MINIREVIEW

Leukotriene B₄ Receptor and the Function of Its Helix 8*

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More than 30 lipid ligands, which express their biological activities through G-protein-coupled receptors (GPCRs), have been reported. Among them, leukotriene B₄ (LTB₄) is a potent lipid mediator involved in host defense, inflammation, and the immune responses. Two GPCRs for LTB₄ (BLT1 and BLT2) have been cloned and analyzed. Recent studies using genetically engineered mice suggest that BLT1 plays an important role in several inflammatory diseases including ischemic reperfusion tissue injury, atherosclerosis, and bronchial asthma. BLT1 is also a good tool to study the molecular mechanism of GPCR activation and inactivation in vitro. In this brief review, we focus on the biological and biochemical properties of BLT1 with special attention to the putative helix 8 of the receptor.

Prostaglandins, leukotrienes (LTs), platelet-activating factor (PAF), lysophosphatidic acid, sphingosine 1-phosphate, endocannabinoids, and free fatty acids all exert a variety of biological activities through GPCRs (Fig. 1). These neurotransmitters and hormone receptors are involved in the regulation of the immune response and the control of cell proliferation and differentiation. Ligands and enzymes that are involved in the biosynthesis of these lipids (Fig. 2) are expressed in a variety of cell types and have been shown to play a role in a variety of biological processes.

Physiological Roles of BLT1

BLT1 receptor mice have been established and analyzed by two independent groups. LTB₄-induced chemotaxis is abrogated in BLT1 receptor-deficient mice, and LTB₄-induced calcium influx is absent in BLT1 receptor-deficient mice and peritoneal macrophages (20, 21), clearly indicating that BLT1 is a functional receptor in these cells. BLT1 receptor mice exhibited reduced lethality induced by PAF, showing that LTB₄ functions as a downstream effector of PAF in anaphylactic shock. LTB₄ is produced more rapidly by serum granulocytes than by peptide chemokines, which require transcription and translation for their biosynthesis. Recent reports showed that the LTB₄/BLT1 axis controls the early phase of immunological reactions by activating and recruiting T lymphocytes (22–25). Tager and co-workers (25–27) reported that BLT1 is not expressed in naive T cells but is induced in Th1- or Th2-skewed CD4⁺ T cells and mediates early T cell recruitment into the airway in an asthma model. Ott et al. (24) proposed that mast cell-derived LTB₄ (upon cross-linking of the Fce receptors) is the initial trigger for migration of CD8⁺ effector T cells into the inflamed lesion. Previously, Kato et al. (28) showed that methylation at the CpG sites in the BLT1 promoter is related to leukocyte-specific transcription of the BLT1 gene, but the molecular mechanism of induction of BLT1 during Th1 and Th2 differentiation remains unclear. Identification of the cells that produce LTB₄ other than mast cells, also is an important issue to be addressed in this atherogenic model. Leukocytes (especially monocytes) invade the arterial intima are critical in the development of atherosclerotic lesions (29). Aiello et al. (30) reported that a BLT1 antagonist, CP-105696, reduced the formation of atherosclerotic plaques in both apoE⁻/⁻ and LDLR⁻/⁻ mice. Studies using BLT1-deficient mice on an apoE⁻/⁻ background also showed the importance of the LTB₄/BLT1 interaction in atherogenesis (31). Although much information on the LTB₄/BLT1 axis has accumulated using various animal disease models, further study is required for identification of the cells that are affected by BLT1 deficiency (TABLE ONE). The preceding are several key studies that aim to identify the cells that are involved in the mechanism of leukocyte trafficking and transcriptional regulation of BLT1 in differentiated T cells.

