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Review

Identification, signaling, and functions of LTB_4 receptors

Kazuko Saeki, Takehiko Yokomizo*

Department of Biochemistry, Juntendo University School of Medicine, Tokyo, Japan

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ABSTRACT

Leukotriene B_4 (LTB_4), a lipid mediator produced from arachidonic acid, is a chemoattractant for inflammatory leukocytes. We identified two receptors for LTB_4, the high-affinity receptor BLT1 and the low-affinity receptor BLT2. BLT1 is expressed in various subsets of leukocytes, and analyses of BLT1-deficient mice revealed that the LTB_4/BLT1 axis enhances leukocyte recruitment to infected sites, and is involved in the elimination of pathogens. Hyperactivation of the LTB_4/BLT1 axis induces acute and chronic inflammation, resulting in various inflammatory diseases. BLT2 was originally identified as a low-affinity receptor for LTB_4, and we later identified 12(*S*)-hydroxy-5*Z*,8*E*,10*E*-heptadecatrienoic acid (12-HHT) as a high-affinity ligand for BLT2. BLT2 is highly expressed in epithelial cells in various tissues including intestine and skin. Large quantities of 12-HHT are produced by activated platelets during skin injury, and activation of BLT2 on epidermal keratinocytes accelerates skin wound healing by enhancing cell migration. BLT2 signaling also enhances cell–cell junctions, protectes against transepidermal water loss, and preventes entry of environmental substances into the body.

1. Introduction

Leukotriene (LT) B_4 [5(*S*),12(*R*)-dihydroxy-6*Z*,8*E*,10*E*,14*Z*-eicosatetranoic acid, LTB_4] is a classical lipid chemoattractant historically known to activate granulocytes. The word "leukotriene" is derived from "leukocytes", in which it is mainly produced, and "triene" refers to the presence of three conjugated double bonds that are a common structural feature of all LTs [1]. Most enzymes involved in the biosynthesis and degradation of LTs and receptors for LTs have been cloned, and corresponding genetically engineered mice have been generated and analyzed to clarify the *in vivo* roles of LTs [2]. Numerous studies clarified that BLT1, the high-affinity receptor for LTB_4 is expressed more widely than expected, and LTB_4 attracts a variety of leukocyte subsets including granulocytes, eosinophils, differentiated T cells, and some subsets of macrophages and dendritic cells. Studies using mouse disease models indicate that inhibition of the LTB_4/BLT1 axis is important in many inflammatory and immunological diseases, and BLT1 is therefore considered an important drug target. Unlike BLT1, BLT2 is a low-affinity LTB_4 receptor that is activated by various oxidized fatty acids such as 12(*S*)-hydroxy-5*Z*,8*E*,10*E*-heptadecatrienoic acid (12-HHT), and BLT2 engages in a variety of biological roles in epithelial cells.

2. Biosynthesis and metabolism of LTB_4

Arachidonic acid (5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid, AA) is an essential fatty acid nutrient in the diet, and an important component of cell membrane. Generally, AA consists of 5−10% of unsaturated fatty acids that are esterified at the *sn*-2 position of the membrane phospholipids, and AA is released by activated phospholipases A_2 (PLA_2), especially cytosolic PLA_2 [3–5]. AA is metabolized by oxygenation into various important lipid mediators such as prostaglandins (PGs) and LTs (Fig. 1). Oxygenation of AA at the C-5 position to form 5(*S*)-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid [5(*S*)-HETE] was first reported in rabbit granulocytes [6], and this study was soon followed by the identification of various derivatives including LTB_4 [7–9], originally known as a potent chemoattractant for granulocytes [1] and now known to attract eosinophils, differentiated T cells, and dendritic cells [10]. Biosynthesis of LTB_4 requires several enzymic reactions (Fig. 1); AA liberated by $cPLA_2$ is oxygenated at the C-5 position by 5-lipoxygenase (5-LOX) to form 5(*S*)-hydroperoxy-6*E*,8*Z*,11*Z*,14*Z*-

