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Leukotriene Receptors: Classification, Gene Expression, and Signal Transduction[1]

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Leukotrienes (LTs) are potent pro-inflammatory mediators derived from arachidonic acid by the action of 5-lipoxygenase. There are two groups of LTs: LTB_4 and cysteinyl LTs (LTC_4, LTD_4, and LTE_4). Both of them play important roles in many inflammatory diseases and allergic responses. Recently, their G-protein coupled receptors have been cloned. The identification of these receptors enables us to analyze their gene structures, regulation of expression, and signal transduction in the cells, and it also leads to the development of useful antagonists. Some LT receptors have been disrupted by gene targeting. Such studies may reveal novel functions of leukotrienes, confirming deeper viewpoints for further research.

Key words: allergy, chemotaxis, G-protein coupled receptor, inflammation, lipid mediator.

In a biological system, arachidonic acid plays a unique role as a precursor for a series of mediators with wide-ranging effects, known as eicosanoids (from the Greek eicosa = twenty; for twenty carbon fatty acid derivatives). Eicosanoids consist of two major groups. Prostaglandins (PGs) are generated by the action of cyclooxygenases, and leukotrienes (LTs) by the action of 5-lipoxygenase (*1, 2*). PGs are formed in most cells of our body, while LTs are made mainly in inflammatory cells as polymorphonuclear leukocytes, macrophages, and mast cells. The synthetic pathways of LT and PG are shown in Fig. 1. Both mediators are not stored in the cells, but synthesized from arachidonic acid released from the membrane (mainly of the nucleus) by the action of phospholipase A_2 in response to various stimuli such as mechanical trauma, cytokine, growth factor, and other mediators that evoke intracellular calcium mobilization. Arachidonic acid is metabolized to LTA_4 by the two-step reaction of 5-lipoxygenase, and further converted by hydration to LTB_4, or by addition of glutathione to LTC_4, further metabolized to LTD_4 and LTE_4. The slow-reacting substance of anaphylaxis consists of LTC_4, LTD_4, and LTE_4, generically named as cysteinyl LTs (*1*). LTB_4 is mainly produced in leukocytes, and acts as one of the most potent chemoattractants and stimulants for leukocytes. LTB_4 is involved in many diseases in which leukocytes may play important roles. Cysteinyl LTs are produced in mast cells, eosinophils, and basophils, and cause constriction of various smooth muscle cells, increased vascular permeability, and enhanced mucus secretion. Cysteinyl LTs are involved in many allergic disorders like bronchial asthma, allergic rhinitis, and atopic dermatitis. These actions of LTs are mainly mediated by their specific cell-surface receptors, while LTs could potentially enter the nucleus and activate nuclear receptors such as peroxisomal proliferator-activated receptors (*3, 4*).

General features of LT receptors

There are four known G-protein coupled receptors (GPCRs) that act as cell-surface receptors for LTs in human. Two of them are receptors for LTB4 (named BLT1 and BLT2), and the other two for cysteinyl LTs (named CysLT1 and CysLT2). The classification with their ligands, the distribution in the body, the number of amino acids, and the chromosome localization of these four LT receptors are summarized in Table I. There are more than 25 reported GPCRs for lipid mediators (*5*). The analysis of a phylogenic tree indicated that GPCRs for lipid mediators could be classified into four groups: (i) classical prostanoid receptors, (ii) chemoattractant receptors, (iii) nucleotide receptors, and (iv) lysophosphatidic acid receptor family (Fig. 2). BLT1 and BLT2 belong to the chemoattractant receptor group. Other chemoattractant receptors like C5a receptor, fMLP receptor, and CRTH2, recently identified as the second PGD_2 receptor (*6*), are in this group (II). Thus, the GPCRs in this group are functionally related. CysLT1 and CysLT2 belong to the nucleotide receptor group (III). P2Y receptors and a platelet-activating factor (PAF) receptor

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Abbreviations: Dex, dexamethasone; GPCR, G-protein coupled receptor; H(P)ETE, hydro(pero)xyeicosatetraenoic acid; HIV, human immunodeficiency virus; IL, interleukin; IP, inositol phosphate; LT, leukotriene; MAPK, mitogen-activated protein kinase; ORF, open reading frame; PAF, platelet-activating factor; PG, prostaglandins; PLC, phospholipase C; PTX, pertussis-toxin.

belong to this group. The ligands of group III (nucleotides, PAF, and LTD_4) can also cause a chemotactic response in some cells. Recently, CysLT1 has been shown to act as a pyrimidinergic receptor for UDP (*7*). Such facts may indicate the validity of this classification.

