CHEMICAL REVIEWS

REVIEW

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Leukotriene Receptors

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1. INTRODUCTION

Arachidonic acid (*all-cis*-5,8,11,14-eicosatetraenoic acid, AA) is an essential constituent of cellular membranes, which is released by tightly regulated phospholipase cleavage. Additionally, AA is a key biological intermediate that is converted to a large number of eicosanoids with potent physiological activities (Figure 1). These eicosanoids are synthesized de novo from AA by pathways of oxidative lipid metabolism in a controlled fashion. In 1976, it was found that AA is oxygenated at the C-5 position in a study of the transformation of polyunsaturated fatty acids in rabbit leukocytes.[1] The major metabolite in this reaction, which is mediated by 5-lipoxygenase (5-LO), is 5(*S*)-hydroxy-6,8,11,14-eicosatetraenoic acid [5(*S*)-HETE]. Subsequently, various derivatives, including leukotriene B_4 (LTB_4), were identified.[2−4] LTB_4 is well-known as one of the potent chemoattractants and activators of leukocytes and is involved in inflammatory diseases.[5,6] The 5-LO pathway is also involved in the production of five series of LTs, e.g., LTB_5, from EPA [eicosa-5(*Z*),8(*Z*),11(*Z*),14(*Z*),17(*Z*)-pentaenoic acid].[7,8] These lipids have less pro-inflammatory and vasoactive activities compared to LTB_4.[9] Moreover, extensive studies led to the discovery of the pivotal epoxide intermediate, LTA_4,[10] and the identification of a cysteinyl-leukotriene (cys-LT) family, i.e., LTC_4, LTD_4, and LTE_4, also known as SRS-A (slow-reacting substance of anaphylaxis).[11] LTC_4 is converted from AA, namely, formation of LTA_4 from AA via 5(*S*)-HpETE, followed by glutathione conjugation of LTA_4 with opening of the epoxide at the allylic position C-6.[11,12] LTC_4 is further metabolized to LTD_4 by enzymatic elimination of glutamic acid by γ-glutamyltranspeptidase.[13] The remaining peptide bond in LTD_4 is further hydrolyzed by a dipeptidase to produce LTE_4.[14] Moreover, further studies on the 5-LO cascade led to the discovery of a novel group of related biologically active lipids, lipoxins (LXs).[15]

The term "leukotriene" was chosen because these lipids were discovered in leukocytes and the common structural feature is a conjugated triene.[16] Various members of the group have been designated alphabetically: LTA is 5,6-oxido-7,9-*trans*-11-*cis*-eicosapolyenoic acid; LTB, 5(*S*),12(*R*)-dihydroxy-6-*cis*-8,10-*trans*-eicosapolyenoic acid; LTC, 5(*S*)-hydroxy-6(*R*)-*S*-γ-glutamyl-cysteinyl-glycyl-7,9-*trans*-11-*cis*-eicosapolyenoic acid; LTD, 5(*S*)-hydroxy-6(*R*)-*S*-cysteinyl-glycyl-7,9-*trans*-11-*cis*-eicosapolyenoic acid; and LTE, 5(*S*)-hydroxy-6(*R*)-*S*-cysteinyl-7,9-*trans*-11-*cis*-eicosapolyenoic acid. Since precursor acids containing the $\Delta^{5,8,11}$ double bond system (5,8,11-eicosatrienoic acid, AA, and EPA) can be converted to LTs containing three to five double bonds, a subscript denoting this number is used.[13] These lipid mediators are shown to play important roles in the regulation of cell proliferation and differentiation, as well as the reproductive, gastrointestinal, and cardiovascular systems. In contrast, the excessive productions of these lipids are associated with pathological manifestations of various diseases, such as asthma, allergic inflammation, and innate immunity. There are two important pathways of the enzymatic oxidation of AA: the lipoxygenase (LO)-cascade and the cyclooxygenase (COX)-cascade. In the former cascade, various LTs and LXs, e.g., LTB_4, LTC_4, LTD_4, LTE_4, 5(*S*),6(*R*),15(*S*)-trihydroxyeicosa-7(*E*),9(*E*),11(*Z*),13(*E*)-tetraenoic acid (LXA_4), 5(*S*),14(*R*),15(*S*)-trihydroxyeicosa-6(*E*),8(*Z*),10(*E*),12(*E*)-tetraenoic acid (LXB_4), 5(*S*)-HETE, and 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE), are synthesized under tight regulations. These lipid mediators exert their biological effects by binding to cognate G protein-coupled receptors (GPCRs), classified into four main groups: BLT receptors with affinity to LTB_4, cys-LTs receptor family, LX receptor, and receptors for other lipids such as 5-oxo-ETE.[17] Consequently, several pharmaceutical companies started to develop inhibitors for the enzymes in the LO-cascade or antagonists for LT receptors for treatment of various diseases, and some of these inhibitors and antagonists are now available as drugs. Currently, substantial progress has been made in understanding the regulation of LTs and LXs biosyntheses and signaling via cognate receptors at cellular and molecular levels. The aim of this review is to summarize the recent information on the biosyntheses and metabolism of LTs and LXs and on the physiological effects of these lipids via their specific GPCRs. Moreover, we discuss the effects of genetic or pharmacological ablation of the receptor functions on various pathophysiological processes and the development of various synthetic ligands for the treatment of various diseases.

2. OVERVIEW

LTs are biologically active metabolites derived from AA and are produced predominantly by inflammatory cells, such as polymorphonuclear leukocytes, macrophages, and mast cells (Figure 2). Cellular activations by various stimuli elicit a sequence of events that include the translocations of cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$) and 5-LO to the nuclear envelope. These events lead to conversion of a membrane phospholipids-derived AA to the principal intermediates, e.g., 5(*S*)-HpETE, 5(*S*)-HETE, and LTA_4, for the productions of LTs and other bioactive lipids with the concerted efforts of 5-LO-activating protein (FLAP). FLAP may retrieve AA and transfer it to 5-LO, but the mechanisms still remain unclear. LTA_4 undergoes transformation by one of three possible fates depending on the cellular context: hydrolysis, conjugation with glutathione, or transcellular transport to generate bioactive eicosanoids.[18] Hydrolysis of LTA_4 by LTA_4 hydrolase yields LTB_4, a potent neutrophil chemoattractant.[19] The conjugation with glutathione to LTA_4 is catalyzed by LTC_4 synthase, resulting in the production of LTC_4.[20] LTC_4 is transported out of the cells via transporters such as the multidrug-resistance-associated protein (MRP1).[21] The peptide moiety of LTC_4 is further subjected to extracellular metabolism, forming LTD_4 and LTE_4. Moreover, the transcellular metabolism of LTA_4 is associated with LXs

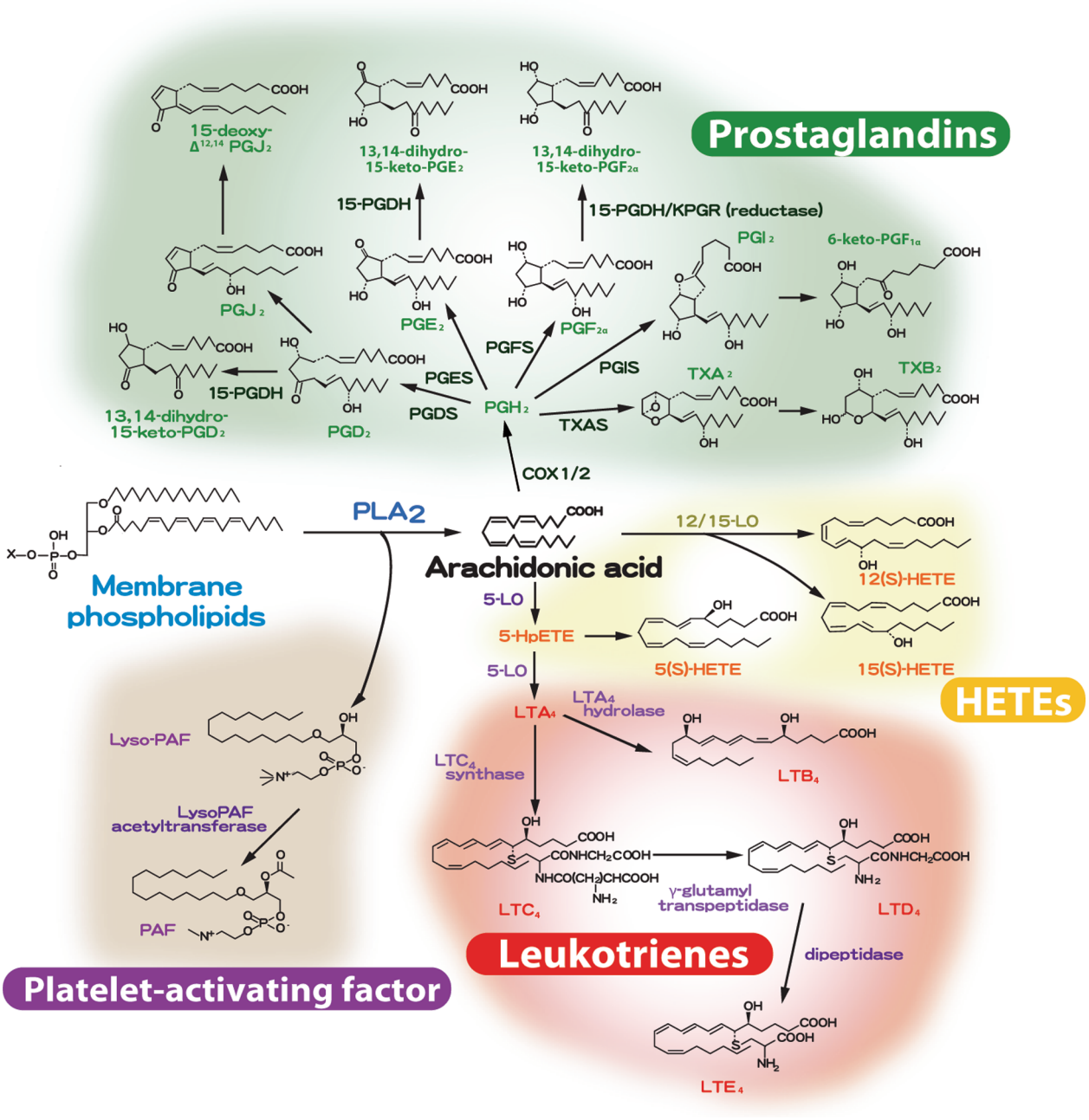

Figure 1. Biosynthetic pathways of eicosanoids and PAF. Enzyme names are also described: 5-LO, 5-lipoxygenase; 12-LO, 12-lipoxygenase; 15-LO, 15-lipoxygenase; 15-PGDH, 15-hydroxy-PG dehydrogenase; COX1/2, cyclooxygenase-1 and cyclooxygenase-2; PGDS, PGD synthase; PGES, PGE synthase; PGFS, PGF synthase; PGIS, PGI synthase; PLA_2, phospholipase A_2; and TXAS, TX synthase.

formation. For instance, LXs are produced during platelets–leukocytes interaction; LTA_4 transported from neutrophils is converted to LXA_4 through the action of 12-LO in platelets. These LTs and LXs exert their biological effects via specific GPCRs, many of which have been recently identified. These lipid mediators have physiological and pathological roles in a variety of immune responses and inflammatory and allergic diseases, such as rheumatoid arthritis, inflammatory bowel disease, allergic rhinitis, and bronchial asthma.[22] Anti-LT therapy has been proven to be efficient in the treatment of these diseases, either through the inhibition of LT syntheses or the selective antagonism of the receptors. In this section, we provide a brief overview of the biosyntheses and metabolism of LTs and LXs and the actions of these bioactive lipids via specific receptors.

2.1. Release of AA from Membrane Phospholipids by $cPLA_2\alpha$

Eicosanoids are synthesized from AA released from membrane phospholipids by $cPLA_2\alpha$. In the membrane phospholipids, the asymmetrical distribution of saturated or monounsaturated fatty acids at the *sn*-1 position and polyunsaturated fatty acids, including AA, at the *sn*-2 position is maintained in part by the rapid deacylation/reacylation proposed by William E. Lands in the late 1950s (the Lands' cycle) (Figure 3).[23−26] The Lands'

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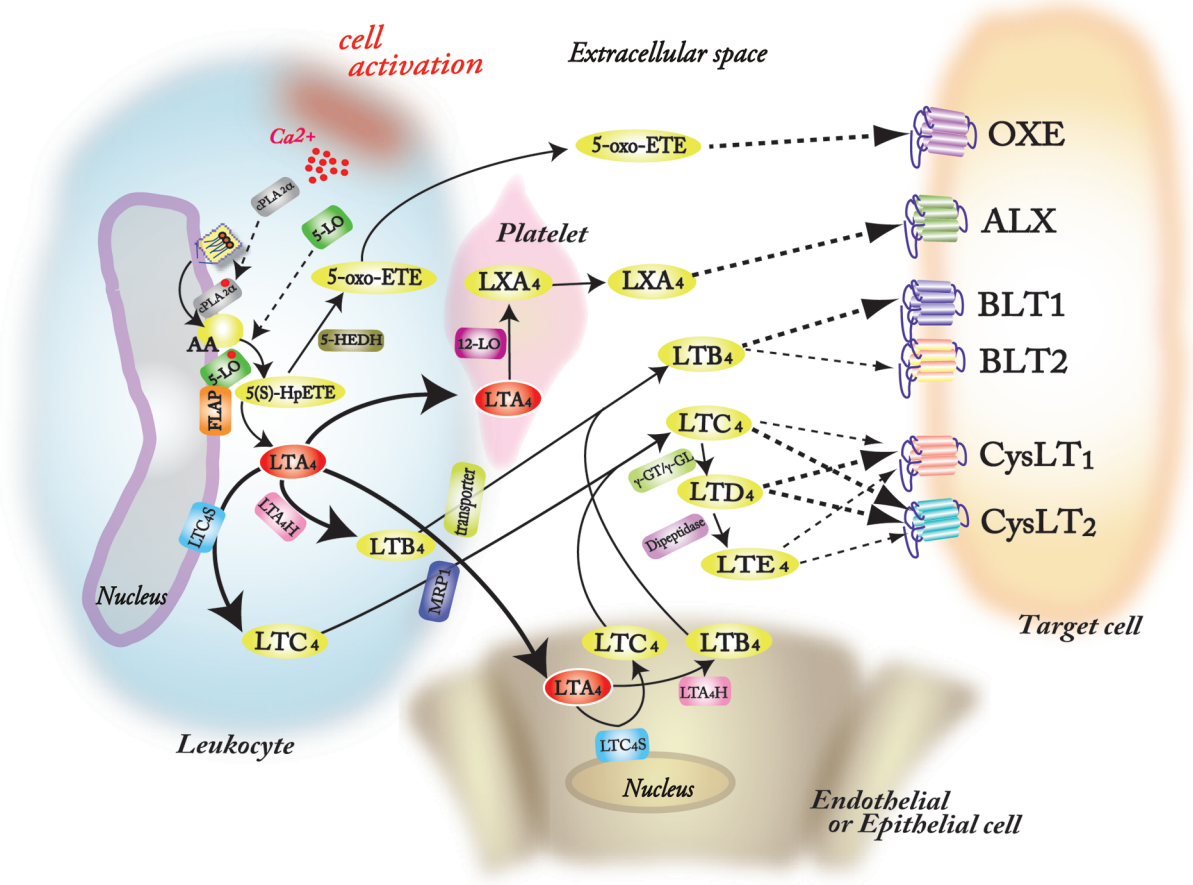

Figure 2. Overview of the synthesis and actions of LTs and LX pathways. See the text for a description. 5-HEDH, 5-hydroxyeicosanoid dehydrogenase; FLAP, 5-LO activating protein; AA, arachidonic acid; $cPLA_2$, cytosolic phospholipase A_2; LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; LTA_4H, LTA_4 hydrolase; LTC_4S, LTC_4 synthase; LXA_4, lipoxin A_4; MRP1, multidrug resistant protein 1; γ-GT, γ-glutamylpeptidase; γ-GL, γ-glutamylleukotrienese; ALX, LXA_4 receptor; BLT1, LTB_4 type-I receptor; BLT2, LTB_4 type-II receptor; $CysLT_1$, cys-LT receptor type-I; $CysLT_2$, cys-LT receptor type-II; OXE, 5-oxo-ETE receptor. The binding affinities to each receptor are indicated by the thickness of the dashed arrows from each agonist.

cycle has been widely received among researchers who are interested in the mechanisms of phospholipid remodeling and the functional significance of PLA_2s and acyl-CoA:lysophospholipid acyltransferases (termed LPLATs) in various cells and tissues. Currently, several LPLAT families have been identified by our laboratory and others and have been shown to possess broad and somewhat overlapped substrate specificities.[6,27] In many cases, the fatty acids at the *sn*-2 position are replaced by AA, which is stored until it is required for eicosanoid biosynthesis. More detailed information about individual enzymes can be found in several previously published reviews.[6,27,28]

After cellular activation by various stimuli, PLA_2 cleaves membrane glycerophospholipids at the *sn*-2 position, resulting in the production of AA and lysophospholipids. Lysophospholipids function as bioactive molecules or serve as precursors of other lysophospholipid mediators, e.g., lysophosphatidic acid (LPA) and platelet-activating factor (PAF). Depending on the molecular structure and biochemical properties, PLA_2 isoforms can be classified into four main categories: (i) the secreted PLA_2 ($sPLA_2$) isoform containing multiple disulfide bonds;[29,30] (ii) the calcium-dependent (micromolar concentration) cytosolic PLA_2 ($cPLA_2$) isoform;[31,32] (iii) the calcium-independent cytosolic PLA_2 ($iPLA_2$) isoform;[33,34] and (iv) the PAF acetylhydrolases, which inactivate PAF and oxidized phospholipids to yield lysophospholipids.[35−37] As for the $cPLA_2$ isoform, calcium is not required for the catalytic activity, but it is important for the interaction with the phospholipids membrane. There are at least six types of $cPLA_2$, i.e., $cPLA_2\alpha$, $cPLA_2\beta$, $cPLA_2\gamma$, $cPLA_2\delta$, $cPLA_2\varepsilon$, and $cPLA_2\zeta$, of which $cPLA_2\alpha$ is a pivotal enzyme in the production of eicosanoids and PAF.[38−40]

$cPLA_2\alpha$-null mice show a lack of LTB_4 production, suggesting the importance of this enzyme for the biosynthesis of LTB_4.[41−43] This enzyme is widely expressed in many types of tissues and cells, e.g., brain, lung, kidney, heart, spleen, platelets, macrophages, neutrophils, endothelial cells, vascular smooth muscle cells, alveolar epithelial cells, renal mesangial cells, and keratinocytes.[44] Significant advances have led to a better understanding of the regulation of the AA release by $cPLA_2\alpha$ that may be the rate-limiting step in eicosanoid formation. The intracellular localization of $cPLA_2\alpha$ using immunofluorescence microscopy revealed that upon cell activation $cPLA_2\alpha$ translocates to the endoplasmic reticulum and the nuclear membranes.[45,46] Moreover, there is evidence demonstrating that 5-LO translocates to a perinuclear site in the stimulated cells.[47] Thus, in the activated cells $cPLA_2\alpha$ may be ideally positioned to provide free AA to the enzymes of the eicosanoid cascade. The stimuli, which can activate $cPLA_2\alpha$,

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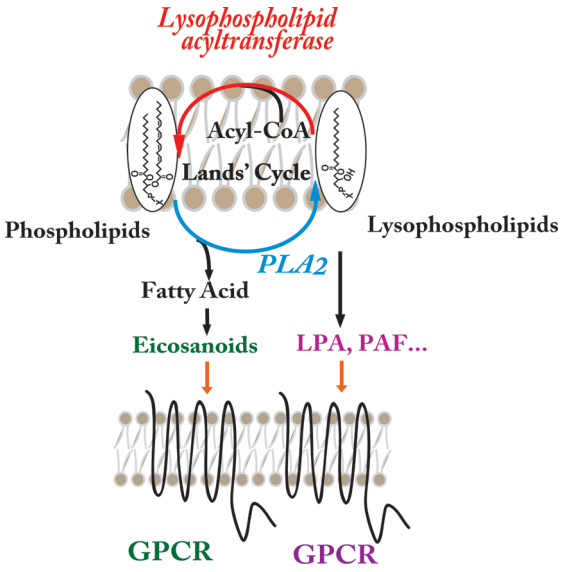

Figure 3. Production of lipid mediators during the deacylation−reacylation cycle (Lands' cycle). Fatty acids of phospholipid are liberated by PLA_2s and converted to eicosanoids. Lysophospholipids are also precursors of a different class of lipid mediators, including LPA, PAF, and endocannabinoids. Lysophospholipids are converted to phospholipids in the presence of acyl-CoA by lysophospholipid acyltransferases (LPLATs). X in the phospholipid structures indicates several polar head groups of phospholipids. See the text for details.

include not only receptor ligands, e.g., growth factors, mitogens, and cytokines, but also nonreceptor-mediated stressful stimuli, e.g., oxidation, hyperglycemia, and shear stress.[48] It is noteworthy that calcium ionophores induce maximal release of AA from cellular phospholipids, suggesting that the increase in cytosolic free calcium is a predominant factor causing $cPLA_2\alpha$ activation. A variety of extracellular stimuli were found to cause rapid phosphorylation of $cPLA_2\alpha$, revealing that phosphorylation also plays a crucial role in the $cPLA_2\alpha$ activation.[49]

2.2. Biosynthetic Pathways of LTs and LXs

LTs and LXs are synthesized by the enzymatic oxidation of the released AA through several LO cascades, e.g., 5-LO, 8-LO, 12-LO, and 15-LO cascades. Structural differences divide the products of the LO cascades into four separate classes: (i) LTB_4, which contains two hydroxy groups; (ii) LTC_4, LTD_4, and LTE_4, referred to as cys-LTs based on the cysteine residue in their structures; (iii) LX group consisting of LXA_4, LXB_4, and aspirin-triggered LX, containing trihydroxytetraene; and (iv) other eicosanoids, such as 5(*S*)-HETE and 5-oxo-ETE. All these mediators are synthesized in a tightly regulated fashion under the direction of specific enzymes. With some exceptions, they have a relatively short half-life (seconds to minutes) and can be degraded both enzymatically and nonenzymatically.

2.3. Comprehensive Analyses of Lipid Mediators by LC−MS/MS

For understanding the roles of each lipid mediator in vitro and in vivo, a simultaneous method with sensitivity and reliability is necessary. An enzyme-linked immunosorbent assay (ELISA) and a radioimmunoassay (RIA) have been the most widely used to determine the quantitation of small amounts of lipid mediators, although a major limitation of these assays is incompatibility with multiplex analysis. They require much sample when multiple target compounds are quantitated. Mass spectrometry (MS)-based quantitation techniques such as gas chromatography (GC)−MS (GC−MS/MS) and liquid chromatography (LC)−MS (LC−MS/MS) have advantages in specificity over immunoassays. Furthermore, these techniques are well-suited to multiplex analysis, which minimizes the sample amount required. Currently, we have established a quantitation system for multiplex lipid mediators by column-switching LC−tandem MS.[50,51] This system has improved sensitivity relative to the previous method with a broader dynamic range, namely, the rapid analysis of 14−20 different lipid mediators in a single sample with a throughput of 96 samples/24 h, lower limits of quantitation of 5 pg on column, and linear calibration ranges up to 2000−5000 pg. Indeed, we successfully detected dynamic changes in a series of lipid mediators in some pathologic tissues of rodents. We used this method for the quantitative analysis of lipid mediators in disease models to illustrate the utility of this comprehensive lipidomics approach. First, we profiled eicosanoids in the hippocampus and cerebral cortex at various times in the kainite-induced seizures model. After the application of kainite, we found robust production of $PGF_2\alpha$ and PGD_2 in the hippocampus and a relatively smaller level of other PGs and HETEs.[52] Next, we measured multiple eicosanoids in animal models of experimental autoimmune encephalomyelitis (EAE). In an AA cascade-targeted lipidomics approach, we recently demonstrated the significant role of microsomal PGE_2 synthase 1 (mPGES-1) in the development of EAE. Eicosanoids (mainly PGD_2) are produced constitutively in the spinal cord of naive mice. However, in EAE lesions, the production of PGE_2 is favored, whereas the syntheses of other eicosanoids, e.g., PGD_2, PGI_2, PGJ_2, 5(*S*)-HETE, LTB_4, LTC_4, and LTD_4, are attenuated.[53] Thus, we concluded that the mPGES-1−PGE_2−PGE_2 receptors axis of the AA cascade exacerbates EAE pathology. While this technique requires further improvements and wider coverage (for example, measurements of lysophospholipids and major metabolites of eicosanoids), this method will be useful to analyze the roles of lipid mediators in vitro and in vivo.

2.4. Action of Lipid Mediators via Cognate GPCRs

Generally, GPCRs stimulated with agonist activate heterotrimeric GTP-binding proteins (G-proteins), composed of α-, β-, and γ-subunits, associate with a group of conventional cellular events. Upon the receptor activation by its specific ligands, Gα- and G$\beta\gamma$-subunits stimulate a variety of intracellular molecular systems. Namely, G-protein activation leads to an increase in intracellular calcium, cAMP production, Rho activation, and/or modification in a number of membrane ion channels (Figure 4). In humans, on the basis of the genome database, nearly 400 nonolfactory GPCRs have been identified.[54,55] Twenty years ago, we reported the first successful cloning of a GPCR for PAF.[56,57] Since the cloning of the PAF receptor, various GPCRs for lipid mediators, e.g., nine different PG receptors (EP1, EP2, EP3, EP4, DP, CRTH2, IP, FP, and TP), four distinct LT receptors (BLT1, BLT2, $CysLT_1$, and $CysLT_2$), a LX receptor (ALX), six LPA receptors (LPA_{1-6}), five S1P receptors ($S1P_{1-5}$), and two endocannabinoid receptors (CB1 and CB2), have been identified and characterized. As of the writing of this review, more than 30 GPCRs for lipid mediators have been clarified. The phylogenic tree for the eicosanoid and bioactive lipid GPCRs, including other proteins with seven transmembrane helices, is illustrated in Figure 5. In these GPCRs, certain families, e.g., PGs, $S1P_{1-5}$, and LPA_{1-3} receptor families, appear to have evolved from the same

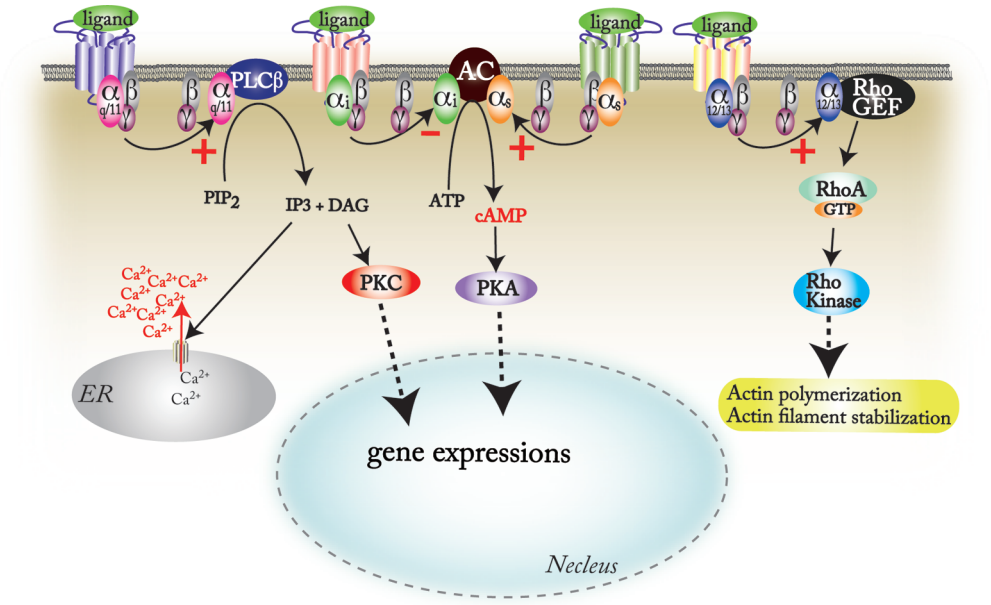

Figure 4. Signaling via GPCRs. Signal transduction pathways regulated through various receptor-coupled G proteins. At least, there are four types of G protein α-subunits, α_s, α_i, $\alpha_{q/11}$, and $\alpha_{12/13}$, involved in the following important signaling: the elevation of intracellular calcium via $G\alpha_{q/11}$, the production of cAMP by the activation of AC via $G\alpha_s$, the inhibition of AC by $G\alpha_i$, and activation of Rho-GEF through $G\alpha_{12/13}$. AC, adenylyl cyclases; PLCβ, phospholipase Cβ; PKA, protein kinase A; PKC, protein kinase C; Rho GEF, Rho GTP/GDP exchange factor.

ancestor, because of the structural similarity of their ligands, whereas other lipid GPCR families are evolved on the basis of functional similarity. For instance, two LTB_4 receptors, BLT1 and BLT2, are evolutionarily distant from PG or cys-LT receptors but in the same family as the $G\alpha_i$-coupled chemoattractant receptors, which include receptors for LXs, formyl peptides, and complements (C5a and C3a). GPCRs represent the most important class of drug targets both in terms of therapeutic benefit and pharmaceutical sales, with at least 30% of marketed drugs being GPCR modulators.[58] Thus, they are major therapeutic targets in the pharmaceutical industry's drug discovery programs. Many GPCR agonists and antagonists have been identified in ligand binding assays and interact with the receptor in a competitive manner with the natural ligand. In the following sections, we also summarize the binding characteristics of natural ligands to the cloned LTs and LXs receptor and the latest developments in LTs and LXs receptor agonists and antagonists for the various diseases.

3. DIHYDROXY LT RECEPTORS

3.1. Biosynthesis of Dihydroxy LTs

When peritoneal leukocytes derived from rabbits were incubated with ^{14}C-labeled AA, the major metabolite was found to be 5(*S*)-HETE, a lipoxygenase product.[1] However, more polar lipids were also synthesized and the major derivative of them was LTB_4.[2−4] LTB_4 is well-known as one of the potent activators of leukocytes[59,60] and is involved in various diseases, including inflammatory ones.[5,6,61,62] LTB_4 is synthesized from AA released from membrane phospholipids by the action of $cPLA_2\alpha$. Two enzymes, 5-LO[63,64] and LTA_4 hydrolase,[65,66] are required for LTB_4 biosynthesis from AA. 5-LO catalyzes the first two steps, namely, conversion of AA to 5(*S*)-HpETE and 5(*S*)-HpETE to LTA_4.[67−70] During activation, a multiprotein complex assembles on the outer and inner nuclear membranes centered on FLAP that serves as a scaffold protein for 5-LO (Figure 6). FLAP is a prerequisite protein for the action of 5-LO.[71,72] Even though the crystal structure of FLAP was currently reported,[73] a precise mechanism by which this protein activates 5-LO remains elusive. Currently, a potent inhibitor of FLAP, AM103 {3-[3-*tert*-butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic acid}, has been developed by Amira Pharmaceuticals.[74] Doses of 50−1000 mg of AM103, administered orally once daily for 11 days, were shown safely and effectively to inhibit LTs production in healthy subjects. This compound is being investigated for the treatment of respiratory disorders such as asthma and allergic rhinitis (Evans et al., in the abstract book of 2010 Keystone Symposia Meeting, "Bioactive Lipids: Biochemistry and Diseases"). AA is transferred to 5-LO by FLAP, initiating the oxygenation of AA to 5(*S*)-HpETE, followed by dehydration to the unstable epoxide LTA_4. Interestingly, FLAP shares significant homologies with other enzymes of the AA cascade, including two types of microsomal LTC_4 synthases[75−77] and glutathione-dependent PGE synthase.[78−80] These proteins constitute a family collectively called "membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEGs)".[81]

5-LO is mainly expressed in leukocytes, e.g., granulocytes, monocytes/macrophages, mast cells, dendritic cells, and B lymphocytes, but not in platelets, endothelial cells, T-cells, and erythrocytes.[64] In the skin, Langerhans cells strongly express 5-LO.[82] Currently, aberrant expression of 5-LO has been observed in a variety of cancer cells.[83,84] In certain cells, e.g., the circulating PMNs, although 5-LO was initially purified as a cytosolic protein, this enzyme is known to translocate to the nuclear envelope[85−87] and to eosinophil lipid bodies[88] upon stimulation (Figure 6). Similar nuclear localization of 5-LO that can be reversed upon ex vivo culture was observed in alveolar macrophages.[89,90] Because two isozymes of COX (COX-1 and COX-2) are also present in the nuclear membrane, it is now

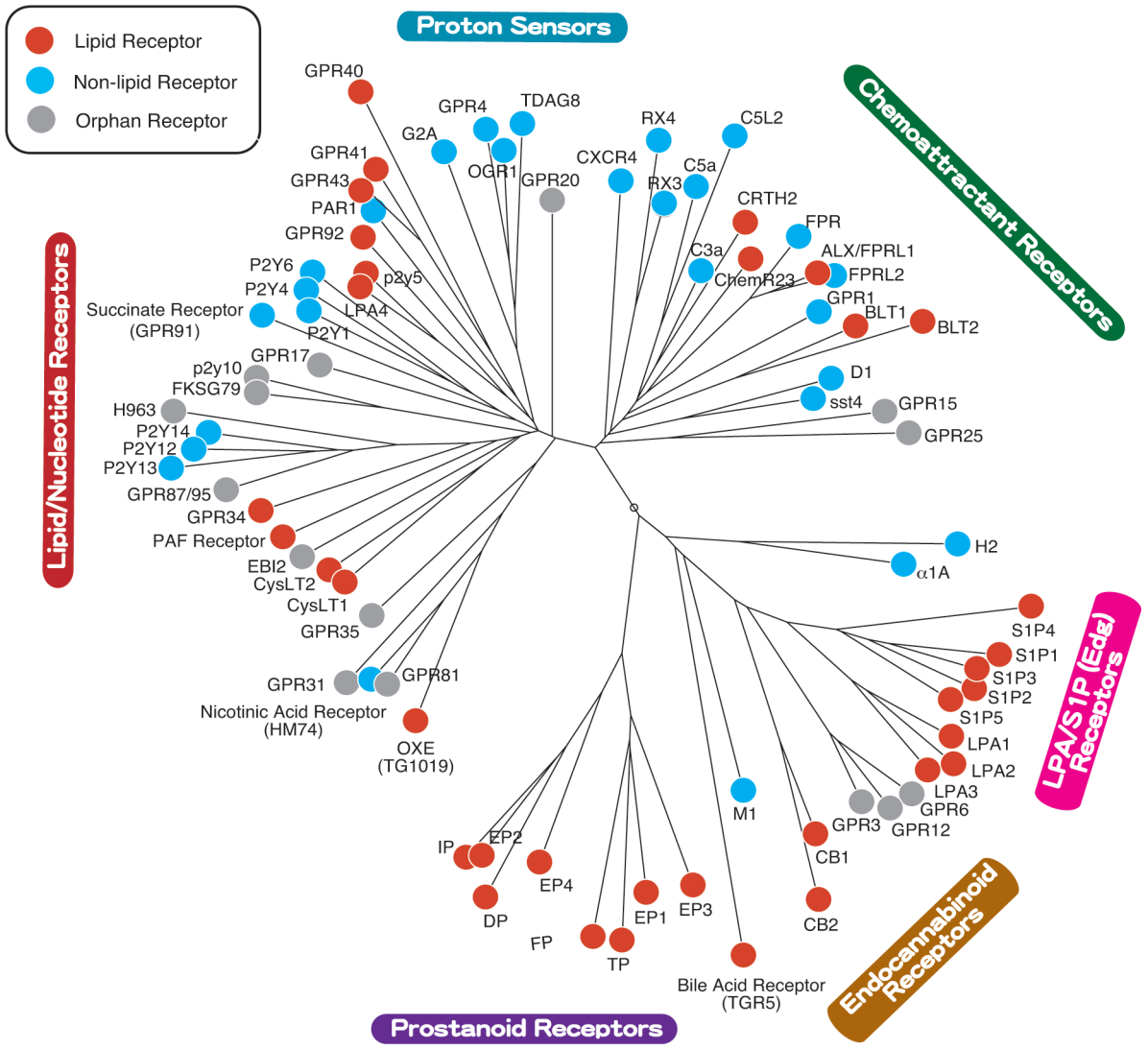

Figure 5. Evolutional tree of lipid GPCRs and orphan receptors. Lipid GPCRs, nonlipid GPCRs, and orphan receptors are shown in red circles, blue circles and gray circles, respectively. This tree was constructed using the "All Program" at The Computational Biochemistry Server at ETHZ (http://cbrg.inf.ethz.ch/ServerBooklet/chapter2_3.tml). Branch lengths represent the evolutional distance between each pair of sequences.

widely accepted that nuclear membrane is a major site of eicosanoid production.[91] The molecular mechanism and domains of 5-LO required for the translocation have been studied extensively. Recently, the green fluorescent protein (GFP) has been used to visualize in real time various proteins as fusion proteins. Using this system, 5-LO was shown to utilize a monopartile nuclear localization signal (NLS) at its N-terminus[92] and a bipartite NLS at its C-terminus[93] for the translocation to the nucleus. Regardless of the 5-LO localization in either cytoplasm or nucleoplasm, cell activation that elevates intracellular calcium initiates translocation of 5-LO to the perinuclear envelope.

Although the expression of 5-LO is limited to hematopoetic cells, LTA_4 hydrolase is ubiquitously expressed.[94] In inflammation, however, enhanced LTB_4 production is reported both in leukocytes and other cells. Regulation of LT biosynthesis also involves transport of LTA_4 from the perinuclear region of the synthetic cells, through the plasma membrane, ultimately into a completely different cells containing either LTC_4 synthase or LTA_4 hydrolase. This is the process of transcellular biosynthesis and is known to occur rather efficiently between certain cells (Figure 7).[95] However, very little is known about the transport of substrates, including AA, specifically within cells such as the human neutrophil that can synthesize LTs. Calcium ion is necessary for the 5-LO action, but the precise basis for this requirement remains elusive. Recently, however, the N-terminus of 5-LO was reported to bind calcium via a putative β-barrel domain with a stoichiometry of 1−2 mol of calcium per mole of 5-LO.[96] Another approach to clarify the mechanism of the translocation and activation of 5-LO has involved isolating 5-LO interacting proteins by yeast two-hybrid experiments. Screening of a lung cDNA library with 5-LO as a bait resulted in the isolation of three putative 5-LO interacting proteins.[97] Among them, CLP protein is homologous to coactosin, an actin-binding protein of *Dictyostelium discoideum*; thus, 5-LO may move along the cytoskeleton by forming a protein−protein complex with CLP. The enzymatic addition of molecular oxygen to polyunsaturated fatty acids is a reaction that occurs in plants as well as humans.[67] Much information about the three-dimensional structure of LOs, e.g., soy bean 15-LO,[98,99] a coral 8-LO,[100,101] and rabbit reticulocyte 15-LO,[102] has been reported.

