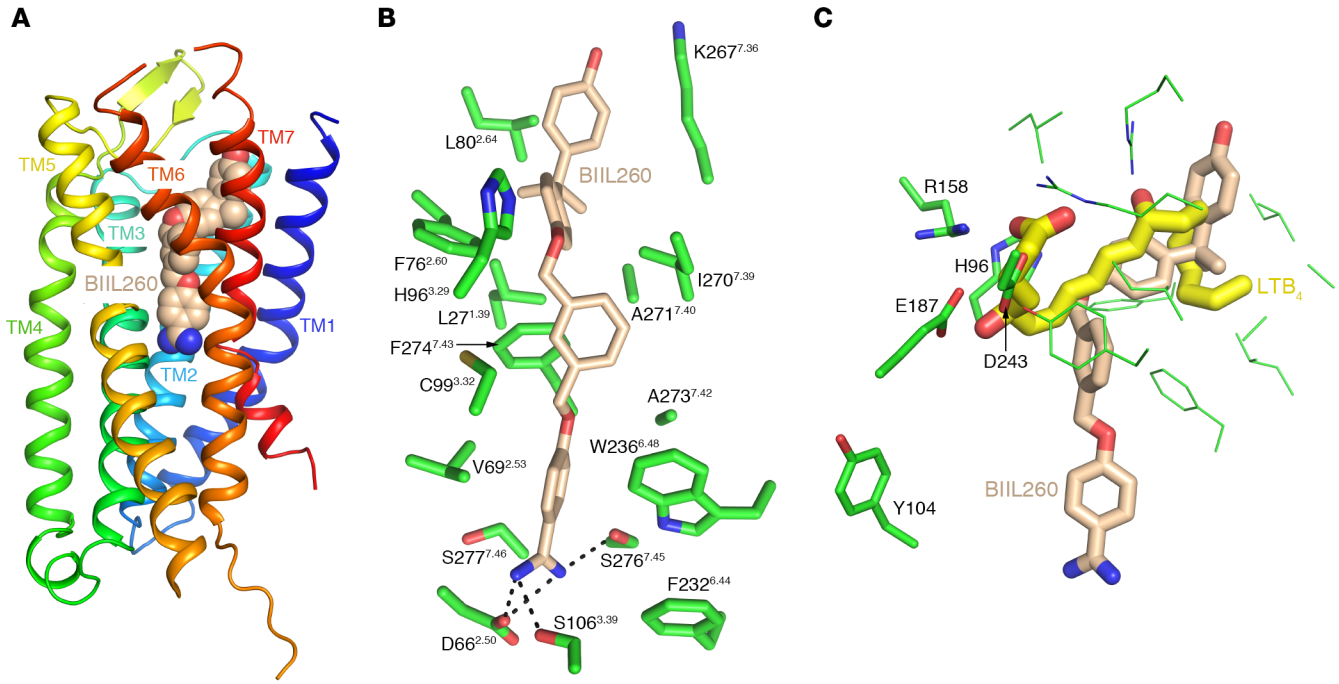

Figure 1. Structure of guinea pig BLT_1 bound with a BLT antagonist, BIIL260. (**A**) Overall structure of BLT_1 and BIIL260 complex (35). BLT_1 is presented as a rainbow-colored cartoon model, and BIIL260 is presented as pink (carbon), blue (nitrogen), and red (oxygen) spheres. (**B**) Mode of BIIL260 binding by BLT_1. BLT_1 side chains within 4 Å from BIIL260 are presented by stick models. Carbon and sulfur atoms of BLT_1 are colored green and gold, respectively. The salt bridge and hydrogen bond interactions are indicated by black dashed lines. (**C**) Docking of LTB_4 in the orthosteric binding site of BLT_1. The docking study was performed with the program AutoDock 4 (184). Carbon atoms of LTB_4 are colored yellow. The residues involved in the LTB_4 binding shown by the mutation study (36) are presented by stick models.

proved requisite for the development of arthritis (66). BLT_1-KO mice were also tested in a collagen-induced arthritis (CIA) model. BLT_1-KO and BLT_1/BLT_2 double-KO mice were completely protected from CIA, despite normal accumulation of serum anti-collagen antibody (67). BLT_1 antagonists (66, 68) were also effective for the attenuation of arthritis.