Receptors for LTB_4

(1) Molecular cloning of LTB_4 receptors. In 1997, our group isolated a cDNA for a cell-surface LTB_4 receptor (BLT1, originally termed BLT) in a subtraction cDNA library from retinoic acid-differentiated HL-60 cells (*8*). The pharmacological properties of BLT1 observed in the transfected cells agreed well with those of native LTB_4 receptors in granulocytes and macrophages. The cDNAs of mouse, rat and guinea pig BLT1s have been identified (*9–13*). During the course of analyzing the structure of human BLT1 gene, we found a putative open reading frame (ORF) of a GPCR with an identity of 45% at the amino acid level to BLT1 (*14*). This GPCR was found to be a low-affinity receptor for LTB_4, termed BLT2. Mouse BLT2 was also identified (*14*). The amino acid identity of BLT2 between human and mouse is 92.7% much higher than that of BLT1 between the two species (78.6%).

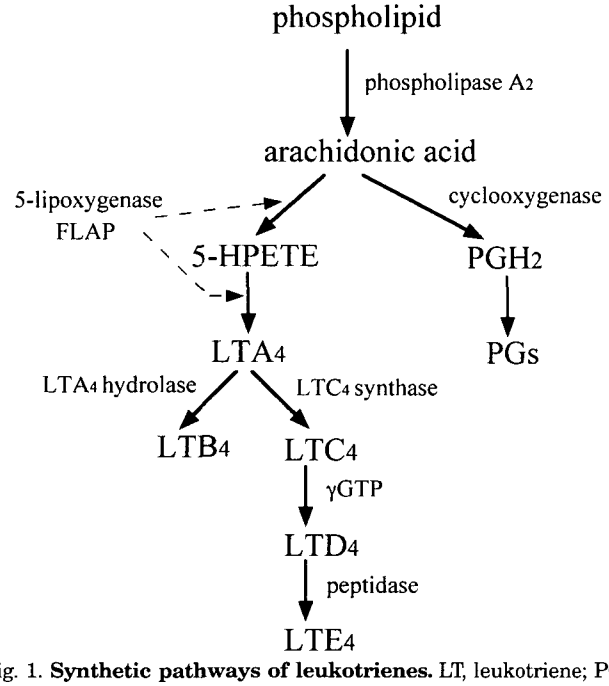

Fig. 1. **Synthetic pathways of leukotrienes.** LT, leukotriene; PG, prostaglandin; 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; γGTP, γ-glutamyl transpeptidase; FLAP, 5-lipoxygenase activating protein.

(2) Genomic structure of BLT1 and BLT2. Human BLT1 gene is a small gene consisting of three exons that is located in a region of 5 kbp on chromosome 14 (*15, 16*). The ORF of BLT1 is in the last exon, exon 3. The basal promoter activity is localized within 100 bp upstream of the transcriptional initiation site. There is no TATA or CAAT element around the transcription initiation site, but an Sp-1 binding site at –50 bp plays a pivotal role in the basal transcription of BLT1. The promoter region of BLT1 is enriched in GC sequences, which are methylated in non-leukocyte cells but not methylated in leukocyte-like cells expressing BLT1 (*16*). The state of methylation in the BLT1 promoter region may control the cell-specific transcription of the BLT1 gene.

In the human gene, the ORF of BLT2 is localized upstream of BLT1 and contains the promoter region of BLT1 as shown in Fig. 3 (*5, 14*). This is a very rare case of a "promoter in ORF" in higher eukaryocytes, but its physiological significance remains to be elucidated. BLT1 and BLT2 might be generated by gene duplication.

(3) Expression of BLT1 and BLT2. Northern analysis revealed almost exclusive expression of BLT1 mRNA in peripheral leukocytes (*8*). Low level of BLT1 expression was observed in human spleen and thymus, and rat resident peritoneal macrophages. However, rat proteosepeptone-induced peritoneal macrophages had an enhanced level of BLT1 mRNA (*10*). Induction of BLT1 mRNA was reported in interleukin (IL) 5- treated eosinophils (*9*).