Discovery and Signaling of BLT2

Binding studies using [³H]LTB₄ had suggested the presence of a low affinity LTB₄ receptor in addition to a high affinity receptor. BLT2, another G-protein-coupled receptor, was independently identified by four groups (32–35). Yokomizo and co-workers (28, 32) identified human BLT2 while analyzing the promoter of BLT1. The human BLT2 gene is located ~3 kb upstream of the human BLT1 open reading frame, and the primary structure of BLT2 is most similar to BLT1 (45% amino acid identity, Fig. 1) among the superfamily of GPCRs (28). BLT2 is highly conserved between mice and human with 93% identity, compared with 78% identity for BLT1, suggesting important but as yet undefined functions for BLT2. Whereas human and mouse BLT2 are expressed exclusively in leukocytes, human BLT2 is distributed more widely (32, 33). Membrane fractions of HEK293 cells transfected with human BLT2 exhibited LTB₄ binding, with a Kd of 5 10⁻⁹ M, ~20-fold higher than that of BLT1 transfectants. BLT2 coupled to both the G and Gₛ family of G-proteins as did BLT1, and CHO cells expressing BLT2 exhibited LTB₄-induced increases in intracellular calcium and chemotactic responses. BLT2 binds various hydroxyeicosatetraenoic acids in addition to LTB₄ (36). Murine BLT2 has been characterized and is highly expressed in keratinocytes (47). The biological roles of BLT2 have not been reported. However, the analysis of BLT2 is expected to reveal novel functions of LTB₄ and structurally related eicosanoids.

Biochemical Characterization of BLT1: Roles of Helix 8

GPCRs form a large superfamily of seven-transmembrane helix proteins that mediate responses to various ligands. Some examples are shown in Fig. 1. Although the first and sole high resolution structure of a GPCR, rhodopsin (37), is very helpful in understanding the intramolecular mechanism of GPCR activation, the mechanisms of receptor activation and inactivation remain unclear. In response to extracellular ligands, GPCRs undergo conformational changes, promote the exit of GDP for GTP on the G-protein α subunit, and initiate the dissociation of the α- and βγ-subunits. After G-protein activation, GPCRs are inactivated by several mechanisms. The best characterized mechanism of inactiva-

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Phosphorylation of cytoplasmic Ser/Thr residues of GPCRs by GPCR kinase, protein kinase C, and cAMP-dependent protein kinase, which leads to β-arrestin-dependent internalization of GPCRs (38). GPCRs are also known to switch into a low affinity state following exposure to ligands. This is caused by GDP-GTP exchange on the subunit of heterotrimeric G-proteins, because addition of GTPγS (a nonhydrolyzable GTP analogue) to membrane preparations induces a structural change of GPCR into a low affinity state.

Given that BLT1 is a very specific GPCR for LTB4 and is not expressed in cultured cell lines commonly used for GPCR transfection, BLT1 is a unique tool for analyzing the molecular mechanism of activation and inactivation of GPCRs in vitro. BLT1 is expressed and sorted to the plasma membrane in most cells after transfection, and examination of [3H]LTB4 binding by overexpressed BLT1 is straightforward because of its low nonspecific binding. Baneres and Parello (39, 40) have reported that only one G-protein trimer binds to a dimerized receptor to form a pentameric complex using BLT1 protein expressed in Escherichia coli.

Analysis of the crystal structure of rhodopsin confirmed the presence of 7 transmembrane (TM) helices and revealed the existence of an 8th helix (helix 8) that projects at a right angle from the C terminus of TM7 (37) (Fig. 2). This short helix of rhodopsin is anchored by a palmitoyl group to the cytoplasmic leaflet of the cell membrane, and a hypothetical model has predicted that helix 8 interacts with the N-terminal helix of the G and G subunits (41). BLT1 belongs to the rhodopsin subfamily of GPCRs and is speculated to contain the helix 8 in its intracellular C terminus (42, 43). However, BLT1 lacks the cysteine residue, which is thought to be palmitoylated in many GPCRs. BLT1 showed little internalization following exposure to LTB4 in HEK293 or CHO cells, in contrast to the robust internalization of the PAF receptor (42, 44).

BLT1 mutants with a truncated or substituted helix 8 showed much higher LTB4 binding than wild-type (WT) receptor in HEK293 and CHO cells, albeit with comparable expression on the cell surface (42). Similar to the WT receptor, LTB4 promoted GTPγS binding in these mutants following exposure to LTB4. Unlike WT-BLT1, the addition of GTPγS did not inhibit LTB4 binding to the mutant receptors. The mutant receptors maintained a high affinity for LTB4 even in the presence of an excess amount of GTPγS, as determined by Scatchard analyses. Consistent with this observation, the mutant receptors showed more prolonged intracellular signaling (e.g. calcium mobilization and metabolic activation) after LTB4 treatment. Fig. 3 is a molecular model of BLT1 (based on the rhodopsin structure as a reference) and shows a close-up of the residues in the vicinity of helix 8. The BLT1 model predicts a helix 8 extending from TM7 similar to the one observed in rhodopsin.