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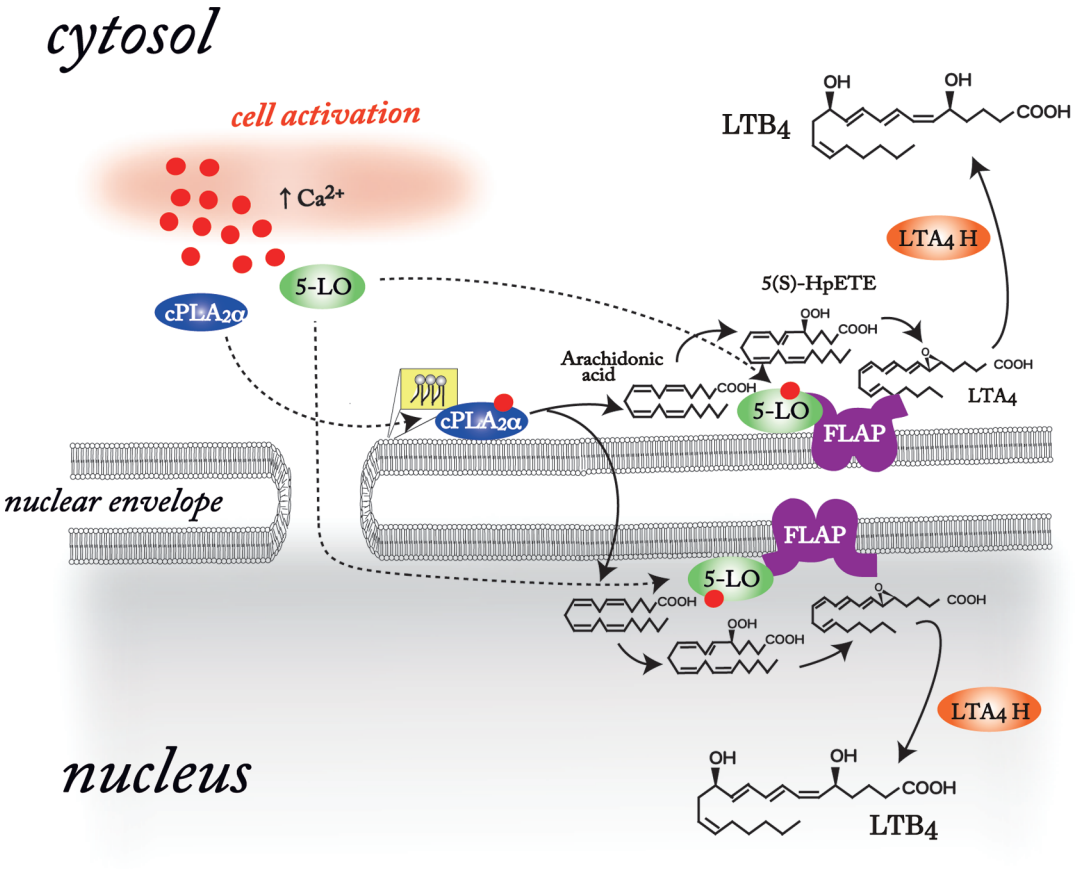

Figure 6. Intracellular organization of the pivotal enzymes involved in LT biosynthesis. Reorganization includes translocation of cPLA$_2$α and 5-LO to the perinuclear region, where FLAP also resides, as a result of the elevation of intracellular calcium. In some cells, 5-LO and LTA$_4$ hydrolase (LTA$_4$H) are found within the nucleoplasm. The elevation of intracellular calcium causes the translocation of 5-LO to the inner envelope of the dual nuclear bilayer. After conversion of the liberated AA into LTA$_4$, LTB$_4$ is then synthesized in both cytosol and nucleoplasm.

Interestingly, all these LOs have similar three-dimensional structures in spite of only modest amino acid sequence homology. Most recently, Newcomer et al. presented the crystal structure at 2.4 Å resolution of human 5-LO.[103] For this analysis, they created the mutant 5-LO, which has the mutations of Lys residues in the C-terminal, resulting in the increased enzymatic stability. These data provide a context for the development of 5-LO blockers and clarify a molecular model for the LT synthesis together with the structural imformation of FLAP[73] and LTC$_4$ synthase.[104,105] In contrast to 5-LO, there is limited information on the activation and translocation of LTA$_4$ hydrolase. The ubiquitous expression of LTA$_4$ hydrolase suggests that this enzyme is constitutively expressed and awaits the substrate provided by 5-LO. AA and AA-derived intermediates such as LTA$_4$ are permeable across plasma membranes, allowing transcellular metabolism of eicosanoids. Intriguingly, 5-LO-null mice transplanted with immune cells deficient in either LTA$_4$ hydrolase or LTC$_4$ synthase produce near normal quantities of LTB$_4$ and cys-LTs, respectively. These results demonstrate that 5-LO-containing immune cells transfer the intermediates that restore LT synthetic capacity by transcellular metabolism and that structural cells play an important role in LT synthesis.[106] LTA$_4$ hydrolase has two interesting characteristics, i.e., a bifunctional zinc-containing enzyme with epoxide hydrolase and aminopeptidase activities and suicide inactivation.[107,108] LTA$_4$ hydrolase has structural similarities to other zinc metalloproteases, and the purified recombinant enzyme contains aminopeptidase activity against synthetic peptide substrates in vitro.[109−111] LTA$_4$ hydrolase is inactivated by its substrate LTA$_4$ (suicide inactivation).[112,113] Although the molecular mechanism of the enzyme inactivation remains to be clarified, Tyr378 was reported to be important for LTA$_4$ binding and subsequent inactivation of this enzyme.[114] Three important reports appeared on LTA$_4$ hydrolase. One is the regulation of the activity by phosphorylation. Rybina et al. found that Ser415 of LTA$_4$ hydrolase is phosphorylated under basal conditions in endothelial cells, and this phosphorylation inhibits epoxide hydrolase activity but does not affect the aminopeptidase activity of LTA$_4$ hydrolase.[115] This finding is well consistent with the findings that the catalytic centers of epoxide hydrolase and aminopeptidase of LTA$_4$ hydrolase overlap but are different.[116,117] Nonetheless, the biological importance of the phosphorylation is still unclear. The second finding is the cloning of an LTA$_4$ hydrolase-like molecule from *Saccharomyces cerevisiae*,[118] predicting the existence of other LT biosynthetic enzymes. This cloned enzyme is 42% identical to human LTA$_4$ hydrolase and exhibits epoxide hydrolase activity, i.e., conversion of LTA$_4$ into 5(*S*), 6(*S*)-diHETE. This yeast enzyme also possesses significant aminopeptidase activity, which is enhanced in the presence of LTA$_4$, demonstrating that the lipid-binding site is located at its active center. The crystal structure of human LTA$_4$ hydrolase, with a catalytic domain highly related to thermolysin and a C-terminal domain with armadillo-like repeats, suggests an interesting

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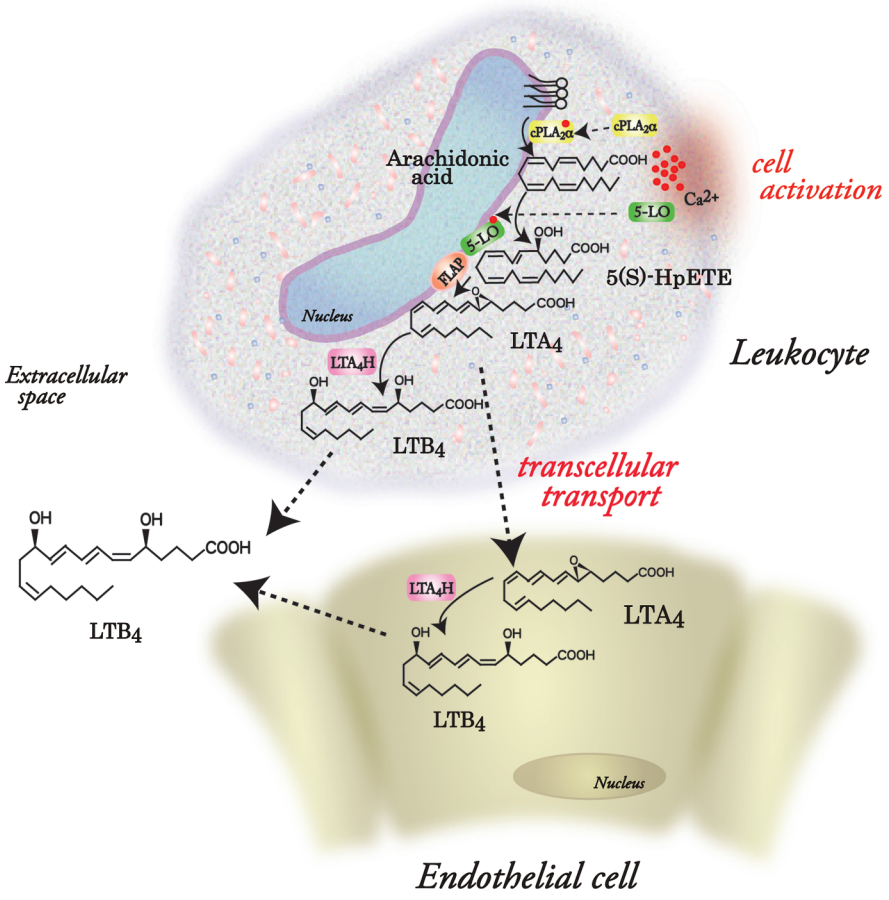

Figure 7. Biosynthesis pathways of LTB_4. AA released from membrane phospholipids is metabolized by 5-LO in the presence of FLAP first to 5(*S*)-HpETE and then to LTA_4. LTA_4 is hydrolyzed by LTA_4 hydrolase (LTA_4H) to form LTB_4 in the cytoplasm. LTB_4 is further generated in the nucleus (Figure 6). LTA_4 is converted to LTB_4 intracellularly and is exported for the transcellular synthesis.

evolutionary heritage.[119] The third important finding is the critical role of this enxyme in limiting chronic pulmonary neutrophilic inflammation. Currently, Snelgrove et al. identified the neutrophil chemoattractant proline-glycine-proline (PGP) as a substrate for LTA_4 hydrolase. PGP is known as a biomarker for chronic obstructive pulmonary disease (COPD) and is involved in neutrophil persistence in the lung. In acute neutrophil-driven inflammation, PGP is normally degraded in the lungs by LTA_4 hydrolase, which is released from neutrophils and epithelial cells, leading to the resolution of inflammation. In contrast, cigarette smoke, a major risk factor for the development of COPD, selectively inhibited the aminopeptidase activity of LTA_4 hydrolase, resulting in the accumulation of PGP and neutrophils.[120] These findings imply that therapeutic strategies blocking LTA_4 hydrolase to prevent LTB_4 generation would be less effective because of elevated levels of PGP.

3.2. Metabolism of LTB_4

3.2.1. ω-Oxidation of LTB_4. Although several inactivating pathways for LTB_4 are known, ω-oxidation of LTB_4 by human neutrophils has been studied most extensively. Neutrophils metabolize LTB_4 into 20-hydroxy-LTB_4 (20-OH-LTB_4) by LTB_4 ω-hydroxylases and then 20-carboxy-LTB_4 (20-COOH-LTB_4) (Figure 8).[121] Initially, two isozymes of LTB_4 ω-hydroxylases, which belong to the membrane-bound cytochrome P450 family 4F3 (CYP4F3), have been cloned.[122−124] So far, more than 14 different types of this enzyme have been identified, including five human CYP4F isoforms.[125] The neutrophil enzymes are CYP4F3A and its splice variant CYP4F3B, which metabolize LTB_4 with K_m values of 3.9 and 90 μM, respectively.[123,126,127] The expressions of these enzymes are enhanced by stimulation with various reagents. For example, in human neutrophils CYP4F3A is induced by retinoic acid and phorbol ester.[125] Since 20-OH-LTB_4 binds to the LTB_4 type-1 receptor, BLT1, with similar affinity for LTB_4 (K_i of 0.54 nM compared with 0.7 nM for LTB_4),[128] ω-oxidation itself does not inactivate LTB_4. Further oxidation of the ω-hydroxy moiety to 20-COOH-LTB_4, an inactive metabolite, by the neutrophils can be catalyzed by CYP4F3A, which proceeds in two steps via the 20-oxo-LTB_4 intermediate.[129] An alternative pathway for subsequent ω-oxidation of 20-OH-LTB_4 was found to involve ADH (alcohol dehydrogenase) and AldDH (aldehyde dehydrogenase) in other tissues, e.g., liver.[130]

3.2.2. β-Oxidation of LTB_4. The major metabolites of eicosanoids are produced by mitochondrial and peroxisomal β-oxidation. For this metabolism, LTB_4 requires initial ω-oxidation to form ω-COOH-LTB_4, and CoA ester formation proceeds from the newly formed ω-carboxy group. The biochemical mechanism has not been clarified but perhaps relates to the affinity of both of these carboxylic acids for the enzymes responsible for CoA ester synthesis. Moreover, LTB_4 has a C-5 hydroxy group as opposed to PGs, which have a double bond at

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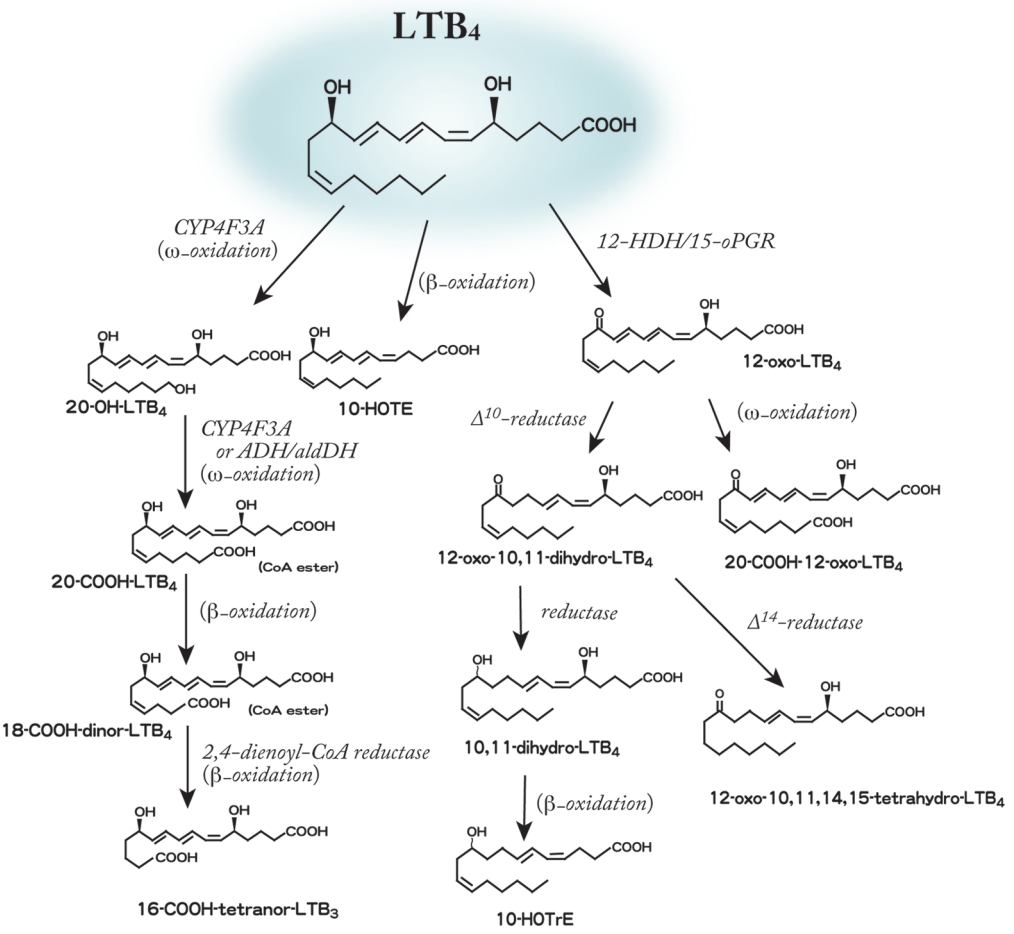

Figure 8. Metabolism of LTB_4. The pathway is catalyzed by CYP4F3A in human neutrophils to form 20-OH-LTB_4, which can be oxidized further either by the P450 enzyme or by ADH and AldDH to form 20-COOH-LTB_4. β-Oxidation of 20-COOH-LTB_4 as a CoA ester leading to 18-COOH-dinor-LTB_4 and 16-COOH-tetranor-LTB_3, which have been found as urinary metabolites. LTB_4 is further metabolized by the 12HDH/15oPGR pathway to the reduced metabolite 10,11-dihydroxy-LTB_4, which was found to be β-oxidized after C-1 CoA ester formation, ultimately forming 10-HOTrE.

C-5. Formation of CoA esters of ω-COOH-LTB_4 has not been studied in detail, even if these derivatives are the critical intermediates for β-oxidation. 20-COOH-LTB_4 is metabolized via the peroxisomal and the mitochondrial β-oxidation pathways. The steps of chemical transformation that take place in peroxisomes start with 20-COOH-CoA esters, which are β-oxidized to a Δ^{18}-intermediate, catalyzed by acyl-CoA oxidase. This is followed by hydration of the C-18 double bond and then oxidation to form the 18-oxo-CoA ester by the peroxisomal bifunctional enzyme having both CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities. The penultimate carbon atoms of LTB_4 are then removed by 3-oxoacyl CoA thiolase, yielding the CoA ester of 18-COOH-LTB_4 (Figure 8). This process is repeated for a second round of β-oxidation. The insertion of a new double bond adjacent to the 18-carboxy-CoA ester results in a conjugated diene involving the double bond at carbon atoms 14−15 in LTB_4. The peroxisomal enzyme 2,4-dieneoyl-CoA reductase reduces this diene to a monoene at C-16. This multifunctional enzyme also has Δ^3,Δ^2 acyl-CoA isomerase activity,[131] which moves the Δ^{16} double bond to Δ^{17}, as required for subsequent β-oxidation to proceed. The removal of the two terminal carbon atoms from 18-COOH-LTB_4 as acetyl-CoA leads to generation of the metabolites 16-COOH-tetranor-LTB_3.

3.2.3. Metabolism of LTB_4 by 12-Hydroxydehydrogenase/15-Oxo-prostaglandin-13-reductase. LTB_4 is also inactivated by the action of 12-hydroxydehydrogenase (12-HDH) (Figure 8).[132,133] The metabolite, 12-oxo-LTB_4, is at least 100 times less potent than LTB_4 in stimulating calcium mobilization.[132] Recently, 15-keto-prostaglandin 13-reductase (15-oPGR) was identified and shown to be identical to 12-HDH.[134] The 12-HDH/15-oPGR pathway for LTB_4 metabolism is fairly specific in human and porcine tissues and was first discovered in studies of LTB_4 metabolism in porcine leucocytes, where several metabolites that did not retain the conjugated triene structure typical for LTs were found.[135] The distribution of this enzyme is more ubiquitous than that of CYP4F3, with the highest expression in liver and kidney. The expression was shown to be inducible in rat liver upon administration of a carcinogen, dithiolethione.[136] LXA_4 is also metabolized by the action of this enzyme, suggesting that this enzyme is functional against various eicosanoids. This enzyme appears to recognize the structural motif [R−CH(OH)−(*trans*)-CH═CH−R′]. This pathway had three separate structural conversions of the starting substrate. The first step is an oxidization of the 12(*R*)-hydroxyl group of LTB_4 to the 12-oxo moiety catalyzed by 12-HDH/15-oPGR. The mechanism of this oxidation has been demonstrated following elucidation of the X-ray crystallographic structure of guinea pig 12-HDH/15-oPGR.[137]

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The product of 12-hydroxy-oxidation is a conjugated ketone with a structural motif [R−CO−(*trans*)-CH═CH−R′] common to many LTs and PGs and as such is a structural unit that can be reduced in two steps to [R−CH(OH)−CH_2CH_2−R′]. 12-HDH/15-oPGR is known as a bifunctional enzyme, which performs the reduction of 15-oxo-PGs into 15-oxo-13,14-dihydro-PG metabolites. However, it is still unclear whether the human or porcine enzyme can reduce 12-oxo-LTB_4 to 12-oxo-10,11-dihydro-LTB_4 or whether a separate reductase carries out this step. Interestingly, human keratinocytes metabolize LTB_4 by the 12-HDH/15-oPGR pathway and convert into a rather unusual metabolite, 10-HOTrE (10-hydroxyoctadecatrienoic acid), suggesting that a C-1 CoA ester can be formed from 10,11-dihydro-LTB_4.[138] In addition, because very few of the observed metabolites are ω-oxidized, there seems to be a low CYP4F activity in the human keratinocytes. The 6-*trans*-LTB_4 isomers formed by nonenzymatic hydrolysis of LTA_4 have low affinity for the CYP4F3A in human neutrophils, which catalyzes ω-oxidation of LTB_4. However, human neutrophils have an enzymatic capacity to oxidize the 6-*trans*-LTB_4 isomers into 5-oxo-Δ^6-*trans*-LTB_4 isomers that are reduced further to the motifs [R−CO−CH_2CH_2−R′] and [RCH(OH)−CH_2CH_2−R′].[139] This metabolism is similar to the 12-HDH/15-oPGR pathway described before. Further studies are needed to address whether other proteins are responsible for these metabolic conversions. Liver also has the LTB_4 β-oxidation activity, which is important for the LTB_4 inactivation by shortening the carbon backbone of LTB_4.[140] Thus, the metabolic fates of LTB_4 seem to differ among the cells and tissues where LTB_4 is biosynthesized.

3.3. Existence of LTB_4 Receptors

As early as 1975, certain monohydroxy eicosanoids, e.g., 5(*S*)-HETE, 12(*S*)-HETE, and 15(*S*)-HETE, were shown to be neutrophil chemoattractants.[141−143] In addition, several derivatives of LTB_4, i.e., 12-oxo-LTB_4 and 20-OH-LTB_4, also have the chemoattractant activity.[132,133] However, these lipids require higher concentrations than LTB_4 to exert their activities.[59] Thus, the differences in relative potencies between LTB_4 and other derivatives facilitated the identification and classification of the specific receptors. Radioligand binding studies using various tissues and cells, e.g., human leukocytes and guinea pig lung and eosinophils, provided more significant evidence for the existence and distribution of the specific receptors for LTB_4. However, the LTB_4 receptors had not been identified until 1997. In 1997 and 2000, two types of LTB_4 receptors, i.e., BLT1 and BLT2, were identified.[144,145] These findings are very meaningful not only for the characterization of each receptor but also the development of specific antagonists to regulate these GPCRs.

3.4. LTB_4 Receptor Type-I (BLT1)

3.4.1. Molecular Cloning of BLT1. In human and mouse, spleen membranes have been shown to contain LTB_4 binding sites. Various attempts were made to identify the LTB_4 receptors, such as purification of the receptor,[146,147] including photoaffinity labeling of the protein,[148] generation of a monoclonal antibody for the LTB_4 binding sites,[149] and expression cloning using *Xenopus laevis* oocytes and mammalian cells (unpublished). The cloning and characterization of the first LTB_4 receptor, termed BLT1, were achieved in our laboratory by a cDNA subtraction technique using human promyelocytic leukemia cell line (HL-60 cells), which were differentiated into granulocytic cells by retinoic acid.[144] The human BLT1 receptor was identified as a putative seven transmembrane receptor with 352 amino acids (Figure 9). The characteristics of BLT1 are summarized in Table 1. BLT1 shares low homology to P2Y receptor group and belongs to a family of receptors for chemoattractants including complement receptors and a recently identified novel PGD_2 receptor, CRTH2 (Figure 5).[150] In human, BLT1 is exclusively expressed in inflammatory cells, e.g., neutrophils,[151] eosinophils,[152] dendritic cells,[151] macrophages,[153] B-cells,[154] and T-cells.[151,155−157] Human spleen and thymus also express BLT1 mRNA, but the amounts are much lower than that of peripheral leukocytes.[5,144] In mouse, the abundant expression of BLT1 was found in eosinophils and casein-elicited peritoneal macrophages, but the precise analysis of BLT1 expression in various subpopulations of leukocytes has not been reported.[152] BLT1 expression in lymphoid tissues was not observed in rat[158] and guinea pig.[159] However, BLT1 expression is enhanced in the proteose peptone-induced peritoneal cells (most of them macrophages), although residential peritoneal macrophages express a small amount of BLT1 mRNA.[158] BLT1 mRNA is also expressed at high levels in IL-5-treated eosinophils and to a lesser extent in IFNγ-stimulated macrophages.[152] Moreover, the expression of BLT1 is greatly enhanced during the differentiation of naive T-cells into $CD4^+$ T-cells or $CD8^+$ T-cells in vitro.[155−157] Nonmyeloid cells, such as vascular smooth muscle and endothelial cells, skeletal muscle satellite cells, and neural stem cells, also express functional BLT1.

3.4.2. Binding Affinities of Natural Ligands to BLT1. The membranes of human BLT1-expressing COS-7 cells exhibit [^{3}H]LTB_4 binding with a K_d of 0.15 nM, comparable to the K_d of 0.14 nM for the differentiated HL-60 cells.[144,160] Various eicosanoids also compete for the [^{3}H]LTB_4 binding to the COS-7 membranes expressing human BLT1, and the potency (K_i values) ranking is LTB_4 (0.38 nM) > 20-OH-LTB_4 (7.6 nM) = 12-oxo-LTB_4 (7.6 nM) > 12(*R*)-HETE (30 nM) > 20-COOH-LTB_4 (190 nM). This profile is consistent with the previously characterized LTB_4 binding sites in human granulocytes.[161−164]

3.4.3. Signal Transduction via BLT1. Although high-affinity binding of LTB_4 to BLT1 is found essentially in leukocytes and macrophages, the G-proteins associated with the functions in these cells have not been clearly established. The intracellular signaling pathways for BLT1 may depend on the G-proteins expressed in the different cells. For example, most of the LTB_4-dependent signals in granulocytes appear to be mediated by $G\alpha_i$ (granulocytes express mainly $G\alpha_{i2}$), whereas in the nervous system $G\alpha_{i1}$ and $G\alpha_o$ are abundantly present.[165] In several cell types, LTB_4 signals via BLT1-$G\alpha_i$-proteins are inhibited by pretreatment with pertussis toxin (PTX). Though chemotaxis and inhibition of adenylyl cyclase by LTB_4 are completely PTX-sensitive in CHO-BLT1 cells, LTB_4-elicited intracellular calcium mobilization in CHO-BLT1 cells is not inhibited by PTX, demonstrating the coupling with $G\alpha_q$-like proteins in these cells. The coupling of BLT1 with various Gα-subunits was further examined by cotransfection studies using COS-7 cells, and BLT1-mediated phospholipase C activation was shown to be mediated by $G\alpha_{16}$- and G$\beta\gamma$-subunits released from $G\alpha_i$.[166]

The LTB_4−BLT1 pathway activates a number of kinases that are involved in the phosphorylation of downstream signaling proteins and the receptor desensitization.[17] For example, activation of mitogen-activated protein kinases (MAPKs) is important for the LTB_4-induced proliferation of RAW 264.7 macrophages and bronchial smooth muscle cells.[167,168] Extracellular signal-regulated kinases (ERKs) may also potentially be involved in LTB_4-induced proliferation, as demonstrated in vascular smooth

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                                    I
hBLT1     1  M--NTTSSAAPPSLGVEFISLLAIILLSVALAVGLPGNSFVVWSILKRMQK--RSVTALM  56
hBLT2     1  .SVCYRP.GNETL.SWKTSRATGTAF.LL.ALL.....G.....LAGWRPARG.PLA.T.  60

               II                                  III
hBLT1    57  VLNLALADLAVLLTAPFFLHFLAQGTWSFGLAGCRLCHYVCGVSMYASVLLITAMSLDRS  116
hBLT2    61  ..H.....G....LT.L.VA..TRQA.PL.QA..KAVY...AL........TGLL..Q.C  120

                                  IV
hBLT1   117  LAVARPFVSQKLRTKAMARRVLAGIWVLSFLLATPVLAYRTVVPWKTNMSLCFPRYPSEG  176
hBLT2   121  ...T...LAPR..SP.L...L.LAV.LAAL...V.AAV..HLW----RDRV.QLCH..PV  176

                    V                                          VI
hBLT1   177  HRAFHLIFEAVTGFLLPFLAVVASYSDIGRRLQARRFRRSR---RTGRLVVLIILTFAAF  233
hBLT2   177  .A.A..SL.TL.A.V...GLMLGC..VTLA..RGA.WGSG.HGA.V....SA.V.A.GLL  236

                                        VII
hBLT1   234  WLPYHVVNLAEAGRALAGQAAGLGLVGKRLSLARNVLIALAFLSSSVNPVLYACAGGGLL  293
hBLT2   237  .A...A...LQ.VA...PPEGA.AKL.GAGQA..AGTT....F.........VFTA.D..  296

hBLT1   294  RSAGVGFVAKLLEGTGSEASSTR-RGGS--LGQTAR-----SGPAALEPGPSESLTASSP  345
hBLT2   297  PR..PR.LTR.F..S.EARGGG.S.E.TME.RT.PQLKVVGQ.RGNGD..GG----MEKD  351

hBLT1   346  LKLNELN   352
hBLT2   352  GPEWDL-   358
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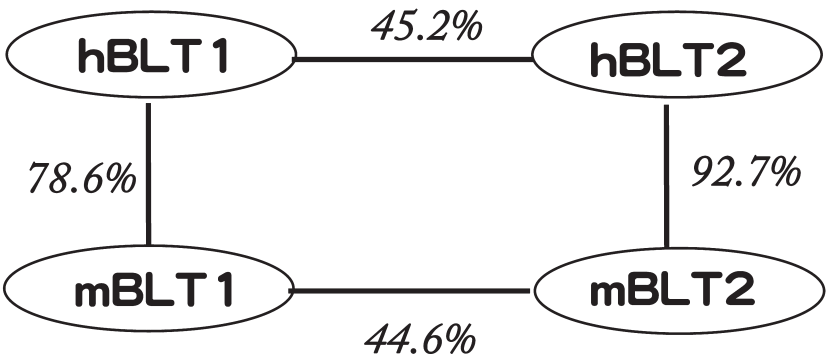

Figure 9. Amino acid sequence alignment of human BLT1 and BLT2. The putative transmembrane domains predicted from a Kyte−Doolittle hydrophobicity analysis are labeled as I−VII. (Upper) Conserved amino acids are indicated by periods (.), and gaps in the alignment are indicated by dashes (−). (Lower) The relative sequence homologies (percentage identities) among human and murine of BLT1 and BLT1 are shown.

muscle cells[169] and in the signaling leading to delayed neutrophil apoptosis.[170] Wortmannin, a phosphatidylinositol 3-kinase (PI3K) inhibitor, was further reported to block LTB_4-induced chemotaxis, suggesting the importance of this kinase, while calcium mobilization remains intact.[171,172] Moreover, tyrosine kinases have been implicated in LTB_4-signaling in neutrophils[173] and BLT1-transfected HeLa cells.[172] Recently, Okamoto et al. reported that BLT1 is necessary for the common γ-chain of Fc receptor (FcγRs) dependent macrophage phagocytosis.[174] They found the crosstalk between LTB_4−BLT1 and FcγRs signaling pathway at the level of PI3K and Rac, downstream of Syk.

With respect to the effects on transcription in monocytes, stimulation with LTB_4 elicits the transcriptional activation of the IL-6 gene.[175] A reporter gene assay identified two core regions within the IL-6 promoter containing binding sites for NF-IL6 and NF-κB, which are critical for this response. Because exogenous addition of LTB_4 increases the DNA binding of these transcription factors in monocytes,[175,176] and the LTB_4-induced NF-κB DNA binding is abolished by CP-105,696,[176] the transcriptional alterations by LTB_4 is transduced via BLT1. This transcriptional activation by LTB_4 seems to be through LTB_4-induced intracellular H_2O_2 formation,[177] since the oxidant scavenger *N*-acetyl-L-cysteine (NAC) completely blocks the LTB_4-mediated transcription factor binding in monocytes.[175] However, another group reported that other antioxidants do not show the effects on IL-6 secretion.[178]

3.4.4. Gene Structure and Transcriptional Regulation of BLT1. Human BLT1 gene consists of three exons located in a region of 5 kbp on chromosome 14q11.2-q12, with the ORF in the last exon (exon-III) (Figure 10).[179] We previously determined the transcriptional start site (TSS) on human BLT1 gene and found the existence of the basal promoter activity at 100 bp upstream from the TSS.[145,179] EMSA (electrophoretic mobility shift assay) and mutagenesis studies revealed that a transcriptional factor, Sp1, binds to the BLT1 core-promoter and plays a crucial role in the basal transcription of human BLT1. Some leukocyte-specific genes contain GC-rich promoters, which are methylated in nonleukocyte cells. The promoter region of BLT1 is enriched in GC sequences and highly methylated in non-BLT1-expressing cells, but not in BLT1-expressing cells. Since

Table 1. Characteristics of BLT1 and BLT2

	BLT1	BLT2
structure (amino acids)	human (352)	human (358)
	mouse (351)	mouse (360)
	rat (351)	rat (359)
accession number (GenBank)	human (D89078)	human (AB029892)
	mouse (AF044030)	mouse (AB029893)
	rat (AB025230)	rat (AB052660)
ligand	LTB_4 > 20-OH-LTB4 > 12(*R*)-HETE	12HHT ≫ LTB_4 > 12(*S*)-HETE > 12(*S*)-HpETE > 15(*S*)-HETE
antagonist	CP105696, CP195543, U75302, ONO4057, ZK158252, Biomed-101, RO5101576	CP195543, ONO4057, ZK158252, LY244283, Biomed-101, RO5101576, Compound-A (agonist)
expression (human)	leukocytes ≫ thymus, spleen	spleen > ovary, liver, leukocytes > ubiquitous
coupled G-protein	G_{16}, G_{i2}	G_q-like, G_i-like, G_z-like
chromosome	human (14q11.2-q12)	human (14q11.2-q12)
	mouse (14 C2)	mouse (14 C1)
	rat (15p13)	tat (15p13)

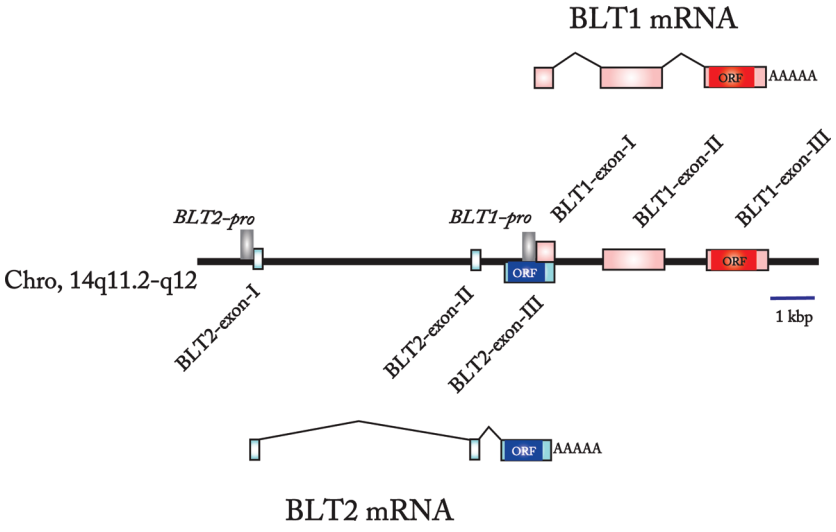

Figure 10. Structure of the human genes for BLT1 and BLT2. Transcribed segments are indicated by boxes and putative ORFs by filled boxes. Note that the promoter region of BLT1 overlaps the ORF of BLT2. The sequence data are available from EMBL/GenBank/DDBJ under accession nos. AB029892 (hBLT2 mRNA), D89079 (hBLT1 mRNA), and AB008193 (Genome sequence).

Sp1 is a ubiquitously expressed transcriptional factor, the methylation status of CpG dinucleotides in the promoter region could be of crucial importance for the cell-specific expression of BLT1. However, the precise mechanism of the enhanced BLT1 expression in leukocytes is to be elucidated. For example, during the differentiation of naive T-cells into $CD4^+$ T-cells or $CD8^+$ T-cells, the BLT1 expression is strongly enhanced.[157,180] A significant increase in BLT1 expression has also been reported in dexamethasone- or LTB_4-treated human neutrophils.[181] Moreover, in HL-60 cells the expression of BLT1 is markedly increased by stimulation with various stimuli, e.g., retinoic acid and dimethyl sulfoxide.[144,182] Recently, we found an activated region by retinoic acid in human BLT1 gene, termed AE-BLex, at the intron-I:exon-II boundary (Figure 11).[183] AE-BLex acts as an enhancer for the BLT1 promoter and possesses two acute myeloid leukemia 1 (AML1; also known as Runx1) recognition sites. We demonstrated that the enhancement of the BLT1 expression during the retinoic acid-induced differentiation of HL-60 cells is due to a loosening of the chromatin structure around AE-BLex, which leads to the incremental binding of AML1. The AML1/AE-BLex complex was further confirmed in other BLT1-expressing leukemia cell lines and human peripheral leukocytes; thus, AML1 enhances the BLT1 expression by binding to AE-BLex, which is accessible in leukocytes. It has been well-established that AML1 plays a pivotal role in the transcriptional regulation of many genes involved in hematopoietic development, implying that AML1 is required for lineage- and stage-specific aspects of adult hematopoiesis.[184,185] Hence, AML1 is also an important factor in the enhanced expression of BLT1, a crucial gene in the activation of the immune and inflammatory responses.

3.5. Structural Features of BLT1

3.5.1. Significant Residues for LTB_4 Binding. Sabirsh et al. reported crucial residues involved in ligand binding in transmembrane domain-3 (TM3) and TM5 of human BLT1.[186] They found that the polar carboxylate group of LTB_4 is recognized by the extracellular surface of human BLT1 and the hydrophobic

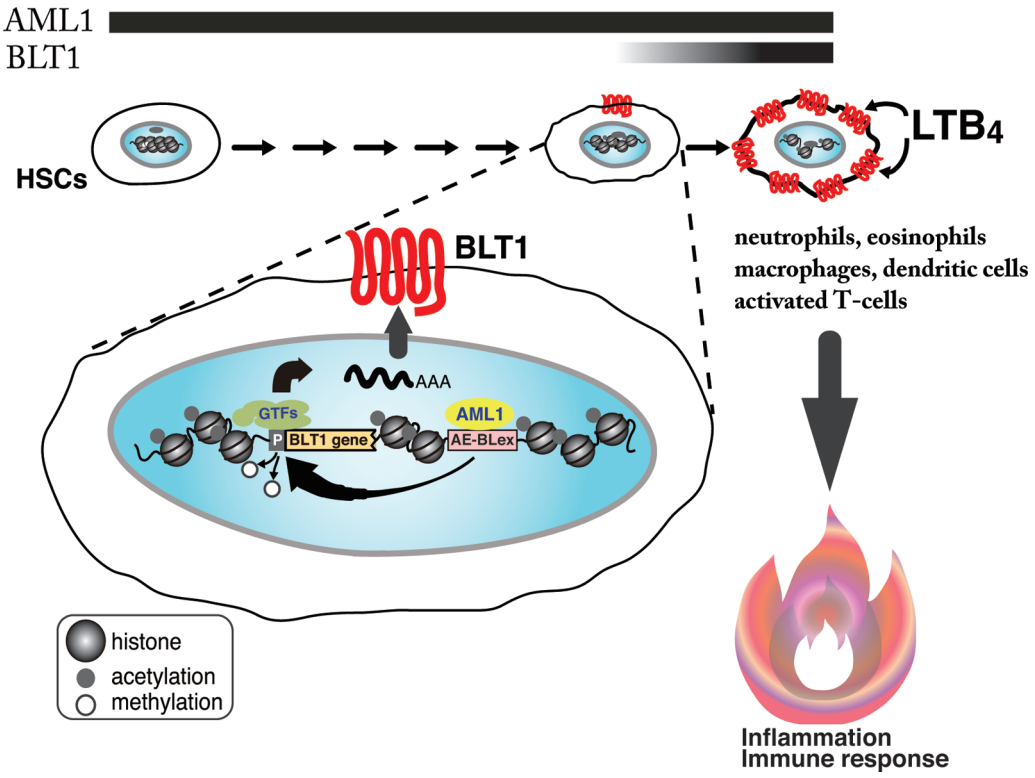

Figure 11. Schematic model of the enhanced expression of BLT1 in leukocytes. The gradation of each bar indicates the expression level of AML1 or BLT1 during hematopoiesis. In HSCs (hematopoietic stem cells), BLT1 gene is silenced due to inactivation of BLT1 promoter and AE-BLex. The chromatin structures of BLT1 promoter and AE-BLex are altered by histone acetylation during the terminal differentiation. Following recruitment to AE-BLex, AML1 facilitates the activity of BLT1 promoter. Upregulated BLT1 expression is crucial for inflammation and the immune response in leukocytes (e.g., neutrophils, eosinophils, activated T-cells, dendritic cells, and macrophages). GTFs, general transcription factors; P, BLT1 promoter.

LTB_4 tail binds to the TM regions of the receptor protein. The carboxylate group and the two hydroxyls of LTB_4 interact with Arg178 and Glu185 in TM5. Residues in TM3, Val105 and Ile108, also line the pocket deeper inside the receptor. Thereafter, Basu et al. examined the ligand binding site and activation mechanism for human BLT1 using in silico molecular dynamics simulations combined with site-directed mutagenesis.[187] Mutations of residues predicted as potential ligand contact points, His94Ala and Tyr102Ala in TM3, Glu185Ala in TM5, and Asn241Ala in TM6, led to reduction of LTB_4 binding affinity. Analysis of Arg residues in extracellular loop 2 revealed that mutating Arg156, but not Arg171 or Arg178, to Ala results in complete loss of LTB_4 binding to human BLT1. Structural models for the ligand-free and ligand-bound states of human BLT1 reveal the activation core formed around Asp64 in TM2, displaying multiple dynamic interactions with Asn36 in TM2, Ser100 in TM3, Asn281 in TM7, and a triad of Ser residues, Ser277, Ser278, and Ser279, in TM7. Mutagenesis of many of these residues in human BLT1 results in loss of signaling capacity while retaining normal LTB_4 binding function. Thus, the polar residues within TM3, TM5, and TM6 and extracellular loop 2 are critical for ligand binding, whereas the polar residues in TM2, TM3, and TM7 play a central role in transducing the ligand-induced conformational change to activation.