Atherosclerosis. The relationship between LTB_4 and atherosclerosis has been documented in several reports. LTB_4 triggered adhesion of human monocytes to endothelial cells in a β_1 and β_2 integrin–dependent fashion (69), and strongly increased monocyte chemoattractant protein-1 (MCP-1) (70). In $LDLr^{-/-}$ and $apoE^{-/-}$ mice, the BLT antagonist CP-105,696 reduced atherogenesis without affecting the plasma lipid concentrations (71). Atherogenesis was attenuated in BLT_1-KO mice crossed with $apoE^{-/-}$ mice, which was explained by the lack of LTB_4-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_2, in carcinogenesis has been extensively studied (73), the contribution of LTB_4 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_4 and BLT_1 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_4 production (74). Hypoxic ovarian cancer cells express higher levels of LTB_4-biosynthetic enzymes, and produced a larger amount of LTB_4, leading to the recruitment of tumor-associated macrophages (75). A 5-LOX inhibitor, zileuton, was shown to inhibit polyp formation in the APC^{d468} mouse colon (76) and the growth of ovarian cancer xenografts (75), possibly by inhibiting local inflammation. Recently, Jala et al. reported that BLT_1 deficiency in $Apc^{Min/+}$ 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_4/BLT_1 axis acts as either a tumor-promoting or a tumor-suppressing factor.

Clinical studies on BLT_1-targeted therapy. Several BLT_1 antagonists were tested in a few inflammatory diseases. Oral administration of a BLT_1 antagonist, LY293111, attenuated LTB_4-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_1 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_2 in health and disease

Identification of BLT_2 was reported in 2000 (9), and the first report on BLT_2-KO mice appeared in 2010 (55). In some cases, it is difficult to distinguish the roles of BLT_1 and BLT_2, because both receptors are activated by LTB_4, transduce similar intracellular signaling, and are antagonized by most BLT antagonists; however, different tissue distribution gives a clue to distinguish biological

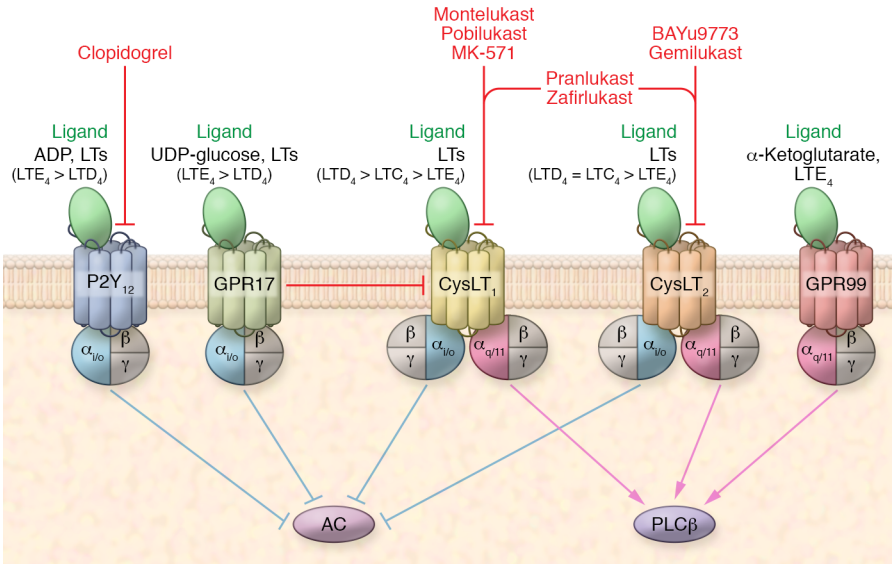

Figure 2. Signaling via $CysLT_1$, $CysLT_2$, GPR17, GPR99, and $P2Y_{12}$. Synthetic antagonists for each receptor are given in red text. Ligands and relative affinities are described adjacent to their respective receptor. GPR17 is a negative regulator for $CysLT_1$. Shown below each receptor is its downstream coupling to $G_{i/o}$ and adenylyl cyclase (AC) and/or $G_{q/11}$ and phospholipase Cβ (PLCβ).