Northern analysis showed that human BLT2 mRNA is most abundantly expressed in spleen, followed by liver, ovary, and leukocytes, with weak signals in most tissues (*14*).

(4) Up-regulation of BLT1 by glucocorticoid. A recent study showed up-regulation of BLT1 by dexamethasone (Dex) and prevention of the effects by pretreatment with an LTB_4 antagonist LY255283 (*17*). It also showed the synergic effects of Dex and LTB_4 on enhanced neutrophil survival, proposing that glucocorticoids can prevent neutrophil apoptosis by up-regulating their expression of BLT1. We also observed up-regulation of BLT1 expression by Dex in HL-60 cells differentiated with retinoic acid into a neutrophilic phenotype, and in human polymorphonuclear leukocytes with enhanced functions of BLT1 (Obinata *et al.*, unpublished data). These findings revealed unexpected pro-

TABLE I. **Ligands, distribution, structure, and chromosome localization of leukotriene receptors.**

Receptor	Ligands	Distribution (Human)	Structure (Amino acids)	Chromosome (Human)
BLT1	LTB_4	Leukocytes	Human (352)	14q11.2-q12
	> 20-OH-LTB_4	≫ spleen, thymus, lung	Mouse (351)	
	≫ 12-HETE		Rat (351)	
			Guinea pig (348)	
BLT2	LTB_4	Spleen	Human (358)	14q11.2-q12
	> 12-HETE,	> liver, ovary, leukocytes	Mouse (360)	
	15-HETE	> ubiquitous		
CysLT1	LTD_4	Spleen, leukocytes	Human (337)	Xq13-21
	> LTC_4	> small intestine, thymus, prostate, lung, ubiqitous	Mouse (339, 352)[a]	
CysLT2	LTC_4 = LTD_4	Heart, leukocytes, spleen, placenta	Human (346)	13q14
	≫ LTE_4	≫ kidney, ovary, brain	Mouse (309)	

[a]Two forms that result from alternative splicing were reported.