3.5.2. Importance of Phosphorylation in BLT1. In an attempt to understand the mechanisms involved in BLT1 desensitization, Gaudreau et al. have reported some initial molecular evidence.[188] They found that the cytoplasmic tail of BLT1 is intimately involved in the desensitization and that the phosphorylation at Thr308 residue in this region is implicated in this phenomenon in COS-7 cells. In contrast, Mollerup et al. observed the ligand-dependent phosphorylation of mouse BLT1 on another site, Ser127, in the second cytoplasmic loop in *X. laevis* oocytes.[189] In general, the GPCR phosphorylation is a flexible and dynamic regulatory process in which GPCRs are phosphorylated in a unique manner that is associated with the cell type. The phosphorylation of GPCRs by specific kinase(s) results in uncoupling of the receptor from G-proteins, followed by the association with the cytoplasmic adaptor, β-arrestins. The receptor$-\beta$-arrestin complex interacts with clathrin and accessory proteins involved in the formation of clathrin-coated pits, ultimately leading to receptor internalization.[190,191] Interestingly, Jala et al. have recently reported the phosphorylation-independent β-arrestin recruitment and internalization of human BLT1.[192] In this study, they generated a phosphorylation-deficient mutant by conversion of all of the Ser/Thr residues in the C-terminal tail of human BLT1 to Ala. Moreover, these Ser/Thr residues were also mutated to Asp/Glu, respectively, to mimic constitutive phosphorylation. The former mutant showed normal β-arrestin recruitment and internalization of the receptor, even with the lack of phosphorylation. In contrast, the latter phosphomimic mutant showed great reduction of BLT1 activation, e.g., decreases in chemotaxis and calcium mobilization, suggesting the importance of the phosphorylation in the signaling.

3.5.3. Importance of the Third Intracellular Region in G-Protein Coupling. Many GPCRs can activate more than two G-proteins. For example, EP3, one of the PGE_2 receptors, couples to $G\alpha_s$ and $G\alpha_o$,[193] PAF receptor activates $G\alpha_i$ and

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$G\alpha_q$,[194,195] and the sphingosine-1-phosphate receptors $S1P_1$ and $S1P_2$ couple to $G\alpha_i$ and $G\alpha_{12/13}$.[196] With respect to BLT1, previous studies have shown that this receptor utilizes different G-proteins, namely, $G\alpha_{i/o}$ and $G\alpha_q$.[144,146] In addition, we and another group have reported that truncation of the cytoplasmic tail of BLT1 does not impair the activation of $G\alpha_i$ and $G\alpha_{16}$.[197,198] We currently performed an extensive mutagenesis study of its intracellular loops to determine the responsible regions of human BLT1 for G-protein coupling.[199] Three intracellular loops (the first intracellular region, i1; second region, i2; and third region, i3) of BLT1 were found to be important for both $G\alpha_i$ and $G\alpha_{16}$ couplings. Interestingly, BLT1 distinguishes $G\alpha_i$ from $G\alpha_{16}$ at the N-terminus of the i3 region, as the lack of this region exhibits greatly reduced 5′-3-*O*-(thio)-triphosphate (GTPγS) binding but intact inositol phosphate accumulation triggered by LTB_4 stimulation.[199] These results suggest that the N-terminus in i3 is required for $G\alpha_i$ activation. A three-dimensional model of human BLT1 suggests that this region forms an extended helical structure proximate to the membrane, which projects toward the cytoplasm in order to interact with $G\alpha_i$. Thus, the extended cytoplasmic helix connected to the TM5 of BLT1 might be a key region for selective activation of $G\alpha_i$ proteins.[200]

3.5.4. Importance of Helix 8 in Inactivation of BLT1. Analysis of the crystal structure of rhodopsin confirmed the presence of seven TM helices and revealed the existence of an eighth helix (H8) that projects at a right angle from the C-terminus of TM7.[201] This short helix is anchored via a palmitoyl group to the cytoplasmic leaflet of the cell membrane, and a hypothetical model has predicted that the H8 interacts with the N-terminal helix of the Gα and Gγ subunits. A comparison of ~180 human rhodopsin-type GPCRs revealed that many of them, including BLT1, bear their H8 domains at the proximal end of their C-terminal tails, although BLT1 lacks the cysteine residue, which is thought to be palmitoylated in many GPCRs. The BLT1 mutants with a truncated or substituted H8 show much higher LTB_4 binding than wild-type (WT) receptor and maintain the high affinity, even in the presence of an excess amount of GTPγS in HEK293 and CHO cells.[197] Consistent with these observations, the mutant receptors show more prolonged intracellular signaling, e.g., intracellular calcium mobilization and metabolic activation, after LTB_4 stimulation. The BLT1 model predicts that a pair of aromatic residues (Tyr285 and Phe300), which are positioned similarly to the conserved Tyr306 and Phe313 pair in rhodopsin, may stabilize the inactive form of the receptor by holding TM7 and the H8 at almost a right angle to each other. Hydrophobic amino acid residues (Val301, Leu304, and Leu305) in the H8 may anchor this helix to the plasma membrane like palmitoylated cysteine. A phosphorylation site (Thr308) is located just after the amphiphilic H8,[188] and phosphorylation of Thr308 is predicted to weaken the interaction between the H8 and the plasma membrane. Gaudreau et al. proposed that Thr308 is involved in GRK6-mediated desensitization of BLT1, suggesting that this phosphorylation may play a role in inactivation of BLT1.[188] Thus, the H8 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, although one will have to wait for the three-dimensional structures of BLT1 and the BLT1−G-protein complex.

3.5.5. Structural Analyses Using Recombinant BLT1. Baneres et al. reported on the production of human BLT1 in *Escherichia coli* in milligram quantities (per liter of bacterial culture) isolated as a functional protein in detergent solution.[202] This has allowed to carrying out the structural characterization of this receptor in solution, as well as of its interactions with LTB_4 or antagonistic molecules.[203] They further demonstrated that formation of a stoichiometrically well-defined complex LTB_4:BLT1 is associated not only with a conformational adaptation of the receptor but also with a change in the conformation of the agonist molecule that is likely to be essential for optimal binding to the receptor. However, to clarify the precise structure of BLT1 by crystallization analysis, establishment of a high-yield expression system of this receptor is necessary. Currently, Hori et al. have established the high yield expression of a glycosylation deficient BLT1 mutant in a *Pichia pastries* expression system for crystallization studies.[204] Due to the artificial lack of post-translational modifications, the BLT1 protein does not require enzymatic treatments for the elimination of the oligosaccharide and the phosphate groups in the purification step, and this allows for a rapid and simple purification scheme, which is highly advantageous for further characterization and structure studies, including crystallization analysis of BLT1.

3.6. LTB_4 Receptor Type-II (BLT2)

3.6.1. Molecular Cloning of BLT2. During the analysis of human and mouse BLT1 genes, we identified a putative ORF for a novel GPCR upstream of BLT1 gene. A blast search shows that this gene is highly homologous to human and mouse BLT1, with the amino acid identities of 45.2% and 44.6%, respectively (Figure 9). This novel receptor was also found in a human genome sequence database and reported to function as a low-affinity receptor for LTB_4.[128,205,206] Thus, this receptor is referred to as a type-II LTB_4 receptor, BLT2.[5] The characteristics of BLT2 are summarized in Table 1. The ORFs of human and mouse BLT2 encode proteins with 358 and 360 amino acids, respectively, with 92.7% amino acid identity.[207] This homology is higher than that of BLT1, which exhibits 78% identity between human and murine, suggesting that BLT2 has been conserved during evolution. Searching EST database revealed that human BLT2 mRNA is expressed in various human tissues, including skeletal muscle, heart, lung, and mammalian gland. Northern blot analyses showed that human BLT2 mRNA is expressed most abundantly in spleen, followed by liver, ovary, and leukocytes, with weak signals detected in most human tissues.[145] The size of the major transcript is 2.5 kb in these tissues, but longer transcripts are also detected.[145] Thus, human BLT2 expression is distinct from BLT1, which is expressed almost exclusively in leukocytes.[144] In mouse, quantitative real time RT-PCR revealed the highest expression in small intestine followed by skin, with low expression in colon and spleen. In contrast, the expression levels of mouse BLT2 in macrophages and neutrophils are under the detection limit. Tissue expression pattern of mouse BLT2 is quite different from that of human BLT2.[179] In mouse skin, BLT2 mRNA was detected in follicular and interfollicular keratinocytes by in situ hybridization.[208]

3.6.2. Binding Affinities of Natural Ligands to BLT2. With respect to the affinity of LTB_4 to human BLT2, K_d values are 23 and 0.17 nM for the membranes of BLT2-expressing HEK293 cells[145] and BLT2-expressing COS-7 cells,[128] respectively. Although there is a difference in the reported K_d values, generally BLT2 is considered as a low-affinity receptor, since the EC_{50} value of LTB_4 required for adenylyl cyclase inhibition and the concentrations required for LTB_4-dependent chemotaxis are higher than those for BLT1 activation.[145,205] As described above,

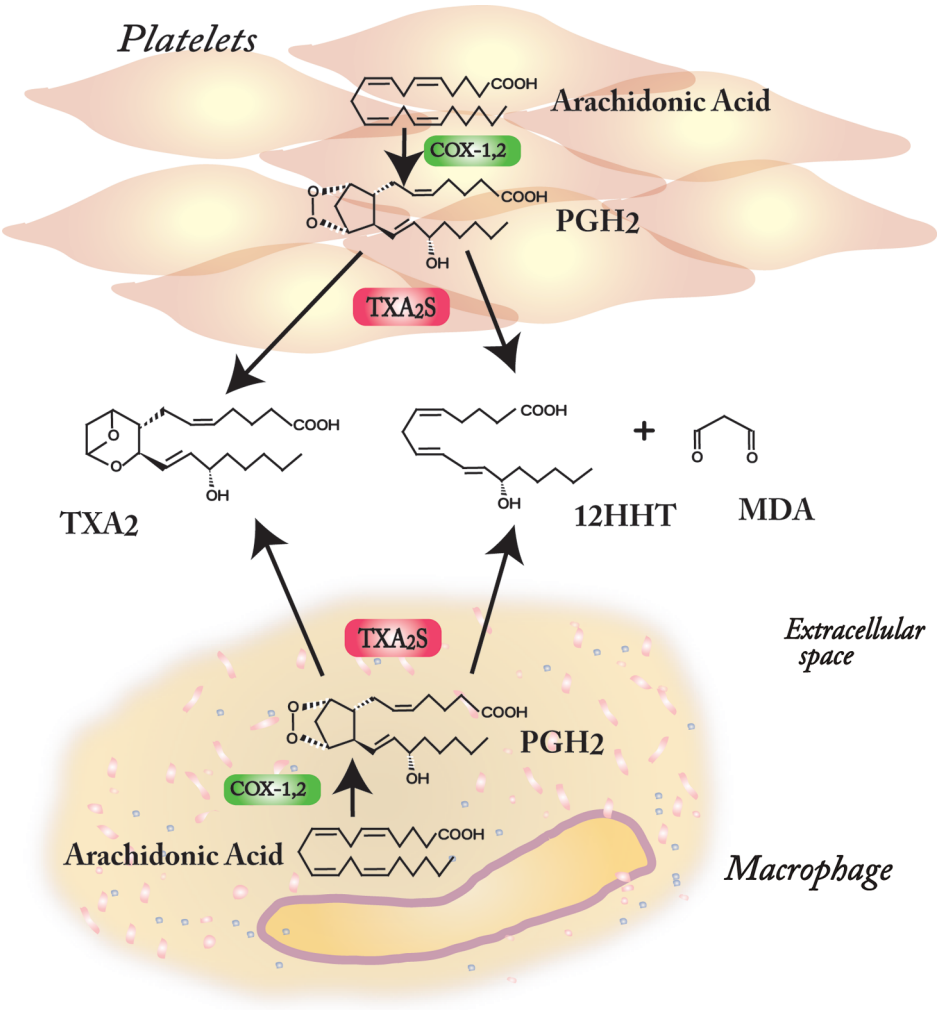

Figure 12. Schematic representation of the production and action of 12-HHT. COX-1, -2, cyclooxygenase-1 and cyclooxygenase-2; 12HHT, 12(*S*)-hydroxyheptadeca-5(*Z*),8(*E*),10(*E*)-trienoic acid; MDA, malondialdehyde; PGH_2, prostaglandin H_2; TXA_2, thromboxane A_2; TXA_2S, TXA_2 synthase.

the highest expression of human BLT2 was found in spleen, followed by leukocytes and ovary. The abundance of low-affinity binding sites for LTB_4 in spleen membrane supports the data showing BLT2 expression in spleen.[209] Of interest, the rank order of potency of several eicosanoids for human BLT2 activation is LTB_4 > 12-epi-LTB_4 > 12(*S*)-HETE > 12(*S*)-HPETE > 12(*R*)-HETE > 20-OH-LTB_4, which is quite different from that of BLT1.[210]

3.6.3. Identification of a Bona Fide Ligand for BLT2. Activated leukocytes, platelets, and macrophages metabolize PGH_2 into thromboxane A_2 (TXA_2) and 12(*S*)-hydroxyheptadeca-5(*Z*),8(*E*),10(*E*)-trienoic acid (12-HHT) in an equimolar ratio by the action of TXA_2 synthase (Figure 12). Although 12-HHT is abundant in tissues and bodily fluids, this lipid has long been viewed as a byproduct lacking any specific function. Recently, we identified 12-HHT as a novel BLT2 agonist in the lipid fractions from rat small intestine.[211] Exogenously expressed BLT2 in mammalian cells is activated by synthetic 12-HHT, as assessed by the activation of intracellular signaling pathways and chemotaxis assay.[211] In membrane preparations from human BLT2-expressing CHO cells, the IC_{50} values of 12-HHT and LTB_4 in the presence of 5 nM [^{3}H]LTB_4 are 2.8 and 25 nM, respectively,[211] suggesting that BLT2 is a high-affinity receptor for 12-HHT and that 12-HHT and LTB_4 occupy the common binding site on BLT2. Bone marrow-derived mast cells (BMMCs) isolated from WT mice migrated toward a low concentration of 12-HHT, whereas BMMCs from BLT2-null mice did not.[211] Hence, 12-HHT is a natural lipid agonist of BLT2 in vivo and induces chemotaxis of mast cells.

3.6.4. Signal Transduction via BLT2. When expressed heterologously in CHO, HeLa, and COS-7 cells, human BLT2 activation leads to the inhibition of adenylyl cyclase and the increase in intracellular calcium. However, the human BLT2 activation is less potent in the calcium mobilization than BLT1 activation.[145] Human BLT2 was also shown to mediate LTB_4-dependent chemotaxis through $G\alpha_i$-like G-proteins.[145,205] Recently, Woo et al. suggested that the activation of human BLT2 by LTB_4 evokes the Rac-extracellular signal-regulatory kinases (Rac-ERKs) pathway followed by the reactive-oxygen-species-mediated chemotaxis in Rat-2 cells.[212] In these cells the generation of the reactive oxygen species by LTB_4 was observed at high concentrations (0.3−1 μM), which are within the range for BLT2 activation. Using mouse BLT2-expressing CHO cells, we further examined several signaling pathways through this receptor. These cells exhibited LTB_4-induced calcium mobilization in a dose-dependent manner (EC_{50} = 170 nM).[208] Activation of

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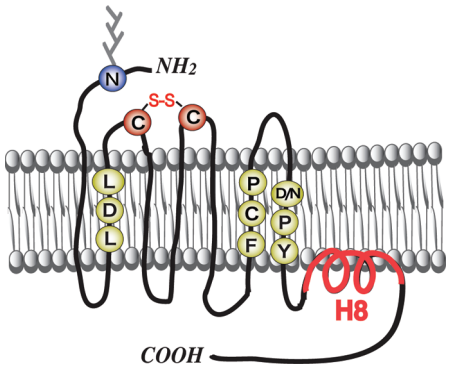

Figure 13. Common characteristics of rhodopsin-type GPCRs. Conserved residues in TM2, TM6 and TM7, and the H8 in the C-terminal tail are represented. Other properties, including disulfide-bond and *N*-glycosylation(s) in the extracellular regions, are shown. The amino acid residues are identified by a single-letter amino acid code.

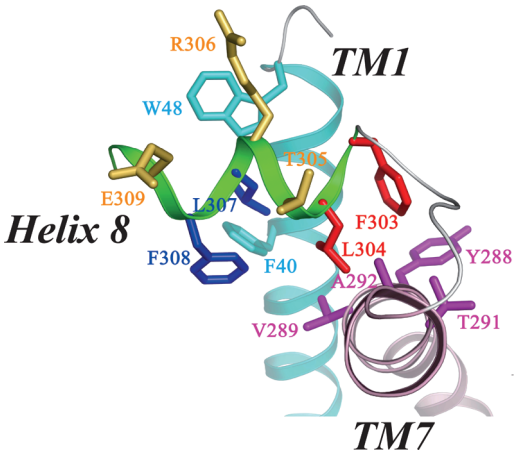

Figure 14. Predicted helix 8 model of human BLT2 based on squid rhodopsin. The atomic model of hBLT2 was constructed based on that of squid rhodopsin (PDB code 2Z73). The H8 is colored green, TM1 is cyan, TM7 is light pink, and the other loop region is gray. Only the side chains related to the interaction with Phe308, Leu304, Leu307, and Phe308 are shown. The side chains interacting with Phe303 and Leu304 (red) are in magenta, and these interacting with Leu307 and Phe308 (blue) are in cyan. The hydrophilic residues of the H8 domain are colored gold. The figure was created using PyMol (http://www.pymol.org). For more detail, see ref 216.

ERKs was also observed in these cells by stimulation with LTB_4. Minimum concentration of LTB_4 required for ERK phosphorylation is 10 nM in mouse BLT2-expressing CHO cells, although 1 nM LTB_4 is enough for similar ERK activations in mouse BLT1-expressing cells. Furthermore, LTB_4 dose-dependently inhibits forskolin-activated adenylyl cyclase in BLT2-expressing CHO cells, with an IC_{50} value of 80 nM. Pretreatment of these cells with PTX abolishes LTB_4-dependent adenylyl cyclase inhibition, suggesting that the mouse BLT2 coupled with $G\alpha_{i/o}$ in inhibiting adenylyl cyclases.[208] In line with these findings in CHO cells, murine BLT2-expressing 300.19 cells show higher affinity for 12-HHT than for LTB_4, and 12-HHT induces intracellular calcium flux with the same efficacy as LTB_4.[213] However, in these cells, 12-HHT is only a weak agonist for chemotaxis with 3% activity relative to LTB_4,[213] suggesting a ligand-specific coupling to chemotaxis.

3.6.5. Gene Structure and Transcriptional Regulation of BLT2. The gene structure for BLT2 has also been established.[5] BLT1 and BLT2 genes are located within 10 kbp of each other on human (chromosome 14q11.2-q12) and mouse (chromosome 14C2) genomes, suggesting that these receptors may be generated by gene duplication (Figure 10). Intriguingly, the exon-III of BLT2 gene containing ORF overlaps the promoter region of BLT1 gene.[5,179] This represents the "promoter in ORF", as has been reported in prokaryotes, but the biological significance of this gene structure is presently not clear.

3.7. Structural Features of BLT2

3.7.1. Pivotal Residues for Endoplasmic Reticulum Export in BLT2. Many GPCRs in the rhodopsin-type family possess several conserved amino acids located in the TMs, e.g., Asp and Leu in TM2; Phe, Cys, and Pro in TM6; and Asp/Asn, Pro, and Tyr in TM7 (Figure 13).[214,215] A three-dimensional structural analysis suggests that changes in these residues are likely to result in misfolding of GPCRs. Previously, we identified several important residues in TM2, TM6, and TM7 of PAF receptor, including Leu59, Asp63, Pro247, Asp289, and Pro290. In particular, mutations of Asp in TM2 (Asp63Ala) and Pro in TM6 (Pro247Ala) lead to a significant decrease in the cell surface expression of the receptors and their accumulation in the endoplasmic reticulum (ER).[216] The importance of the corresponding residues was further confirmed in human BLT2.[216] Namely, mutations of Asp in TM2 (Asp68Ala) and Pro in TM6 (Pro239Ala) of human BLT2 lead to a drastic decrease in the cell surface expression and their accumulation in the ER. Helices bend at Pro residues, and defects in ligand binding due to Pro mutations have been previously reported,[217,218] suggesting the pivotal role in the correct folding of GPCRs. Therefore, it is likely that mutation of Pro in TM6 causes a marked conformational change of the receptor. Consistently, the crystal structure of rhodopsin suggests that the Asp in TM2 makes a hydrogen bond with Asn in TM1 and Ala in TM7;[201] thus, it too might contribute to the correct folding of GPCRs.

3.7.2. Importance of Helix 8 (H8) in Correct Folding of BLT2. Although there are several reports describing the significance of the H8 in GPCR functionality (see section 3.5.4), we currently demonstrated that it is also required for GPCRs to fold properly. Human BLT2 that lacks the H8 (BLT2/Δ*H*8) accumulates in the ER, suggesting that the H8 is necessary to pass the quality control checkpoint mechanism in the ER.[219] A molecular model of BLT2 predicts that Phe303, Leu304, Leu307, and Phe308 form the hydrophobic region of the H8 that interacts with some residues in the TM1 and TM7 of BLT2 (Figure 14). Phe303 may interact with Tyr288 in the last helical turn of TM7, like the inactive forms of bovine rhodopsin, turkey β_1-adrenergic receptor, and human A_{2A} adenosine receptor.[201,220,221] Furthermore, Phe303 may also interact with Thr291-Ala292 (hydrophobic interaction). Leu304 may have a local influence on receptor folding by hydrophobic interactions with Leu307, Phe308, and Thr291-Ala292. Leu307 and Phe308 are predicted to face toward both TM1 and TM7. Because Phe40 and Trp48 in TM1 and Val289 in TM7 are close to Leu307 and Phe308, hydrophobic interactions may be generated among Phe40 and Trp48 in TM1 and Val289 in TM7. These interactions may determine the relative position of TM1 and TM7, and contribute to the formation of the correct structure of BLT2, which is required to pass through the ER quality control.

The surface expression of BLT2/ΔH8 is significantly enhanced by addition of chemical ligands, such as an agonist, Compound-A,

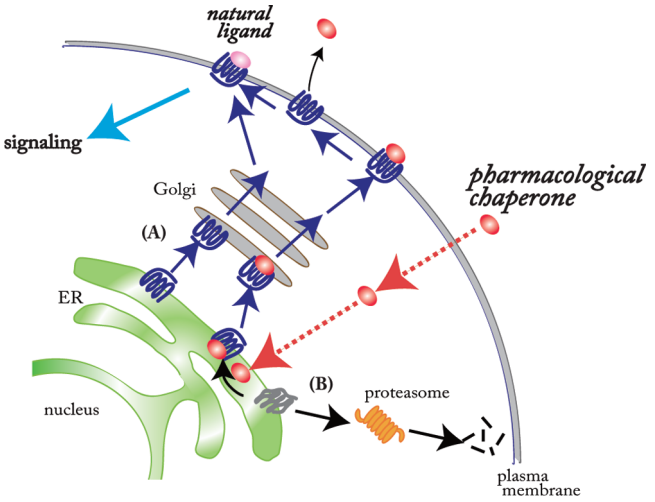

Figure 15. Transport of the ER-accumulated mutant GPCRs by pharmacological chaperones. (A) After synthesis in the ER, correctly folded GPCRs are trafficked to the cell surface via the Golgi. (B) Misfolded GPCRs are retained in the ER by quality control mechanisms. The chaperones are sufficiently hydrophobic to gain access to the ER lumen by passing through the plasma and ER membranes. Thereafter, the chaperones bind misfolded GPCRs and stabilize a conformation that is suitable for transport to the cell membrane. At the cell surface, GPCRs can bind their agonists and evoke signaling via coupled G-proteins.

or an antagonist, ZK 158252, suggesting the action of these ligands as pharmacological chaperones (Figure 15).[219,222] The surface-trafficked BLT2/ΔH8 evokes the intracellular calcium mobilization by stimulation with 12-HHT, with a similar efficiency as the WT receptor; thus, the H8 is not critical for the agonist binding and following G-protein activation.[219] Although the H8-deficient human BLT2 is "functional", it is nevertheless retained by the ER quality control system. These findings suggest that some diseases caused by a deficiency of GPCR trafficking due to a mutation in the H8, e.g., Ile317Thr/human melanocortin-4 receptor, which has been detected in patients with morbid obesity, may be ameliorated by treatment with its specific ligand.[223]

3.8. Synthetic Ligands for BLT Receptors

Several inflammatory diseases, including asthma, chronic obstructive pulmonary disease (COPD), arthritis, and inflammatory bowel disease (IBD), have been associated with elevated levels of LTB_4. The molecular cloning, expression profiling, and pharmacological characteristics of LTB_4 receptors represent a significant milestone in the history of this research field. As a result, pharmacological strategies to modulate the synthesis of LTB_4 (e.g., inhibitions of PLA_2, 5-LO, and FLAP) or the effects of LTB_4 itself (antagonism of BLT receptors) are being developed by several pharmaceutical companies. On the basis of the information, there have been several clinical developments in the use of synthetic LTB_4 receptor antagonists for inflammatory diseases. This section summarizes the characteristics of latest preclinically and clinically developed BLT antagonists for inflammatory diseases and discusses potential future developments.

3.8.1. BIIL-284 (Amelubant). In 1998, the structure of BIIL-284 was disclosed in a patent publication of Boehringer Ingelheim. BIIL-284 {carbamic acid, [[4-[[3-[[4-[1-(4-hydroxyphenyl)-1-methylethyl]phenoxy]methyl]phenyl]methoxy]phenyl]-iminomethyl]ethyl ester} is a prodrug formulated for oral

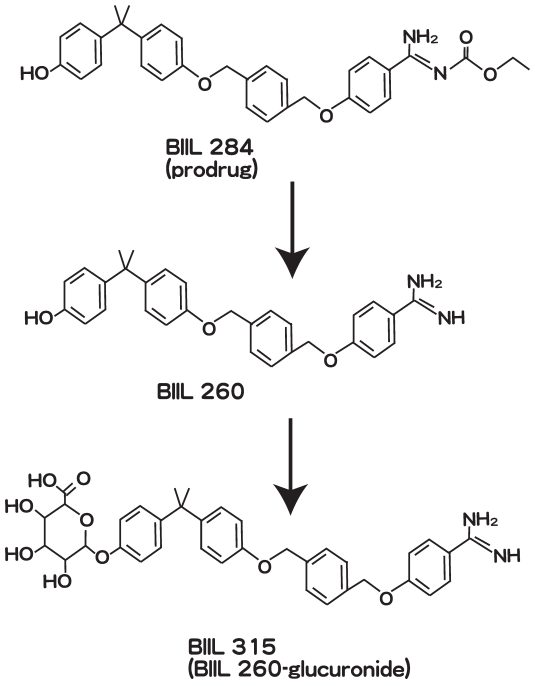

Figure 16. Structures of BIIL 284 and its metabolites.

administration with negligible binding to the LTB_4 receptors. In vivo, BIIL-284 is converted by ubiquitous esterases to the active metabolites BIIL-260, the free guanidine, and BIIL-315 {β-D-glucopyranosiduronic acid, 4-[1-[4-[[3-[[4-(aminoiminomethyl)phenoxy]methyl]phenyl]methoxy]phenyl]-1-methylethyl]phenyl}, the phenoxy α-D-glucuronidate conjugate (Figure 16). The biological activity of these compounds has been extensively described.[224] Due to the prodrug character of BIIL-284, i.e., the masking of the benzamidine moiety, no high-affinity binding to the LTB_4 receptors was measured. Both metabolites, BIIL-260 and BIIL-315, have high affinity for the LTB_4 receptors on isolated human neutrophil membranes (K_i = 1.7 and 1.9 nM, respectively) and potently inhibited LTB_4-induced chemotaxis of human neutrophils (IC_{50} = 2.9 and 0.65 nM, respectively). In addition, BIIL-260 has been reported as a dual BLT1 and BLT2 antagonist.[225] The efficacy of BIIL-284 has been demonstrated in various in vivo models. For example, when dosed orally, BIIL-284 inhibited LTB_4-induced neutropenia in rats, guinea pigs, and monkeys at submilligram doses (e.g., 0.0041 mg/kg for rhesus monkeys). Inhibition of LTB_4-induced neutropenia by BIIL-284 is of longer duration when dosed at 0.03 mg/kg in monkeys compared with the inhibition observed with 3 mg/kg of other LTB_4 antagonists, LY-293111 or CGS-25019C.[224] Moreover, treatment with BIIL 284 (10 mg/kg, once daily) for 14 days after carotid artery balloon injury in vivo inhibited initial hyperplasia in rats.[226] Several clinical development activities for BIIL-284 for the potential treatment of inflammatory diseases, such as rheumatoid arthritis, COPD, and cystic fibrosis, have been reported (see section 3.11.1).

3.8.2. LY-293111 (VML-295, Etalocib Sodium). LY-293111-{2-[2-propyl-3-[3-[2-ethyl-4-(4-fluorophenyl)-5-hydroxyphenoxy]propoxy]phenoxy]benzoic acid}, a diaryl ether carboxylic acid derivative, is a BLT1 antagonist (Figure 17).[227] However, this compound also acts as a 5-LO inhibitor and a PPARγ agonist.[228] The in vitro actions of LY-293111, a potent and selective LTB_4 receptor antagonist, on human neutrophils,

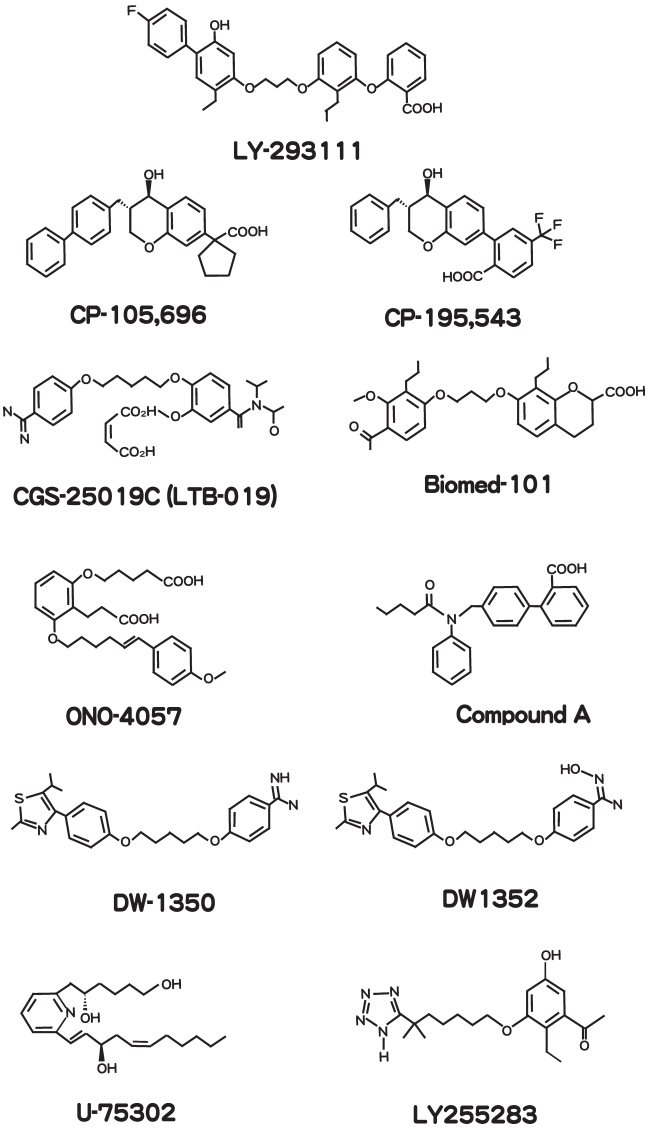

Figure 17. Chemical structures of synthetic ligands for BLT receptors.

guinea pig lung membranes, and guinea pig parenchymal and tracheal strips were confirmed.[229] An IC_{50} value for the inhibition of [^{3}H]LTB_4 binding to human neutrophils is 17.6 nM. LY-293111 inhibited LTB_4-induced human neutrophil aggregation (IC_{50} = 32 nM), luminol-dependent chemiluminescence (IC_{50} = 20 nM), chemotaxis (IC_{50} = 6.3 nM), and superoxide production by adherent cells (IC_{50} = 0.5 nM). This compound also inhibited LTB_4-induced contraction of guinea pig lung parenchyma. At micromolar concentrations, LY-293111 inhibits production of LTB_4 and TXB_2 by plasma-depleted human blood by stimulation with fMLP and thrombin. In addition, at these higher concentrations, formation of LTB_4 by A23187-activated whole blood and conversion of AA to LTB_4 by a human neutrophil cytosolic fraction are inhibited.[229] LY-293111 also demonstrated marked anti-inflammatory effects in a murine model.[230] This agent is orally available with reproducible pharmacodynamic effects in man.[227]

3.8.3. CP-105,696 and CP-195,543. Pfizer has developed two potent antagonists, CP-105,696 {1-[3-(4-phenylbenzyl)-4-hydroxychroman-7-yl]cyclopentane-1-carboxylic acid} and CP-195,543 {(+)-2-(3-benzyl-4-hydroxychroman-7-yl)-4-trifluoromethylbenzoic acid} (Figure 17). CP-105,696 has been in development for rheumatoid arthritis and IBD and entered phase I single- and multiple-dose trials in the early 1990s. Under conditions of low protein, CP-105,696 exhibits high potency at LTB_4 receptors; however, the potency is significantly decreased when assays are performed in whole blood, demonstrating a high level of protein binding.[209] As a consequence, Pfizer focused its efforts on the identification of a second-generation compound and found that replacement of the cyclopentyl group with an aromatic ring provided potent compounds. CP-195,543 is the result of these efforts. This compound inhibits [^{3}H]LTB_4 binding to LTB_4 receptors on human neutrophil and mouse spleen membranes with the IC_{50} values of 6.8 and 37 nM, respectively. LTB_4-evoked human and mouse neutrophil chemotaxis is attenuated by CP-195,543 with IC_{50} values of 2.4 and 7.5 nM, respectively. Key data of CP-105,696 is the observation that the potency of this molecule is not altered in the presence of plasma protein.[209] Recently, we demonstrated that CP-105,696 is highly selective for BLT1 and that CP-195,543 is a potent antagonist of both BLT1 and BLT2.[210] In vivo, oral administration of CP-195,543 attenuates LTB_4-evoked neutrophil infiltration in guinea pig and mouse skin with ED_{50} values of 0.1 and 2.8 mg/kg, respectively.[209] Furthermore, CP-195,543 reduces the clinical symptoms and weight loss in an IL-1 exacerbated murine model of collagen-induced arthritis.

3.8.4. CGS-25019C (LTB-019). Novartis has developed CGS-25019C {4-[5-[4-(aminoiminomethyl)phenoxy]pentoxy]-3-methoxy-*N*,*N*-bis(1-methylethyl) 2-butanedioate} for the potential treatment of asthma, rheumatoid arthritis, and COPD (Figure 17).[231] Other LTB_4 receptor antagonists bear an acidic functional group at one end, resembling LTB_4 itself; in contrast, CGS-25019C possesses a basic amidine moiety. The selectivity of CGS-25019C at BLT1 and BLT2 has not been reported. In 2002, Novartis presented the results of a 4-week double-blind, randomized, crossover trial of CGS-25019C in 24 patients with moderate COPD.[232] No statistically significant differences were reported between CGS-25019C and placebo in any measured end points (neutrophil numbers, myeloperoxidase activity, IL-8, and TNF-α) despite plasma concentrations of this compound similar to those shown previously to prevent the ex vivo LTB_4-induced upregulation of Mac-1 on the surface of neutrophils (see section 3.11.4).

3.8.5. Biomed-101 (SC-41930, Intarcia). Biomed-101 {7-[3-(4-acetyl-3-methoxy-2-propylphenoxy)propoxyl]-3,4-dihydro-8-propyl-2*H*-1-benzopyran-2-carboxylic acid} inhibits LTB_4-induced neutrophil chemotaxis and degranulation (Figure 17). The compound also inhibits 12(*R*)-HETE-evoked neutrophil chemotaxis and 12(*S*)-HETE binding to human epidermal cells, suggesting that it is a dual BLT1 and BLT2 antagonist.[233,234] In vivo, this compound attenuates acetic acid-evoked colonic inflammation with ED_{50} values of 20, 24, and 30 mg/kg for rats, guinea pigs, and rabbits, respectively.[235] Biomed-101 inhibits the binding of [^{3}H]LTB_4 to isolated human neutrophils with a K_i of 17 nM.[227] This compound prevents LTB_4-induced calcium mobilization with an IC_{50} of 808 nM. Biomed-101 is a less potent than LY-293111 in blocking LTB_4-induced CD11b upregulation on isolated neutrophils.

3.8.6. ONO-4057. ONO-4057 {5-[2-(2-carboxyethyl)-3-[6-(4-methoxyphenyl)-5*E*-hexenyl]oxyphenoxy]valeric acid} is an orally active, dual antagonist for both BLT1 and BLT2, which has been developed by Ono Pharmaceutical Co. Ltd. for the potential treatment of ulcerative colitis, psoriasis, IBD, and Behcet's

disease (Figure 17).[236,237] ONO-4057 displaces the binding of [^{3}H]LTB$_4$ to the human neutrophil membranes with a K_i value of 3.7 nM.[236] ONO-4057 inhibits the LTB$_4$-induced intracellular calcium increase (IC_{50} = 0.7 μM) and inhibits human neutrophil aggregation, chemotaxis, and degranulation induced by LTB$_4$ (IC_{50} = 3.0, 0.9, and 1.6 μM, respectively) without showing any agonistic activity at concentrations up to 30 μM.[236] In in vivo studies, ONO-4057 given orally prevents LTB$_4$-induced transient neutropenia or intradermal neutrophil migration in guinea pigs (ED_{50} = 25 or 5.3 mg/kg). Furthermore, ONO-4057 given topically suppresses phorbol-12-myristate-13-acetate (PMA)-induced neutrophil infiltration in guinea pig ear (the effective dose of 1 mg/ear).

3.8.7. DW-1350 and DW-135. DW-1350 and DW-1352 {4-[5-[4-(5-isopropyl-2-methylthiazol-4-yl)phenoxy]pentoxy]-benzamidine} are in development by Procter & Gamble under license from Dong Wha Pharmaceuticals for the treatment of osteoporosis (Figure 17). Recent patents describe the use of these compounds for the prevention and treatment of allergic inflammatory diseases and bone fractures, although no clinical development for these indications has been reported. Osteoporosis is caused by an imbalance between the amount of bone produced by osteoblasts and the amount absorbed by osteoclasts, thus leading to a decrease in bone density or mass.[238] LTB$_4$ stimulates bone absorption via production of osteoclasts.[239,240] Moreover, using BLT1-null mice, we recently suggested that autocrine/paracrine LTB$_4$ increases the osteoclastic activity through the BLT1-Gα_i-Rac1 signaling pathway (see section 3.9.7).[241] Thus, LTB$_4$ receptor antagonists may be useful for suppressing osteoclastic bone absorption. DW-1350 and DW-1352 inhibit osteoclast proliferation and differentiation (IC_{50} = 20 and 1.3 nM, respectively), osteoclast fusion (IC_{50} = 0.81 and 0.74 μM, respectively), and bone reabsorption of osteoclasts (IC_{50} = 0.08 and 0.1 μM, respectively).