roles of these two receptors. Currently, BLT_2 agonists are attracting interest as new drugs for skin, corneal, and intestinal ulcers.

Small intestine. The first phenotype of BLT_2-deficient mice was susceptibility to drug-induced inflammatory colitis. Because of the lack of BLT_2-specific antibody at that time, in situ hybridization was used to show BLT_2 expression in epithelial and cryptic cells of mouse intestine. BLT_2-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_2-KO intestine that may be linked to observations of increased STAT3 phosphorylation (55). In vitro study using BLT_2-overexpressing Madin-Darby canine kidney II (MDCKII) cells showed that BLT_2 expression increased transepithelial electrical resistance and decreased FITC-dextran leakage through MDCKII monolayers, suggesting the barrier-enhancing activity of BLT_2 (55). The intracellular mechanism of BLT_2-dependent barrier function will be described in the next section.

Skin and cornea. In skin, BLT_2 expression is restricted to dermal keratinocytes. Wound-healing assays after skin punching were performed using BLT_2-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_2 deficiency and aspirin treatment delayed skin wound healing with the attenuated re-epithelialization, and the aspirin effect was not seen in BLT_2-KO mice. Thus, BLT_2 stimulates keratinocyte migration after skin injury, and BLT_2 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_2 is expressed in corneal epithelial cells and stimulates corneal wound healing (54). BLT_2-KO mice also showed enhanced transepidermal water loss and antigen uptake, suggestive of attenuated skin barrier function in BLT_2-KO mice. BLT_2-dependent barrier function involves the enhanced expression of the tight junctional protein claudin-4 downstream of BLT_2 (88).

Lung. Small but significant BLT_2 expression was observed in mouse lung, and BLT_2-KO mice were evaluated in the pneumolysin-dependent (PLY-dependent) acute lung injury model. BLT_2-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 $CysLT_1$ antagonist montelukast prevented the death of BLT_2-KO and NSAID-treated mice, suggesting the possible drug repositioning of $CysLT_1$ antagonists for acute lung injury (56). BLT_2-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_2-deficient $CD4^+$ T cells (89).

Characterization of CysLT receptors

So far, five CysLT receptors have been identified: $CysLT_1$, $CysLT_2$, $P2Y_{12}$, GPR99, and GPR17 (Figure 2 and Table 1). $CysLT_1$ is widely expressed in spleen, leukocytes, lung, small intestine, colon, and skeletal muscle (90–92). $CysLT_2$ exhibits 37.3% amino acid identity with $CysLT_1$ (93), and is exclusively expressed in heart, adrenals, leukocytes, spleen, lymph nodes, and brain (93–96). $CysLT_1$ is preferentially activated by LTD_4, whereas $CysLT_2$ binds both LTC_4 and LTD_4 with equal affinity. Recently, $P2Y_{12}$, GPR99, and GPR17 were reported as receptors for LTE_4 (97–99). Moreover, GPR17 has been proposed as a putative negative regulator of $CysLT_1$. This section serves to give information on these receptors.

$CysLT_1$. Consistent with the clinical effectiveness of CysLT antagonists in asthma, $CysLT_1$ is expressed in a variety of inflammatory cells, i.e., neutrophils, mast cells, and monocytes/macrophages (100, 101). Human $CysLT_1$-expressing cells respond selectively to CysLTs with rank order of potency $LTD_4 > LTC_4 > LTE_4$ (90, 91). Antagonist affinities are similar to those investigated in other functional assays for $CysLT_1$ as well as in binding experiments (90, 102). Activation of $CysLT_1$ by LTD_4 results in the production of several second intracellular messengers through phospholipase Cβ (103, 104). Several reports demonstrated that CysLTs elicit Ca^{2+} 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 $CysLT_1$ in G protein coupling (108).