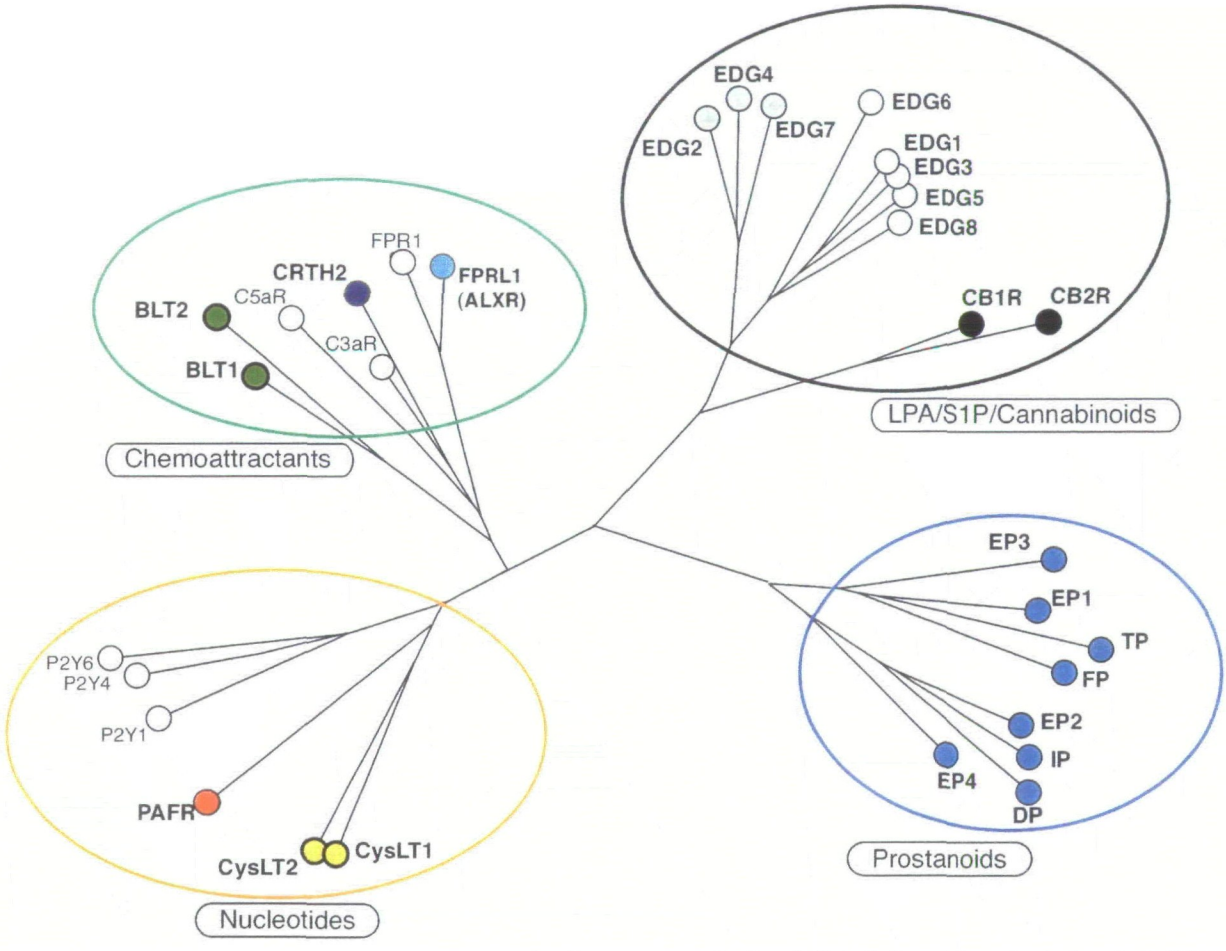

Fig. 2. **Phylogenic tree for leukotriene receptors and other GPCRs.** Filled circles indicate the receptors for eicosanoids and other lipid mediators. GPCRs for lipid mediators could be classified into four groups: (i) classical prostanoid receptors, (ii) chemoattractant receptors, (iii) nucleotide receptors, and (iv) lysophosphatidic acid (LPA) receptor family, which includes sphingosine-1-phosphate (S1P) receptors and cannabinoid receptors. This tree was constructed using the "All All program" at Computational Biochemistry Research Group Server at (http://cbrg.inf.ethz.ch/Server/AllAll.html) with some modifications.

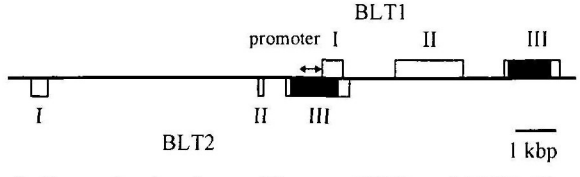

Fig. 3. **Genomic structure of human BLT1 and BLT2.** Chromosome 14 is shown by a line. Transcribed sequences are indicated by boxes and ORFs by filled boxes, above the line for BLT1 and below for BLT2. The promoter region for BLT1 is shown by an arrow. Note that the third exon for BLT2 contains the promoter region and the first exon for BLT1.

inflammatory effects of glucocorticoids other than anti-inflammatory ones in neutrophils.

(5) Physiological characters of BLT1 and BLT2. [^{3}H]LTB_4 binding to the membrane fractions of HEK 293 cells transiently expressing BLTs was analyzed (*8, 14*). Scatchard analysis revealed that the K_d values of BLT1 and BLT2 were 1.1 nM and 22.7 nM, respectively. Both ONO 4057 and U 75302 inhibited [^{3}H]LTB_4 binding to BLT1, but only ONO 4057 inhibited the binding to BLT2. Using the membrane fractions of CHO cells stably expressing BLT1 and BLT2 (CHO-BLT1 and CHO-BLT2 cells, respectively), inhibition by various eicosanoids (5 μM) to 5 nM of [^{3}H]-LTB_4 binding was examined. Only LTB_4 and 20-hydroxy LTB_4 inhibited [^{3}H]LTB_4 binding to BLT1, while almost all eicosanoids examined exhibited inhibition of [^{3}H]LTB_4 binding to BLT2 with a rank order of LTB_4 > 12(*S*)-hydroxyeicosatetraenoic acid (HETE)> 12(*S*)-HPETE > 12(*R*)-HETE > 15(*S*)-HETE > 20-hydroxy LTB_4 > 5(*S*)-HETE (*18*). Thus, BLT2 is a pharmacologically distinct LTB_4 receptor from BLT2.