3.8.8. U-75302. U-75302 {6-[6-(3*R*-hydroxy-1*E*,5*Z*-undecadien-1-yl)-2-pyridinyl]-1,5*S*-hexanediol} is an LTB$_4$ receptor antagonist with a K_i of 159 nM on guinea pig lung membranes (Figure 17).[242−244] This activity is specific for BLT1. U-75302 does not antagonize the binding of [^{3}H]LTB$_4$ to human BLT2.[128] Boie et al. observed that U-75302 possesses both agonistic and antagonistic properties for BLT1.[245] Moreover, Johansson et al. recently reported that U-75302 has intrinsic agonist activity for adhesion of neutrophils; up-regulation of E-selectin, ICAM-1, and VCAM-1; and release of MCP-1 in human umbilical vain endothelial cells (HUVEC).[246]

3.8.9. LY255283. LY255283 {1-[5-ethyl-2-hydroxy-4-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]phenyl]ethanone} was originally developed as a BLT1 antagonist and inhibits BLT1 signaling as well (Figure 17).[247,248] However, this compound is also a competitive antagonist of BLT2. It displaces [^{3}H]LTB$_4$ from guinea pig lung membrane, with an IC_{50} of about 100 nM.[210] LY255283 exhibits IC_{50} values of ∼950 and >10 μM at human recombinant BLT2 and BLT1, respectively (competitive for BLT2 and allosteric antagonist for BLT1).[249] LY255283 inhibits eosinophil chemotaxis by 80% at a concentration of 10 μM and inhibits the binding of [^{3}H]LTB$_4$ to eosinophil membranes with an IC_{50} of 260 nM.[248] Recently, Kim et al. reported that LY255283 significantly blocked several biological responses via BLT2, e.g., invasion and metastasis of aggressive bladder cancer cells,[250] Th2-cytokine production in allergen-stimulated mast cells,[251] and UVB (280−320 nm)-induced reactive oxygen species generated in human keratinocytes.[252]

3.8.10. RO5101576. RO5101576 {4-[3-[6-(3-benzo-[1,3]dioxol-5-yl-5-thiophen-3-ylphenoxy)hexyl]-2-(2-carboxyethyl)phenoxy]butyric acid} was developed at Roche Pharmaceuticals. Hicks et al. reported that RO5101576 inhibits LTB$_4$-evoked chemotaxis of human neutrophils with an IC_{50} of 8.0 nM.[253] In retinoic acid-stimulated HL-60 cells, this compound inhibits LTB$_4$-evoked calcium mobilization with an IC_{50} of 0.42 nM. In HEK293 cells expressing human BLT1 and BLT2, RO5101576 dose-dependently inhibits LTB$_4$-evoked calcium mobilizations with IC_{50} values of 187 and 379 nM, respectively. The inhibitory effects of RO5101576 on human BLT1 and BLT2 are not significantly different from each other, suggesting a dual antagonistic property of this compound.[253] In BHK21 cells expressing human PPARs, RO5101576 acts as a weak agonist for PPARα and PPARγ with no activity for PPARδ.[253] Aerosol challenge of LTB$_4$ to guinea pigs evokes a significant influx of eosinophils to the airway when compared to sham animals. Oral pretreatment with RO5101576 significantly attenuates LTB$_4$-evoked eosinophilic pulmonary inflammation in a non-dose-dependent manner with high potency, exhibiting significant effects down to 0.03 mg/kg po.[253] Ozone inhalation to cynomolgus monkeys results in significant increases in the numbers of neutrophils in the bronchoalveolar lavage fluid (BALF), although this challenge has no effect on eosinophil and lymphocyte populations.[253] Pretreatment with RO5101576 (30 mg/kg po) significantly attenuates ozone-evoked increases in the numbers of neutrophils compared to vehicle-treated animals.

3.8.11. Other Synthesis Ligands. In the light of our knowledge, the actions of LTB$_4$ receptor antagonists that have been developed as anti-inflammatory drugs have to be re-evaluated. To distinguish the effects of the antagonists on BLT1 and BLT2, we recently examined their inhibitory effects on [^{3}H]LTB$_4$ binding using the membrane fractions of CHO cells expressing either human BLT1 or BLT2.[210] The results show the competition of 5 nM [^{3}H]LTB$_4$ binding by various BLT antagonists. LTB$_4$ binding to BLT1 is inhibited by CP 105696,[254] ZK 158252,[5] CP 195543,[209] and U75302[255] in a dose-dependent manner, but not by 10 μM LY-255283[256] or LY-223982.[257] In contrast, LTB$_4$ binding to BLT2 is inhibited by ZK 158252, LY-255283, and CP 195543, but not by 10 μM U75302, CP105695, or LY-223982. We also examined their agonistic activities on these receptors by calcium mobilization and found that 1 and 10 μM U75302 acts as a weak agonist on human BLT1, and 1 and 10 μM CP 195543 acts as a weak agonist on human BLT2. Recently, Ono Pharmaceutical Co. Ltd. has developed a synthetic ligand for the LTB$_4$ receptor, named Compound A {4′-[[pentanoyl(phenyl) amino]methyl]-1,1′-biphenyl-2-carboxylic acid} (Figure 17).[208] We tested the competitive effects of this compound on [^{3}H]LTB4 binding to membrane fractions of mouse BLT1-expressing CHO and mouse BLT2-expressing CHO cells.[208] Compound A inhibits the binding of [^{3}H]LTB$_4$ to BLT2 with an IC_{50} value of 20 nM, even though there is no effect on the [^{3}H]LTB$_4$ binding to BLT1.We further found that this compound induces calcium mobilization and phosphorylation of ERKs through BLT2 in CHO cells and in primary mouse keratinocytes. Furthermore, Compound A induces chemotaxis in primary mouse keratinocytes as well as LTB$_4$.[208]

3.9. LTB$_4$ and Cognate Receptors in Health and Diseases

The binding of LTB$_4$ to its specific receptors on immune cells, e.g., neutrophils, macrophages, T-cells, mast cells, and DCs, initiates a number of these cell functions, e.g., adhesion to vascular

Table 2. Physiological Roles of LTB_4

cell types/tissues	bioactivities of LTB_4
leukocytes	chemotaxis (neutrophils, monocytes/ macrophages, eosinophils, effector CD4+ T-cells and CD8+ T-cells)
	adhesion to endothelial cells
	activation (CD11b up-regulation, release of lysosomal enzymes, generation of reactive oxygen species)
	activation of natural killer cells
	IL-2 and IFN-γ productions in CD4+ T-cells
endothelium	adhesion of neutrophils
respiratory system	constriction of lung parenchyma
brain and nervous system	modulation of ryanodine receptor
	activation of capsaicin receptors
skin	melanocyte pigmentation

Table 3. Pathophysiological Roles of LTB_4

	related diseases
respiratory system	bronchial asthma (confirmed in BLT1-null mice)
	• development of airway hyperresponsiveness
	• migrations of eosinophils, DCs, and IL-13 producing Th2 cells
	• IL-12p70 production in DCs
	• Th1 development
	• delayed-type hypersensitivity
kidney	glomerulonephritis
	ischemic renal injury
	lupus nephritis
joints	rheumatoid arthritis (confirmed in both BLT1-null and BLT1/BLT2-null mice)
brain and nerve system	allergic encephalomyelitis
	multiple sclerosis (confirmed in BLT1-null mice)
	• migration of inflammatory cells
	• Th1/Th17 immune responses
colon	inflammatory bowel disease (confirmed in BLT2-null mice)
	• protection of DSS-induced colitis
skin	atopic dermatitis
	psoriasis
blood vessel	atherosclerosis
	• reduction of vascular lesions by BLT1-antagonists in apo-E-null/ LDLR-null mice
	• gene expression for development and progression of atherosclerosis
	• arrest of monocytes on vascular endothelium
bone	osteoporosis (confirmed in BLT1-null mice)
	• regulation of osteoclast function

endothelial cells, chemotaxis, release of lysosomal enzymes, and production of reactive oxygen species.[15] For example, exogenous LTB_4 elicits production of IL-2 and IFN-γ in $CD4^+$ T-cells,[258] and BLT1 antagonists inhibit T-cell proliferation and IFN-γ production in vitro.[259,260] The evidence for the importance of LTB_4 in disease development has been obtained from the detection of this mediator in inflammatory exudates and the efficacy of the receptor antagonists in abolishing or reducing the inflammatory condition. To data, many studies have associated LTB_4 with several inflammatory events, including asthma, atopic dermatitis, rheumatoid arthritis, septic peritonitis, psoriasis, and IBD.[261−263] The physiological and pathophysiological roles of LTB_4 are summarized in Tables 2 and 3. In this regard, several antagonists for LTB_4 receptors, as well as inhibitors of LT biosynthesis, have been developed for clinical use. In this section, we summarize the current knowledge about various physiological and pathophysiological roles of LTB_4 and its receptors, BLT1 and BLT2.

3.9.1. Inflammation. Mice deficient in 5-LO or LTA_4 hydrolase have all indicated the importance of LTs in inflammation and certain types of anaphylactic reactions.[264−268] In BLT1-null mice, leukocytes exhibit a normal response to C5a and PAF; however, there is a selective loss of responsiveness to exogenous LTB_4.[269,270] Tager et al. reported that adhesion to the endothelium in response to LTB_4 is diminished in leukocytes from BLT1-null mice.[270] These findings are in line with earlier observations with LTB_4 in the hamster cheek pouch and suggest that BLT1 is pivotal for the LTB_4-induced leukocyte activation. Two groups have provided evidence that peritoneal inflammation is suppressed in these null mice.[269,270] Interestingly, Haribabu et al. investigated a loss of this protection at 72 h between knockout and WT animals,[269] whereas Tager et al. observed an increase in protection between knockout and WT animals with time intervals greater than 50 h.[270] The latter investigators also reported a marked diminution in the number of eosinophils; in contrast, the former group noted reductions in both PMN and macrophage populations.[269] The reasons for these differences are still unclear. Conversely, transgenic mice overexpressing human BLT1 (driven by CD11b promoter) exhibit enhanced leukocyte responsiveness in acute dermal inflammation, with leukocyte trafficking to remote organs (as in secondary organ reperfusion injury), or leukocyte recruitment following a peritoneal challenge.[271] Expression of human BLT1 in vivo leads to an upregulation of 5-LO expression and LTs biosyntheses,[271] suggesting that receptor expression may amplify pro-inflammatory circuits in vivo. In the model of PAF-induced anaphylaxis, only the female BLT1-null mice are protected, consistent with the loss of a male survival advantage for 5-LO-null of MRL-*lpr/lpr* mice.[269,272] In either case, the basis for sex-related differences remains to be investigated. Studies on BLT1-null mice have also greatly contributed to the understanding of BLT1 function in the recruitment of T-cells.[155−157] The inflammatory response can be initiated by direct trauma to mast cells or cross-linking of their surface IgE by allergen, followed by the immediate releases of various mediators including LTB_4 from these cells. LTB_4 attracts myeloid leukocytes, such as macrophages, eosinophils, and neutrophils, and also plays a potent chemoattractant for effector $CD4^+$ T-cells and $CD8^+$ T-cells expressing BLT1,[155−157] demonstrating that the LTB_4−BLT1 axis is a fundamental immune regulator for recruitment of these cells.

3.9.2. Bronchial Asthma. Although much of the focus on LTs in asthma is on cys-LTs, recent studies have defined an important regulatory role of LTB_4 in models of asthma.[273] Immunohistochemical staining of human bronchi revealed colocalization of both BLT1 and BLT2 proteins with smooth muscle α-actin positive airway smooth muscle cells.[168] Furthermore,

both receptors were also identified on human airway smooth muscle cells in culture by RT-PCR, Western blot analysis, and flow cytometry.[168] In these cells, LTB_4 induces proliferation and migration, which is inhibited by the selective BLT1 antagonist U75302.[168] We and others have recently demonstrated that BLT1-null mice fail to develop airway hyperresponsiveness (AHR) in a Th2-biased bronchial asthma model, and this is accompanied by reduced migration of eosinophils and IL-13 producing Th2 cells into the airway.[274−276] In the mast-cell-dependent model, which bypasses the need for host-derived IgE, BLT1-null mice also impair the development of AHR due to ablation of the $CD8^+$ T-cell function.[277] These studies mainly focused on the roles of the LTB_4−BLT1 axis in T-cell recruitment, but practically little information is available on the function of LTB_4−BLT1 in DCs, even though they are important antigen-presenting cells that control Th1/Th2 development. Currently, increasing numbers of reports have shown the importance of several lipid mediators, e.g., PGE_2, PGD_2, TXA_2, LTC_4, and LTD_4, in DC functions such as trafficking.[21,278−281] Most recently, LTB_4 was reported to activate migration of DCs by upregulating CCR7.[282] Bone-marrow-derived DCs (BMDCs) from BLT1-null mice develop significantly lower AHR to inhaled methacholine, lower goblet cell metaplasia and eosinophilic infiltration in the airway, and decreased levels of Th2-type cytokines in the BALF, suggesting the requirement of BLT1 in DCs for the allergen-induced AHR.[283] We further found that in murine BMDCs, BLT1-null DCs produce less IL-12p70 than WT DCs, leading to attenuated Th1 cytokine production, e.g., IFN-γ, in an allogeneic mixed lymphocyte reaction.[284] In this study, BLT1-null mice have also attenuated delayed-type hypersensitivity (DTH), consistent with the reduced Th1-inducing ability of BLT1-null DCs in adoptive transfer experiments. Thus, the LTB_4−BLT1 pathway is a fundamental immune regulator as well as a terminal mediator of inflammation and allergic reaction.

There is relatively little information about the role of BLT2 in asthma. Bone-marrow-derived mast cells from WT mice migrate toward the BLT2 ligand 12-HHT, but not cells from BLT2-null mice. In a murine asthma model, expression of BLT2 is increased in lungs after sensitization and challenge, and antisense oligonucleotide inhibition of BLT2 expression or pharmacological inhibition of BLT2 with LY-255283 attenuates airway inflammation and AHR.[285] In this model, inhibition of BLT2 reduces the generation of reactive oxygen species (ROS) and the expression of NF-κB.

3.9.3. Atherosclerosis. Atherosclerosis is a complex vascular disease that is now recognized as an inflammatory disease. Effects of antagonists and gene knockouts in mouse models have all revealed a major role for LTs in atherosclerosis. Mehrabian et al. have identified 5-LO as one of the genes conferring resistance to atherosclerosis in the WT mouse strain CAST.[286] Deriving this locus from CAST, they constructed a congenic strain (CON6) and found that CON6 mice express only 15% of 5-LO levels compared to C57BL/6J mice. They also generated 5-LO-null mice onto low-density lipoprotein receptor (LDLR)-null background and found that the heterozygotes for the gene encoding 5-LO in this background had reduced lesions by over 95%, indicating a dominant role of 5-LO in atherogenesis.[287] Recent studies have further identified association of human 5-LO promoter variants with increased risk for atherosclerosis.[288] Among the several atherosclerosis loci on the human genome, another locus might also relate to the function of LTs.[289] This locus maps to chromosome 14. The nearest marker, D14Mit63, is 0.34 Mb away from BLT1 and BLT2 genes.

Human atherosclerotic plaques have the capacity to produce LTB_4 ex vivo. Recent studies have demonstrated high levels of all components involved in LT biosynthesis, i.e., 5-LO, FLAP, and LTA_4 hydrolase, but failed to identify 12/15-LO, in human vascular lesions.[290] Moreover, HUVECs express $CysLT_2$, BLT1, and BLT2; macrophages express BLT1, BLT2, $CysLT_1$, and $CysLT_2$; smooth muscle cells express CysLTs; and recent findings demonstrated the expression of BLT1 in activated T-cells.[157,291,292] The expressions of all biosynthetic enzymes of LTs, as well as their target receptors, in cells involved in atherogenesis are consistent with their involvement in atherosclerosis. A major finding on the involvement of LTs in atherogenesis comes from a study by Aiello et al., who showed that antagonists of BLT1 significantly reduce vascular lesions in both apo-E-null and LDLR-null backgrounds.[293] Treatment with the BLT1 antagonists results in lesions that contain fewer macrophages and shows a decrease in CD11b expression both in lesions and in peripheral blood.[293] Studies with BLT1-null mice in apo-E-null background also show significant reduction in lesions at 4 and 8 weeks, when the animals are kept on an atherogenic high-fat diet.[294] However, when mice are fed a high-fat diet for 19 weeks, no difference in lesions is observed between WT and BLT1-null mice. Of note, macrophages derived from both WT and BLT1-null mice express BLT2, and BLT1-null macrophages show chemotaxis to high concentrations of LTB_4.[294] In view of the complete protection observed in 5-LO-null animals and limited resistance to atherogenesis in BLT1-null animals, it is possible that other LTs (LTC_4 and LTD_4) and receptors (BLT2 and CysLTs) might be involved in complete expression of the vascular disease.

3.9.4. Rheumatoid Arthritis. Rheumatoid arthritis is a chronic inflammatory disease involving multiple joins and remains an autoimmune disease of unknown etiology. The importance of LTB_4 in rheumatoid arthritis is suggested by the high level of LTB_4 in synovial fluid and serum,[295] as well as the enhanced capacity for release of LTB_4 by neutrophils.[296] Furthermore, the BLT1 expression has been demonstrated on neutrophils in synovial tissues and fluids derived from patients with rheumatoid arthritis.[297] A current report has demonstrated that in neutrophils derived from the synovial fluids of patients with rheumatoid arthritis, BLT1 antagonism inhibits LTB_4-induced calcium influx and produces a rightward shift of the peak chemotactic response. In contrast, LY-255283, a BLT2 antagonist, only inhibits these responses at high concentrations because this compound may also act as an antagonist of BLT1.[213] Collagen-induced arthritis (CIA) is a model for rheumatoid arthritis that is induced in susceptible mouse strains by intradermal immunization with collagen type II emulsified in a complete adjuvant.[298] In the mouse model, $cPLA_2\alpha$-null mice are resistant to CIA, and their clinical scores are equally as good as those of TNF-α-null mice.[299] The role of LTB_4 was further confirmed with BLT antagonist CP-105,696, which improves the symptoms of CIA mice.[261] Recently, several reports demonstrated that genetic ablation of BLT1 protects mice from developing arthritis.[213,300,301] Shao et al. developed mice deficient in both BLT1 and BLT2 (BLT1/BLT2-null mice) by simultaneous, targeted disruption of these genes and showed complete protection of these mice from CIA development.[300] In WT mice, their disease severities are correlated well with histopathology, including loss of joint architecture, inflammatory cell infiltration, fibrosis, pannus formation, and bone erosion in joints, whereas a total absence of these disease pathologies is confirmed in

BLT1/BLT2-null mice. Kim et al. demonstrated a nonredundant role for BLT1 in neutrophil recruitment into the joint in the K/BxN mouse model of inflammatory arthritis.[301] Adoptive transfer of WT neutrophils restores arthritis and chemokine production in BLT1-null mice. Interestingly, the effect of the transferred WT neutrophils into BLT1-null mice is to promote the entry of endogenous BLT1-null neutrophils into the joints of these mice, suggesting the importance of BLT1-activated neutrophils in the BLT1-null neutrophil recruitment by other chemoattractants.[301] Furthermore, Chou et al. currently reported that in the K/BxN model, the LTB_4−BLT1 pathway delivers neutrophils producing IL-1 and chemokines into the joint, which in turn amplifies and sustains neutrophil recruitment and inflammation through both neutrophil-derived and IL-1-inducible chemokines.[302]

3.9.5. Aortic Abdominal Aneurysms. Circulating neutrophils derived from patients undergoing surgery for abdominal aortic aneurysms produce increased levels of LTB_4,[303] and the expressions of LTB_4 synthesis enzymes are enhanced in neutrophils infiltrating the intraluminal thrombus covering the aneurysm.[304] The local LTB_4 production has further been demonstrated to transduce the major part of the chemotactic activity derived from the intraluminal thrombus.[304] In addition to neutrophils, macrophages and T-lymphocytes may be involved in the adventitial inflammation, which is part of the pathogenesis of abdominal aortic aneurysms.[304] The importance of BLT1 signaling has also received support from animal studies. Either genetic or pharmacological disruption of BLT1 signaling reduces the incidence of experimental abdominal aortic aneurysms induced.[305,306] For example, BLT1 and Apo-E-double null mice are protected from abdominal aortic aneurysm development induced by angiotensin II infusion.[305]

3.9.6. Multiple Sclerosis. To data, little is known about the neuroimmune functions of BLT1. Multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis (EAE), are demyelinating diseases of the central nervous system.[307] We currently demonstrated a distinct role for BLT1 in the pathology of EAE and Th1/Th17 immune responses.[308] BLT1 mRNA is highly upregulated in the spinal cord of EAE mice, especially during the induction phase. BLT1-null mice have delayed onset and less severe symptoms of EAE than WT mice. Additionally, inflammatory cells are recruited to the spinal cord of asymptomatic WT mice, but not BLT1-null mice, before the onset of disease. Ex vivo studies showed that both the proliferation and the production of IFN-γ, TNF-α, IL-17, and IL-6 are impaired in BLT1-null cells, as compared with WT cells. Thus, BLT1 exacerbates EAE by regulating the migration of inflammatory cells and Th1/Th17 immune responses. These findings provide a novel therapeutic option for the treatment of multiple sclerosis and other Th17-mediated diseases.

3.9.7. Bone Metabolism. Although LTB_4 is produced in various inflammatory diseases, its functions in bone metabolism remain unknown. Using BLT1-null mice, we recently evaluated the roles of BLT1 in two bone resorption models, namely, bone loss induced by ovariectomy and lipopolysaccharide.[241] Through observations of bone mineral contents and bone morphometric parameters, the bone resorption in both models is significantly attenuated in BLT1-null mice. Furthermore, osteoclasts from BLT1-null mice show reduced calcium resorption activities compared with WT osteoclasts. Osteoclasts express BLT1, but not BLT2, and significantly produce LTB_4. LTB_4 changes the cell morphology of osteoclasts through the BLT1−$G\alpha_i$−Rac1 signaling pathway. Given the causal relationship between osteoclast morphology and osteoclastic activity, these findings suggest that autocrine/paracrine LTB_4 increases the osteoclastic activity via the BLT1−$G\alpha_i$−Rac1 pathway. Inhibition of BLT1 functions may represent a strategy for preventing bone resorption diseases.

3.9.8. Colitis. Despite the well-defined pro-inflammatory roles of BLT1, the in vivo functions of BLT2 remain elusive. On the basis of the finding that BLT2 is expressed in mouse intestinal tissues and 12-HHT is abundantly present in small intestine, we investigated the roles of BLT2 in dextran sodium sulfate (DSS)-induced colitis using mice lacking BLT2.[309] BLT2-null mice show increased susceptibility to a low dose of DSS with severe body weight loss and inflammation than WT and BLT1-null mice. Expression of inflammatory cytokines IFN-γ, IL-1β, IL-6; chemokines CXCL9 and CCL19; and metalloproteinases is upregulated with the enhanced accumulation of activated macrophages in DSS-treated BLT2-null colon. Phosphorylation of signal transducers and activator of transcription 3 (Stat3) is also accelerated in crypts in DSS-treated BLT2-null mice. Madin−Darby canine kidney II (MDCKII) cells overexpressing BLT2 exhibit enhanced transepithelial electrical resistance and attenuate leakage of FITC−dextran through monolayers, demonstrating that BLT2 is important in the maintenance of mucosal integrity both in vitro and in vivo. BLT2 is expressed in colon cryptic cells; thus, this receptor protects from DSS-induced colitis, possibly by enhancing barrier function of epithelial cells in colon.

3.9.9. Others. The BLT2 expression is highly induced by VEGF. Transgenic BLT2 overexpressing mice have been studied in a model of VEGF-induced angiogenesis.[310] In these mice, the blood vessel formation is augmented in response to LTB_4 and 12(*S*)-HETE. The induced BLT2 plays an essential role in mediating VEGF-induced angiogenesis, suggesting a potential interplay among VEGF, BLT2, and BLT2 ligands in vascular angiogenesis.

3.10. Topics for LTB_4 and Its Receptors

Recently, a novel function of BLT1 was reported. Owman et al. demonstrated that human BLT1 acts as a coreceptor for macrophage-tropic human immunodeficiency virus type 1 (HIV-1) strains in the same manner as several chemokine receptors, CCR5, and CXCR2.[311] Therefore, BLT1 expressing on human macrophages can be a target for the inhibition of HIV entry, and various BLT1 antagonists should be tested for their ability to prevent HIV infection. However, another group currently obtained conflicting data using different strains of HIV and other assay systems;[312] thus, extensive experiments are necessary to discuss the coreceptor activity of BLT1.

In addition, there are two interesting findings regarding actions of LTB_4 besides the activation of BLT receptors. Although the classical LTB_4 receptors are cell-surface proteins, the first molecule identified as an LTB_4 receptor was the nuclear protein peroxisome proliferators-activated receptor (PPAR) α.[313] PPARs, i.e., PPARα, PPARβ, and PPARγ, are known to bind to the promoters of several lipid-metabolizing enzymes at their consensus sequence, PPAR responsive elements. Interestingly, PPARs can be activated by a variety of eicosanoids: PPARα by 8(*S*)-HETE, PPARβ by prostacyclin analogs, and PPARγ by 15-deoxy-δ-12,14-PGJ_2.[313−316] Devchand et al. reported that recombinant PPARα binds to LTB_4 with a K_d value of 90 nM, and extracellular application of LTB_4 at micromolar concentrations induces adipogenesis.[313] A recent study has also demonstrated that mice lacking LTB_4 biosynthesis through 5-LO-null

exhibit reduced PPARα activation in response to LPS administration in vivo.[317] If the LTB_4—PPAR pathway actually functions in vivo, LTB_4 is the unique ligand that functions via dual cell surface and intranuclear receptor systems. Whether eicosanoids are bona fide endogenous PPAR ligands has yet to be resolved with rigorous analytical methods and testing of COX, 5-LO, and other eicosanoid-null mice.

The other finding is the modulator function of LTB_4 and related lipids for ion channels, such as the ryanodine receptor and the transient receptor potential vanilloid type-1 receptor (TRPV1).[318−320] The rank order of potency is 12(*S*)-HpETE > 15(*S*)-HpETE > LTB_4.[318] These activations lead to excitation of nociceptors and evoke pain-related behavior.[321] TRPV1 is necessary for the development of inflammatory hyperalgesia to thermal stimuli and is important in the pathophysiology of neurogenic inflammation. Researchers have recently described novel compounds that are dual antagonists of BLT1 and TRPV1 as potential therapies in inflammatory diseases. For example, McHugh et al. recently reported two compounds, O-3367 {*N*-[2-fluoro-4-[3(11 hydroxyheptadec-8-enyl)thioureiomethyl]phenyl]-methanesulfonamide} and O-3383 {*N*-[4-[3-(11 hydroxyheptadec-8-enyl)thioureiomethyl]phenyl]methanesulfonamide}, that interact with both BLT1 and TRPV1.[322] In human neutrophils, both O-3367 and O-3383 act as antagonists, significantly attenuating the LTB_4-induced migration and dorsal root ganglion neurons. These compounds also attenuate the capsaicin-induced increase in intracellular calcium.[322]

3.11. Clinical Studies on LT Receptors Targeted Therapy

Zileuton, a 5-LO inhibitor, is efficacious and approved for the treatment of chronic asthma and has shown some clinical benefit in IBD and COPD.[323,324] Although not studied in all diseases, small studies have shown that 5-LO inhibitors demonstrate superior efficacy in asthma and COPD compared to the $CysLT_1$ antagonist zafirlukast, suggesting that specific modulation of LTB_4 pathways may mediate this efficacy. As a result, pharmacological strategies to regulate the synthesis and/or action of LTB_4 are being developed by several pharmaceutical companies. Despite strong supporting evidence from preclinical studies and elevated levels of LTB_4 in various inflammatory diseases, clinical trials with LTB_4 receptor antagonists have not shown significant positive efficacy results so far.

3.11.1. Clinical Trial of BIIL-284. Several clinical activities of BIIL-284 for the potential treatment of inflammatory diseases, such as rheumatoid arthritis, COPD, and cystic fibrosis, have been reported.[325] Results from these trials show doses of 2.0−750 mg to be well tolerated and to inhibit the LTB_4-evoked expression of CD11b/CD18 (Mac-1) on neutrophils ex vivo in a dose-dependent manner. In February 2004, the data were published from a double-blind, randomized, and placebo-controlled parallel trial involving 26 patients with rheumatoid arthritis.[326] Doses of 25−150 mg of BIIL-284, administered orally, once daily for 14 days, were shown safely and effectively to inhibit LTB_4-evoked Mac-1 expression on neutrophils ex vivo, the primary end point of this trial. However, secondary clinical end points, including tender and swollen joint counts and pain, were not affected. Adverse events included dyspnea, fatigue, and headache. It was concluded that longer duration of treatment could result in a clinical benefit for rheumatoid arthritis patients. The results of such follow-on studies were published in 2007.[225] Doses of 5, 25, or 75 mg of BIIL-284 were administered orally, once daily for 3 months. In this report, the investigators demonstrated that LTB_4 is not a major contributor to the inflammatory process in rheumatoid arthritis, because BIIL-284 evoked only modest improvements in disease activity.

3.11.2. Clinical Trial of LY-293111. In 2002, this compound was reported by Lilly to be in development for asthma, IBD, and rheumatoid arthritis. Interestingly, the data from a double-blind, placebo-controlled trial in atopic asthmatic subjects show that pretreatment with LY-293111 prior to an allergen challenge results in an attenuation of neutrophil influx, myeloperoxidase activity and levels of LTB_4, LTC_4, and IL-8, consistent with anti-inflammatory activity.[327] Moreover, LY-293111 proved to be a potent inhibitor of LTB_4-induced cutaneous inflammation and hyperproliferation.[328] Conversely, in psoriasis, LY-293111 is not effective in preventing relapse, either clinically or at the cellular level, following an 8-week treatment of 200 mg twice daily,[329] resulting in the compound having been discontinued for inflammatory indications. Recent information from Lilly showed that the compound is in development for the treatment of cancer based on the ability of LTB_4 to modulate cancer cell migration in vitro and in vivo.[330−332] Thus, clinical development has been initiated in pancreatic cancer, chondrosarcoma, and melanoma.[333] In September 2003, a double-blind, randomized, and placebo-controlled parallel assignment, safety, and efficacy phase II study began investigating LY-293111 in combination with gemcitabine and cisplatin in patients for the treatment of stage IIIb or IV non-small-cell lung cancer (NSCLC). Primary end points indicated that LY-293111 in combination with gemcitabine and cisplatin does not increase median progression-free survival when compared with NSCLC patients taking the placebo with gemcitabine/cisplatin. The development of LY-293111 has subsequently been discontinued for NSCLC. Furthermore, the double-blind and randomized phase II trial of gemcitabine plus LY-293111 in advanced adenocarcinoma of the pancreas did not show any benefit of this compound.[334]

3.11.3. Clinical Trial of CP-195,543. In June 2006, Pfizer initiated a double-blind, randomized, placebo-controlled clinical trial for the study of CP-195,543 and celecoxib, a selective COX-2 inhibitor, as combination therapy in subjects with rheumatoid arthritis who are inadequately controlled with methotrexate.[325]

3.11.4. Clinical Trial of LTB019. Novartis initiated tests to see the effect of LBT019 on inflammatory markers in induced sputum, in particular sputum neutrophilia, in ex-smokers with moderate, stable COPD.[335] The trial followed a double-blind, randomized, placebo-controlled, crossover design including two treatment periods (4 weeks) separated by a 4-week washout period. No significant differences occurred between LTB019 and placebo regarding the percentage of sputum neutrophils, total cell count, and the levels of myeloperoxidase, IL-8, and TNF-α. Therefore, a 4-week treatment with LTB019 had no effect on sputum neutrophil numbers and related cytokine levels in these patients, despite the plasma concentrations achieved being similar to those shown to prevent the ex vivo LTB_4-induced upregulation of Mac-1 on neutrophils.

3.11.5. Future Implications. There are several possible reasons for the minimal efficacy of the BLT antagonists in inflammatory diseases. Many researchers took advantage of the well-established action of LTB_4 to evoke Mac-1 expression on neutrophils and used this assay ex vivo in patients treated with BLT antagonists. For instance, BIIL-284 (25 mg/kg) achieved drastic inhibition of the LTB_4-evoked Mac-1 expression in peripheral neutrophils ex vivo, but failed to demonstrate any

clinical benefit in patients with rheumatoid arthritis. However, this peripheral pharmacodynamic marker may not correlate with LTB_4-mediated effects in vivo and subsequent concentrations of antagonists required for inhibition at the site of inflammation. Additionally, these effects are mediated by BLT1 and, therefore, do not provide evidence for inhibition of BLT2, which may be relevant for efficacy. The possibility also remains that dual inhibition of BLT1 and BLT2 may exert a lack of effect if the functions of these two receptors are opposing. Indeed, we currently found the protective role of BLT2 in the murine model of inflammatory colitis (see section 3.9.8). The identification of several selective BLT2 antagonists recently disclosed by several Japanese groups exemplifies methods to overcome this issue. Another reason for the lack of clinical effect of LTB_4 antagonists may lie in the choice of patient population in trials conducted so far. Most inflammatory disease populations are heterogeneous with involvement of a large variety of mediators. Hence, LTB_4 may not be elevated in all patients with disease. So far, BLT antagonists, although safe and well-tolerated, have not shown significant positive efficacy results for the therapy of inflammatory diseases. However, there is strong evidence that LTB_4 and its signaling via BLT1 and BLT2 play a significant role in inflammatory diseases. Future directions need to include controlled clinical trials with novel measures of pharmacodynamic effect, patient selection, end points, and disease indications. In addition, further understanding of the roles of BLT1 and BLT2, development of selective antagonists for these receptors, and exploration of potential combination approaches may lead to more effective therapies in the treatment of inflammatory diseases in the future.

4. CYS-LT RECEPTORS

About 70 years ago, Kellaway et al. demonstrated that when antigen-sensitized guinea pig lungs were stimulated with cobra venom they produced a substance that contracts smooth muscle.[336] Thereafter, several studies showed that this substance is important as a mediator in asthma and other types of immediate hypersensitivity reactions.[337] This material was initially called "slow reacting substance (SRS)". Immunologically generated SRS is usually referred to as "slow reacting substance of anaphylaxis (SRS-A)".[338,339] SRS-A is released together with other mediators, e.g., histamine and chemotactic factors, after interaction between IgE molecules bound to membrane receptors and antigens such as pollen. In contrast, this substance has been clarified as a sulfur-containing polar lipid with ultraviolet (UV) absorption,[340] and further studies demonstrated the incorporation of labeled AA into SRS-A.[341] Moreover, the UV absorbance of purified SRS-A was found to be similar to that of the dihydroxy acids derived from leukocytes.[4,342,343] The physicochemical and biological properties of this substance were shown subsequently to reside in cys-LTs, whose biosynthetic pathway was elucidated in 1983.[19] By comparison with active and inactive standards with different peptide adducts, the three components of authentic SRS-A were defined as LTC_4 and two additional theoretic structures, LTD_4 and LTE_4. LTC_4 possesses the sulfur-linked glutathione tripeptide adduct composed of glutamic acid, glycine, and cysteine, whereas LTD_4 lacks the glutamic acid residue and LTE_4 is deficient in both the glutamic acid and glycine residues.

The notion of specific receptors for these cys-LTs originates in the acetophenon FPL-55712, which was described as an antagonist of SRS-A.[339] Subsequently, the structural requirements for their high potency and the development of several specific antagonists made it clear that these lipid mediators exert their biological actions through the specific receptors. Although these lipids were originally identified on the basis of their contractile properties for intestinal and bronchial smooth muscle, they now have clearly defined roles in asthma, perpetuating airway inflammation, leading directly to airflow obstruction through effects on vascular permeability, and mucus production, and they have contributed to airway remodeling in murine asthma models. Though a considerable amount of evidence demonstrates that all the radiolabeled cys-LTs bind to the membrane preparations from a number of tissues, there are data suggesting the existence of at least two types of cys-LT receptors.[344] This section serves to give information on the biosynthesis and metabolism of cys-LTs and the molecular cloning of the receptors for these lipids, $CysLT_1$ and $CysLT_2$. The possibility of a specific receptor for LTE_4, termed $CysLT_E$, is also discussed. Moreover, we describe the significance of cys-LTs in health and disease and the development of various cys-LT receptor antagonists for clinical uses.

4.1. Biosynthesis of Cys-LTs

Cys-LTs are rapidly generated de novo from membrane-phospholipid-derived AA, which is liberated by $cPLA_2\alpha$ in response to cell activation (Figure 18). AA is converted to the intermediate 5(*S*)-HpETE and quickly to LTA_4 by 5-LO in concert with FLAP. In neutrophils, LTA_4 is hydrolyzed to LTB_4 by LTA_4 hydrolase, whereas eosinophils, basophils, mast cells, and macrophages preferentially form LTC_4 through conjugation of a reduced glutathione to LTA_4 by LTC_4 synthase or microsomal glutathione *S*-transferase-II (MGST-II), the terminal enzyme involved in cys-LT synthesis.[75,76,345,346] LTC_4 synthase is also expressed in platelets, which cannot synthesize LTA_4 on their own. However, platelets are able to synthesize LTC_4 using LTA_4 released by 5-LO-containing cells via transcellular transport.[347,348] LTC_4 synthase, an 18 kDa integral nuclear membrane protein, is the key enzyme for the biosynthesis of LTC_4.[349,350] LTC_4 synthase is a member of the membrane-associated proteins in the eicosanoid and glutathione metabolism enzyme (MAPEG) family, including MGSTs, microsomal PGE synthase-1 and -2, and FLAP.[351,352] In contrast with MGSTs, LTC_4 synthase conjugates glutathione with a high degree of substrate selectivity for LTA_4, but it does not conjugate glutathione to xenobiotics. LTC_4 synthase exhibits approximately 31% amino acid identity with FLAP and is inhibited by the FLAP inhibitor MK886. Both FLAP and LTC_4 synthase constitutively localize to the perinuclear envelope, where LTC_4 synthesis is thought to occur. The importance of LTC_4 synthase in the biosynthesis of LTC_4 has been found by generating the knockout mice. The homogenates of most tissues from the mice with a targeted deletion of LTC_4 synthase are unable to perform the glutathione conjugation to LTA_4, and mast cells derived from LTC_4 synthase-null mice generate no cys-LTs when activated ex vivo.[349] Moreover, in inflammatory models, there is a reduction in IgE-mediated passive cutaneous anaphylaxis of the ear in the LTC_4 synthase-null mice, suggesting the prominent role of LTC_4 synthase and cys-LTs in augmenting mast cell-dependent permeability.[349] LTC_4 is exported to the extracellular space via multidrug-resistance-associated protein 1 (MRP1).[353] The transcellular metabolism of LTC_4 is associated with formations of LTD_4 and LTE_4. Namely, LTC_4 is converted extracellularly to LTD_4 by a γ-glutamyltranspeptidase (γ-GT)[354] or by a more

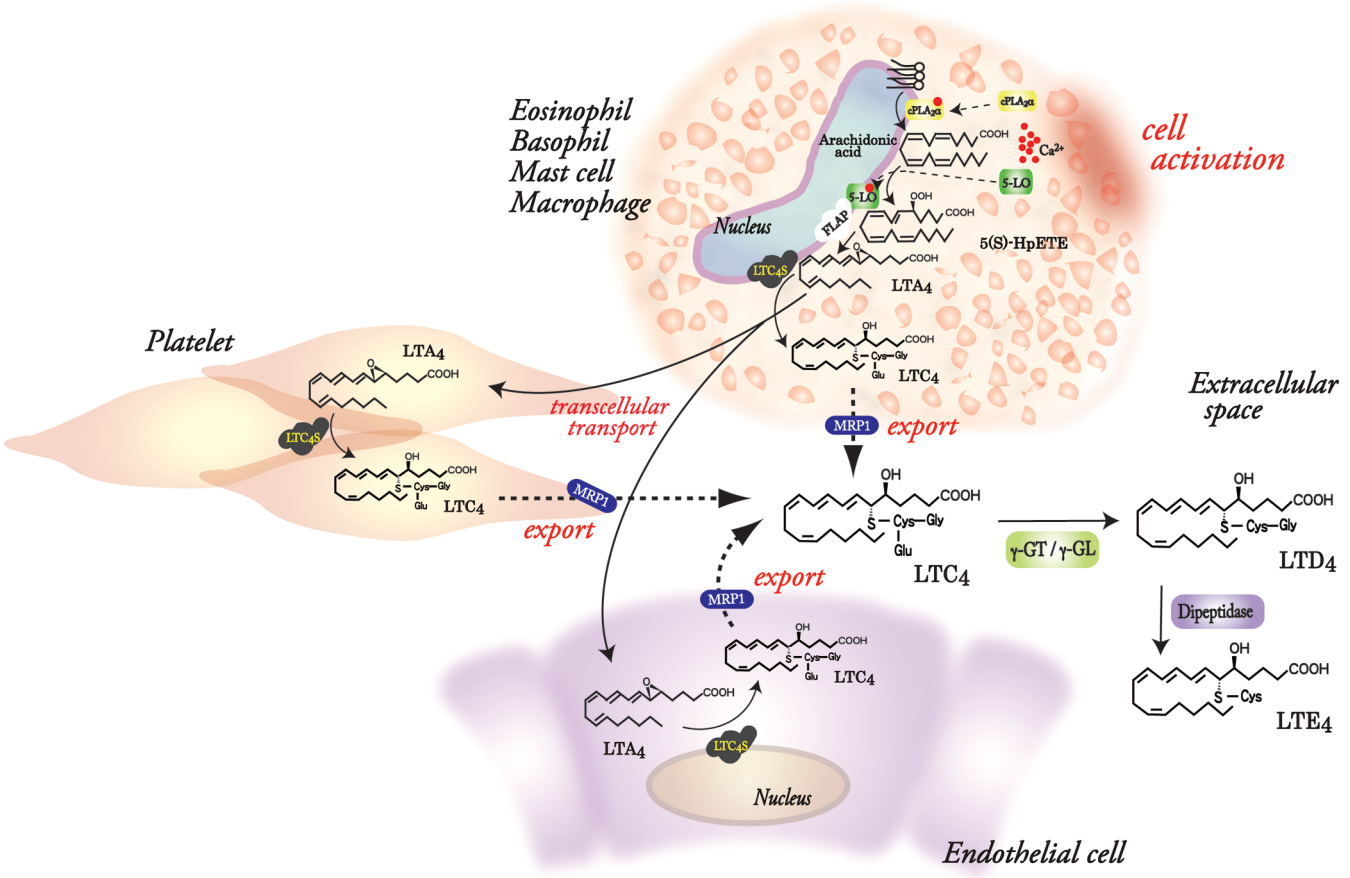

Figure 18. Biosynthesis pathway for the conversion of AA into cys-LTs. AA released from membrane phospholipids is metabolized by 5-LO in the presence of FLAP first to 5(*S*)-HpETE then to LTA_4. LTC_4 synthase, which is a nuclear membrane protein, conjugates glutathione (GST) to this intermediate to form LTC_4. In the extracellular space, further generation takes place by γ-glutamyltransferase (γ-GT) or γ-glutamylleukotrienese (γ-GL) to yield LTD_4 and then dipeptidase to give LTE_4.

functionally specific enzyme, γ-glutamylleukotrienase (γ-GL),[355] and then to LTE_4 by a dipeptidase.[356] LTE_4 is the most stable cys-LT and can be measured after excretion into the urine. Although the enzymes in the 5-LO/LTC_4 synthase pathway are expressed constitutively, their activity and expression in human and mouse mast cells is subject to modulation by exogenous cytokines. Mouse mast cells derived from bone marrow cells cultured in the presence of stem cell factor and IL-10 respond to exogenous IL-3 with marked proliferation and progressive increments in their expression of 5-LO, FLAP, and LTC_4 synthase.[357]

A genetic variant of LTC_4 synthase gene has been described in human. A polymorphism in human LTC_4 synthase gene, namely, an A-C substitution at the 444 bp upstream of the translational start site, was identified. This allelic variant in the LTC_4 synthase core promoter results in the enhancement of the promoter activity. Thus, the presence of this polymorphism correlates with increased levels of LTC_4 generation in calcium ionophore-stimulated peripheral blood eosinophils,[358] diminished lung function in asthmatic children,[359] increased clinical responses to $CysLT_1$ antagonists,[358,360] and an increased incidence of aspirin sensitivity in some,[361] but not all, human ethnic populations.[362]

Recently, there were two important reports demonstrating the crystal structure of human LTC_4 synthase in its apo and glutathione (GSH)-complexed forms.[104,105] The LTC_4 synthase monomer has four transmembrane segments and forms a homotrimer as a unit with functional domains across each interface. The structure of the enzyme in complex with substrate revealed that the active site enforces a horseshoe-shaped conformation on GSH and effectively positions the thiol group for activation by a nearby Arg at the membrane−enzyme interface. These data provide new structural insights into the mechanism of LTC_4 formation and a structural basis for the development of LTC_4 synthase inhibitors for a proinflammatory cascade mediated by three cys-LTs.