$CysLT_2$. Comparison of human $CysLT_1$ and $CysLT_2$ revealed negligible existence of $CysLT_1$ but high expression of $CysLT_2$ 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 $CysLT_2$ is expressed in interstitial macrophages and smooth muscle cells (93). Moreover, the presence of human $CysLT_2$ mRNA was determined in atrium, ventricle, and intermediate coronary arteries by in situ hybridization (110). The potency ranking for the competition with tritiated $[^3H]LTD_4$ binding to human $CysLT_2$ is $LTD_4 = LTC_4 >> LTE_4$ (93). The $CysLT_1$ antagonists are either weak (zafirlukast and pranlukast) or inactive (montelukast and pobilukast) at competing for $[^3H]LTD_4$ binding to human $CysLT_2$ (Figure 2 and refs. 93, 111, 112), whereas full competition was observed with the dual $CysLT_1/CysLT_2$ antagonist BAY-u9773 (112). LTC_4 and LTD_4 evoke a dose-dependent activation of Ca^{2+} flux through a PTX-insensitive G protein ($G_{q/11}$) in human $CysLT_2$-expressing oocytes (Figure 2 and ref. 93). On the other hand, in human mast cells, the $CysLT_2$ 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 $CysLT_2$ negatively regulates the development of Th2 pulmonary inflammation by inhibiting the $CysLT_1$ functions on dendritic cells (114). Furthermore, LTC_4, but not LTD_4 and LTE_4, activates mouse platelets via $CysLT_2$, although these cells express both $CysLT_1$ and $CysLT_2$ (115).

Other receptors related to CysLTs. Some of LTE_4-mediated responses are resistant in mice deficient in $CysLT_1$ and $CysLT_2$, implying the existence of LTE_4 receptors. Based on the similarity among $CysLT_1$, $CysLT_2$, and the nucleotide P2Y receptors, LTE_4 receptors seemed to be P2Y-like GPCRs.