One of the most remarkable features of CHO-BLT cells is their chemotactic response to LTB_4. Because LTB_4 is a potent chemoattractant for neutrophils, we tried to examine the chemotactic activity of LTB_4 in CHO-BLT cells (*8*). CHO-BLT cells showed unexpectedly strong responses that allowed the migrated cells to be evaluated by staining instead of counting the cell number. The dose-response curves of chemotaxis were bell-shaped, with the maximum doses of 10 nM and 1 μM for CHO-BLT1 and CHO-BLT2 cells, respectively. 12(*S*)-HETE also served as a chemoattractant of CHO-BLT2 cells with the same maximum response as LTB_4, but needed a 10-fold higher dose of 10 μM (*18*).

In vivo chemotaxis of CHO-BLT1 cells was observed in a rat renal ischemia-reperfusion injury model (*19*). Fluorescence-labeled CHO-BLT1 cells were able to accumulate along with neutrophils in the postischemic kidney, in contrast to vector control CHO cells. Furthermore, BLT1 antagonists that protect against the decrease in renal function and diminish the tissue myeloperoxidase activity also led to the marked decrease in the number of CHO-BLT cells and neutrophils. Thus, LTB_4 alone appears sufficient to cause cells to migrate into postischemic tissues, and its dominant role in reperfusion injury has been demonstrated. This is the first report of the migration of CHO cells expressing a chemotactic receptor to affected tissues. This method can be useful to analyze the roles of chemoattractants and their receptors that might be difficult to be otherwise confirmed.

Previous studies reported that in most inflammatory cells, the LTB_4-induced signals are mediated by a G_i-like G-protein, as they are inhibited by pertussis-toxin (PTX) pretreatment. Intracellular signals *via* BLTs were studied in CHO-BLT1 and CHO-BLT2 cells. In these cells, LTB_4 increases intracellular inositol phosphates (IPs) levels and leads to calcium mobilization that is relatively unaffected by PTX pretreatment, suggesting that LTB_4-induced activation of phospholipase C is mediated by a G_q-like G-protein in CHO cells. Co-transfection of human BLT1 with various a subunits of G-protein in COS-7 cells showed that BLT1 is able to couple with both G_{i2} and G_{16}, but not G_{11}, leading to IPs production (*20*). LTB_4 inhibited forskolin-activated adenylyl cyclase activities in both CHO-BLT1 and CHO-BLT2 cells. This inhibition is sensitive to PTX pretreatment in CHO-BLT1 cells but insensitive in CHO-BLT2 cells, indicating the coupling to G_i protein and G_z protein, respectively (*14*). Chemotactic activities of LTB_4 are completely abolished by PTX pretreatment in both CHO-BLT1 and CHO-BLT2 cells. These data show that both BLT1 and BLT2 couple with G_i and other G-proteins, but the types of G-protein are divergent among cell-types.

Human BLT1 has been reported to act as a coreceptor for macrophage-tropic human immunodeficiency virus (HIV) type 1 strains in the same manner as chemokine receptors like CCR5 and CXCR4, both of which have significant sequence homology with BLT1 (*21*). BLT1 expressed on human macrophages can be a target for the inhibition of HIV entry, and various BLT1 antagonists should be examined for their ability to prevent HIV infection. However, another study reported that among the 16 HIV strains tested, the human BLT1 did not act as a coreceptor for virus entry into CD4-expressing cells based on infection and cell–cell fusion assays (*13*).

In conclusion, BLT1 is a high-affinity receptor for LTB_4, only expressed in leukocytes and macrophages. On the other hand, BLT2 is a low-affinity receptor for LTB_4, that can respond to other eicosanoids, and is expressed in many tissues, not only in leukocytes.

(6) Genetically engineered mice for BLT1. BLT1 transgenic mice showed enhanced leukocyte trafficking to skin microabscesses and to the lung after ischemia-reperfusion. These mice showed enhanced expression of 5-lipoxygenase, suggesting that there is a positive feedback in the LT pathway (*22*). There are two reports on targeted disruption of mouse BLT1 (*23, 24*). BLT1-deficient mice developed normally. Leukocytes from these mice did not respond to LTB_4. It was revealed that BLT1 plays an important role in the recruitment of eosinophils to the inflamed peritoneum *in vivo* (*24*). Additionally, female BLT1-deficient mice displayed selective survival relative to male mice of PAF-induced anaphylaxis (*23*). These results demonstrate the role of BLT1 in LT-mediated acute inflammation and an unexpected involvement in PAF-induced anaphylaxis.