4.2. Metabolism of Cys-LTs

4.2.1. Metabolism of LTC_4. Initially, metabolism of LTC_4 proceeds by peptide cleavage reactions of glutathione adducts. The peptide bonds contained in LTC_4 are the unusual γ-glutamyl peptide bond to the N-terminus of cysteine and the glycinyl amino group forming the amide with the cysteinyl carboxy group. LTC_4 is exported to the extracellular space by MRP1,[21] where it is converted to LTD_4 by cleavage of the γ-glutamic acid amide bond. Another cys-LT has been reported and named LTF_4, which retains the γ-glutamic acid in the cys-LT structure but no glycine.[363] However, this metabolite has not been observed either in vitro or in vivo in systems that generate LTC_4. The importance of γ-GT in this LTC_4 metabolism pathway was recognized quite early after the discovery of LTC_4.[354] γ-GT is a heterodimeric glycosylated enzyme located

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on the external side of the plasma membrane.[364,365] In the studies of human γ-GT genes, a related gene, termed γ-GT-rel, that had only 40% amino acid identity to human γ-GT was found. γ-GT-rel was found to have an ability to convert LTC_4 into LTD_4 efficiently.[366] This finding also raised the possibility of other γ-GT isoenzymes that metabolize LTC_4 in vivo. Support for this hypothesis came from the studies of γ-GT-null mice, which were found to have substantial capacity to metabolize LTC_4 to LTD_4.[367] An enzyme responsible for the residual peptidase activity was further isolated from the γ-GT-null mice, partially purified, and found to cleave LTC_4, but not the normal γ-GT substrates used for biochemical assays.[367] This protein, termed γ-GL,[355,368] is similar to γ-GT and is thus considered to be a member of the γ-GT family. γ-GT-null mice were found to accumulate LTC_4; however, double mutant mice with deficiency in both γ-GT and γ-GL prevent conversion of all LTC_4 into LTD_4.[368] In mice, γ-GL is expressed in endothelial cells and plays a central role in catalyzing the initial peptide cleavage of LTC_4 to LTD_4.[369] Several human subjects with deficiency in γ-GT were previously studied for their ability to metabolize cys-LTs.[370] In these experiments, cys-LTs excreted into urine were determined as well as the capacity of circulating monocytes to produce cys-LTs after stimulation with A23187 in vitro.[370] In the γ-GT-null human subjects, LTC_4, but not LTD_4 and LTE_4, could be readily detected in the urine. This is in sharp contrast with normal subjects where no LTD_4 or LTC_4 has ever been detected in urine, whereas LTE_4 is readily measured in urine or in bile.[371,372] Stimulated monocytes from normal human subjects resulted in equal quantities of LTC_4, LTD_4, and LTE_4; in contrast, the γ-GT-null monocytes could produce only LTC_4. These data suggest that γ-GT plays a central role in converting LTC_4 into LTD_4 in humans, although the exact gene defect in these deficient human subjects has not been described yet. Whether γ-GT-rel is deficient as well in these patients is also obscure. The significant increases in LTC_4 metabolism and γ-GT-rel mRNA, but not γ-GT mRNA, were observed in bronchoepithelial cells (16-HBE cells) stimulated with dexamethasone,[373] suggesting a role for γ-GT-rel in the metabolism of LTC_4 to LTD_4. Extensive studies are necessary to clarify the significance of γ-GT and γ-GT-rel for the metabolism of LTC_4 in human.

4.2.2. Metabolism of LTD_4. The half-life of LTD_4 is short (minutes) because of its rapid conversion to LTE_4, resulting in effectively limiting its duration of action in vivo. The conversion of LTD_4 to LTE_4 is carried out by the hydrolysis of the Cys-Gly amide bond in LTD_4, which is catalyzed by a number of dipeptidases.[356] This conversion leads to alteration of the biological activity,[15] as well as the affinity for $CysLT_1$ and $CysLT_2$.[374] Leucine aminopeptidase, which is known as a metabolic enzyme for GSH, was found to have this activity.[375] However, current studies have demonstrated a fairly low activity of leucine aminopeptidase toward hydrolysis of LTD_4.[376] The enzymatic activity that converts LTD_4 into LTE_4 was detected in membrane or microsomal fractions from human neutrophils;[377] rat neutrophils, lymphocytes, and macrophages;[378] and sheep lungs,[379] indicating that the responsible enzyme is a membrane-bound dipeptidase in vivo. A specific membrane-associated dipeptidase, referred to as a microsomal membrane-bound dipeptidase (MBD), was purified from human kidney. This protein is a zinc metalloprotein with a unique zinc-binding motif[380] and has considerable activity in metabolizing LTD_4 to LTE_4, suggesting a central role in cys-LT metabolism.[381] Sheep lung dipeptidase with high similarity to human kidney dipeptidase was subsequently cloned,[379,382] further supporting MBD as an important protein in mediating LTE_4 formation. Interestingly, the MBD-null mice still retained considerable ability to convert LTD_4 into LTE_4.[383] In this study, the lung and heart LTD_4 metabolizing activities were reduced to 40%, whereas other tissues, such as the small intestine, retained 80−90% of the activity compared with WT mice. These results indicate that MBD is partially responsible for the conversion of LTD_4 and that several different peptidases might be responsible for this activity in vivo, although a potent inhibitor for MBD, called cilastatin, has been generated.[384] Two additional candidate genes related to MBD, termed MBD-2 and MBD-3, were identified by search of a gene database.[385] MBD-2, which shows 33% identity with mouse MBD, is capable of metabolizing LTD_4 to LTE_4 with a very similar activity to that of MBD. In contrast, MBD-3 does not have any ability to metabolize LTD_4. Although there is strong evidence for MBD and MBD-2 as having a pivotal role in the synthesis of LTE_4, extensive studies will reveal whether these are the only two dipeptidases that are responsible for the critical termination of the biological activity of cys-LTs.

4.2.3. Metabolism of LTE_4. LTE_4 is metabolized via similar pathways to LTB_4 in rat hepatocytes and human subjects (Figure 19).[371] After initial ω-oxidation to 20-COOH-LTE_4 and formation of the 20-CoA ester, metabolism proceeds through at least three stages of β-oxidation exclusively within the peroxisome.[372,386] In rat hepatocytes, the most abundant metabolite observed is *N*-acetyl-16-COOH-tetranor-LTE_3. *N*-Acetylation is a major metabolic step for LTE_4 in the rat even before ω/β-oxidation; however, it is not a major metabolic pathway in human tissues.[371] Intact LTE_4 is also excreted into urine as a major metabolite due to its stability in vivo,[371] accounting for approximately 4% total LTC_4 production in human subjects.[387] Consequently, LTE_4 levels can be monitored in the urine,[388] sputum,[389] and exhaled breath condensate[390] as an index of the cys-LT synthesis in human diseases such as asthma, where its concentrations can be markedly elevated.

4.3. Existence of Cys-LT Receptors

The existence of cys-LT receptors was suggested even before the structures of cys-LTs were elucidated in 1979. In human and guinea pig airway preparations, the compound FPL-55712 selectively blocked the contractile actions of cys-LTs, at that time known as SRS-A.[339] In these studies, FPL-55712 inhibited the contractions induced by LTD_4, but not those induced by LTC_4, suggesting the action of LTC_4 at a FPL-55712-insensitive site.[391,392] These guinea pig receptors became known as LTD_4 and LTC_4 receptors, respectively. In human bronchi, however, FPL-55712 blocked the contractions induced by both LTC_4 and LTD_4, demonstrating that in human bronchial smooth muscle, LTC_4 and LTD_4 appeared to act at the common receptor, which is termed $CysLT_1$. Dose−response analysis showed that LTD_4 is the most potent agonist at human $CysLT_1$, with LTC_4 and LTE_4 being significantly less active. Evidence for a second cys-LT receptor ($CysLT_2$) in humans came from studies of vascular tissue. Labat et al. reported that a number of antagonists that block LTC_4- and LTD_4-induced airway smooth muscle contractions failed to inhibit the cys-LT-induced contractile responses of venous preparations from human lung.[393] Unlike airway tissues, LTC_4 and LTD_4 were equally potent in causing vasoconstriction. Contractions of human pulmonary vessels were antagonized by BAY-u9773, a nonselective dual antagonist of $CysLT_1$ and $CysLT_2$, but not by selective $CysLT_1$ antagonists.[393] In contrast to vasoconstriction at higher concentrations (10^{-6}−10^{-5} M),

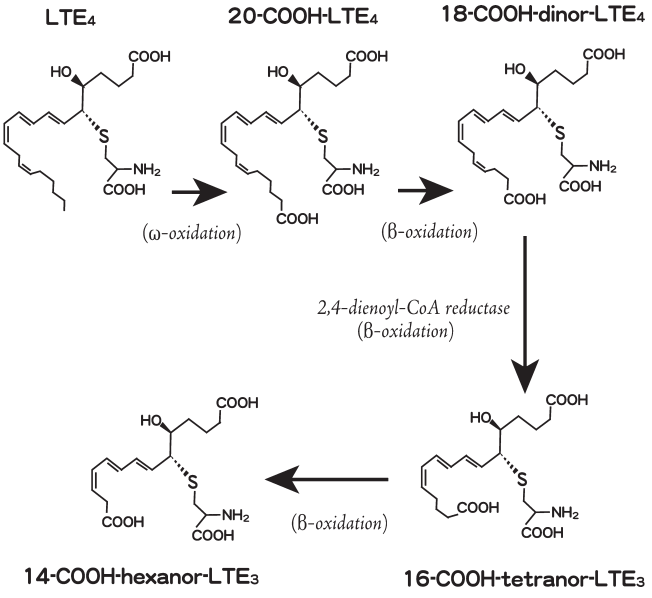

Figure 19. Metabolism of LTE_4. Pathway of the ω- and β-oxidation of LTE_4 showing the chemical structure of metabolites identified in the urine of human subjects.

cys-LTs at lower concentrations (10^{-8}–10^{-7} M) relax many vascular preparations from dogs, guinea pigs, and humans.[394−396] In pulmonary vessels, this effect has been proposed to involve $CysLT_2$ on the vascular endothelium that produce indirect relaxation via the syntheses of nitric oxide and prostanoids. The existence of other receptors, which designated the $CysLT_E$ receptor with high specificity for LTE_4, is proposed in mice lacking both $CysLT_1$ and $CysLT_2$. LTE_4 also mediates its effects through the adenosine diphosphate-reactive purinergic receptor, $P2Y_{12}$. Although cys-LT receptor subtypes were first identified pharmacologically, the recent clonings and molecular characterizations of $CysLT_1$ and $CysLT_2$ represent an important advance in defining their respective roles in airway inflammation.[397−400]

4.3.1. Binding Studies Using Radiolabeled Cys-LTs. Three important points, which should be considered, became apparent from the radioligand binding experiments. First, interpretation of the results of $[^3H]LTC_4$ binding studies are complicated by the rapid metabolic transformation of LTC_4 to LTD_4, if this is not controlled by enzymatic inhibition. Inhibitors of this metabolism must frequently be incorporated in $[^3H]LTC_4$ binding experiments. In addition, recent reports have also suggested the necessity to include *S*-decylglutathione (a high-affinity ligand for nonreceptor LTC_4 binding sites) in the membrane preparations.[401] Second, there was often no correlation between the $[^3H]LTC_4$ binding data and the results of functional studies, e.g., contraction, as in the case of either the guinea pig ileum,[402] guinea pig lung strip,[403,404] or guinea pig uterus.[405,406] In contrast, a correlation was observed between the antagonist effects in the $[^3H]LTD_4$ binding studies and the ability of the antagonists to block LTD_4-induced contractions. Furthermore, in the rat lungs the binding data provided evidence for the presence of two sites,[407,408] whereas the functional studies (contraction) showed the existence of single receptor for LTC_4.[409,410] Third, $[^3H]LTC_4$ may bind to other cellular proteins, such as glutathione *S*-transferase (GST),[411−413] specific exporters in human eosinophils,[414] ATP-dependent carriers in rat liver,[415,416] and MRP1.[417] Therefore, the $[^3H]LTC_4$ binding to nonreceptor proteins may mask the LTC_4 receptors present in the preparations and explain the reason for the lower affinity for LTC_4 binding sites when compared with the functional data.

4.3.2. Binding Sites of $[^3H]LTC_4$ on Tissues. Despite the high nonspecific bindings of $[^3H]LTC_4$, the $[^3H]LTC_4$ binding has been determined in the central nervous system,[418] brain capillaries,[419,420] endothelial cell,[421] human erythrocytes,[422] and leukocytes.[423] Cheng et al. reported that $[^3H]LTC_4$, but not $[^3H]LTD_4$, binds to the membranes from the guinea pig uterus,[424] and these data were also confirmed in kinetic binding experiments.[403] The $[^3H]LTC_4$ binding affinity to the membrane fractions in the uterus (K_d = 10 nM)[406] is similar to that of intact cultured smooth muscle cells from the hamster vas deferens[425] but slightly lower than those observed in the guinea pig and rat lung membranes.[407,426] Thus, a specific high-affinity receptor is present in the uterus. Further studies were performed using brain homogenates, which contain the highest activity of the $[^3H]LTC_4$ binding sites. The potency order of cys-LTs for inhibition of the brain $[^3H]LTC_4$ binding is identical to their inhibitory order in the uterine homogenate. Additionally, these studies have demonstrated that the relative abilities of LTC_4, LTD_4, and LTE_4 to inhibit the $[^3H]LTC_4$ binding in the brain and uterine membranes are the same as their ability to reduce binding in the smooth muscle cell and lung homogenates.[407,424,425] In contrast, the IC_{50} of LTC_4 to inhibit brain $[^3H]LTC_4$ binding differs from that value obtained in the uterine homogenate, indicating a difference in the K_d values of the $[^3H]LTC_4$ binding between brain and uterus. Data from initial studies have also shown that the K_d of lung $[^3H]LTC_4$ binding apparently differed from that of the smooth muscle cell.[407,425] Whether these differences are due to the existence of high- and low-affinity LTC_4 receptor sites awaits further works.[425] Interestingly, Cheng and Townley also demonstrated that LTC_4 was more potent than LTD_4 in its ability to produce uterine contractions.[427] These results suggest that in the guinea pig uterine preparations, $[^3H]LTC_4$ binds to a receptor that may be responsible for cys-LT-induced uterine contraction in this species.

4.3.3. Binding Sites of $[^3H]LTD_4$ and $[^3H]LTE_4$ on Tissues. The initial studies in a variety of membrane preparations demonstrated the presence of $[^3H]LTD_4$ and $[^3H]LTE_4$ binding sites. These data also showed a correlation between the binding distribution of $[^3H]LTD_4$ and $[^3H]LTE_4$.[424] In guinea pig lung preparations, Aharony et al. found that the rank order of LTC_4, LTD_4, and LTE_4 for the inhibition of the $[^3H]LTD_4$ and $[^3H]LTE_4$ binding is similar; however, the potencies of inhibitions by cys-LTs is higher against $[^3H]LTE_4$ than those observed with $[^3H]LTD_4$.[428] Although ICI 198,615 exhibited inhibitory effect on the binding of the $[^3H]LTE_4$ and $[^3H]LTD_4$, there were subtle differences in potency. Namely, LTD_4 was significantly less potent than ICI 198,615 for inhibiting the $[^3H]LTD_4$ binding, whereas no such difference was observed against $[^3H]LTE_4$. These data provide evidence for the existence of heterogeneous LTD_4 receptors. While these studies indicate that tissues may contain different receptors,[429,430] the binding sites for the same radioligand in one tissue may also be different from that observed in another preparation. For example, in guinea pig lung membranes, $[^3H]LTD_4$ exhibited a K_d value of 0.4 nM, whereas in the membranes obtained from the guinea pig myocardium the K_d was 3.4 nM.