Human mast cells express $P2Y_{12}$, an adenosine diphosphate receptor, and knockdown of this receptor impaired the LTE_4-elicited production of MIP-1β and PGD_2 in LAD2 human mast cells without altering their responses to LTD_4 (116). LTE_4 induces the activation of ERK1/2 in CHO cells expressing $P2Y_{12}$, which is sensitive to PTX (Figure 2 and ref. 97). Furthermore, administration of LTE_4 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 $CysLT_1/CysLT_2$-null mice but are completely blocked by administration of clopidogrel, a $P2Y_{12}$-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 $G_{q/11}$ pathway (Figure 2 and ref. 118). Kanaoka et al. reported that GPR99 is a high-affinity receptor for LTE_4 (99). The binding study revealed a specificity of GPR99 to $[^3H]LTE_4$ 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 $CysLT_1/CysLT_2$-KO mice (99). GPR99, which is also expressed in respiratory epithelial cells, mediates mucin release and submucosal swelling in response to LTE_4 induced by *Alternaria* fungi (121). GPR99-KO mice are protected from epithelial cell mucin release and swelling by *Alternaria* or intranasal administration of LTE_4. Moreover, GPR99 regulates a baseline number of mucin-containing goblet cells. Because LTE_4 elicits airflow obstruction and lung inflammation in asthmatics, inhibition of LTE_4/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^{2+} 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 $CysLT_1$ 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 $CysLT_1$-mediated signaling on the cell surface through heterodimerization, proposing CPR17 as a negative regulator for $CysLT_1$ (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 $CysLT_1$ antagonist (125). Furthermore, they recently reported the negative regulation of $CysLT_1$ 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_4 and LTD_4 are equipotent in guinea pig tracheal smooth muscle, while LTD_4 is more effective in peripheral airways (134). For example, the potency of LTD_4 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_4 elicits smooth muscle constriction in isolated guinea pig trachea in preference to LTC_4 and LTD_4, which required an intact epithelium (136). Moreover, patients with bronchial asthma show an increased sensitivity to LTE_4 leading to airflow obstruction (137–139). Similarly, LTE_4 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 $CysLT_1$ and $CysLT_2$ promote LTC_4- or antigen-induced bronchoconstriction. In humans, both $CysLT_1$ and $CysLT_2$ 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 $CysLT_1$ antagonist, effectively ameliorates regional air trapping due to small airway obstruction in asthma, although contribution of $CysLT_2$ in this disease has not been fully clarified (144). Intriguingly, Sekioka et al. recently suggested that inhalation of LTC_4 causes $CysLT_2$-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 $CysLT_1$ and $CysLT_2$. 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_4, LTD_4, or LTE_4 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 $CysLT_1$ pathway, initiate features of chronic inflammation (150). Furthermore, several $CysLT_1$ antagonists decreased LTD_4-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_4 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 $CysLT_2$ in OVA-induced airway inflammation. A recent study further demonstrated that intranasal administration of LTC_4 to OVA-sensitized mice induces airway eosinophilia via a platelet- and $CysLT_2$-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 $CysLT_1$. Zymosan A–induced plasma protein leakage and IgE-dependent passive cutaneous anaphylaxis are reduced in both $CysLT_1$-KO mice and LTC_4 synthase–KO mice (160). These results indicate the pivotal role of $CysLT_1$ 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_4 synthase–KO or $CysLT_1$-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 $CysLT_2$-KO mice, although the zymosan A–induced peritoneal inflammation is not altered (162). $CysLT_2$-mediated vascular permeability via transendothelial vesicle transport was further investigated in a $CysLT_2$-KO LacZ mouse model (163). In this model, $CysLT_2$ mediated inflammatory reactions in a vascular bed–specific manner by altering transendothelial vesicle transport–based vascular permeability. Further reports corroborate CysLT/$CysLT_2$-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_4 synthase–KO mice (166). Although these injuries are not prevented by $CysLT_1$ deficiency in mice, $CysLT_1$-KO mice do show exaggerated alveolar septal thickening with reticular fiber deposition when compared with WT or LTC_4 synthase–KO mice (166). Additionally, CysLT levels in the BALF recovered from $CysLT_1$-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 $CysLT_2$. Intriguingly, alveolar septal thickening after intratracheal injection of bleomycin is significantly reduced in $CysLT_2$-KO mice (166). Because the amount of CysLTs in BALF is similar in $CysLT_2$-KO mice and WT mice, $CysLT_2$ 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_4 and LTD_4 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 $LTD_4 > LTC_4 > LTE_4$ (172).

Although $CysLT_2$ is predominantly expressed in vascular smooth muscle cells, the expression of $CysLT_1$ is also induced by stimulation with lipopolysaccharide in human coronary artery vascular smooth muscle cells (173). Intriguingly, in these cells, $CysLT_1$ is localized in the perinuclear region of human aortic valve myofibroblasts, and its activation is coupled to a predominantly nuclear Ca^{2+} signaling (173, 174). Furthermore, a recent report suggested that CysLTs elicit inflammation and proliferation of endothelial cells through $CysLT_2$ and $CysLT_1$, respectively (175). In this study, the authors further demonstrated that $CysLT_2$ 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 $CysLT_1$, 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

$CysLT_1$ 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_1 antagonists failed to attenuate arthritis and psoriasis, studies using BLT_1-KO mice and BLT_1 antagonists are expanding the BLT_1-related inflammatory diseases, suggesting the usefulness of BLT_1 antagonists in the future. BLT_2 agonists will target intractable skin and corneal ulcers, including bedsores. Thus, leukotriene receptors are still important drug targets.

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