Abbreviations: AA, arachidonic acid; CLDN4, claudin-4; COX, cyclooxygenase; DGS3, desmoglein-3; DSS, dextran sodium sulfate; 5-LOX, 5-lipoxygenase; 5(*S*)-HETE, 5(*S*)-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid; 5(*S*)-HpETE, 5(*S*)-hydroperoxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid; FLAP, 5-LOX-activating protein; GPCR, G-protein coupled receptor; HPLC, high-performance liquid chromatography; $InsP_3$, inositol 1,4,5-trisphosphate; LT, leukotriene; LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; MAPEG, Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism; MDCK, Madin-Darby canine kidney; MMP, matrix metalloproteinase; MRP1, multidrug resistant protein 1; NSAID, nonsteroidal anti-inflammatory drug; PAF, platelet-activating factor; PG, prostaglandin; PGE_2, prostaglandin E_2; PLA_2, phospholipase A_2; PLC, phospholipase C; PTX, pertussis toxin; RAGE, receptor for advanced glycation end products; TNFα, tumor necrosis factor α; 12-HHT, 12(*S*)-hydroxy-5*Z*,8*E*,10*E*-heptadecatrienoic acid; 12(*S*)-HETE, 12(*S*)-hydroxy-5*Z*,8*Z*,10*E*,14*Z*-eicosatetraenoic acid; 12(*S*)-HpETE, 12(*S*)-hydroperoxy-5*Z*,8*Z*,10*E*,14*Z*-eicosatetraenoic acid; TxA_2, thromboxane A_2

* Corresponding author at: Department of Biochemistry, Juntendo University School of Medicine, Hongo 2−1−1, Bunkyo−ku, Tokyo 113−8421, Japan.
E-mail address: yokomizo-tky@umin.ac.jp (T. Yokomizo).

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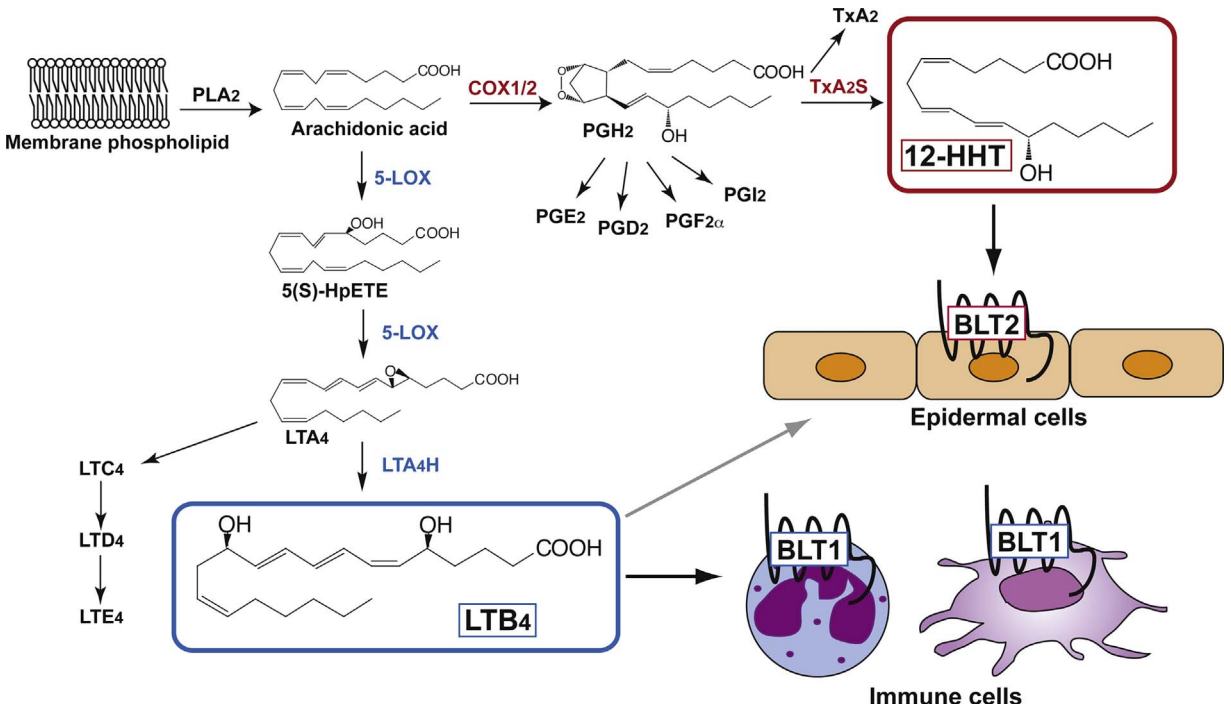

Fig. 1. Production of leukotrienes, prostanoids, and 12-HHT. Arachidonic acid cleaved from membrane phospholipids is converted to leukotrienes, prostanoids, and 12-HHT. LTB_4 serves as a high-affinity ligand of BLT1 and induces chemotaxis and/or activation of various subsets of leukocytes. BLT2, originally identified as a low-affinity receptor for LTB_4, is a high-affinity receptor for 12-HHT, and enhances epithelial barrier function. Enzyme names are abbreviated as follows: PLA_2, phospholipase A_2; COX1/2, cyclooxygenase-1 and cyclooxygenase-2; TxA_2S, thromboxane A_2 synthase; 5-LOX, 5-lipoxygenase; LTA_4H, leukotriene A_4 hydrolase.