The BLT1 model suggests that a pair of aromatic residues...
(Tyr-285 and Phe-300), which are positioned similarly to the conserved Tyr-306 and Phe-313 pair in rhodopsin, may stabilize the inactive form of the receptor by holding TM7 and helix 8 at almost a right angle to each other. Hydrophobic amino acid residues (Val-301, Leu-304, and Leu-305) of the short helix 8 may anchor this helix to the plasma membrane like palmitoylated cysteine. A phosphorylation site (Thr-308) is located just after the amphiphilic helix 8, and phosphorylation of Thr-308 is predicted to weaken the interaction between helix 8 and the plasma membrane. Gaudreau et al. (43) also proposed that helix 8 is involved in a hydrophobic core containing other hydrophobic residues in helix 1. Disruption of this hydrophobic core may facilitate the irreversible activation of BLT1. Cell surface expression levels of helix 8 mutants of BLT2 are considerably reduced. The helix 8 of BLT2 might also be important for receptor sorting. Although one will have to wait for the three-dimensional structure of the

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**FIGURE 2.** Characteristic amino acid residues of helix 8 of rhodopsin family GPCRs. Alignments of helix 8 of 180 human rhodopsin family GPCRs are summarized using GPCRDB (www.gpcr.org/7tm). The numbers of amino acid residues from Tyr-306 of the NPXXY sequence to Phe-313 of the N terminus of helix 8 are 6 (163 receptors), 5 (7 amine receptors), 7 (8 prostanoid receptors), or 14 (2 BLTs). The most and second most frequent amino acids are shown with the corresponding amino acids of bovine rhodopsin, and the frequency is shown in parentheses. Hydrophobic amino acids are Ala, Phe, Ile, Leu, Met, Pro, Val, Trp; hydrophilic amino acids are Cys, Gly, Asn, Gln, Ser, Thr, Tyr; basic amino acids are His, Lys, Arg; acidic amino acids are Asp, Glu. H, hydrophobic; A, aromatic; P, possibly palmitoylated; B, basic amino acids.

**FIGURE 3.** BLT1 structure model. A, the BLT1 atomic model is based on that of bovine rhodopsin (Protein Data Bank entry 1F88) with homology modeling as a rhodopsin subfamily member of GPCRs. Helix 8 with side chains in a stick model is shown. The vicinity of helix 8 is presented in the color green, helix 8 is in violet, transmembrane helix 7 is in pink, and helix 1 is in blue. In helix 8, the hydrophobic side chains of Val-301, Leu-304, and Leu-305 are colored in violet and extrude to the plasma membrane, and a pair of aromatic residues, Tyr-285 and Phe-300, with the transparent Corey-Pauling-Koltun surface may restrain helices 7 and 8. B, Edmundson helical wheel projection of helix 8. The hydrophobic and hydrophilic amino acids cluster on opposite sides.

**FIGURE 4.** A model depicting functions of BLT1 helix 8. Although wild-type BLT1 can change its conformation from a high affinity state to a low affinity state after G-protein activation (A), the BLT1 mutant that lacks helix 8 cannot change its conformation to the low affinity state (B). The helix 8 of BLT1 may function in sensing the status of its coupling Gsubunit as being GTP-bound or being anchored in the plasma membrane; as a consequence the receptor may change its conformation. Therefore, helix 8 mutants may remain in a high affinity state and exhibit higher LTB4 binding and more prolonged intracellular signaling (42).
BLT1-G-protein complex, these studies suggest that helix 8 of BLT1 may play an important role in the inactivation of BLT1 after G-protein activation, possibly by sensing Gα subunits as being GTP-bound (Fig. 4). Using reconstitution of a budded baculovirus expression system, Masuda et al. (46) showed that trimeric G-proteins were required for maintenance of the high affinity state of BLT1. The binding affinity of GPCR is critically dependent on the status of G-proteins, and biochemical studies of GPCRs are important in understanding the molecular mechanisms of receptor activation and inactivation.

Conclusion
In addition to the physiological and pathophysiological importance of BLT1 as revealed by analyses of BLT1-deficient mice, BLT1 is a useful molecular device for analyzing the mechanism of activation and inactivation of GPCRs in vitro. The C terminus of BLT1 plays an important role in sensing GDP and GTP on the G-protein α subunit to which BLT1 couples and in switching the affinity states of BLT1 for LTB₄. It will be important to know whether this sensing mechanism (via the 8 helix) is common to the other GPCRs or only limited to a small population of GPCRs, including BLT1.

REFERENCES