4.4. Cys-LT Receptor Type-I ($CysLT_1$)

4.4.1. Molecular Cloning of $CysLT_1$. In 1999, the molecular cloning and characterization of human $CysLT_1$ were successfully

```
                                                                  I
hCysLT1     1  MD----------ETGNLTVSSATCHD-----TIDDFRNQVYSTLYSMISVVGFFGNGFVL  45
hCysLT2     1  .ERKFMSLQPSISVSEMEPNGTFSNNNSRNC..EN.KREFFPIV.LI.FFW.VL...LSI  60

                          II                              III
hCysLT1    46  YVLIKTYHKKSAFQVYMINLAVADLLCVCTLPLRVVYYVHKGIWLFGDFLCRLSTYALYV  105
hCysLT2    61  ..FLQP.K.STSVN.F.L...IS...FIS...F.AD..LRGSN.I...LA..IMS.S...  120

                                                IV
hCysLT1   106  NLYCSIFFMTAMSFFRCIAIVFPVQNINLVTQKKARFVCVGIWIFVILTSSPFLMAKPQK  165
hCysLT2   121  .M.S..Y.L.VL.VV.FL.M.H.FRLLHVTSIRS.WIL.GI...L-.MA..IM.LDSGSE  179

                                        V
hCysLT1   166  DEKNNTKCFEPPQDNQTKNHVLVLHYVSLFVGFIIPFVIIIVCYTMIILTLLKKSMKKN-  224
hCysLT2   180  QNGSV.S.L.LNLYKIA.LQTM--N.IA.V..CLL..FTLSI..LL..RV...VEVPESG  237

                          VI                                    
hCysLT1   225  LS-SHKKAIGMIMVVTAAFLVSFMPYHIQRTIHLHFLHNETKPCDSVLRMQKSVVITLSL  283
hCysLT2   238  .RV..R..LTT.IITLII.FLC.L...TL..V..TTWKVGL--.KD--.LH.AL....A.  293

                     VII
hCysLT1   284  AASNCCFDPLLYFFSGGNFRKRL-STFRKHSLSSVTYVPRKKASLPEKG--EEICKV  337
hCysLT2   294  ..A.A..N....Y.A.E..KD..K.AL..----GHPQKAKT.CVF.VSVWLRKETR.  346
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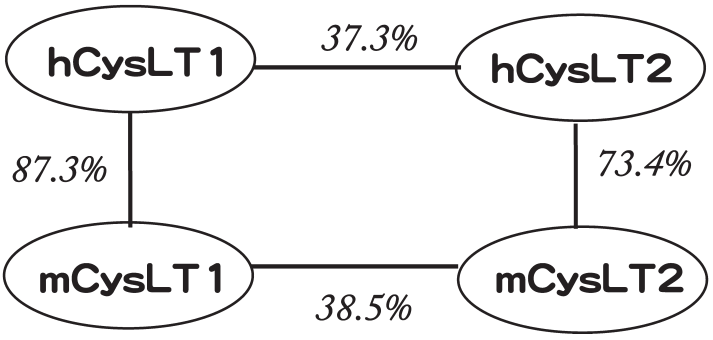

Figure 20. (Upper) Amino acid sequence alignment of human $CysLT_1$ and $CysLT_2$. The putative transmembrane domains predicted from a Kyte−Doolittle hydrophobicity analysis are labeled as I−VII. Conserved amino acids are indicated by periods (.), and gaps in the alignment are indicated by dashes (−). (Lower) The relative sequence homologies (percentage identities) among human and murine of $CysLT_1$ and $CysLT_2$ are shown.

performed under the general program of identifying cognate ligands for orphan GPCRs.[397,399] Human $CysLT_1$ was identified as a 337 amino acid putative GPCR, referred to as either HG55[397] or HMTMF81[399] (Figure 20). The characteristics of $CysLT_1$ are summarized in Table 4. The former investigators observed the functional expression of HG55, namely LTD_4-elicited activation of a calcium-activated Cl^- channel in HG55-expressing *X. laevis* oocytes. In contrast, the other group at SmithKline Beecham Pharmaceuticals identified HMTMF81, which responded selectivity with calcium mobilization to LTC_4, LTD_4, and LTE_4 (0.1−10 μM) when expressed in HEK293 cells. Northern analyses of human tissues showed that human $CysLT_1$ mRNA was detected as an approximately 2.8 kb species and is widely expressed in spleen and peripheral blood leukocytes; less strongly in lung, bronchus, small intestine, colon, skeletal muscle, pancreas, and placenta; and weakly in the prostate, heart, brain, liver, kidney, and adipose tissues.[397,399,431] This receptor was also detected in U937 and HL-60 cells, with an enhanced expression in dimethyl sulfoxide-differentiated HL-60 cells.[399] In the sodium butyrate-stimulated HL-60 cells differentiated to eosinophilic-like cells, IL-5 treatment leads to a 5-fold increase in the expression of $CysLT_1$ mRNA.[432] In agreement to the action of cys-LTs in asthma, $CysLT_1$ is expressed in a variety of inflammatory cells, i.e., eosinophils, neutrophils, mast cells, monocytes/macrophages, B-lymphocytes, and pregranulocytic $CD34^+$ cells, but not $CD4^+$ or $CD8^+$ peripheral T-lymphocytes.[433,434] Northern analysis in normal and asthmatic lungs indicates no differences in $CysLT_1$ mRNA expression, although precise analyses, such as in situ studies, are needed to conclude.[399] In situ hybridization demonstrated that human $CysLT_1$ mRNA is also expressed in lung smooth muscle cells and interstitial macrophages, with little or no expression in epithelial cells.[397] In mouse, two isoforms of $CysLT_1$ cDNA have been cloned from a mouse lung cDNA library.[435−437] The short isoform of $CysLT_1$ containing two exons encodes a polypeptide of 339 amino acids with 87.3% amino acid identity to human $CysLT_1$.[397,399,435] On the other hand, the long isoform has two additional exons and an in-frame upstream start codon, resulting in a 13 amino acid extension at the N-terminus of the receptor.[435] Northern blot analysis of mouse $CysLT_1$ detecting both isoforms revealed production of a 3.5 kb transcript in lung and skin, whereas RT-PCR showed wide expression of the long

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Table 4. Characteristics of $CysLT_1$ and $CysLT_2$

	$CysLT_1$	$CysLT_2$
structure (amino acids)	human (337)	human (346)
	mouse (short, 339; long, 352)	mouse (309)
	rat (352)	rat (309)
accession number (GenBank)	human (AH119711)	human (AB038269)
	mouse (AC021992)	mouse (AF331658)
	rat (AB052685)	rat (BAB60816)
ligand	$LTD_4 > LTC_4 \gg LTE_4$	$LTD_4 = LTC_4 \gg LTE_4$
antagonist	montelukast, zafirlukast, pranlukast, MK571, BAY-u9773, LY171833	BAY-u9773
expression (human)	leukocytes, spleen, smooth muscle > lung, intestine	leukocytes, spleen, adrenal medulla, lung, heart, brain
coupled G-protein	$G_{q/11}$	$G_{q/11}$
chromosome	human (Xq13.2-Xq21.1)	human (13q14.12-q21.1)
	mouse (X D)	mouse (14 D2)
	rat (Xq31)	rat (15p11)

isoform, with strongest expression in lung and skin. The long form rather than the short type of mouse $CysLT_1$ is the closer counterpart to the human receptor, and that is expressed more abundantly in the mouse tissues.

4.4.2. Binding Affinities of Natural Ligands to $CysLT_1$. Determination of the binding affinity of cys-LTs to the cloned human and mouse $CysLT_1$ was carried out.[397] Scatchard analyses of $[^3H]LTD_4$ binding to human $CysLT_1$ in COS-7 membranes demonstrated a K_d value of 0.3 nM,[397] and the IC_{50} values of LTD_4, LTC_4, and LTE_4 for the labeled ligand are 0.9, 350, and 200 nM, respectively. The affinities of antagonists are similar to those demonstrated in other functional assay systems for $CysLT_1$ as well as in the binding experiments on human lung or U937 cells.[397,438] Likewise, human $CysLT_1$-expressing HEK293 cells respond selectively to cys-LTs with a rank order of potency (EC_{50} value) of LTD_4 (2.5 nM) > LTC_4 (24 nM) > LTE_4 (240 nM).[399] In these studies, LTE_4 was shown to be a partial agonist of human $CysLT_1$-expressing HEK293 cells.[399] This is similar to evidence derived from human bronchus,[393] sheep trachea,[439] and U937 cells.[440] The $[^3H]LTD_4$ binding and LTD_4-induced calcium mobilization in these cells are potently inhibited by several cys-LT receptor antagonists. The rank order of the potency in this system is pranlukast = zafirlukast > montelukast > pobilukast.[399]

4.4.3. Signal Transduction via $CysLT_1$. Initial studies demonstrate that activation of $CysLT_1$ by LTD_4 leads to G-protein activation and the release of several second intracellular messengers, namely, diacylglycerol, inositol phosphates, and calcium ion, which are followed by activation of protein kinase C (PKC).[441,442] Clark et al. demonstrated that $CysLT_1$ activation by LTD_4 also leads to the release of AA via stimulation of $cPLA_2\alpha$, which is associated with an enhanced transcription of $cPLA_2\alpha$ activating protein.[443] In CHO cells expressing mouse $CysLT_1$, LTD_4-induced intracellular calcium mobilization is blocked by the treatment with the $CysLT_1$ antagonist MK-571.[435] The rank orders of agonist functional potency for calcium mobilization and the potency for competition of the $[^3H]LTD_4$ binding to the recombinant mouse receptor are similar to those of the human receptor, except that LTC_4 is relatively less potent for mouse than for human $CysLT_1$.[397,399,435] In circulating peripheral blood mononuclear cells (MNCs), cys-LTs have been demonstrated to modulate calcium responses via a PTX-sensitive G-protein ($G_{i/o}$)[444] or through two distinct G-proteins, PTX-sensitive and -insensitive ($G_{q/11}$), in monocyte/macrophage U937 cells[445] or human epithelial cell.[446] These data suggest the $CysLT_1$ promiscuity in G-protein coupling, at variance with results obtained so far in recombinant systems, and are in good agreement with the findings that LTD_4 activates distinct signaling pathways differently coupled to G-proteins in MNCs or intestinal epithelial cells. Among these pathways, several groups, including ours, have demonstrated the activation of MAPK by $CysLT_1$ in THP-1 cells,[447] renal mesangial cells,[448] intestinal epithelial cells,[449] monocyte/macrophage U937 cells,[450] astrocytes,[451] colon cancer cells,[452] mast cells,[453] and airway smooth muscle cells.[454] Interestingly, LTD_4 activates MAPK through a PKC−Raf-1-dependent pathway in THP-1 cells,[447] whereas in dU937 cells ERK1/2 activation involves a RasGTP-dependent pathway, PLC, and calcium-dependent tyrosine kinase(s).[450] More recently, LTD_4 has been shown to induce proliferation and migration of mouse embryonic stem cells through a mechanism involving STAT-3, PI3K, glycogen synthase kinase-3β/β-catenin phosphorylation, and calcineurin expression.[455] In U937 cells and in primary human monocytes, a series of inflammatory mediator-activating $G\alpha_i$-coupled receptors (e.g., FPR1 and BLT1) desensitizes $CysLT_1$-induced calcium response unidirectionally through activation of PKC.[456] Conversely, PAF receptor, exclusively coupled with $G\alpha_q$, cross-desensitizes $CysLT_1$ without the apparent involvement of any kinases.[456] Interestingly, $G\alpha_s$-coupled receptors [e.g., β_2-adrenergic, hitamine$_{1/2}$, and PGE_2 (EP_2 and EP_4) receptors] are also able to desensitize $CysLT_1$ response through activation of PKA. Heterologous desensitization seems to affect mostly the $G\alpha_i$-mediated signaling of the $CysLT_1$. Cys-LTs (LTD_4 and LTC_4) induces an increased production of a potent monocyte chemoattractant CCL2 (MCP-1) in IL-4-primed THP-1 cells in a dose-dependent manner.[431] This effect is effectively inhibited by the $CysLT_1$-selective antagonist MK571 and only partially by a nonselective $CysLT_1$/$CysLT_2$ inhibitor BAY-u9773, implying a $CysLT_1$-mediated mechanism.[431] Thus, cys-LTs signaling through $CysLT_1$ might contribute to inflammatory reactions by cooperating with IL-4 in enhanced CCL2 production in human monocytic cells.[431] LTD_4 enhances the adhesion of eosinophils[457] and PMNs[458] to ICAM-1 via β2 integrins. Recently, LTD_4 has been shown to rapidly induce focal adhesion kinase-related tyrosine kinase (Pyk2) phosphorylation and significantly upregulate

α4β1 and α5β1 integrin-dependent adhesion of both primitive and committed hematopoietic stem and progenitor cell.[459] These effects are inhibited by treatment with montelukast[458,460] and pranlukast,[457] suggesting the involvement of $CysLT_1$. Nielsen et al. recently reported a localization of $CysLT_1$ in the nucleus and the activation of ERK1/2 via the nuclear localizing $CysLT_1$ (see section 4.8.3).[461] It is interesting that over the past few years, some GPCRs have been shown to reside not only in the plasma membrane but also in the nuclear envelope.[462] The amount of nuclear $CysLT_1$ is increased in colorectal adenocarcinoma cells, suggesting involvement of the signaling via $CysLT_1$ in the proliferation of colon cancer cells.

4.4.4. Gene Structure and Transcriptional Regulation of $CysLT_1$. The gene for human $CysLT_1$ is located on chromosome Xq13.2-q21.1.[397] This gene consists of five exons, which are variably spliced, and has a single promoter region with multiple transcriptional start sites.[435] Mouse $CysLT_1$ gene also maps to the chromosome X at band XD.[463] In human, four different $CysLT_1$ transcripts, transcript-I, transcript-II, transcript-III, and transcript-IV, are identified.[431] Especially, transcript-I (approximately 2.8 kb) represents the major transcript present in human leukocytes and smooth muscle cells, as well as in other studied human tissues. A similar structure of 5′-UTR in transcript-I was found in a peripheral blood mononuclear cell (PBMC) cDNA library[399] and in a human lung cDNA library.[435] A BLAST search of GenBank sequences revealed only sequences representing the 5′-UTR sequence of transcript-I, suggesting that transcript-I is a major mRNA for human $CysLT_1$. Although transcript-II is less expressed, it is also produced in blood leukocytes, smooth muscle cells, and tissues showing high $CysLT_1$ mRNA expression, such as heart, lung, and spleen. Comparison of human and mouse $CysLT_1$ cDNAs shows that human transcript-II contains sequences equivalent to the short isoform of mouse $CysLT_1$.[435] Both human transcript-II and mouse short isoform cDNAs are less preferentially expressed. However, all human transcripts lack the equivalent of mouse exon-III. In addition, rare human transcripts-III and -IV contain exons-II and -III, equivalents of which have not been identified in mice. The role of each splice variant of $CysLT_1$ is still unclear. All identified transcripts are generated from the common region of exon-I, indicating that the 5′ end of exon-I is the primary start site of the $CysLT_1$ transcription. Reporter gene analyses using THP-1 cells revealed that the region immediately upstream of TSS, approximately 650 bp, possesses a core promoter activity. Like other GPCR promoters, human $CysLT_1$ promoter is TATA-less and contains several binding sites for transcription factors, such as AP-1 and GATA. It has been reported that IL-4 regulates $CysLT_1$ expression in human monocytes and macrophages.[464] Moreover, in THP-1 cells, IL-4 stimulation significantly enhances the transcription of $CysLT_1$ gene, followed by a change in the surface $CysLT_1$ protein level.[431,464] The primary pathway of the IL-4 receptor signaling relates to the activation of cytoplasmic Janus kinases and the subsequent phosphorylation of STAT6, which is translocated to the nucleus.[465] In the nucleus, STAT6 binds preferentially to the consensus sequence TTC(N4)GAA[466] and drives expression of many IL-4-responsive genes.[467] In human $CysLT_1$ promoter, there are two putative STAT6 response elements, which are necessary for IL-4-induced upregulation of the $CysLT_1$ expression.[431]

4.5. Other Features of $CysLT_1$

4.5.1. Importance of the C-Terminus Region for Signaling and Internalization. Naik et al. have found that rapid and profound LTD_4-stimulated internalization was observed for WT $CysLT_1$. In contrast, a C-terminal truncated $CysLT_1$ exhibited impaired internalization yet signaled robustly, suggesting a region within amino acids 310−321 as critical for internalization of this receptor.[468] Although the overexpression of arrestins significantly increases the WT $CysLT_1$ internalization, the internalization of WT $CysLT_1$ normally occurs in murine embryonic fibroblasts lacking both arrestin-2 and arrestin-3, indicating that arrestins are not the primary physiologic regulators of $CysLT_1$. Moreover, mutation of putative PKC phosphorylation sites within the C-terminus of $CysLT_1$, Ser313−316Ala, reduces their internalization, phosphatidylinositol production, and calcium mobilization by LTD_4, suggesting the importance of the phosphorylation of these sites for the signaling and internalization of $CysLT_1$.[468]

4.5.2. Amino Acid Residues Involved in the Constitutively Active Form of $CysLT_1$. It has been established that several GPCRs have been shown to be spontaneously active without stimulation by its agonists in vivo.[469,470] Actually, we found that GPR20, which has been known as an orphan GPCR, constitutively transduces signals intracellularly without any stimulation.[471] These constitutively active receptors have been a valuable tool to demonstrate that certain ligands convert the receptor into inactive conformations. These compounds are known as "inverse agonists", given they have the opposite effect of agonists.[472] Because the inverse agonists may have preferred therapeutic applications,[472−475] generation of the constitutively active mutants of GPCRs is a useful strategy to develop these compounds. Dupre et al. reported that in human $CysLT_1$, the substitution of Ile232 in the third intracellular loop to Arg (I232R) and of Asp106 in the TM2 to Ala (N106A) stabilizes the active form and leads to a significant increase in the basal production of inositol phosphates (IPs), demonstrating the construction of constitutively active mutants of human $CysLT_1$.[476] Using these mutant human $CysLT_1$, they suggested that montelukast, zafirlukast, and MK-571 have the inverse agonist activities on these mutant receptors.[476]

4.5.3. Single Nucleotide Polymorphisms of $CysLT_1$ Associated with Asthma. Human $CysLT_1$ promoter polymorphism is associated with aspirin-intolerant asthma (AIA) in males.[477,478] Kim et al. found the $CysLT_1$ promoter polymorphism in patients with AIA by comparison with aspirin-tolerant asthma (ATA) and normal healthy controls. They have shown that three single nucleotide polymorphisms (SNPs), −634C>T, −475A>C, and −336A>G, in the $CysLT_1$ promoter are associated with AIA risk in males. Males with AIA have significantly higher frequencies of the minor alleles (T, C, G) at the three SNPs than male control subjects.[477] The different association found between the sexes is consistent with a previous report that male subjects more often show higher levels of $CysLT_1$ than female subjects in colorectal adenocarcinomas.[479] The observed differences in association between males and females may be related to allele-dependent dysregulation of $CysLT_1$ gene in AIA patients. Consequently, Kim et al. suggested that the nucleotide substitutions at the three SNPs create an unknown transcription factor binding sequence, which induces an increase in $CysLT_1$ protein.[477] In fact, due to these SNPs the $CysLT_1$ expression is upregulated in a Th2 cytokine-rich environment, promoting the proinflammatory effects of cys-LTs.[432,464,480] Although there is not enough supporting biological evidence, it is speculated that in patients with the SNPs of $CysLT_1$, Th2 cytokine production is enhanced, which may lead to IgE production. Interestingly, a

recent report studying the British 1958 birth cohort found that the $CysLT_1$ gene promoter polymorphisms might influence the risk of atopy in the female white population with suggestive evidence of heterozygote vigor.[481] In a high prevalence asthmatic population with a founder effect, Thompson et al. observed the minor allele of the coding polymorphism 899G>A (Gly300Ser) to be associated with both asthma and atopy in a population of Tristan da Cunha subjects.[482,483] Other studies have detected an association of the promoter or coding SNPs with allergic phenotypes such as high total IgE[477] and atopic dermatitis,[484] which could not be reproduced elsewhere.[485,486] Overall, most studies agree that there is an association between $CysLT_1$ genetic variation and allergic phenotypes and that this association is gender specific.

4.6. Cys-LT Receptor Type-II ($CysLT_2$)

4.6.1. Molecular Cloning of $CysLT_2$. The $CysLT_2$ identification was made through the analysis of a rat cys-LT receptor homologue, RSPBT32, followed by the cloning of a human homologue.[398] Human $CysLT_2$ shows 37.3% amino acid identity with human $CysLT_1$, and the extreme C-termini of these proteins have little homology (Figure 20).[398] The characteristics of $CysLT_2$ are summarized in Table 4. In the human $CysLT_2$ cloning, Heise et al. demonstrated the pharmacological characterization of this receptor in several tissues, the relative potencies of cys-LTs, the lack of responses to classical $CysLT_1$ receptor antagonists, and the antagonist activity of BAY-u9773.[393,398,487] Subsequently, the group of Yamanouchi Pharmaceutical Co. reported the identification of human $CysLT_2$,[400] and then a third report on the characteristics of human $CysLT_2$ was published by Nothacher et al.[488] The same clone was also identified from the Helix Research Institute database.[400] Currently, the clonings of mouse[489,490] and guinea pig[491] $CysLT_2$ have also been reported. Northern blot analyses of human $CysLT_2$ showed high expression in heart, adrenals, peripheral blood leukocytes, placenta, spleen, and lymph nodes, with weaker expression in the brain.[398,400,488] By RT-PCR, comparison of two cys-LT receptors revealed negligible human $CysLT_1$ expression but high expression of human $CysLT_2$, in the heart and eosinophils,[492] whereas only human $CysLT_1$ is expressed in tracheal tissue.[400] Both receptors are highly expressed in spleen.[398,400,488] In situ hybridization analyses of human lung demonstrated that $CysLT_2$ mRNA is strongly expressed in interstitial macrophages and slightly expressed in smooth muscle cells.[398] In human peripheral blood monocytes, about 30% of cells produce human $CysLT_2$, whereas greater than 90% of purified human eosinophils express the receptor.[398] In mouse, Northern blot analysis of poly(A^+) RNA from various tissues failed to find a detectable hybridization band of $CysLT_2$, indicating low level expression of this receptor.[489] A more sensitive ribonuclease protection assay revealed that the highest expression was found in spleen, thymus, and adrenal gland, with weaker expression in kidney, brain, and buffy coat (primarily peripheral blood leukocytes). However, mouse $CysLT_2$ expression is not apparent in lung, liver, heart, aorta, skeletal muscle, uterus, and ovary. Using amplified in situ hybridization, the $CysLT_2$ mRNA was detected in mouse heart. Mouse $CysLT_2$ mRNA was detected selectively on larger cells concentrated at the apical portions of the ventricle. This pattern is suggestive of expression on conducting Purkinje cells and is consistent with the mouse $CysLT_2$ expression seen in human heart, particularly in Purkinje fiber cells, myocytes, and fibroblasts derived from atrium and ventricle.[398]

Recently, we reported different pharmacological properties of $CysLT_2$ between human and mouse and showed that the tissue expressions of mouse $CysLT_1$ and $CysLT_2$ are different in some strains of mice, being higher in C57BL/6 inbred strain than in 129 inbred strain.[490] These data suggest that receptor distributions are different between and within species. Differential tissue expression between two mouse strains raises the possibility that regulatory polymorphism is present. In C57BL/6 strain, Northern analysis showed the highest expression of $CysLT_1$ mRNA in skin, lung, small intestine, and macrophages, and moderate expressions were found in other tissues. In contrast, the expression of $CysLT_2$ is ubiquitous, with higher expressions in spleen, lung, and small intestine. Given the importance of cys-LTs in skin diseases including atopic dermatitis,[493] we investigated the distribution of cys-LT receptors in mouse skin by in situ hybridization. In 129 inbred strain, for which the potent inflammatory response of the skin has been reported,[494] the high expressions of $CysLT_1$ and $CysLT_2$ mRNAs were detected mostly in fibroblasts of the subcutaneous connective tissues.[490] Consistent with these data, it has been reported that cys-LTs increase collagen synthesis in fibroblasts and the expressions of $CysLT_1$ and $CysLT_2$ in the fibroblasts.[495]

4.6.2. Binding Affinities of Natural Ligands to $CysLT_2$. The [^{3}H]LTD_4 binding to the membranes of human $CysLT_2$-expressing COS-7 cells revealed the presence of high and low affinity binding sites (K_d = 0.4 and 51 nM, respectively).[398] The rank order potency of LTs to compete with the [^{3}H]LTD_4 binding is $LTD_4 = LTC_4 \gg LTE_4$, with no competition up to 10 μM by LTB_4. The $CysLT_1$ antagonists are either weak (zafirlukast and pranlukast) or inactive (montelukast) at competing for the [^{3}H]LTD_4 binding to human $CysLT_2$. In contrast, full competition was observed with the dual $CysLT_1$/$CysLT_2$ antagonist BAY-u9773 with an IC_{50} value of 0.6 μM.[397] The membranes from mouse $CysLT_2$-expressing HEK293 cells were also used for radioligand binding studies.[489] The rank order potency of LTs to compete with [^{3}H]LTD_4 binding is $LTC_4 = LTD_4 >$ BAY-u9773 $> LTE_4$, with IC_{50} values of 8.5, 17.6, 325, and 1985, respectively. LTB_4 and MK-571 do not compete with [^{3}H]LTD_4 even at 10 μM.

4.6.3. Signal Transduction via $CysLT_2$. Due to the absence of selective antagonists, at least until a short time ago,[496] it has been difficult to obtain detailed information about signal transduction via $CysLT_2$. In the human $CysLT_2$-expressing *X. laevis* oocytes, either LTC_4 or LTD_4 elicit a calcium-dependent chloride flux that is not blocked by the selective $CysLT_1$ antagonist MK-571 but is inhibited by the dual $CysLT_1$/$CysLT_2$ antagonist BAY-u9773.[398] In addition, LTC_4 and LTD_4 evoke a concentration-dependent activation of calcium flux in human $CysLT_2$-expressing HEK293 cells, demonstrating the coupling with $G\alpha_{i/o}$ and/or $G\alpha_q$ types of G-proteins.[398] As expected, this activation is blocked by BAY-u9773 but not by MK-571, montelukast, zafirlukast, and pranlukast.[398] Kamohara et al. showed the presence of human $CysLT_2$ mRNA in atrium, ventricle, and intermediate coronary arteries by in situ hybridization.[497] These investigators also reported that human coronary smooth muscle cells stimulated with LTC_4 cause an increase in the intracellular calcium mobilization, suggesting the existence of the functional $CysLT_2$ in the human heart.[497] We have recently cloned mouse $CysLT_2$ to further examine the signaling via this receptor.[490] The cys-LTs-induced intracellular calcium mobilizations were observed by a fluorescence-based imaging plate (FLIPR) assay in the HEK293T cells expressing

mouse $CysLT_2$. LTC_4 and LTD_4 were equipotent agonists for this receptor, with EC_{50} values of 13.4 and 14.4 nM, respectively. In this assay LTE_4 behaved as a partial agonist, whereas LTB_4 failed to elicit a significant calcium response at concentrations up to 3 μM. BAY-u9773 could elicit a partial calcium mobilization response by itself, even though pretreatment with BAY-u9773 decreased the response to LTC_4 and LTD_4.[490]

4.6.4. Gene Structure and Transcriptional Regulation of $CysLT_2$. The chromosomal localization of the human $CysLT_2$ gene is on 13q14.12-q21.1.[498,499] Woszczek et al. reported the human $CysLT_2$ gene structure consisting of six major exons, including two variants of exon-II (named exons-II and -II′).[500] Of there exons, five exons (exon-I−exon-V) are corresponding to the 5′-untranslated region (5′UTR), and exon-VI contains the ORF without interrupting intronic sequences. In human vascular endothelial cells (HUVECs), human monocytes, and U937 and THP-1 cells, 17 different TSSs were found, all in the first exon. Eight different transcripts (transcript-I−transcript-VIII) of human $CysLT_2$ are generated from this gene; all of them contain the same exon-I and exon-VI.[500] Especially, transcript-III is the most abundant product in HUVECs, representing 90% of detected sequences. Woszczek et al. further found that the expression of the $CysLT_2$ mRNA, mainly transcript-III, is enhanced by the stimulation with IFN-γ in HUVECs and human lung microvascular endothelial cells (HMVEC-Ls).[500] In HUVECs, pretreatment with the Jak2 inhibitor AG490 significantly inhibits the IFN-γ-mediated $CysLT_2$ mRNA induction.[500] Moreover, the IFN-γ-mediated increase in $CysLT_2$ mRNA is significantly inhibited in the presence of the dominant-negative STAT1, demonstrating that the IFN-γ-dependent $CysLT_2$ expression is strongly related to activation of Jak and STAT1 proteins.[500]

The chromosomal location of the mouse $CysLT_2$ gene was initially determined by interspecific backcross analysis using progeny derived from matings of ([C57BL/6J × *Mus spretus*] F_1 × C57BL/6J) mice.[489,501] This interspecific backcross mapping panel has been typed for more than 3200 loci that are well-distributed among all of the autosomes as well as the X chromosome. These mappings indicated that $CysLT_2$ gene is located in the distal region of mouse chromosome 14. The distal region of mouse chromosome 14 shares regions of homology with human chromosomes 8p and 13q, consistent with the assignment of $CysLT_2$ to 13q14.12-q21.1 in humans. The mouse $CysLT_2$ gene is composed of six exons; exon-I−exon-V contain the 5′-UTR, resulting in the coding region (exon-VI) being intronless like many other GPCR genes. The mouse $CysLT_2$ gene produces long and short splice variants.[435,436] The alternative splicings of mouse $CysLT_2$ occur in the 5′-UTR region; thus, there should be no difference in protein structure. The significance of the alternative splicing remains to be clarified.

4.7. Other Features of $CysLT_2$; SNPs of $CysLT_2$ Associated with Atopy Disease

Several markers on chromosome 13 have shown to be linked to phenotypes of asthma or atopy in two genome-wide searches.[498] $CysLT_2$ has also been implicated in atopic asthma, since it is localized on chromosome 13q14.12-q21.1, which has been linked to these diseases in several populations.[499] Slutsky et al. analyzed a genetically isolated founder population on Tristan da Cunha, an island in the South Atlantic Ocean, where the incidence of asthma is greater than 50% (compared to 6% in the non-Tristan da Cunha Caucasian population).[502−505] They found four variants of human $CysLT_2$, Met202Val, Ser237Leu, Ala293Gly, and Arg315Lys, with the asthma phenotype by single-stranded conformational polymorphic (SSCP) analysis.[482] Among these, the Met202Val variant is associated with atopy (21%) on Tristan da Cunha compared with those who are nonatopic (7%). The Met202Val variant was shown to confer a partial inactivation of the $CysLT_2$ signaling, e.g., activation by 4-fold higher concentration of LTD_4 in a calcium flux assay.[482] The Met202Val mutation is located within the extracellular side of the TM5 in $CysLT_2$, a position that may alter ligand binding and signaling, suggesting the structural importance of $CysLT_2$ in the atopic phenotype. If $CysLT_1$ and $CysLT_2$ form the functional dimer with unique pharmacological properties in inflammatory cells (see section 4.8.1), they may functionally alter the cys-LT signaling; thus, the pharmacology of $CysLT_2$ ligands could be important. While specific $CysLT_2$ ligands have not yet been reported, the development of drugs that target $CysLT_2$ may be significant, given that approximately 20% of patients fail to respond to $CysLT_1$ agents, particularly in cases where $CysLT_2$ is also polymorphic.

4.8. Topics for Cys-LT Receptors

4.8.1. Homo/Heterodimerization of Cys-LT Receptors. In some cases, GPCRs appear to assemble into dimers or oligomers.[506−509] Much evidence indicates the possibility that $CysLT_1$ might exist as a homodimer. Figueroa et al. found by Western blot that $CysLT_1$ is mostly present in homodimeric and oligomeric forms, even in the presence of denaturing agents.[434] They suggested a substantial oligomerization because, they observed the punctate appearance of the immunohistochemical signal in peripheral blood leukocytes.[434] Like many hematopoietic cells found in inflammatory lesions, mast cells express both $CysLT_1$ and $CysLT_2$.[510,511] Recently, Jiang et al. demonstrated the interaction of $CysLT_1$ with $CysLT_2$ in cord-blood-derived human mast cells.[512] Although the precise domain of this interaction is still unclear, the physical interaction of $CysLT_1$ and $CysLT_2$ at the plasma membrane was demonstrated by a FLIM (fluorescent lifetime imaging microscopy) assay.[512] The knockdown of $CysLT_2$ enhances the surface expression of $CysLT_1$, LTD_4-induced ERK1/2 activation, and $CysLT_1$-dependent proliferation of the mast cells. Thus, they suggested that the presence of $CysLT_2$ on the $CysLT_1$-expressing cells, e.g., mast cells, may attenuate the cys-LT-mediated signaling by limiting the formation of $CysLT_1$ homodimers and/or controlling their surface expression, although additional mechanisms, e.g., heterologous desensitization and competition for ligand, may contribute as well. The counterbalance of $CysLT_1$/$CysLT_2$ may regulate cys-LT-dependent function in immune and inflammatory responses.[512] This information is of interest because increasing evidence suggests that many GPCRs have the propensity to dimerize, offering new horizons to study an important aspect of GPCR biology and possibly to develop new drugs. Whether the homo- and/or heterodimer formations between $CysLT_1$ and $CysLT_2$ have yet to be established, how these dimers influence their pharmacology and function, and how these are of any importance in cell physiology are issues that will clearly need to be addressed.

4.8.2. GPR17. An orphan GPCR, GPR17, was identified by homologous screening with the IL-8 receptor in the human genomic DNA library.[513] Subsequently, GPR17 and its variant form were cloned from a human hippocampus cDNA library with the nucleotide chicken $P2Y_1$ and murine $P2Y_2$ receptors.[514] Phylogenic analysis for P2Y-related receptors reveals that human

GPR17 is homologous to the cys-LT receptors (Figure 5),[515] with 31% and 36% identities at amino acid sequence levels to those of human $CysLT_1$ and $CysLT_2$, respectively. Human GPR17 is also 90% identical to both mouse and rat orthologs.[515] Human, rat, and mouse GPR17 have recently been identified as dual receptors for uracil nucleotides and cys-LTs, LTC_4, and LTD_4, based on [^{35}S]GTPγS binding assays.[515,516] The original report showed that 1321N1 cells and COS-7 cells expressing human GPR17 could respond to 100 nM LTD_4 and 100 μM UDP-glucose by single-cell calcium imaging analysis. Moreover, the survival and differentiation of PC12 cells are regulated through the activation of GPR17 by its agonists, UDP-glucose and LTD_4.[517] However, Maekawa et al. currently reported that GPR17 is a ligand-independent, negative regulator for $CysLT_1$ that suppresses $CysLT_1$-mediated signaling on the cell surface.[518] The activities of $CysLT_1$, e.g., ligand binding, intracellular calcium flux, and ERK1/2 phosphorylation by LTD_4-stimulation, are abolished by coexpression with GPR17 in several cells, such as CHO, HEK293, and 1321N1 cells. $CysLT_1$ and GPR17 expressed in the transfected cells are coimmunoprecipitated, suggesting the heterodimerization of these receptors.[518] In vivo, they demonstrated that the vascular leak following IgE-dependent, mast-cell-mediated passive cutaneous anaphylaxis (PCA) is increased in GPR17-null mice and that this response is blocked by administration of a $CysLT_1$ antagonist.[518] Furthermore, they currently reported a constitutive negative regulation of $CysLT_1$ function by GPR17 in both the antigen-presentation and downstream phases of allergic pulmonary inflammation.[519] Thus, these data provide physiologic evidence for the regulatory role of GPR17. It is necessary to perform extensive studies to clarify the GPR17 function.

4.8.3. Intracellular Localization of $CysLT_1$. The actions of cys-LTs are thought to be primarily dependent on their specific cell surface receptors. However, several reports demonstrated that $CysLT_1$ could also be localized at the nuclear level, suggesting major unanticipated roles for this receptor in cell signaling and function. Bandeira-Melo et al. provided evidence for an intracrine $CysLT_1$-mediated signaling in eosinophils.[520] This signaling, which is PTX-sensitive, leads to vesicular transport-mediated IL-4 secretion. Furthermore, $CysLT_1$ was found to be located in the outer nuclear membrane in colon cancer cells or to be translocated into the nucleus after prolonged exposure to LTD_4 in nontransformed intestinal epithelial cells.[461] Nielsen et al. demonstrated that a nuclear localization sequence is crucial for the $CysLT_1$ translocation to the nucleus,[461] as already demonstrated for other GPCRs.[521,522] The existence of functional intracellular GPCRs has also been observed for other eicosanoid receptors, e.g., EP3 and EP4.[462,523] For example, perinuclear EP3 has been recently demonstrated to induce eNOS expression via PTX-sensitive G-proteins.[524] Since other lipid mediators have been demonstrated to exert biological effects though their nuclear GPCRs, e.g., transcriptional regulation of COX-2 and inducible NOS,[525] it is possibly that the roles for nuclear $CysLT_1$ are different from those on plasma membrane. Interestingly, Neves et al. currently reported that $CysLT_1$, $CysLT_2$, and the purinergic receptor $P2Y_{12}$ are expressed on human eosinophil granule membranes. The isolated eosinophil granules secrete eosinophil cationic protein (ECP) by stimulation with cys-LTs, and this response is inhibited by the treatment with montelukast and a $P2Y_{12}$ antagonist, MRS-2395, suggesting the functional expression of cys-LT receptors on the granules.[526]

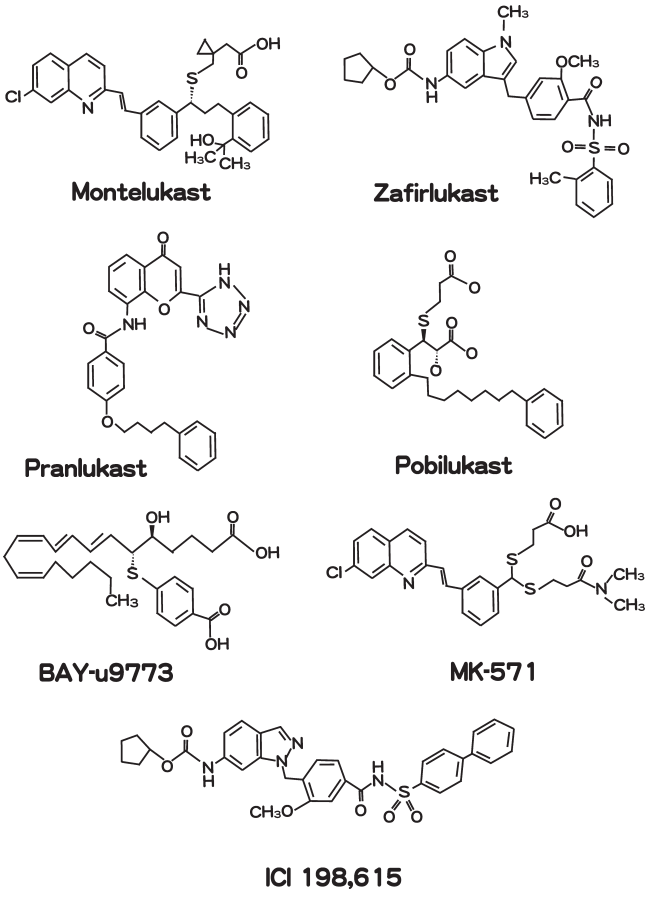

Figure 21. Chemical structures of synthetic ligands for cys-LT receptors.

4.9. Synthetic Ligands for Cys-LT Receptors

As described above, LTD_4 is the preferred ligand for $CysLT_1$, whereas LTC_4 and LTD_4 bind with approximately equal affinity to $CysLT_2$. $CysLT_1$ is highly expressed in the bronchial mucosa of asthmatic patients and clearly mediates many of the pathophysiological effects of cys-LTs in asthma. In contrast, $CysLT_2$ is found on leukocytes, smooth muscle cells, and endothelial cells and may have a role in fibrosis and vascular injury. On the basis of this information, many drugs specifically recognizing cys-LT receptors have been clinically developed and characterized by pharmaceutical companies. This chapter summarizes the latest developments of cys-LT receptor antagonists for the treatment of diseases and discusses potential future developments.

4.9.1. Montelukast (Singulair, Kipres). Montelukast {2-[1-[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanylmethyl]-cyclopropyl]acetic acid}[527] is a potent and selective inhibitor for [^{3}H]LTD_4 binding to guinea pig lung (K_i = 0.18 nM), sheep lung (K_i = 4 nM), and DMSO-differentiated U937 cell plasma membrane preparations (K_i = 0.52 nM) (Figure 21). However, it was essentially inactive against the [^{3}H]LTC_4 binding to DMSO-differentiated U937 cell membranes (IC_{50} = 10 μM) and [^{3}H]LTB_4 binding to THP-1 cell membranes (IC_{50} = 40 μM). Montelukast, a potent and selective LTD_4 receptor antagonist with excellent in vivo activity, is currently used for the treatment of asthma and related diseases. Radioligand-binding studies using the human $CysLT_1$-expressing HEK293 cell membranes, which showed binding affinity of [^{3}H]LTD_4

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(1.5 nM) with an IC_{50} for cold LTD_4 of 9 nM, demonstrated the competitive activity of montelukast with an IC_{50} value of 4.9 nM.[399] Montelukast inhibits the LTD_4-induced calcium mobilization in $CysLT_1$-expressing HEK293 cells with a respective IC_{50} value of 2.3 nM. Montelukast is inactive at competing for $[^3H]LTD_4$ binding to $CysLT_2$ in COS-7 cells.[398] This compound also inhibits specific binding of $[^3H]LTD_4$ to guinea pig lung in the presence of human serum albumin, human plasma, and squirrel monkey plasma with K_i values of 0.21, 0.19, and 0.26 nM, respectively. Functionally, montelukast antagonizes contractions of guinea pig trachea induced by LTD_4.[527] In contrast, montelukast does not antagonize contractions of guinea pig trachea induced by LTC_4, serotonin, acetylcholine, histamine, or PGD_2. Intravenous montelukast antagonizes bronchoconstriction induced in anesthetized guinea pigs by iv LTD_4, but it does not block bronchoconstriction to AA, histamine, serotonin, or acetylcholine. Oral administration of montelukast blocks LTD_4-induced bronchoconstriction in conscious squirrel monkeys, ovalbumin-induced bronchoconstriction in conscious sensitized rats (ED_{50} = 0.03 mg/kg), and also ascaris-induced early- and late-phase bronchoconstriction in conscious squirrel monkeys (0.03−0.1 mg/kg).

4.9.2. Zafirlukast (Accolate). Zafirlukast {4-(5-cyclopentyloxycarbonylamino-1-methylindol-3-ylmethyl)-3-methoxy-*N*-[(2-methylphenyl)sulfonyl]benzamide} is the first cys-LTs antagonist approved in the US and Europe (Figure 21).[528] Zafirlukast is an orally active, potent, and selective antagonist that has been approved for the prophylaxis and treatment of chronic asthma.[529] This compound exerts its action through the binding to $CysLT_1$, both in guinea pig and human lungs.[530] Radioligand binding studies showed that increasing concentrations of zafirlukast compete with $[^3H]LTD_4$ for binding in guinea pig lung membranes.[531] Zafirlukast also competes for receptor binding with $[^3H]LTE_4$. Radioligand binding studies using the human $CysLT_1$-expressing HEK293 cell membranes demonstrated the competitive activity of zafirlukast with an IC_{50} value of 1.8 nM for the binding of $[^3H]LTD_4$.[399] Furthermore, zafirlukast inhibits the LTD_4-induced calcium mobilization in $CysLT_1$-expressing HEK293 cells with a respective IC_{50} value of 0.26 nM. In contrast, zafirlukast showed a weak competing activity for $[^3H]LTD_4$ binding to $CysLT_2$.[399] LTD_4 or ovalbumin challenge into sensitized guinea pigs provokes mucus secretion from the trachea in vitro, and this effect is inhibited by zafirlukast by 65%.[532] In humans, zafirlukast antagonizes the effects of exogenously administered LTD_4 and endogenously released cys-LTs in response to physical and chemical stimuli. Zafirlukast antagonizes LTD_4-induced bronchoconstriction, still being effective 12 h after drug administration.

4.9.3. Pranlukast (Onon). Pranlukast {4-oxo-8-[[4-(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran-8-yl]-4-(4-phenylbutoxy)benzamine} is a potent, selective, and orally active $CysLT_1$ antagonist also known as Onon, Azlaire, SB 205312, and ONO-1078 (Figure 21).[533−536] Clinical studies in Japan, Europe, and North America all showed that pranlukast significantly attenuates bronchoconstriction in response to a variety of allergen challenges as well as to inhaled LTD_4.[537] Radioligand binding studies using the membranes from human $CysLT_1$- and $CysLT_2$-expressing cells demonstrated that pranlukast binds to human $CysLT_1$ and $CysLT_2$ with IC_{50} values of approximately 4−7 nM and 3.6 μM, respectively.[397−399] Pranlukast inhibits the LTD_4-induced calcium mobilization in $CysLT_1$-expressing HEK293 cells with a respective IC_{50} value of 0.1 nM.[399] The effect of pranlukast was investigated on guinea pig lung anaphylaxis in vitro and in vivo.[538] It was demonstrated that this compound could inhibit the late phase of contractile responses in Schultz−Dale reactions and endogenous LT-mediated bronchoconstrictions in passively sensitized guinea pigs. Additionally, this compound significantly reduces antigen-induced airway microvascular leakage in ovalbumin-sensitized guinea pigs.[539] Several reports demonstrated the efficacy of this compound on the brain diseases in animal models. For example, pranlukast has a long-lasting protective effect on focal cerebral ischemia in mice and rats and inhibits the ischemia-induced glial scar formation, providing further evidence of the therapeutic potential of pranlukast in the treatment of ischemic stroke.[540] This drug exerts an anti-inflammatory effect on focal cerebral ischemia in the subacute phase that is limited to neutrophil recruitment through the disrupted blood−brain barrier.[541] Furthermore, pranlukast also protects mice against brain cold injury, especially on brain−blood barrier disruption, as evidenced by the decreases in lesion volume, brain edema, IgG exudation, and neuron degeneration and the inhibition of neuron loss.[542]