eicosatetraenoic acid [5(*S*)-HpETE], and subsequently to form leukotriene A_4 (LTA_4) [11,12]. For this oxygenation, 5-LOX-activating protein (FLAP) is required [13]. FLAP is a membrane-spanning protein with three transmembrane domains [14] belonging to the Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism (MAPEG) family [15] that includes leukotriene C_4 (LTC_4) synthase [16] and microsomal prostaglandin E_2 (PGE_2) synthase. The precise roles of FLAP in 5-LOX reactions remain unknown, but it is suggested that FLAP might present AA to 5-LOX or function as a scaffold for 5-LOX [13,17]. Although 5-LOX was originally purified as a cytosolic protein, it was later shown to translocate to the nuclear envelope after phosphorylation [18,19]. It is now accepted that the nuclear membrane is the major site for production of LTs. 5(*S*)-HpETE is further hydrolyzed by LTA_4 hydrolase to generate LTB_4 [20–24]. LTA_4 hydrolase is a cytosolic protein with both LTA_4 hydrolase activity and zinc-dependent peptidase catalytic activity [25,26]. Although the biological role of the peptidase activity of LTA_4 hydrolase is unknown, it limits pulmonary inflammation by degrading the chemotactic peptide PGP (proline-glycine-proline) [27]. Thus, LTA_4 hydrolase is a bi-functional enzyme in inflammation because it generates the chemotactic lipid mediator LTB_4 and degrades chemotactic peptide PGP.

In contrast to the restricted expression of 5-LOX in hematopoietic cells, LTA_4 hydrolase is ubiquitously expressed [23]. In inflammatory tissues, LTA_4 generated in inflammatory cells is transferred to adjacent cells expressing LTA_4 hydrolase [28] and converted to LTB_4, which can explain the enhanced LTB_4 production in inflammation. This phenomenon is referred to as the “transcellular biosynthesis of leukotrienes” [29], and LTC_4 is also produced in a same way [30]. LTB_4 produced in cells is released through a putative ATP-dependent LTB_4 transporter [31] that is yet to be isolated, whereas, for LTC_4, multidrug resistant protein 1 (MRP1, ABCC1) was identified as a possible transporter [32]. Recently, neutrophil exosome was shown to mediate LTB_4 release during chemotaxis [33].

Given the potent chemotactic activity of LTB_4, its inactivation is important for limiting inflammation. Two major pathways of LTB_4 inactivation are known, and responsible enzymes have been identified. Granulocytes and hepatocytes inactivate LTB_4 through the omega oxidation pathway [34] in which C-20 of LTB_4 is oxidized by several cytochrome P450 enzymes, CYP4F3 in granulocytes [35] and CYP4F1 and 2 in hepatocytes [36]. In other tissues, LTB_4 is inactivated by conversion into 12-keto-LTB_4 by the cytosolic enzyme LTB_4 12-hydroxydehydrogenase [37,38], which is also involved in the inactivation of various eicosanoids including PG [39] and lipoxin A_4 [40].

3. Identification of BLT1, a high-affinity receptor for LTB_4

Numerous studies using radiolabeled LTB_4 revealed the presence of a high-affinity LTB_4 receptor in granulocytes in human, guinea pig, and other mammalian species. Pharmacological studies showed that LTB_4 receptor is a G-protein coupled receptor (GPCR) that couples with pertussis toxin (PTX)-sensitive Gi-protein. Despite the fact that many laboratories, including ours, attempted to identify an LTB_4 receptor in granulocytes by conventional strategies including protein purification and expression cloning using *Xenopus* oocytes, all studies proved fruitless until we managed to clone the cDNA in 1997 [41]. Based on the finding that LTB_4 binding activity increased during granulocyte-like differentiation of HL-60 cells by retinoic acid, we tried to identify a LTB_4 receptor among mRNAs that were upregulated by retinoic acid. Using a cDNA subtraction strategy, we isolated two cDNA clones for a GPCR sharing some homology with receptors for chemokines and formyl peptides, and found that these clones encode a GPCR for LTB_4 [41]. We initially designated this receptor as BLTR but this was subsequently changed by the IUPHAR (International Union of Basic and Clinical Pharmacology) nomenclature committee to “high-affinity receptor BLT1” [42]. When exogenously expressed in COS-7 and HEK 293 cells, BLT1 exhibited high-affinity binding to LTB_4, with the *Kd* values of 0.15 and 1.2 nM, respectively, and binding was observed to be competitive with various BLT1 antagonists. LTB_4 competed with [^{3}H]-labeled LTB_4 most efficiently, followed by 20-hydroxy-LTB_4, 12-oxo-LTB_4, and 12(*R*)-HETE, but not by all-trans-LTB_4, suggesting that BLT1