Receptors for cysteinyl LTs

Pharmacological studies had indicated that there are two types of cysteinyl LT receptors. CysLT1 is present in human bronchial smooth muscle and potently contracts it. Before the cloning of CysLT1 cDNA, three CysLT1 antagonists developed had been used for the therapy of bronchial asthma, namely, pranlukast, montelukast and zafirlukast. CysLT2 was pharmacologically demonstrated on pulmonary and saphenous vein preparations. Only BAY u9773 partially antagonizes CysLT2, and no potent CysLT2 antagonists have been reported.

(1) Molecular cloning and expression of CysLT1 and CysLT2. The molecular cloning of human CysLT1 was achieved by two groups independently, during their screening for ligands of orphan GPCRs (*25, 26*). Scatchard analysis of $[^3H]LTD_4$ binding to the membranes of Cos-7 cell expressing CysLT1 demonstrated a K_d value of 0.3 nM (*25*). When expressed in HEK 293 cells, CysLT1 responded selectively to cysteinyl LTs with calcium mobilization; the rank order potency was LTD_4, LTC_4, and LTE_4 (*26*).

Northern analysis revealed that CysLT1 mRNA is strongly expressed in spleen, peripheral leukocytes, and less strongly in lung, small intestine, pancreas, and placenta (*26*). *In situ* hybridization and immunohistochemical study showed strong expression of CysLT1 on lung smooth muscle cells and interstitial macrophages, with little or no expression on epithelial cells (*25*). CysLT1 was up-regulated in dimethyl sulfoxide-differentiated U-937 and HL-60 cells (*26*), IL-5 treated eosinophil-like HL-60 cells, and IL-13 or IL-4 treated human monocytes and macrophages (*27–29*).

The cloning of CysLT2 was achieved by three groups also during searches for ligands of orphan GPCRs (*30–32*). Human CysLT2 consists of 346 amino acids and has 38% identity to the CysLT1. Scatchard analysis of $[^3H]LTD_4$ binding to the membranes of Cos-7 cells expressing CysLT2 demonstrated a K_d value of 4.8 nM (*30*). CysLT2 is selectively activated by nanomolar concentrations of cysteinyl LTs with a rank order potency of $LTC_4 = LTD_4 \gg LTE_4$ (*30, 32*). A dual CysLT1/CysLT2 antagonist, BAY u9773 acted as a partial agonist at CysLT2 (*32*). CysLT1 antagonists, montelukast, zafirlukast, and MK-571 did not inhibit the agonist-induced calcium mobilization *via* CysLT2 at physiological concentrations (*32*).

Northern blotting of CysLT2 mRNA showed high expression in human heart, placenta, adrenal gland, peripheral leukocytes, spleen, and lymph node with weaker expression throughout the CNS. *In situ* hybridization study revealed that CysLT2 is strongly expressed in lung interstitial macrophages, but weakly expressed in lung smooth muscle cells. In the adrenal gland, CysLT2 is localized to the chromaffin-containing medulla cells. In the heart, CysLT2 is observed in myocytes, fibroblasts, vascular smooth muscle cells, and Purkinje fiber conducting cells.

The CysLT1 gene is located on the X chromosome at

Xq13-21. No markers for asthmatic disease have been shown to map to this region (*25*). The CysLT2 gene is in 13q14 that has been identified as a polygenic atopy linkage (*32*).

Mouse CysLT1 (mCysLT1) is a polypeptide of 339 amino acids with an 87.3% identity to the human CysLT1. Recently, a longer form with a 13–amino acid extension at the *N*-terminus was reported (*33*). Northern blot analysis revealed that mCysLT1 mRNA is differently expressed in C57BL/6 and 129 mice (*34*). In general, the expression of CysLT1 is higher in C57BL/6 mice than in 129 mice. In C57BL/6 mice, it is strongly expressed in skin, lung, small intestine, and weakly in other tissues like skeletal muscle, spleen and heart. In 129 mice, weak but the highest expression of mCysLT1 mRNA is observed in small intestine. As mentioned before, in human the strongest expression of CysLT1 is observed in the spleen. Thus, the expression of CysLT1 mRNA is different between human and mouse, and different between mouse strains. These facts may indicate the physiological roles of cysteinyl LTs were evolutionarily varied. Careful choice and interpretation are necessary for a study of CysLTs using animal models.