4.9.4. Pobilukast. Pobilukast {2-hydroxy-3-[(2-carboxyethyl)-thio]-3-[2-(8-phenyloctyl)phenyl]propanoic acid} is a synthetic structural analog of LTD_4 and LTE_4 (Figure 21). Radioligand binding studies using the human $CysLT_1$-expressing HEK293 cell membranes, which showed the binding affinity of $[^3H]LTD_4$ with IC_{50} for cold LTD_4 of 9 nM, demonstrated the competitive activity of pobilukast with an IC_{50} value of 30 nM.[399] Pobilukast inhibits the LTD_4-induced calcium mobilization in $CysLT_1$-expressing HEK293 cells with an IC_{50} value of 5.5 nM.[543] A previous report demonstrated that this compound binds to guinea pig and human lung cys-LT receptors with K_i values of 5 and 10 nM, respectively.[544] Hay et al. described the in vitro and in vivo pharmacologic profile of pobilukast in guinea pig and human airways.[543] In the isolated guinea pig trachea, pobilukast is a potent, competitive antagonist of LTD_4-induced contractions. LTE_4-induced contractions in guinea pig trachea are also inhibited by pobilukast, although pobilukast (10 μM) has no intrinsic contractile activity and is without effect on contractions elicited by KCl, histamine, PGD_2, or PAF.[543] In conscious male Sprague−Dawley rats, administration of *Salmonella enteritidis* endotoxin (30 mg/kg iv; LD_{90}) results in a decrease in the number of circulating platelets, leukopenia, an increase in hematocrit, and 0% survival at 24 h.[545] Pretreatment with pobilukast (1 mg/kg, iv bolus followed by 3 mg/kg/h, iv infusion for 6 h) attenuates the endotoxin-induced thrombocytopenia but has no effect on either the endotoxin-induced early leukopenia or late leukocytosis.[545] Additionally, pobilukast significantly reduces the endotoxin-induced hemoconcentration and improves survival to 30% at 48 h. Moreover, the ability of pobilukast to prevent and reverse LTD_4-induced bronchoconstriction was examined in anesthetized, spontaneously breathing cynomolgus monkeys.[546] Aerosol administration of LTD_4 showed a sustained increase in pulmonary resistance and a decrease in dynamic lung compliance. Aerosolized pobilukast (150 breaths, 0.3 or 4.4 mg/mL) administered 15 min prior to LTD_4 challenge antagonized these changes in a dose-dependent manner.

4.9.5. MK-571. MK-571 {3-[[[3-[2-(7-chloro-2-quinolinyl)-ethenyl]phenyl][[(3-dimethylamino)-3-oxopropyl]thio]methyl]-thio]propanoic acid} is a selective, orally active $CysLT_1$ antagonist and MRP1 inhibitor (Figure 21).[547] This compound inhibits the binding of LTD_4, but not LTC_4, to human and guinea pig lung

membranes with K_i values of 0.22 and 2.1 nM, respectively. MK-571 effectively blocks LTD_4-elicited activation of recombinant human and mouse $CysLT_1$, but it is ineffective at blockade of LTC_4- or LTD_4-induced activation of human or murine $CysLT_2$. For example, Maekawa et al. reported that LTD_4-induced intracellular calcium mobilization in CHO cells expressing mouse $CysLT_1$ is blocked by MK-571.[435] This compound potentially inhibits MRP1 and has been shown to overcome acquired arsenic tolerance. The potential for MK-571 to cause bronchodilation in asthma patients with existing airway obstruction and to evaluate its effect on the bronchodilation response to an inhaled β2-agonist was reported.[548] Although blockade by MK-571 infers cellular responses mediated by $CysLT_1$, MK-571 also have off-target effects, such as interference with sphingosine-1-phosphate transporter[549] and blockade of purinergic receptors.[550]

4.9.6. BAY-u9773. BAY-u9773 {6(*R*)-(4′-carboxyphenylthio)-5(*S*)-hydroxy-7(*E*),9(*E*),11(*Z*),14(*Z*)-eicosatetraenoic acid} is a LTE_4 analogue used to define "atypical" receptors for cys-LTs (Figure 21). This compound is a nonselective antagonist of $CysLT_1$ and $CysLT_2$, having about the same IC_{50} values (20−80 nM) for the inhibition of cys-LT responses in various tissue preparations, for example, human pulmonary veins,[393] guinea pig trachea, rat lung, ferret spleen, and sheep bronchus.[487] Although many of the developed $CysLT_1$ antagonists are either weak (zafirlukast and pranlukast) or inactive (montelukast) at competing for the [^{3}H]LTD_4 binding to $CysLT_2$, full competition was observed with the dual $CysLT_1$/$CysLT_2$ antagonist BAY-u9773 with an IC_{50} value of 0.6 μM. Therefore, BAY-u9773 is the only pharmacological tool available to suggest the presence of $CysLT_2$. Dahlén et al. reported that BAY-u9773 is a competitive antagonist of LTC_4 and LTD_4 in sheep trachealis muscle[551] and of LTC_4 in guinea pig ileum longitudinal muscle.[552] Moreover, BAY-u9773 possesses contractile activity in guinea pig lung parenchyma.[487] The similar properties of BAY-u9773 have also been reported in human pulmonary veins,[393] but they were not observed in other preparations, such as tracheae from guinea pig[487] or sheep[551] or guinea pig ileum.[552] The contractile activity of this compound in guinea pig lung parenchyma is indomethacin (COX inhibitor)-sensitive, suggesting that BAY-u9773 evokes release of spasmogenic prostanoids from the lung.[487] Interestingly, Wikstrom Jonsson et al. found that BAY-u9773 acts as a $CysLT_1$ agonist and in a higher concentration also as a TP receptor agonist.[553] BAY-u9773 in itself causes a concentration-dependent contraction, which is not inhibited by the indomethacin nor by the 5-LO inhibitor zileuton {*N*-[1-benzo-(12)-thien-2-ylethyl]-*N*-hydroxyurea}. The $CysLT_1$ antagonist ICI 198,615 alone blocks the contractile response of 1 μM BAY-u9773, whereas a combination of the TP receptor antagonist BAY-u3405 [(3*R*)-3-(4-fluorophenylsulfonamido)-1,2,3,4-tetrahydro-9-carbazolepropanoic acid] and ICI 198,615 is required to block the contraction induced by 10 μM BAY-u9773.

4.9.7. ICI 198,615. Snyder et al. first reported the in vitro pharmacology of ICI 198,615 {1-[[2-methoxy-4-[[[(phenylsulfonyl)amino]carbonyl]phenyl]methyl]-1*H*-indazol-6-yl]carbamic acid cyclopentyl ester} (Figure 21).[554] Specific binding of [^{3}H]LTD_4 to guinea pig lung parenchymal membranes (K_d = 0.43 nM) is greatly inhibited in the presence of ICI 198,615.[554,555] In competition assays, ICI 198,615 competitively inhibits binding of [^{3}H]LTD_4 (K_i = 0.27 nM) and is 2300- and 3100-fold more potent than LY171883 and FPL55712, respectively. The in vivo pharmacology of ICI 198,615 was also reported.[556] In a conscious guinea pig model, ICI 198,615 provides dose-dependent antagonism of aerosol LTD_4-induced dyspnea when administered po, iv, or by aerosol. The pharmacologic half-lives of ICI 198,615 by these routes of administration are greater than 16 h, 68 min, and 34 min, respectively. When the effects of iv or aerosol administration of LTC_4, LTD_4, and LTE_4 on pulmonary resistance (Rp) and dynamic lung compliance (Cdyn) are evaluated, ICI 198,615 provides dose-related antagonism by po, i.d., iv, or aerosol administration. ICI 198,615 inhibits LTC_4-, LTD_4-, and LTE_4-induced increases in cutaneous vascular permeability in the guinea pig, with the ED_{50} values of 0.083, 0.11, and 0.067 nmol/kg, respectively.[557] This compound inhibits and reverses aerosol ovalbumin antigen-induced increases in Rp in passively sensitized guinea pigs, but it demonstrates little ability to inhibit or reverse ovalbumin antigen-induced decreases in Cdyn.

4.9.8. CR3465. CR3465 {L-tyrosine, *N*-[(2-quinolinyl)carbonyl]-*O*-(7-fluoro-2-quinolinylmethyl), sodium salt} is a potent antagonist of the [^{3}H]LTD_4 binding to guinea pig lung preparations, with a K_i of 4.7 nM being comparable with that of montelukast (5.6 nM).[558] In tracheal strips from naive or ovalbumin-sensitized guinea pigs, CR3465 causes parallel rightward shifts in the concentration−response curves obtained with either LTD_4 or antigen. By iv administration this compound both antagonizes (ED_{50} = 9.9 μg/kg) and reverts LTD_4-induced bronchoconstriction of anesthetized guinea pigs. CR3465 reduces inflammatory infiltrates in the BALF after antigen challenge of sensitized animals and also inhibits phosphodiesterase (PDE)-3 and PDE-4 activities in human platelets and neutrophils (IC_{50} = 2.0 and 4.7 μM, respectively). In line with properties shown by PDE inhibitors, CR3465 reduces the contractile response of guinea pig airways to histamine and decreases fMLP-induced degranulation of human neutrophils (IC_{50} = 13.8 μM). Oral administration (20 mg/kg) of this compound in rats showed a significant ex vivo inhibition of TNF-α release from lipopolysaccharide-stimulated whole blood.

4.9.9. FPL-55712. FPL-55712 {7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxyl]-4-oxo-8-propyl-4*H*-chromene-2-carboxylate, sodium salt}[339] has been used as a conventional pharmacological tool to ascertain the involvement of SRS-A in animal models of allergic asthma.[559−562] Despite its potent SRS-A antagonist activity[563] and apparent antiallergic activity in vivo,[564] FPL-55712 has long been considered to be of limited clinical potential because it is orally inactive and displays a short biological half-life after intravenous administration.[563] Thus, the aerosol rout of administration has been explored with this compound. For example, it has been shown that an aerosol formulation of FPL-55712 before and during antigen challenge of dogs[565] or ragweed-sensitive human patients[566] prevents antigen-induced decreases in tracheal mucous velocity.

4.10. Cys-LTs and Cognate Receptors in Health and Diseases

The cloning of $CysLT_1$ and $CysLT_2$, development of receptor antagonists, and generation of mice deficient in LTC_4 synthase or two receptors have all expanded the scope of functions served by cys-LTs. Cys-LTs participate in the inflammatory component of asthma by increased microvascular permeability, bronchoconstriction, afferent limb of adaptive immunity (maturation and migration of DCs), recruitment of effector cells (especially eosinophils and mast cells), mucus and cytokines secretion, and fibrosis.[567−573] The physiological and pathophysiological

Table 5. Physiological Roles of Cys-LTs

cell types/tissues	bioactivities of cys-LTs
blood cells	chemotaxis of leukocytes (eosinophils, monocytes/macrophages, neutrophils, dendritic cells)
	IL-4 production in eosinophils
	cytokines (IL-5, TNF-α, MIP-1β and IL-8) production in mast cells
respiratory system	bronchospasm
	mucous secretion from goblet cells
	smooth muscle proliferation
	smooth muscle contraction
	interstitial infiltration of fibroblasts
blood vessel	increase in vascular permeability (plasma exudation)
	vasoconstriction
	expression of P-selectin (in HUVECs)
cardiovascular system	coronary vasoconstriction

Table 6. Pathophysiological Roles of Cys-LTs

	related diseases
respiratory system	bronchial asthma
	• airway smooth muscle contraction
	• eosinophil influx, basophil influx
	• edema
	• mucus production
	• goblet cell mataplasia
	• smooth muscle hyperplasia/hypertropy
	• collagen deposition
	• cytokine (IL-13, IL-4, IL-10, and IL-5) production
	• increase in microvascular permeability
	pulmonary fibrosis
	• macrophage and neutrophil recruitment
	• alveolar septal thickening
	• fibroblast accumulation
	• collagen deposition
	COPD
	• neutrophil influx, macrophage influx, CD8+ lymphocyte influx
	• increase in mucus production
	• goblet cell hyperplasia
	• smooth muscle hyperplasia/hypertropy
kidney	ischemis-reperfusion injury
	• erythema, edema, eosinophil influx, cllagen deposition, fibrosis
skin	atopic dermatitis
	• edema, increase in endothelial adhesion molecules
others	atherosclerosis
	• DC influx, mast cell influx
	• smooth muscle cell hyperplasia
	allergic rhinitis
	• chemotaxis of leukocytes
	• nasal congestion
	• edema
	• goblet cell hyperplasia
	• subepithelial fibrosis
	• production of cytokines/chemokines in mast cells

roles of cys-LTs are summarized in Tables 5 and 6. The involvement of cys-LTs in these events has all been supported by animal models and awaits validation in humans. In this section, we summarize the current knowledge about the functions of cys-LTs and their receptors in these events.

4.10.1. Airway Smooth Muscle Contraction. LTC_4 and LTD_4 are equipotent in guinea pig tracheal smooth muscle preparations, while LTD_4 is a more selective agonist for peripheral airway.[391] LTD_4 exhibits a 100-fold greater potency than LTC_4 in the lung parenchyma strip, suggesting the existence of two cys-LT receptors. The heterogeneity of cys-LT receptors was further confirmed by showing that the potency of LTD_4 in the guinea pig lung parenchymal tissues is significantly different from that observed in the tracheal preparations.[574] When contractile studies are performed on human airways, LTC_4, LTD_4, and LTE_4 are equipotent as contractile agonists[393,575] with EC_{50} values of approximately 10 nM, which is about $^1/_{1000}$ of the EC_{50} value for histamine in the same tissues.[576] However, LTE_4 is markedly less active than LTC_4 and LTD_4 in other tissues.[577] Although a comparison of LTC_4, LTD_4, and LTE_4 potencies between different tissues illustrates considerable variation,[402,578] one is unable to surmise whether either a single or several receptors are present in these preparations. This requires additional information from structural, operational, and molecular biological studies. However, several data suggest a marked difference between LTE_4 and the other cys-LTs.

4.10.2. Recruitment of Effector Leukocytes. Functional studies revealed the expression of $CysLT_1$, but not $CysLT_2$, in human $CD34^+$ peripheral blood-derived progenitor cells.[579] Cys-LTs induce transendothelial migration of these cells in vitro, indicating a possible role for $CysLT_1$ in the regulation of hemopoietic progenitor cell trafficking. In human, peripheral blood cells, monocytes,[398,434] eosinophils,[580] and lung macrophages[397,398] all express both $CysLT_1$ and $CysLT_2$. Priming of either human peripheral blood monocytes or monocyte-derived macrophages with recombinant human IL-4 or IL-13 increases their expression of $CysLT_1$ and enhances their chemotactic responses to LTD_4 in vitro.[464] Both $CysLT_1$ and $CysLT_2$ are expressed in eosinophils, mononuclear cells, and resident mast cells in nasal biopsy tissue from humans with seasonal allergic rhinitis.[581] $CysLT_1$, but not $CysLT_2$, also localizes to a subset of lesional neutrophils. Furthermore, the expression of $CysLT_1$ is upregulated in $CD45^+$ leukocytes in nasal biopsy specimens obtained from subjects with aspirin-sensitive chronic rhinitis and nasal polyposis, compared with the same leukocyte subsets in biopsy specimens from non-aspirin-sensitive subjects with rhinitis and nasal polyposis.[582] Inhaled LTE_4 or LTD_4 increases airway eosinophil numbers when assessed in bronchoalveolar lavage from humans.[569,583] Similar results were obtained with LTC_4 or LTD_4 in guinea pigs.[584,585] Single administration of inhaled LTD_4 elicits a marked increase in eosinophils, which is maintained for at least 4 weeks.[585] These data suggest the ability of cys-LTs to either directly or indirectly attract leukocytes to initiate inflammatory responses in vivo. Pobilukast decreases LTD_4-induced chemotaxis of peripheral blood eosinophils from nonasthmatic volunteers.[586] Furthermore, the $CysLT_1$ antagonists pranlukast, MK-571, and ICI

198,615 inhibit antigen-induced eosinophil influx in rats,[587] guinea pigs,[584] and cynomolgus monkeys.[588] Together, these results indicate that cys-LTs may serve as chemotactic mediators and/or activating ligands for human effector leukocytes and that the cys-LT receptor expression profiles of certain leukocytes are modified by available cytokines and other microenvironmentally derived factors in inflammation. In vitro studies support the capacity for cys-LTs to serve as eosinophil chemoattractants through a $CysLT_1$-dependent mechanism.[589]

4.10.3. Activation of Dendritic Cells in Adaptive Immunity. That Cys-LTs are crucial for the maturation of dendritic cells (DCs) emerged from studies of DC migration in mice lacking MRP1, which is required for the export of LTC_4 to the extracellular space.[353,590] In a model of contact hypersensitivity induced by topical application of fluorescein isothiocyanate (FITC), DC migration is significantly impaired in MRP1-null mice compared with that observed in WT control mice.[21] The defect of migration is recovered by the injection of LTC_4 or LTD_4. In a subsequent study, myeloid DCs cultured ex vivo from mouse bone marrow were found to express $CysLT_1$ mRNA, as well as mRNAs encoding 5-LO, FLAP, and LTC_4 synthase.[281] These DCs produce IL-10 and IL-12 when challenged ex vivo with the dust mite antigen Der f. Treatment of these DCs with the $CysLT_1$-selective antagonists significantly blunts their Der f-mediated generation of IL-10, but it enhances their production of IL-12. Mice that received intranasal adoptive transfer of Der f-pulsed DCs, which are costimulated with LTD_4, develop enhanced eosinophilia and increase BALF levels of IL-5 after inhalation challenge with Der f compared with those in mice that received transfer of DCs stimulated with Der f alone. Thus, endogenous LTD_4 produced by DCs during their initial exposure to antigen is a critical determinant of their homing to regional lymph nodes, and their repertoire of cytokines are required to induce T-cell responses. Either or both of these events, which depend at least in part on $CysLT_1$, may play an important role in the afferent limb of acquired immunity.

4.10.4. Cytokine Production in Mast Cells. Resident tissue mast cells in nasal biopsies express both $CysLT_1$ and $CysLT_2$.[581,582] Human cord-blood-derived mast cells also express these receptors,[510,591] and respond to ex vivo stimulation by low nanomolar concentrations of LTC_4 and LTD_4 in a PTX-insensitive manner. In these cells, LTC_4- and LTD_4-mediated calcium fluxes are completely blocked by pretreatment with MK-571, indicating the requirement of $CysLT_1$ in this response. IL-4-primed mast cells secrete several cytokines (IL-5, TNF-α, MIP-1β, and IL-8) when stimulated with LTC_4, LTD_4, or UDP.[510,592] Of these cytokines, IL-8 is unique for the resistance of its secretion to blockade by MK-571, indicating dependence on $CysLT_2$.[592] Blockade of $CysLT_1$ by MK-571, or inhibition of endogenous cys-LT production by MK886, significantly attenuates the generation of IL-5 and TNF-α by mast cells activated by FcRI cross-linkage.[592] These data demonstrate that $CysLT_1$ and $CysLT_2$ have distinct yet complementary functions for mast cells, permitting cytokine generation through both autocrine and paracrine mechanisms.

4.10.5. Allergen-Induced Pulmonary Inflammation. In ovalbumin (OVA)-induced asthma model, the levels of LTC_4 and LTB_4 in the BALF of the challenged mice increase compared with those of the saline controls.[593] These increases are associated with widespread mucus occlusion of the airways, an influx of predominantly eosinophils in airway tissues and BALF, and airway hyperresponsiveness to iv methacholine. The ip administration of LT synthetic inhibitor, i.e., zileuton or MK-886, before nasal allergen challenge impairs eosinophil infiltration into the tissues and BALF and significantly blocks airway mucus release and plugging. In the chronic protocol, treatment with montelukast starting on day 26 significantly reduces the airway eosinophil infiltration, mucus plugging, collagen deposition, and smooth muscle hyperplasia, as determined on day 76. Levels of IL-13, IL-4, IL-10, and IL-5 mRNAs are dramatically reduced in the lungs of the challenged montelukast-treated mice compared with controls. These data indicate that cys-LTs, acting via $CysLT_1$, initiate features of chronic inflammation and matrix remodeling in allergen-induced pulmonary disease, possibly by enhancing cytokine generation by resident inflammatory cells. The smooth muscle hyperplasia in the chronic model could also reflect direct proliferative effects, because LTD_4 is a mitogen for human bronchial smooth muscle cells when provided in vitro in combination with IL-13[594] or EGF.[595]

4.10.6. Microvascular Permeability. Cys-LTs increase microvascular permeability in guinea pig airways.[539,596,597] The mechanism may involve contraction of endothelial cells resulting in gaps in the endothelium of venules.[598] This effect of cys-LTs, which appears to involve both direct and indirect pathways, is inhibited by either FPL-55712[599] or pranlukast,[597,600] suggesting the involvement of $CysLT_1$. Plasma protein leakage after ip challenge with zymosan A or IgE-dependent passive cutaneous anaphylaxis is reduced in LTC_4 synthase-null mice compared with WT mice. Decrements in the plasma protein leakage in $CysLT_1$-null mice are comparable in magnitude to those in LTC_4 synthase-null mice.[463] Thus, these results indicate the prominent role of cys-LTs, acting through $CysLT_1$, in mediating increased vascular permeability in models of both innate and adaptive immunity. Shi et al. reported that ip injection of zymosan A into γ-GL-null mice, which leads to the accumulation of LTC_4 in the peritoneal cavity, showed the reduction of neutrophil infiltration without the effect on the plasma protein extravasation in these mice.[368] These findings suggest that the conversion of LTC_4 to LTD_4 is not required for the increase in vascular permeability, but that LTD_4 may play a role in neutrophil recruitment. However, several controversial data have been reported. For example, neutrophil recruitment is not impaired in either LTC_4 synthase- or $CysLT_1$-null mice in the zymosan A-induced peritonitis model.[349,463] Moreover, the increased vascular permeability associated with IgE-dependent passive cutaneous anaphylaxis is significantly reduced in $CysLT_2$-null mice, whereas zymosan A-induced peritoneal inflammation is not altered.[601] $CysLT_2$-mediated vascular permeability via transendothelial vesicle transport was further investigated in $CysLT_2$-null LacZ mouse model.[602] In this model, $CysLT_2$ can mediate inflammatory reactions in a vascular bed-specific manner by altering transendothelial vesicle transport-based vascular permeability.

4.10.7. Pulmonary Fibrosis. Intratracheal or systemic administration of the chemotherapeutic agent bleomycin induces chronic pulmonary inflammation and fibrosis in mice. These features are significantly blunted with respective disruptions of $cPLA_2$[603] and 5-LO,[604] suggesting a role for LTs in this model. Several features of bleomycin-induced injury, including pulmonary macrophage and neutrophil recruitment, alveolar septal thickening, fibroblast accumulation, and collagen deposition, are also significantly reduced in LTC_4 synthase-null mice compared with WT mice.[605] Unexpectedly, $CysLT_1$-null mice are not protected from this injury. Instead, these mice showed exaggerated alveolar septal thickening with reticular fiber deposition when compared with WT or LTC_4 synthase-null mice.[605]

Additionally, cys-LT levels in the BALF of $CysLT_1$-null mice are roughly 2-fold greater than the levels recovered from the WT mice, with no change in the levels of LTB_4 or PGE_2. These findings suggest that the cys-LTs are crucial for this macrophage-mediated chronic inflammatory and fibrotic insult, likely working through $CysLT_2$. Interestingly, alveolar septal thickening after intratracheal injection of bleomycin, characterized by interstitial infiltration with macrophages and fibroblasts and the accumulation of collagen fibers, is significantly reduced in $CysLT_2$-null mice.[601] Because the amount of cys-LTs in BALF after bleomycin injection is similar in $CysLT_2$-null mice and WT mice, $CysLT_2$ can promote chronic pulmonary inflammation with fibrosis in response to a particular pathobiologic event.

4.10.8. Cardiovascular Effects. After findings that SRS-A markedly reduces coronary blood flow,[606] several groups have explored the cardiovascular effects of cys-LTs in a variety of different animal models. In sheep and pigs, cys-LTs induce not only coronary vasoconstriction but also ischemia and impair left ventricular function.[607,608] In isolated perfused guinea pig heart preparations, LTC_4 and LTD_4 cause a reduction in myocardial contractility concomitant with the vasoconstriction.[609,610] FPL-55712 antagonizes the cardiodepressant effects of cys-LTs, and the potency of FPL-55712 is greater against ventricular contraction induced by LTD_4 compared with those of LTC_4, suggesting the presence of at least two receptors in guinea pig heart.[611] In human heart, the negative inotropic effect of cys-LTs is similar to that of guinea pig, and the rank order potency ($LTD_4 > LTC_4 > LTE_4$) is the same for both species.[611] Furthermore, in perfused rat heart,[612] LTD_4 causes a reduction in flow and impairs the spontaneous heart rate to a greater extent than contractility, demonstrating an action on conductivity in this species.[613,614] The use of $CysLT_1$ antagonists provided an alternate approach for establishing the effects of cys-LTs in the perfused heart preparations. ICI 198,615 selectively inhibits LTD_4-induced increases in coronary vascular resistance with no significant effect against LTC_4.[615] In addition, pobilukast inhibits both LTC_4 and LTD_4 responses, whereas MK-571 does not show the effect on cardiac vascular resistance. However, ICI 198,615 blocks both LTC_4- and LTD_4-induced ventricular contractility. These findings suggest that the reduction in contractility by LTC_4 is more sensitive to the antagonists when compared with their action against the vasoconstrictory effect of LTC_4. In contrast, Falcone et al. showed specific binding of [^{3}H]ICI 198,615 to guinea pig cardiac ventricular membranes, indicating the existence of $CysLT_1$ in this tissue,[244] whereas Egan et al. demonstrated that selective antagonism of LT responses does not reduce myocardial effects.[616] Together, these data indicate that the antagonist profiles in the guinea pig heart and lung preparations are not similar and suggest that the receptors in cardiac vessels may be different from those in the airways of this species. Marone et al. tested the effects of cys-LTs following intracoronary injection in man and found that low doses of exogenous LTD_4 and LTC_4 induce immediate changes of cardiovascular function, i.e., a fall in blood pressure, a rise in heart rate, and sympathoadrenergic activation.[617] In another study, Albazzaz et al. assessed the cardiopulmonary effects of LTC_4 inhalation in nonasthmatic and asthmatic patients.[618] The use of the potent and selective $CysLT_1$ antagonists for such studies in human may provide an alternative understanding for the role of these mediators in cardiovascular disease.

4.10.9. Diverse Effects of Cys-LTs. The effects of cys-LTs on mucus secretion have been explored. LTC_4 and LTD_4 increase the mucus secretion from cultured human airway mucosal explants and the effects are antagonized by FPL-55712.[568,619] Pobilukast inhibits the aerosolized LTD_4-induced mucus secretion from goblet cells in guinea pig respiratory epithelium, indicating that the effects of cys-LTs on mucus secretion are $CysLT_1$-mediated.[620]

Currently, the expression of both $CysLT_1$ and $CysLT_2$ has been reported in human platelets by RT-PCR, Western blotting, and flow cytometry.[621] In this report the authors demonstrated that cys-LTs induce a release of significant amounts of RANTES and that this effect is inhibited by pranlukast, pointing to a novel role for platelets in allergic inflammation.[621]

Although LTD_4 alone has no significant effect on DNA synthesis in human cultured airway smooth muscle cells, there is a marked potentiation in the proliferation of these cells induced by the classical mitogens, epidermal growth factor, or thrombin.[595] This effect of LTD_4 is inhibited by either pranlukast or pobilukast, but not zafirlukast, suggesting the activation of cys-LT receptors other than $CysLT_1$.[595] The influence of cys-LTs on the airway smooth muscle proliferation was further confirmed in in vivo studies in rat following chronic antigen challenge, where airway smooth muscle proliferation and hyperreactivity[622] and eosinophil infiltration[587] are attenuated by $CysLT_1$ antagonists.

In guinea pigs, cys-LTs influence lung function by modulating the afferent nervous system, specifically enhancing activity of capsaicin-sensitive sensory C fibers.[623,624] For example, LTD_4 enhances the release of substance P and potentiates tachykinin-mediated, nerve-induced responses in guinea pig trachea.[625,626] Vagal nerve-induced contraction and microvascular permeability in guinea pig airways are inhibited by $CysLT_1$ antagonists; thus, the effects of cys-LTs on tachykinin-containing sensory C fibers are mediated via $CysLT_1$.[626] Few studies have been performed to examine the role of cys-LTs in angiogenesis, where pobilukast has been reported to block responses to LTD_4, albeit with less potency than those to LTC_4.[627] In addition, studies on the actions of cys-LTs on renal function,[628−630] pulmonary circulation,[631−633] systemic circulation,[634] and microcirculation[567,635−637] to established specific cys-LT receptors have not been pursued.

4.11. Other Receptors Involved in Cys-LT Signaling

Initial reports have shown that the LTC_4-stimulated contractions of guinea pig trachea are resistant to $CysLT_1$ antagonists.[638] In guinea pig lung preparations, MK-571 exhibits a low affinity, and BAY-u9773 does not block the cys-LT contractions.[639,640] In addition, Tudhope et al. observed a residual contraction in guinea pig lung strip subsequent to $CysLT_1/CysLT_2$ antagonism by BAY-u9773.[487] Recently, Bäck et al. reported that the contractions to LTC_4 in porcine and human pulmonary arteries are not blocked by either MK-571 or BAY-u9773.[641,642] Because these data do not fit the classical $CysLT_1$ profile, one interpretation is that there may be a cys-LT receptor subtype. Recently, the results generated from radioligand binding studies in human lung membranes suggested that [^{3}H]LTC_4 binding may be associated with a specific LTC_4 receptor different from $CysLT_1$ and $CysLT_2$.[401,643,644] Furthermore, Panettieri et al. demonstrated that LTD_4 enhances the proliferation of human smooth muscle cells and that zafirlukast cannot block this effect.[595] These data further suggest that other receptor subtypes might be present in different preparations, although this hypothesis is based solely on indirect pharmacological assessment and should be interpreted cautiously.

4.11.1. LTE_4 Response Confirmed in $CysLT_1/CysLT_2$-Null Mice. LTE_4 has received little attention because it binds poorly to $CysLT_1$ and $CysLT_2$ and is much less active on normal airways than LTC_4 and LTD_4. However, recent studies indicate that LTE_4 causes skin swelling in human subjects as potently as LTC_4 and LTD_4, and airways of asthmatic subjects are selectively hyperresponsive to LTE_4. Recently, Maekawa et al. generated deficient mice in both $CysLT_1$ and $CysLT_2$ ($CysLT_1/CysLT_2$-null).[645] They examined the dose-dependent ear edema elicited by each cys-LT in $CysLT_1$-null, $CysLT_2$-null, and $CysLT_1/CysLT_2$-null strains. The dose-dependent ear edema induced by injection of LTC_4, LTD_4, or LTE_4 in $CysLT_1/CysLT_2$-null strain is equivalent to that in the WT animals, suggesting the existence of a distinct LTE_4-reactive receptor.[645] The LTE_4-mediated vascular leak in $CysLT_1/CysLT_2$-null mice is inhibited by pretreatment of the mice with PTX or a Rho kinase inhibitor, supporting that the mechanism involved a GPCR linked to $G\alpha_i$ proteins and Rho kinase.[645] These data prompted many researchers to re-evaluate the existence of the unknown receptor, $CysLT_E$, in WT mice and single-receptor-null strains. The permeability response to LTC_4 or LTD_4 is remarkably reduced in $CysLT_1$-null mice and normal in magnitude but delayed in $CysLT_2$-null mice, suggesting that $CysLT_2$ is a negative regulator for $CysLT_1$.[645] Moreover, LTE_4-induced vascular leak is not reduced in $CysLT_1$-null mice but delayed and sustained in $CysLT_2$-null strain, demonstrating that $CysLT_E$ is the dominant receptor for this ligand and that $CysLT_2$ is again a negative regulator.[512] Additionally, administration of MK-571 to $CysLT_2$-null mice mimics the phenotype of $CysLT_1/CysLT_2$-null strain, namely, the significant increase in vascular leak of the ear to intradermal LTE_4.[645] This also indicates that MK-571 is not an inhibitor for $CysLT_E$. Interestingly, pretreatment with MK-571 showed an enhanced response to cys-LTs in $CysLT_1/CysLT_2$-null mice, suggesting that MK-571 potentiates responses apparently mediated via $CysLT_E$. It is possible that MK-571 might block a yet-to-be-defined receptor with negative regulatory properties for $CysLT_E$.

4.11.2. $P2Y_{12}$ as a Receptor for LTE_4. In human mast cells, which express $CysLT_1$ and $CysLT_2$, both LTC_4 and LTD_4 induce intracellular calcium flux, cytokine and chemokine productions, and cell proliferation.[512] LTE_4 augments the potency of LTC_4 and LTD_4 by increasing the numbers of human mast cells from cultures of cord-blood-derived progenitor cells maintained in the presence of stem cell factor, IL-6, and IL-10.[453] Furthermore, in a human mast cell line, LAD2 cells, LTE_4 not only acts as a mitogen but also far exceeds its potency for the production of MIP-1β, promoting delayed PGD_2 generation.[646] Some of these LTE_4-mediated responses are resistant to knockdowns of $CysLT_1$ and $CysLT_2$, suggesting the existence of the unrecognized LTE_4 receptor $CysLT_E$ in these cells. On the basis of sequence homology among $CysLT_1$, $CysLT_2$, and the P2Y receptor family, it seemed likely that a putative $CysLT_E$ might belong to orphan P2Y-like GPCRs or even a known member (Figure 5). Human mast cells express several such receptors, including $P2Y_{12}$ receptor, a $G\alpha_i$-coupled receptor for adenosine diphosphate. Paruchuri et al. recently reported that LTE_4 elicits the activation of ERK1/2 in CHO cells expressing human $P2Y_{12}$, exceeding the potency of LTD_4.[647] Although $P2Y_{12}$ does not bind LTE_4 directly, knockdown of $P2Y_{12}$ by RNA interference reduces LTE_4-mediated productions of MIP-1β and PGD_2 in LAD2 cells without significantly altering their responses to LTD_4. LTE_4, but not LTD_4, was previously shown to induce bronchial eosinophilia when administered by inhalation to asthmatic human subjects.[648] Administration of LTE_4 to the airways of sensitized BALB/c mice potentiates eosinophilia, goblet cell metaplasia, and expression of IL-13 in response to low-dose aerosolized OVA.[647] These effects are intact in $CysLT_1/CysLT_2$-null mice but are completely blocked by administration of clopidogrel, a $P2Y_{12}$-selective antagonist.[647] Importantly, clopidogrel fails to block the response of the murine skin microvasculature to LTE_4, indicating that $P2Y_{12}$ is separate and distinct from the LTE_4 receptor in the skin.[645]

4.12. Clinical Studies on Cys-LT Receptors Targeted Therapy

As described above, cys-LTs play an important role in the pathogenesis of bronchial asthma and allergic rhinitis. These mediators cause smooth muscle contraction, impair mucociliary clearance, enhance mucus secretion, attract eosinophils to the airways, and increase vascular permeability, producing edema.[15,649] In patients with asthma, the airways are more sensitive to inhaled LTD_4 and LTE_4, and the inhaled LTC_4 and LTD_4 increase bronchial reactivity to methacholine or histamine,[650,651] indicating the biological role of these mediators in asthma. In addition, cys-LTs have been identified in urine, plasma, nasal secretions, induced sputum, and BALF from patients with asthma. Especially, urinary LTE_4 excretion increases during spontaneous exacerbations of bronchial asthma,[652] following exercise,[653] allergen,[654] and aspirin challenge;[655] thus, the measurement of urinary LTE_4 can be used to monitor systemic production of cys-LTs. The effects of blockers for LT biosynthesis (e.g., 5-LO inhibitors) or LT receptors in patients with asthma have suggested that interventions in the 5-LO pathway may be of therapeutic use in the treatment of this disease (Table 7).[656,657] These drugs inhibit not only the early but also the late phases of allergic response, which implicates an anti-inflammatory component of such treatment.

The $CysLT_1$ antagonists used in asthma and rhinitis include montelukast (Singulair, Kipres), zafirlukast (Accolate), and pranlukast (Onon). Montelukast is administered orally once daily and is approved for treatment of asthma in patients 2 years or older. The bioavailability is similar regardless of patient age, and absorption is not affected by food. 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 reversible increases in liver enzymes and reports of rare but significant liver dysfunction have prompted recommendations against prescribing this drug to patients with hepatic dysfunction and recommendations to monitor liver enzymes.[323] Pranlukast is an orally administered, selective, competitive antagonist of LTC_4, LTD_4, and LTE_4. It is indicated for the prophylactic treatment of chronic bronchial asthma in pediatric and adult patients. The efficacy of pranlukast twice daily in adults with mild to moderate asthma was demonstrated in placebo- or azelastine-controlled studies of 4 or 8 weeks duration. Pranlukast (225 mg) twice daily appears to be as effective as montelukast (10 mg) once daily and zafirlukast (40 mg) twice daily in adults with mild to moderate asthma. Experimental data suggested that LTs are also involved in the pathogenesis of atopic dermatitis. Because the majority of children with atopic dermatitis later develop allergic rhinitis and asthma, it is conceivable that early LT inhibitor use could not only treat atopic dermatitis but also modify the disease course of allergic rhinitis and asthma in children. However, there are only a few small studies of the use of LT inhibitors in the treatment of atopic dermatitis, most of

Table 7. LT Modifiers Available on the Market for the Treatment of Asthma

generic name	trade name	daily doses
montelukast sodium	Singulair	≥15 years of age, 10 mg/day
		6−14 years of age, 5 mg/day
		2−5 years of age, 4 mg/day
zafirlukast	Accolate	≥12 years of age, 20 mg twice-daily
		7−11 years of age, 10 mg twice-daily
pranlukast hydrate	Onon (Japan)	≥12 years of age, 225 mg twice-daily
	Azlaire (Mexico)	1−12 (Japan) or 2−12 (Mexico) years of age, 3.5−5 mg/kg twice-daily
zileuton (5-LO inhibitor)	Zyflo (US)	≥12 years of age, 600 mg/four times-daily
		<12 years of age, not approved

which are either case reports or small-randomized crossover trials. Two studies show small but significant improvements in atopic dermatitis with the use of these agents.[658,659] Another study on the use of either montelukast or zafirlukast in seven patients in a nonrandomized, no-control, add-on usage trial shows that LT inhibitors do not lead to a sustained benefit for extensive atopic dermatitis.[660] Thus, the role of cys-LT receptor inhibitors in atopic dermatitis has yet to be defined.

5. 5-OXO ETE RECEPTOR

The 5-LO pathway is also involved in the production of five series of LTs, e.g., LTB_5, from EPA.[7,8] These lipids have less proinflammatory and vasoactive activities compared to LTB_4.[9] Other LO-pathway-derived metabolites are 15-oxo-5(*Z*),8(*Z*),11(*Z*),13(*E*)-eicosatetraenoic acid (15-oxo-ETE) and the macrophage mediators in resolving inflammation (maresins), which derived from AA and DHA, respectively. Not much is known about the physiological and pathophysiological significance of these lipids, but there is evidence that 15-oxo-ETE inhibits endothelial cell proliferation[661] and that maresins are potent mediators for resolving inflammation.[662] AA is converted by 5-LO to 5(*S*)-HpETE, which is then either cyclized to LTA_4 in a second 5-LO-catalyzed reaction or reduced to 5(*S*)-HETE by peroxidase activity.[345] Although 5(*S*)-HETE had been shown to activate neutrophils,[663] its rather modest potency is not consistent with what would be expected of receptors for their preferred ligand. In contrast, a metabolic pathway of 5(*S*)-HpETE to produce 5-oxo-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoic acid (5-oxo-ETE), which is about 100 times more potent than 5(*S*)-HETE in activating neutrophils, was found.[664] The biological actions of 5-oxo-ETE are mediated via its specific receptor, which is expressed on a variety of inflammatory cells as well as tumor cells.[665] Although biological roles for 5-oxo-ETE have not been clearly elucidated, it would seem likely that this substance plays important functions in vivo. This section will introduce current advances in 5-oxo-ETE and its specific receptor, termed OXE.

5.1. Biosynthesis of 5-Oxo-ETE