We investigated the distribution of CysLTs in mouse skin by *in situ* hybridization (*34*), as the importance of cysteinyl LTs in skin diseases including atopic dermatitis was shown. No signals of CysLTs were detected in epidermis, but in the subcutaneous connective tissues, high expressions of CysLT1 and CysLT2 mRNAs were seen mostly on fibroblasts. It has been reported that cysteinyl LTs increase collagen synthesis in fibroblasts (*35, 36*).

(2) Intracellular signals through CysLT1. Despite its potent biological activity, little is known about intracellular signaling pathways *via* CysLT1. Recently, the signal transduction mechanisms through CysLT1 were analyzed in a human monocytic leukemia cell line THP-1 (*37*) and in a human epithelial cell line Int 407 (*38*). When these cells were stimulated with LTD_4, intracellular calcium concentration was increased in a PTX-insensitive manner. In THP-1 cells, LTD_4 activated mitogen-activated protein kinase (MAP kinase) through protein kinase Cα and Raf-1 at the same time, also in a PTX-insensitive manner. However, LTD_4-induced chemotactic response in THP-1 cells was PTX-sensitive. In Int 407 cells, LTD_4 induced tyrosine phosphorylation of phospholipase C (PLC)-γ1 and rapid association of PLC-γ1 with $G_{\beta\gamma}$ subunits in a PTX-insensitive manner. Thus, LTD_4 has at least two distinct signaling pathways in these cells: PTX-insensitive activation of MAPK and PLC-γ1, and a PTX-sensitive chemotactic response. These cellular signaling pathways can explain in part the versatile activities of LTD_4 in macrophages and epithelial cells under inflammatory and allergic conditions. However, the molecular mechanisms for the LTD_4-induced strong and prolonged constriction of bronchial smooth muscle have not been elucidated yet.

(3) Targeted disruption of mouse CysLT1. Recently, the phenotype of CysLT1-deficient mice was reported (*39*). These mice developed normally and were fertile. Peritoneal macrophages from wild-type littermates, which express both CysLT1 and CysLT2, responded to LTD_4 and slightly to LTC_4 at 1 μM, whereas the macrophages from CysLT1-deficeient mice did not respond to either LTD_4 or LTC_4. Plasma protein extravasation was significantly reduced in CysLT1-deficient mice subjected to zymosan-induced peritoneal inflammation and in those undergoing IgE-mediated passive cutaneous anaphylaxis. These data confirmed that CysLT1 plays an important role in the response of the microvasculature during acute inflammation.

Future prospects

The identification of LT receptors, especially the second LT receptors as BLT2 and CysLT2, and the analyses of gene-disrupted mice for LT receptors will help to find new aspects of the physiological and pathophysiological roles of LTs.

Selective CysLT1 antagonists with high affinity have been developed and used as effective anti-asthmatic drugs. The development of specific agonists and antagonists for each of the LT receptors will provide important tools for deciding the biological meanings of LTs and potential therapeutic drugs for diseases in which LTs play important roles. So far, no BLT antagonist is available for clinical use. BLT antagonists are potentially useful for the therapy of many diseases like infectious diseases, ischemic diseases, organ transplantation, and many inflammatory diseases in which leukocytes play a pivotal role. As it has been revealed that BLT1 and BLT2 have distinct functions, development of specific antagonists for each receptor is expected. The tissue distribution of CysLT2 is different from that of CysLT1. Unexpected expression of CysLT2 was found in heart, placenta, adrenal gland, and brain, though its function in these tissues has not been elucidated. Thus, a specific antagonist for CysLT2 is also expected.

There are few reports on genetic variants of LT receptors, but such studies will form an important area for understanding the individual response in allergy and inflammation.

The authors apologize to colleagues whose works were not cited because of the restrictions of the review.

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