5-Oxo-ETE is formed by the oxidation of 5(*S*)-HETE by 5-hydroxyeicosanoid dehydrogenase (5-HEDH) (Figure 22). 5-HEDH is a microsomal enzyme that is highly specific for 5(*S*)-HETE and requires $NADP^+$ as an obligatory cofactor. Because NADPH is a potent inhibitor for the production of 5-oxo-ETE, the 5-oxo-ETE synthesis is regulated by the ratio of $NADP^+$ to NADPH rather than the absolute concentration of $NADP^+$. The 5-HEDH activity is normally suppressed in resting cells due to maintaining of $NADP^+$ at very low levels. However, the synthesis of 5-oxo-ETE is drastically increased under conditions that favor oxidation of NADPH to $NADP^+$, such as oxidative stress. Other closely related eicosanoids, e.g., 5(*R*)-HETE, 12(*S*)-HETE, and 15(*S*)-HETE, are little or not metabolized by 5-HEDH.[666] In addition to neutrophils, 5-HEDH is expressed in various inflammatory and structural cells, including monocytes,[667] dendritic cells,[668] platelets,[669] endothelial cells,[670] epithelial cells,[671] and airway smooth muscle cells.[671] Therefore, like LTA_4 hydrolase and LTC_4 synthase, 5-HEDH is distributed much more widely than 5-LO, which is expressed at high levels only in inflammatory cells. This raises the possibility that transcellular biosynthesis could contribute to the generation of 5-oxo-ETE, analogous to LTs.[672] Resting neutrophils predominantly metabolize 5(*S*)-HETE to 5(*S*),20(*S*)-diHETE by LTB_4 20-hydroxylase (CYP4F3A), which is highly expressed in these cells.[673] However, upon elicitation of the respiratory burst with PMA, which activates NADPH oxidase-2, the metabolism of 5(*S*)-HETE shifts to the 5-oxo-ETE formation due to the rapid generation of $NADP^+$.[674] Similarly, exposure of cells to oxidative stress in the form of H_2O_2 increases the ratio of $NADP^+$ to NADPH, leading to the 5-oxo-ETE synthesis. In contrast, the oxidation of glucose 6-phosphate by the pentose phosphate pathway results in the impairment of the 5-oxo-ETE production due to the reduction of $NADP^+$.[675] Other endogenous PUFA can also be converted to analogous 5-oxo-fatty acids by a combination of 5-LO and 5-HEDH. For example, in human neutrophils, sebaleic acid and Mead acid [5(*Z*),8(*Z*),11(*Z*)-eicosatrienoic acid] are metabolized to 5-oxo-6,8-octadecadienoic acid (5-oxo-ODE) and 5-oxo-6,8,11-eicosatrienoic acid (5-oxo-ETrE), respectively.[676,677] Similarly, EPA is converted to 5-oxo-6,8,11,14,17-eicosapentaenoic acid by these enzymes.[678]

5.2. Metabolism of 5-Oxo-ETE

Murine macrophages metabolize 5-oxo-ETE to 18- and 19-hydroxy derivatives by a combination of 6,7-reduction and ω-oxidation (Figure 23).[679] These cells also convert 5-oxo-ETE to a GSH conjugated metabolite, 5-oxo-7-glutathionyl-8,11,14-eicosatrienoic acid (FOG_7), by the action of LTC_4 synthase.[680] Since 5-oxo-ETE is also a substrate for 12-LO and 15-LO in platelets[669] and eosinophils,[681] respectively, this lipid is consequently metabolized to 5-oxo-12(*S*)-HETE and 5-oxo-15(*S*)-HETE in these cells. 5-HEDH catalyzes a reversible reaction; thus, 5-oxo-ETE can be stereospecifically reduced back to 5(*S*)-HETE, although the oxidation reaction is dominant.[682] In addition, in neutrophils, 5-oxo-ETE is metabolized to 5(*S*),20(*S*)-diHETE via ω-oxidation.[673] Neutrophils also contain a calcium/calmodulin-dependent Δ^6-reductase, resulting in the conversion to 5-oxo-8,11,14-eicosatrienoic acid.[683]

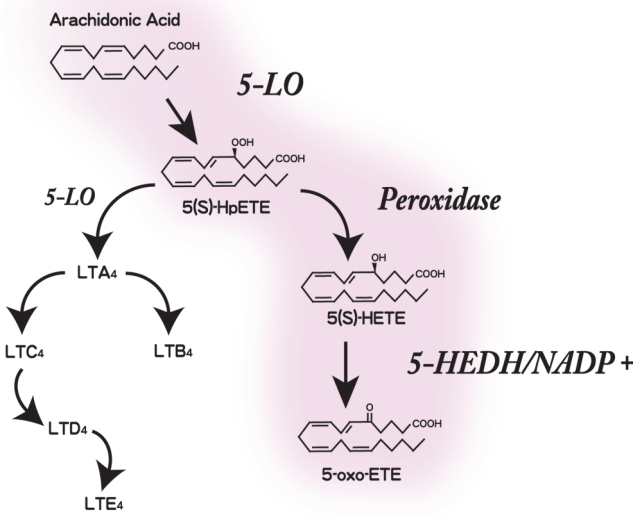

Figure 22. Biosynthesis pathway of 5-oxo-ETE. Formation of 5-oxo-ETE and other 5-LO products from AA. 5-HEDH, 5-hydroxyeicosanoid dehydrogenase.

5.2.1. ω-Oxidation of 5-Oxo-ETE. In murine macrophages, 5-oxo-ETE is converted to 5-oxo-18(S)-HETrE and 5-oxo-19(S)-HETrE, which are formed by a combination of 5-oxoeicosanoid Δ^6-reductase and either ω-2 or ω-1 hydroxylase (Figure 23).[679] In addition, 5(S),18(S)-diHETE and 5(S),19(S)-diHETE are formed by a combination of 5-ketoreductase and either ω-1 or ω-2 hydroxylases. A fifth ω-oxidation product, 5(S),18(S)-diHETrE, is generated by a combination of three enzymes: 5-oxoeicosanoid Δ^6-reductase, 5-ketoreductase, and ω-2 hydroxylase.[679] Neither these metabolic pathways of 5-oxo-ETE nor the precise nature of the responsible enzymes is still clear. Murine macrophages cannot convert 5-oxo-ETE to 20-hydroxy products, which is the major pathway for its metabolism in human neutrophils.

5.2.2. Formation of FOG_7 from 5-Oxo-ETE by LTC_4 Synthase. Murine macrophages also convert 5-oxo-ETE to the glutathione adduct FOG_7.[684] This lipid is formed by the 1,4-Michael addition of glutathione to the 7-position of 5-oxo-ETE by LTC_4 synthase (Figure 23). FOG_7 shows chemoattractant activity on both eosinophils and neutrophils and stimulates actin polymerization in these cells, although it does not induce calcium mobilization.[684] Other glutathione transferases from human placenta and rat liver also catalyze the formation of glutathione adducts with 5-oxo-ETE.[680] These products have similar chromatographic properties and mass spectra to FOG_7; however, they are biologically inactive, suggesting that they are isomers of this substance. It is not yet known whether the glutathione in the 7-position of FOG_7 is in the R- or the S-configuration.

5.2.3. Metabolism of 5-Oxo-ETE by 12-LO and 15-LO. 5-Oxo-ETE is a substrate for 12-LO in platelets.[669] Thus, this lipid is consequently metabolized to 5-oxo-12(S)-HETE in these cells. 5-Oxo-ETE can also be converted to 5-oxo-15(S)-HETE by human eosinophils, which contain high levels of 15-LO.[681] This product is further formed to 5(S),15(S)-diHETE by the action of neutrophil 5-HEDH.[666]

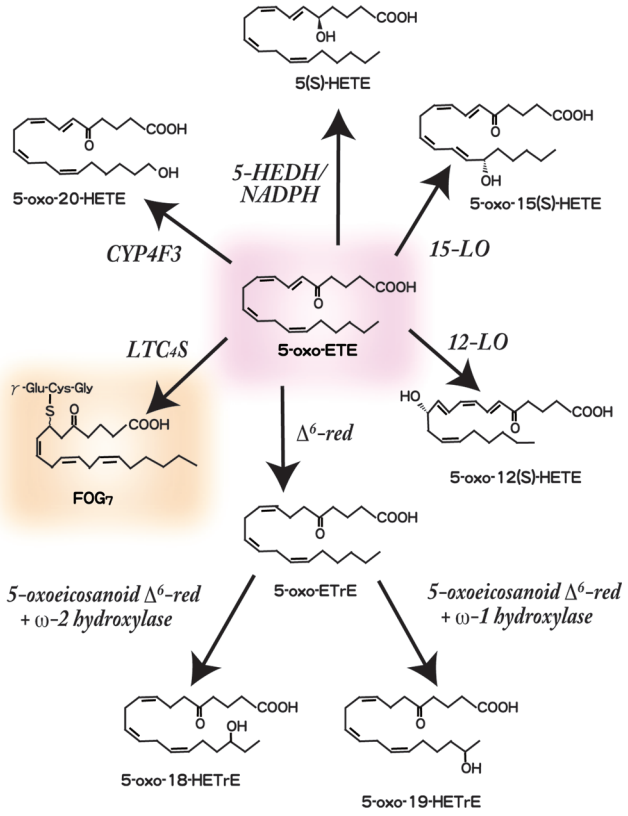

Figure 23. Metabolism of 5-oxo-ETE. Δ^6-red, 5-hydroxyeicosanoid Δ^6-reductase; 12-LO, 12-lipoxygenase; 15-LO, 15-lipoxygenase; CYP4F3, LTB_4 20-hydroxylase; LTC_4S, LTC_4 synthase.

5.3. Existence of 5-Oxo-ETE and FOG_7 Receptors

Although the previous report demonstrated the stimulatory effects of 5(S)-HETE on human neutrophils, 5-oxo-ETE shows about 100 times more potent activity than 5(S)-HETE in calcium mobilization and chemotaxis to these cells.[664] These effects are subject to homologous desensitization by pretreatment with 5-oxo-ETE, but not to heterologous desensitization with LTB_4, PAF, or other chemoattractants, consistent with mediation via a specific receptor for 5-oxo-ETE.[664,681,685] Binding studies with 5-oxo-ETE in neutrophils are complicated by its esterification into cellular lipids. However, O'Flaherty et al. overcame this problem by conducting binding experiments in the presence of the acyl-CoA synthetase inhibitor triacsin C.[686] These studies indicate that the biological actions of 5-oxo-ETE are exerted via the specific receptor.

FOG_7 is highly potent in stimulating eosinophils, as well as neutrophil chemotaxis, and is also capable of initiating actin polymerization, without elevating intracellular calcium.[684] However, the properties of the receptor for FOG_7 are still unclear. Because of the structural differences between FOG_7 and 5-oxo-ETE, it would seem likely that its actions are not mediated via the 5-oxo-ETE receptor.

5.4. Molecular Cloning of 5-Oxo-ETE Receptor, OXE

In the search for the ligand for an orphan GPCR, TG1019, Hosoi et al. screened a library of natural bioactive molecules and related compounds on the basis of the binding of GTPγS to a TG1019−$G\alpha_{i1}$ fusion protein.[687] Of these ligands examined, 5-oxo-ETE is the most potent in activating the binding of GTPγS (EC_{50} = 6 nM).[687] Other fatty acids, e.g., 5(S)-HpETE, AA, and

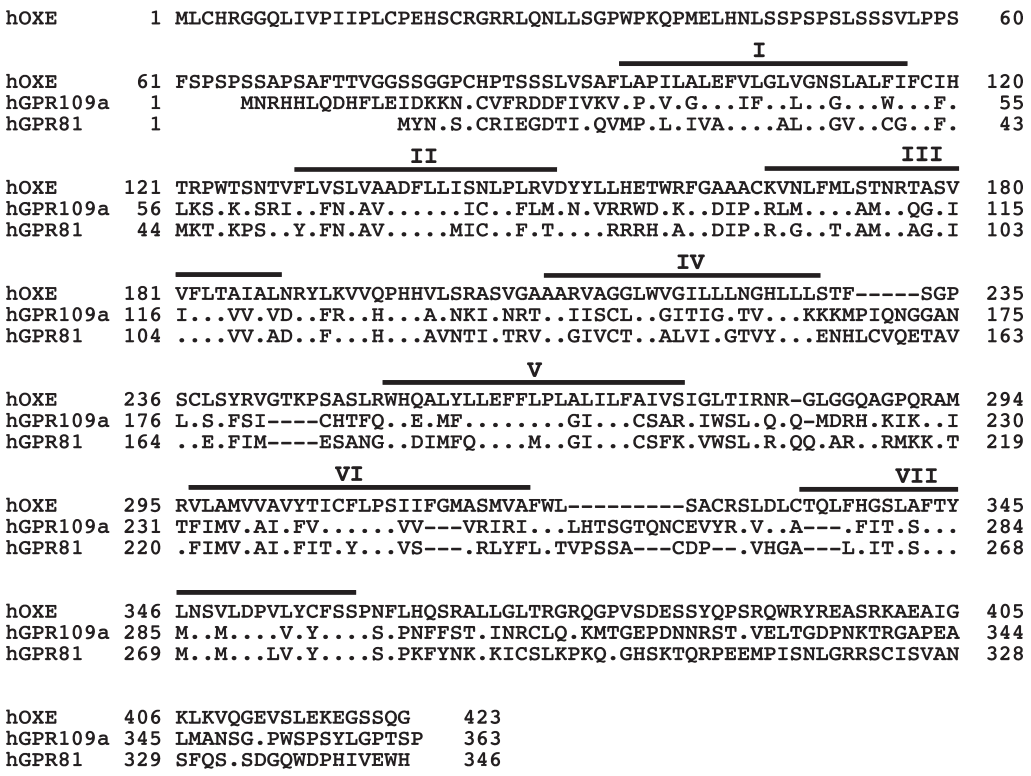

Figure 24. Alignment of human OXE, GPR109a, and GPR81. The putative transmembrane domains predicted from a Kyte−Doolittle hydrophobicity analysis are labeled as I−VII. Conserved amino acids are indicated by periods (.), and gaps in the alignment are indicated by dashes (−).

5(*R* or *S*)-HETE, are much less potent, whereas LTs, PGs, 12(*S*)-HETE, and 15(*S*)-HETE are inactive. Jones et al. independently cloned an orphan GPCR, termed R527. They evaluated about 2000 potential ligands for calcium mobilization in the transfected HEK293 cells and identified 5-oxo-ETE as the most potent agonist, followed by 5(*S*)-HpETE (100 times less potent) and 5(*S*)-HETE.[688] R527 is identical to TG1019 except for the substitution of Val for Leu at position 368 and truncation of the N-terminus by 39 amino acids. These differences do not affect the biological activity of these receptors, e.g., the ligand affinity and tissue distribution. Moreover, Koike et al. identified the other orphan GPCR, hGPCR48, which shows a sequence identity to TG1019, as a 5-oxo-ETE receptor.[689] Hence, the 5-oxo-ETE receptor was named OXE by the IUPHAR Nomenclature Committee for LT and LX receptors (Figure 24).[690,665] The characteristics of OXE are summarized in Table 8. The sequence of OXE shows significant identity at the amino acid level to a nicotinic acid receptor, GPR109a,[691] and a lactate receptor, GPR81.[692]

5.5. Binding Affinities of Natural Ligands to OXE

OXE specifically recognizes 5-oxo-PUFA containing at least two double bonds in the 6- and 8-positions. The most potent ligands are those derived from AA, Mead acid, and sebaleic acid, i.e., 5-oxo-ETE, 5-oxo-ETrE, and 5-oxo-ODE, which have EC_{50} values of 2−3 nM.[677] The product derived from EPA, i.e., 5-oxo-EPE, is also fairly potent, but less so than 5-oxo-ETE. 5(*S*)-HETE, which can be formed by the reduction of 5-oxo-ETE by 5-HEDH, has only about 1% of the potency of 5-oxo-ETE, whereas 6,7-dihydro-5-oxo-ETE and 5(*S*),20-diHETE are only 0.1% and 1% as potent, respectively. Other modifications of 5-oxo-ETE also lead to loss of potency, including esterification of the carboxyl group and isomerization of the Δ^8-cis double bond to the trans configuration.[673,693] Recently, it was reported that C-18 and C-20 5-oxo-$\Delta^{6,8}$ fatty acids are potent activators for OXE.[677] The most potent compounds tested are 5-oxo-20:3 and 8-*trans*-5-oxo-20:3, in addition to 5-oxo-ETE.

5.6. Gene Structure and Transcriptional Regulation of OXE

The OXE gene is localized on the human chromosome 2p21.[687,688] Dot-blotting hybridization analysis revealed that OXE is expressed in various tissues, except brain, although somewhat more intense signals were observed in kidney and liver.[687] Northern hybridization identified three species of OXE-mRNAs with 6.5, 3.3, and 1.8 kb. The transcripts of 6.5 and 3.3 kb are expressed in liver and kidney and in skeletal muscle, respectively, whereas the 1.8 kb transcript is the major species in peripheral leukocytes, lung, placenta, small intestine, spleen, thymus, colon, and heart, and it is also found in skeletal muscle, liver, and kidney. The hybridization signals of peripheral leukocytes, lung, liver, kidney, and spleen seemed to be more intense than those of the other tissues.[687,688] In the blood cells, the relative expression of this receptor in eosinophils, neutrophils, and macrophages is 200:6:1.[688]

5.7. Signal Transduction via OXE

5-Oxo-ETE induces a rapid increase in intracellular calcium in neutrophils[664,694] and inhibits forskolin-stimulated cAMP formation in OXE-expressing CHO cells.[687] A variety of responses to 5-oxo-ETE, including these signalings, are inhibited by PTX, indicating that OXE is coupled to $G\alpha_{i/o}$-proteins.[695−697] The effect of 5-oxo-ETE on calcium mobilization, as well as cell migration, in OXE-expressing CHO cells is blocked by the phospholipase C inhibitor U73122, suggesting the requirement of IP_3 production for these responses.[698] 5-Oxo-ETE also

Table 8. Characteristics of OXE

	OXE
structure (amino acids)	human (423)
accession number (GenBank)	human (AB083055)
ligand	5-oxo-ETE ≫ 5(*S*)-HpETE > 5(*S*)-HETE
expression (human)	leukocytes (eosinophils:neutrophils: macrophages = 200:6:1), lung, placenta, kidney, liver, spleen, small intestine, thymus, colon, skeletal muscle
coupled G-protein	$G_{i/o}$
chromosome (human)	2q21

activates phosphoinositide-3 kinase (PI3K) through OXE, since it elevates the levels of its product phosphatidylinositol (3,4,5)-trisphosphate in neutrophils.[697] Activation of PI3K appears to be involved in the chemoattractant effects of 5-oxo-ETE, because this response is blocked by LY294002, an inhibitor of this enzyme.[698] 5-Oxo-ETE also induces the phosphorylation of ERK1/2 in a variety of cell types, including neutrophils[695] and eosinophils,[696,699] resulting in the activation of cPLA$_2\alpha$ followed by the release of AA,[695,700] which could lead to further production of proinflammatory AA metabolites.

5.8. Other Ligands for OXE

No synthetic OXE antagonists have so far been reported. However, several endogenously formed substances have been reported to possess antagonist properties. The 12-LO metabolites of 5-oxo-ETE, 5-oxo-12(*S*)-HETE and 8-*trans*-5-oxo-12(*S*)-HETE, block 5-oxo-ETE-induced calcium mobilization in neutrophils with IC_{50} values of 0.5 and 2.5 μM, respectively.[669] In agreement with this, these lipids also inhibit 5-oxo-ETE-elicited neutrophil migration. Certain PUFA, including EPA, DHA, dihomo-γ-linolenic acid, and Mead acid, also have antagonistic activities (IC_{50} = 2−6 μM) for OXE on the basis of inhibition of 5-oxo-ETE-induced binding of GTPγS to the cells expressing OXE.[687]

5.9. 5-Oxo-ETE and OXE in Health and Diseases

The roles of the 5-oxo-ETE−OXE pathway have been most extensively studied on eosinophils and neutrophils. It has similar effects on these two cells, inducing chemotaxis, calcium mobilization, actin polymerization, CD11b expression, and L-selectin shedding.[665] Eosinophils and neutrophils pretreated with cytokines such as GM-CSF and TNF-α respond strongly to 5-oxo-ETE, although 5-oxo-ETE has only relatively modest effects on degranulation and superoxide production in the untreated cells.[695,696] OXE is also expressed in prostate tumor cells,[701] in which 5-oxo-ETE induces a proliferative response.[702] The pathophysiological roles of 5-oxo-ETE are summarized in Table 9. Thus, the 5-oxo-ETE−OXE pathway is discussed as a new therapeutic target for several diseases in light of the recent understanding of the roles in eosinophils and neutrophils.

5.9.1. Bronchial Asthma. Among inflammatory cells, OXE is most highly expressed in eosinophils, with lower levels of expression being observed in neutrophils, monocytes,[688] and basophils.[703] Eosinophils strongly respond to 5-oxo-ETE; thus, these cells are one of its primary targets.[681,696,704] There has been some debate about the precise role of eosinophils in asthma, because clinical trials with anti-IL-5 antibodies failed to obtain the expected results.[705,706] In these studies, considerable numbers of eosinophils, which may be sufficient to account for the prolongation of the symptoms, have been shown to persist in the lungs even after anti-IL-5 treatment.[707] In addition, the significant role of eosinophils in lung remodeling was confirmed using transgenic mice with the targeted depletion of eosinophils, revealing that eosinophils remain an attractive target in asthma.[708] Drugs designed to prevent their infiltration into the lungs may have important therapeutic benefits.[709] 5-Oxo-ETE induces the strongest chemotactic response in human eosinophils, although it is a bit less potent than eotaxin.[704,710] Furthermore, it has synergistic effects with eotaxin, RANTES,[711] and PAF[704] in inducing eosinophil migration through filters coated with Matrigel[710] as well as endothelial cell monolayers.[712] In addition to eliciting the eosinophil infiltration, 5-oxo-ETE has effects that are likely to contribute to the pathophysiology of asthma. Although 5-oxo-ETE has only a modest effect on eosinophil degranulation, these cells respond strongly to this lipid by priming with GM-CSF, leading to the release of proteins that may have damaging effects on the airway epithelium.[696] Moreover, 5-oxo-ETE strongly enhances eosinophil degranulation in responses to several mediators, e.g., PAF, C5a, LTB_4, and fMLP.[696] Another important effect of 5-oxo-ETE is its ability to stimulate human monocytes to release GM-CSF, which is a potent survival factor for eosinophils.[713] When eosinophils are cocultured with small numbers of monocytes in the presence of 5-oxo-ETE, the survival of eosinophils is enhanced. 5-Oxo-ETE also shows stimulatory effects on basophils, which play a pivotal role in asthma and other allergic diseases.[714] Although 5-oxo-ETE has relatively modest effects on basophils,[715] this lipid is a potent chemoattractant for these cells.[703,716] Because 5-oxo-ETE has wide-ranging effects on eosinophils as well as more limited effects on basophils, blocking the actions of this lipid may be a useful strategy for the treatment of asthma.

Table 9. Pathophysiological Roles of 5-Oxo-ETE

tissues	associated pathologies
respiratory system	bronchial asthma
	• enhancement of eosinophil influx and degranulation
	• enhancement of neutrophil influx
	• GM-CSF release by monocytes
	• enhanced expression of CD203c and CD11b in basophils
	COPD
	• neutrophil influx, macrophage influx, $CD8^+$ lymphocyte influx
prostate	cancer
	• enhanced proliferation of tumor cells [PC3 prostate cancer cells]
cardiovascular system	atherosclerosis
	• increase in monocyte infiltration, neutrophil infiltration
	• enhanced 5-oxo-ETE production in endothelial cells
kidney	ischemia−reperfusion injury
	• neutrophil influx
skin	atopic dermatitis
	• monocyte/macrophage influx, neutrophil influx, eosinophil influx

5.9.2. Cancer. AA has been reported to increase the proliferation of prostate cancer cells.[717] These effects are mediated by 5-LO products, because increased 5-LO expression has been reported in prostate tumors[718] and 5-LO inhibitors or FLAP inhibitors were found to reduce tumor development in vivo in animal models[719,720] and to induce apoptosis in cancer cells derived from various tissues.[702,717,721−723] Ghosh and Myers found that both 5-oxo-ETE and 5(*S*)-HETE, the former being more potent, reduce the proapoptotic effects of MK886 (FLAP inhibitor) and AA861 (5-LO inhibitor), whereas LTB_4 and cys-LTs are ineffective.[702,717] 5-Oxo-ETE also inhibits selenium-induced apoptosis in prostate cancer cells[724] and increases the rates of proliferation of cancer cells derived from a variety of other tissues.[725] These effects of 5-oxo-ETE appear to be mediated via OXE, which is expressed in a variety of tumor cells, because blocking the OXE expression with siRNA reduces the viability of PC3 prostate cancer cells.[701,725] These data raise the possibility that OXE antagonists could be useful in the treatment of cancer.

5.9.3. Cardiovascular Disease. Because of its effects on neutrophils and monocytes, 5-oxo-ETE could also be involved in cardiovascular disease. The study by Helgadottir et al. linked a SNP in FLAP to increased risk for myocardial infarction and stroke.[726] Since monocyte infiltration of the vessel wall is a key step in the development of atherosclerosis,[727] 5-oxo-ETE could contribute to this process due to its chemoattractant effects on these cells. Furthermore, 5-oxo-ETE has synergistic effects with chemokines, such as MCP-1, which plays a role in the recruitment of monocytes in this disease.[728] Endothelial cells contain a high level of 5-HEDH activity; thus, these cells can synthesize 5-oxo-ETE, especially under conditions of oxidative stress, as might be expected to occur in inflammation.[670] Because endothelial cells do not have appreciable 5-LO activity, these cells could synthesize 5-oxo-ETE by transcellular biosynthesis from neutrophil- or monocytes-derived 5(*S*)-HETE.

5.10. Clinical Studies on 5-Oxo-ETE and OXE

Eosinophils are very prominent in the asthmatic lung, while high levels of neutrophils occur in chronic obstructive pulmonary disease. Mobilization of these cells is dependent on their release into the circulation from the bone marrow under the influence of various mediators and their accumulation in tissues in response to topically released chemoattractants. Among the chemokines, IL-8 is an active factor for neutrophils,[729] whereas eotaxin is selective for eosinophils and basophils.[730] In lipid mediators, 5-oxo-ETE is the most potent in eliciting a chemotactic response in human eosinophils,[704] followed by PAF and PGD_2. For example, intratracheal administration of 5-oxo-ETE to Brown Norway rats induces pulmonary eosinophilia.[731] Recently, Muro et al. reported the cellular infiltration induced by 5-oxo-ETE in patients with atopic asthma and nonatopic control subjects.[732] The intradermal administration of 5-oxo-ETE (1.5 and 5 μg) elicits the infiltration of both eosinophils and neutrophils into the skin in both control and atopic asthmatic subjects. Increased numbers of eosinophils are observed at 6 and 24 h after injection; in contrast, neutrophil numbers are significantly elevated only after 24 h. Eosinophils are more than 3 times higher in patients with atopic asthma compared to control subjects. Since there are no OXE antagonists and synthetic inhibitors for 5-oxo-ETE, it is difficult to evaluate its physiological role in allergic responses. Nevertheless, the stimulatory effects of 5-oxo-ETE on eosinophil infiltration both in vitro and in vivo suggest that it could be an important physiological mediator in diseases such as asthma. Because a variety of mediators, e.g., 5-LO products, PAF, and chemokines, are all released after allergen challenge, interactions among these mediators may be important in eliciting eosinophil infiltration in allergic diseases. These findings may be helpful in designing new therapies.

6. LX RECEPTOR

Various eicosanoids play important roles as local mediators exerting a wide range of actions relevant in immune hypersensitivity and inflammation.[15] An acute inflammatory reaction may either progress to a chronic state or enter a resolution phase for complete healing. In the latter case, recent findings indicate that specific lipid mediators derived from the LO-pathways play a key role in initiating the resolution of acute inflammation. These lipid mediators are generated either from the ω-6 polyunsaturated fatty acid (PUFA), i.e., AA, or from the ω-3 PUFAs, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Serhan et al. characterized these oxygenated derivatives of AA, lipoxins (lipoxygenase interaction products, LXs), isolated from human leukocytes.[733] Multicellular host responses to infection, injury, or inflammation stimuli lead to the generation of LXs, which bear potent anti-inflammatory activities. LXs and their carbon 15-epimer lipoxins (aspirin-triggered lipoxins, ASA-15-epi-LXs) are bioactive and structurally distinct from other eicosanoids in that they carry a conjugated tetraene system and are present in biological matrix in two main forms that are positional isomers, namely, LXA_4 [5(*S*),6(*R*),15(*S*)-trihydroxyeicosa-7(*E*),9(*E*),11(*Z*),13(*E*)-tetraenoic acid] and LXB_4 [5(*S*),14(*R*),15(*S*)-trihydroxyeicosa-6(*E*),8(*Z*),10(*E*),12(*E*)-tetraenoic acid]. Several reports demonstrated that LXs are rapidly and locally generated in response to stimuli and undergo immediate metabolic inactivation. Due to the short half-life of LXs, it has been difficult to confirm the temporal-spatial production of these mediators in vivo. Although LXs clearly have bioactivity, extensive studies, e.g., detection of the temporal and spatial formation of these mediators, will be needed to clarify the significance of LXs as physiologic mediators in vivo. In this section, we review recent developments on the molecular basis of LX bioactions mediated through specific receptor and the accumulating evidence that LXs may have potential as novel anti-inflammatory agents.

6.1. Biosynthesis of LXs

LXs have their carbon-15 (C-15) hydroxyl group mainly in the 15(*S*)-configuration, which is inserted by LO-based mechanisms. LXs are produced by transcellular biosynthesis via the interactions of two or more cell types. Two main classical LX biosynthesis pathways are used in human cells and tissues (Figure 25). The first pathway involves leukocytes/platelets interactions and microaggregates that promote LX formation through transcellular transport of LTA_4. In leukocytes, 5-LO converts AA to LTA_4, which is then transferred into adherent platelets, and LXA_4 and LXB_4 are further generated via the LX synthase activity of human 12-LO.[734] Namely, human platelets cannot produce LXs on their own but become a major source of LXs when the leukocytes/platelets adhesion occurs. The second biosynthetic route is initiated at the mucosal surfaces.[735] In airway epithelial cells or monocytes, eicosanoid products of 15-LO, 15(*S*)-hydroperoxyeicosatetraenoic acid [15(*S*)-HpETE] or the reduced alcohol form 15(*S*)-hydroxyeicosatetraenoic acid [15(*S*)-HETE], can serve as substrates for the leukocytes 5-LO and lead to the

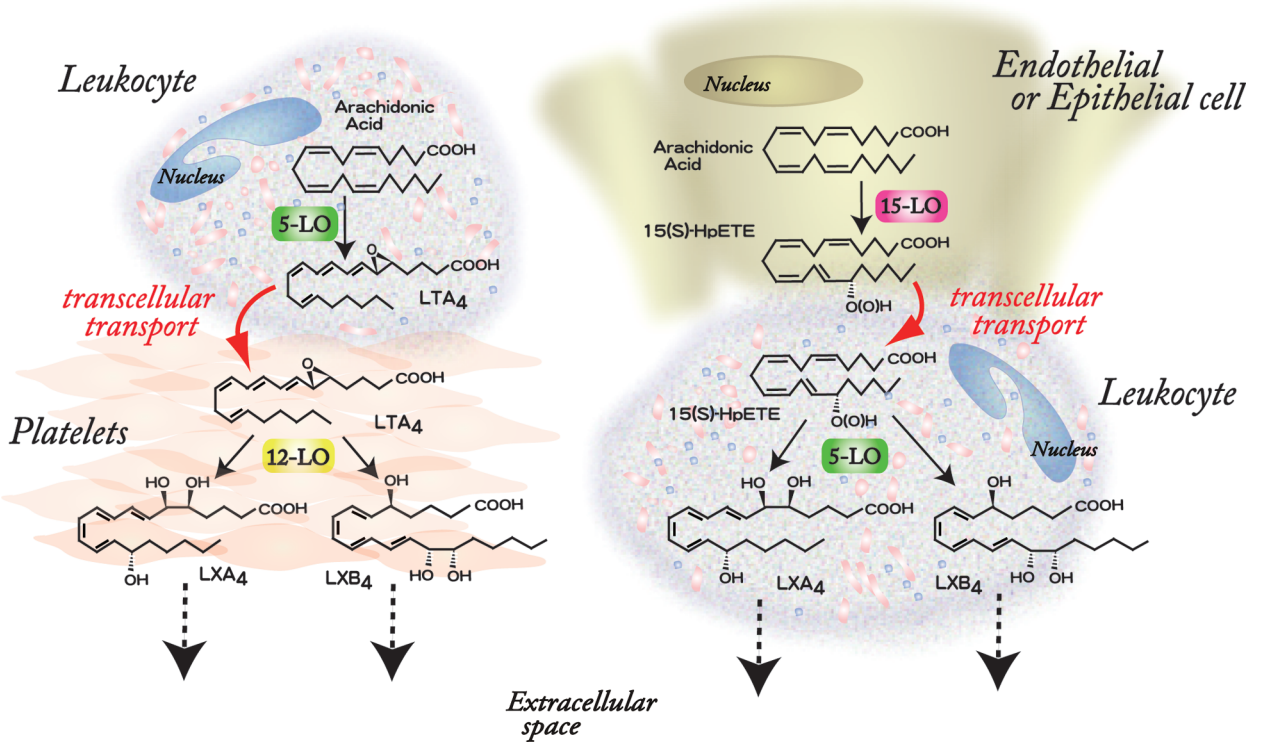

Figure 25. De novo pathways of LXs biosynthesis. During cell−cell interactions, LXs can be amplified by the transcellular biosynthesis via the interactions of two or more cell types. Two major "classic" pathways appear to be used in human cells and tissues.

formation of the trihydroxytetraenes LXA_4 and LXB_4. Importantly, these processes not only lead to LX biosynthesis but also result in the reduction of LT formation in the leukocytes. LXB_4 is a positional isomer of LXA_4, carrying alcohol groups at the carbon 5(*S*), 14(*R*), and 15(*S*) positions, instead of the carbon 5(*S*), 6(*R*), and 15(*S*) positions present in LXA_4. Although LXA_4 and LXB_4 are similar in structure, the biological activities of both mediators are quite distinct. Recently, a third pathway for LX production, which involves aspirin and the actions of COX-2 and 5-LO, was reported (Figure 26).[736] This pathway is called the "aspirin-triggered 15-epi-LX pathway" and is initiated when COX-2 is upregulated and irreversibly acetylated by aspirin. In response to various stimuli, such as cytokines, hypoxia, and bacterial infections, epithelial and endothelial cells inducibly produce COX-2. In addition to the reduction of PGs production, aspirin also acetylates COX-2 in these cells. The acetylated COX-2 remains catalytically active, but the action of this enzyme switches from generating PGs to producing 15(*R*)-HETE. The generated 15(*R*)-HETE is transferred into leukocytes via transcellular routes and converted to 15-epimeric forms of LXs, termed aspirin-triggered LXA_4 (ASA-15-epi-LXA_4, ATL) and ASA-15-epi-LXB_4, by 5-LO.[737] These aspirin-triggered forms bear C-15 alcohol in the *R*-configuration, which is inserted by the acetylated COX-2. The acetylated COX-2 also metabolizes ω-3 fatty acids, EPA and DHA, as substrates to generate the bioactive lipids denoted as resolvin E (RvE) series (derived from EPA) and resolvin D (RvD) series (derived from DHA).[738] Additionally, DHA is converted by LO to 17(*S*)-hydroxyperoxy-DHA and further to protectin D1, also known as neuroprotectin D1.[739] These lipid species have attracted a lot of interest due to their anti-inflammatory and proresolution capacities in vivo.[740,741] LXA_4 and ATL, which have a unique trihydroxytetraene structure, function as stop signals in inflammation and actively participate in dampening host responses to bring the inflammation to a resolution. The route of LX formation depends on the cells and enzymes present therein and can be modulated by cytokines.[62] For instance, IL-4 and IL-13, which are negative regulators of the inflammatory response, both increase 15-LO expression, resulting in enhanced LX generation.

Although the formations of LXs were initially described in human leukocytes, these are also generated in bovine, porcine, and rat cells, including basophils and macrophages. Since more than 50% of LTA_4 is released from the cell of origin,[735] LTA_4 serves as an intermediate for both intracellular and transcellular eicosanoid biosynthesis in several cells (Figure 2). LTA_4 has multiple potential enzymatic and nonenzymatic fates, including (i) conversion by 12-LO to LXA_4 and LXB_4, (ii) conversion to LTB_4 by LTA_4 hydrolase, (iii) formation by LTC_4 synthase to LTC_4, or (iv) nonenzymatic hydrolysis (which occurs in seconds in aqueous environments). Because LTB_4 and cys-LTs possess potent proinflammatory activities and LXs inhibit LT-mediated responses in vivo, the balance between LTs and LXs is critical to cellular responses.

The production of LXs during cell−cell interactions via transcellular biosynthesis may be linked to the pathophysiology of several human diseases. These mediators are rapidly metabolized, the major routes of degradation being dehydrogenation at C-15 and possibly ω-oxidation at C-20. The rapid inactivation and short half-life of LXs result in the difficulty in obtaining the reliable evidence that these mediators are generated in vivo at levels sufficient to exert the physiological function. Further studies, such as establishment of the specific methods for extracting and monitoring of LXs in various diseases, will be needed. Clarifying both the temporal−spatial formation and action of

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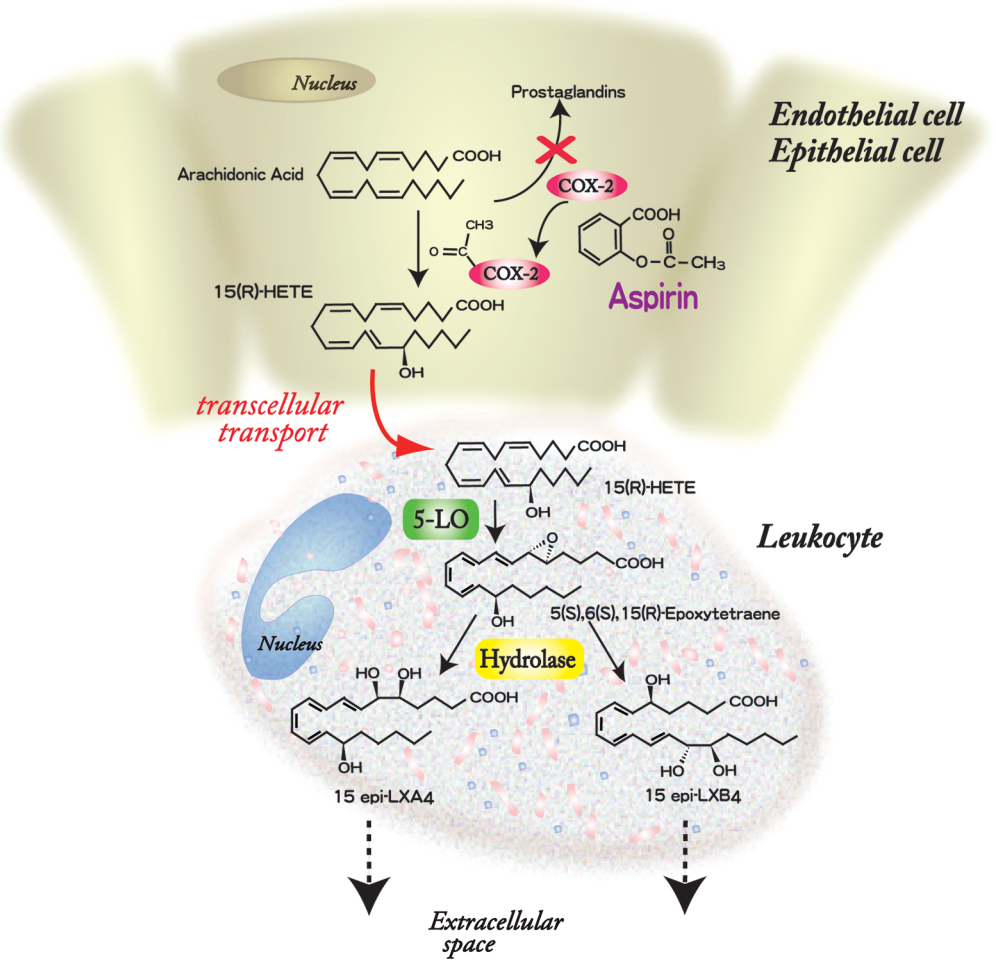

Figure 26. "Aspirin-triggered" pathway of LXs biosynthesis. Aspirin-triggered lipid-generation pathway is initiated when COX-2 is upregulated and irreversibly acetylated by aspirin. The acetylated COX-2 remains catalytically active, but the enzyme activity switches from generating prostaglandin intermediate to producing 15(*R*)-HETE, the precursor of 15-epi-LXs (ATL).

LXs is important for determining their impact during an acute inflammatory reaction.

6.2. LXA_4 Receptor (ALX)

6.2.1. Molecular Cloning of ALX. Because LXA_4 does not compete with [^{3}H]LTB_4 binding to either recombinant human BLT1 or isolated human peripheral blood neutrophils,[742,743] the abilities of LXA_4 and ATL to inhibit the LTB_4-induced responses in vivo and in isolated PMNs may not be through direct interaction with BLT1. Human PMNs show specific [11,12-^{3}H]LXA_4 binding (K_d = 0.5 nM) that is modulated by guanosine stable analogs, suggesting the existence of specific membrane receptors for LXA_4 on human leukocytes.[742] These LXA_4-specific binding sites are inducible in HL-60 cells exposed to various differentiating reagents, e.g., retinoic acid, DMSO, and phorbol 12-myristate 13-acetate.[160] In contrast, although functional studies have indicated the existence of a receptor for LXB_4,[744] this receptor has not been cloned yet. In addition, the receptor binding investigations with LXB_4 must await the synthesis of a radiolabeled LXB_4 with high specificity.

On the basis of the information that the functional LXA_4 receptor is expressed in the differentiated HL-60 cells,[160] several orphan GPCRs isolated from myeloid cells were evaluated for their ability to bind and signal in response to LXA_4.[745] The LXA_4 receptor, termed ALX, was originally identified in the analyses of fMLP receptor (FPR) family in the myeloid lineages (Figure 27). The isolated clone has high sequence homology (approximately 70%) with FPR. In the light of this homology, this receptor was initially termed formyl peptide receptor-like-1 (FPRL1),[746] FPRH1,[747] FPR2,[748] or RFP (receptor related to FPR)[749] by different research groups. The same receptor was also identified by Nomura et al. from the human cDNA library and remained as an orphan receptor, HM63.[750] Although it seemed that the agonist for ALX would be fMLP, this receptor does not effectively respond to fMLP unless stimulated with higher pharmacological doses (i.e., >1−10 mM), suggesting that fMLP is not a physiologically relevant ligand. In CHO cells expressing ALX, this receptor exhibited specific [^{3}H]LXA_4 binding with high affinity (K_d = 1.7 nM) and showed ligand selectivity when compared with LXB_4, LTB_4, LTD_4, and PGE_2.[745] Moreover, in these cells LXA_4-stimulation elicited the activation of GTPase and the release of AA from membrane phospholipids, indicating that ALX is a functional receptor for LXA_4. The characteristics of ALX are summarized in Table 10. Subsequently, the mouse[751] and rat[752] ALX cDNAs were isolated. Both human and mouse ALX cDNAs contain an ORF of 1056 nucleotides that encode a protein of 351 amino acids. The overall identity between human and mouse ALXs is 73% in deduced amino acid (Figure 27).[751]

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                                                                  I
hALX       1 MET---NFSTPLN-EYEEV-------SYES---AGYT-VLRI-LPLVVLG-VTFVLGVLG   43
hChemR23   1 ..DEDY.T.ISYGD..PDYLDSIVVLEDL.PLE.RV.---..F.-V..YSI.C.-.....   55
                                           II
hALX      44 NGLVIWVAGFRMTRTVTTICYLNLALADFSFTATLPFLIVSMAMGEKWPFGWFLCKLIHI  103
hChemR23  56 .....II.T.K.KK..NMVWF....V...L.NVF..IH.TYA..DYH.V..TAM..ISNF  115
                    III                                 IV
hALX     104 VVDINLFGSVFLIGFIALDRCICVLHPVWAQNHRTVSLAMKVIVGPWILALVLTLPVFLF  163
hChemR23 116 LLIH.M.T....LTI.SS....S..L...S....S.R..YMACMVI.V..FF.SS.SLV.  175
                                                                 V
hALX     164 LTTVTIPNGDTYCTFNFASWGGTPEERLKVAITMLTARGIIRFVIGFSLPMSIVAICYGL  223
hChemR23 176 RD.ANLHGKISC--..NF.LS-..GSSSWPTHSQMDPV.YS.HMVVTVTRFLCGFLVPV.  232
                                             VI
hALX     224 IAAKIHKKGMIKSSRP-LRVLTAVVASFF-ICWFPFQLVALLG-TVWLKEMLFYGKYKII  280
hChemR23 233 .ITACYLTIVC.LQ.NR.AKTKKPFKIIVT.IIT-.F.CWCPYH.LN.L.LHHTAMPGSV  291
                           VII
hALX     281 DILVNPTS-SLAFFNSCLNPMLYVFVGQDFRERLIHSLPTSLERALSEDSAPTNDTAANS  339
hChemR23 292 FS.GL.LATA..IA...M..I....M....KKFKVA-.FSR.VN.....TGHSSYPSHR.  350

hALX     340 ASPPAETELQAM          351
hChemR23 351 FTKMSSMNERTSMNERETGML  373
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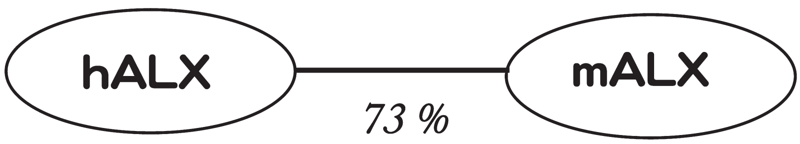

Figure 27. (Upper) Amino acid sequence alignment of human ALX. The putative transmembrane domains predicted from a Kyte−Doolittle hydrophobicity analysis are labeled as I−VII. Conserved amino acids are indicated by periods (.), and gaps in the alignment are indicated by dashes (−). (Lower) The relative sequence homology (percentage identity) between human and murine ALX is also shown.

The molecular evolution assessed from computer-based sequence analyses indicates that ALX belongs to a cluster of chemoattractive peptide and chemokine receptors, exemplified by fMLP, C5a, and IL-8 receptors (Figure 5). BLT1 shares an overall homology of approximately 30% with ALX in deduced amino acid sequences. Human ALX is expressed in several types of leukocytes, including monocytes[753] and T-cells,[754] as well as resident cells, such as macrophages, synovial fibroblasts,[755] and intestinal epithelial cells.[756] Although LXB_4 has also been reported to activate another receptor, the putative receptor activated by LXB_4 has not been cloned yet.

At least nine distinct mouse genes in the FPR family have been cloned and are designated Fpr1, Lxa4r/Fprl1, and Fpr-rs1 to Fpr-rs7; Fpr1 is the ortholog of human FPR[757] and Lxa4r/Fprl1 encodes a functional receptor for LXA_4.[751] Fpr-rs1 and Fpr-rs2 are most similar to Lxa4r/Fprl1 (mouse ALX), sharing 97 and 83% identity, respectively, in the deduced amino acid sequences.[758] Noteworthy, Fpr-rs1 differs from ALX by the deletion of four amino acids at the cytoplasmic end of TM4, which might have an impact on receptor function. Fpr-rs2 was proposed to be a low-affinity receptor for fMLP.[759] Five other genes, Fpr-rs3, Fpr-rs4, Fpr-rs5, Fpr-rs6, and Fpr-rs7, lack human counterparts and are currently considered orphan receptors.

6.2.2. Binding Affinities of Natural Ligands to ALX. In CHO cells expressing human and mouse ALXs, these receptors display specific binding to $[^3H]LXA_4$, with K_d values of 1.7[745] and 1.5 nM,[751] respectively. In the competition study using other eicosanoids, including LXB_4, LTD_4, LTB_4, and PGE_2, only LTD_4 shows competition for $[^3H]LXA_4$ binding with a K_i value of 80 nM.[745] Although ALX shares approximately 70% homology with FPR, ALX binds $[^3H]$fMLP with only low affinity ($K_d = 5\ \mu M$).[760] Intriguingly, it has been recently reported that mitochondria-derived formyl peptides are more potent agonists for ALX than fMLP,[761] suggesting that its primary function may be to recognize host-driven mitochondrial peptides or possibly other bacterially derived formyl peptides.

6.2.3. Gene Structure and Transcriptional Regulation of ALX. The three members of the human FPR gene family, FPR (FPR1), ALX (FPR2), and FPR3, are clustered, spanning approximately 80 kbp, on chromosome 19q13.3−19q13.4, adjacent to the human C5a receptor gene.[746,747] This suggests that these genes arose by relatively recent duplication of a common ancestor. Northern blot analysis of multiple murine tissues demonstrated that ALX mRNA is most abundant in neutrophils, spleen, and lung, with lesser amounts in heart and liver.[751] In humans, ALX mRNA is abundant in PMNs, as well as in spleen, heart, lung, placenta, and liver.[745,751] In human enterocytes, ALX is expressed in crypt and brush-border colonic epithelial cells and inducible by IL-13 and IFN-γ, suggesting that ALX is associated with enterocyte immune functions.[756] The distribution of ALX provides additional evidence that this receptor is not associated with bacterial chemotaxis, as observed for *N*-formyl peptide FPR-signaling, because the gastrointestinal tract always has a high level of bacteria present.

Table 10. Characteristics of ALX

	ALX/FPR2
structure (amino acids)	human (351)
	mouse (351)
	rat (351)
accession number	human (M88107)
	mouse (NM_008042)
ligand (lipid)	LXA_4, ATL (15-epi-LXA_4)
ligand (peptide)	uPAR, HIV peptides, mitochondrial peptides, SAA, $PrP_{106-126}$, $A\beta_{42}$, LL-37
synthetic ligand	ATLa1, ATLa2, ZK-142, ZK-994, WKYMVM, MMK-1, Quin-C1, BML-111, Benzo-LXA4, Compound-11, Compound-43
expression (human)	PMNs, monocytes, activated T-cells, enterocytes, synovial fibroblasts, spleen, lung, heart, placenta, liver
coupled G-protein	$G_{i/o}$
chromosome (human)	19q13.3-19q13.4

6.3. Signal Transduction of ALX

The signaling via ALX is highly specific and selective for different cell types. In human PMNs, the activation of ALX by LXA_4 or ATL reduces cell functions, e.g., impairment of chemotaxis,[762,763] inhibition of natural killer cell cytotoxicity,[764] and reduction of LTB_4- or fMLP-elicited PMN transmigration and β2-integrin-dependent adhesion.[760] In contrast, LXA_4 and ATL activate human monocytes, e.g., increase in intracellular calcium and the phagocytosis of apoptotic PMNs,[765] suggesting a different intracellular signaling pathway from that in PMNs.[766,753] GPCRs are well-known to couple to different G-proteins and/or have different signaling pathways in different cells,[767] especially in comparisons between natural and recombinant systems.[768] Thus, there could be different PTX-sensitive G-proteins that couple to ALX with divergent downstream signaling pathways in different cell types, leading to, for example, activation of monocytes versus reduction of PMNs.[743,753] In addition, LXA_4 modulates MAPK activities in mesangial cells in a PTX-insensitive manner,[448] suggesting the presence of an additional receptor subtype and/or signaling pathway. Interestingly, LXA_4-stimulated monocyte adherence to laminin is not dependent on the increase in intracellular calcium, because a calcium chelator, 1, 2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, acetoxymethyl ester (BAPTA-AM), does not affect the LXA_4-stimulated adherence response.[766] Thus, although LXA_4 stimulates calcium mobilization in monocytes, calcium is not the crucial second messenger of LXA_4 actions in monocytes.

6.4. Synthetic Ligands for ALX

Activation of ALX leads to inhibition of PMN migration, thus preventing neutrophil-mediated tissue injury, while promoting nonphlogistic monocytes emigration, thereby allowing clearance of apoptotic cells by macrophage phagocytosis.[769] The growing evidence supporting the anti-inflammatory and tissue-protective effects of ALX ligands prompted many researchers to search for novel agonists for this receptor. So far, several peptides and small molecular compounds that act as ALX agonists in vitro have been developed. In this section, we summarize the current information about these synthetic ligands for ALX.

6.4.1. Stable Analogs of LX and ATL. One drawback for the therapeutic use of natural LXA_4 is its rapid metabolism in vivo and chemical instability. Therefore, more stable analogs, i.e., 15 (*R*/*S*)-methyl-LXA_4 (ATLa1), 15-epi-16-(*p*-fluorophenoxy)-LXA_4 (ATLa2), and 16-phenoxy-LXA_4, were developed (Figure 28).[770,771] These analogs are still detectable to 90−100% after 3 h of application to mice.[770] ATLa2 and 16-phenoxy-LXA_4 potently inhibit leukocyte recruitment in the mouse ear model of inflammation.[751,770] In addition, Wu et al. showed that ATLa1 exerts a protective effect in a model of mesangioproliferative glomerulonephritis in rat.[772] Furthermore, new 3-oxa-LXA_4 analogues with improved pharmacokinetic characteristics were generated. These second generation analogs, such as [5(*R*),6(*R*),7(*E*),9(*E*),11(*Z*),13(*E*),15(*S*)]-16-(4-fluorophenoxy)-3-oxa-5,6,15-trihydroxy-7,9,11,13-hexadecatetraenoic acid, possess a potency and efficacy comparable to ATLa in diverse animal models after topical, intravenous, or oral delivery.[773] Remarkably, 3-oxa-LXA_4 analogues (ZK-142 and ZK-994) (Figure 28) have potent once daily oral efficacy in preventing and promoting the resolution of established colitis in a model of Crohn's disease.[773] Currently, a new class of stable LX analogues containing a benzo-fused ring system has been designed and studied (Figure 28). These new analogues were shown to exhibit potent anti-inflammatory properties, significantly decreasing neutrophil infiltration in a zymosan A-triggered acute inflammation model in mice.[774]

6.4.2. Peptide Ligands. A synthetic peptide, Trp-Lys-Tyr-Met-Val-Met-NH_2 (WKYMVM), had been identified from a random peptide library as a potent agonist for human ALX.[775−777] Recently, a D-methionine-containing peptide, Trp-Lys-Tyr-Met-Val-D-Met-NH_2 (WKYMVm), was developed with respect to its ability to activate neutrophil effector functions.[778] In the various cells expressing ALX, e.g., human phagocytes and neutrophils, WKYMVm shows more potent activities for mobilizing intracellular calcium and chemotaxis through ALX, suggesting a potent agonist for ALX.[776−778] By measuring calcium mobilization in HL-60 cells expressing FPR1, ALX (FPR2), or FPR3, WKYMVm activates all three receptors, whereas WKYMVM activates exclusively ALX and FPR3.[777] The ED_{50} values of WKYMVM for calcium mobilization in ALX-expressing and FPR3-expressing HL-60 cells are 2 and 80 nM, respectively. In the same assay, the ED_{50} values of WKYMVm for ALX- and FPR3-expressing HL-60 cells are 75 pM and 3 nM, respectively.

By a random screening of a peptide library, Klein et al. identified a small peptide that reacts with ALX, termed MMK-1 (LESIFRSLLFRVM). This peptide elicits calcium mobilization with high efficacy in human monocytes and neutrophils.[779,780] MMK-1 also induces considerable migration of both human monocytes and neutrophils starting at concentrations of 1 nM (EC_{50} = 10 nM).[780] Tsuruki et al. reported that intraperitoneally injected MMK-1 shows an antialopecia effect in neonatal rats.[781] They further demonstrated that oral administration of 100 mg/kg MMK-1 for 6 days suppresses alopecia induced by the anticancer drug etoposide in neonatal rats.[782]

A novel ALX agonist, TIPMFVPESTSKLQKFTSWFM-amide (CGEN-855A), was identified using a computational platform designed to search for novel GPCR peptide agonists cleaved from secreted proteins by proteolysis.[783,784] This peptide displaces the [^{125}I]labeled WKYMVm binding to human ALX on CHO cells with a K_i value of 54.1 nM.[783] In vivo, CGEN-855A shows anti-inflammatory activity manifested as 50% inhibition of PMN recruitment to inflamed air

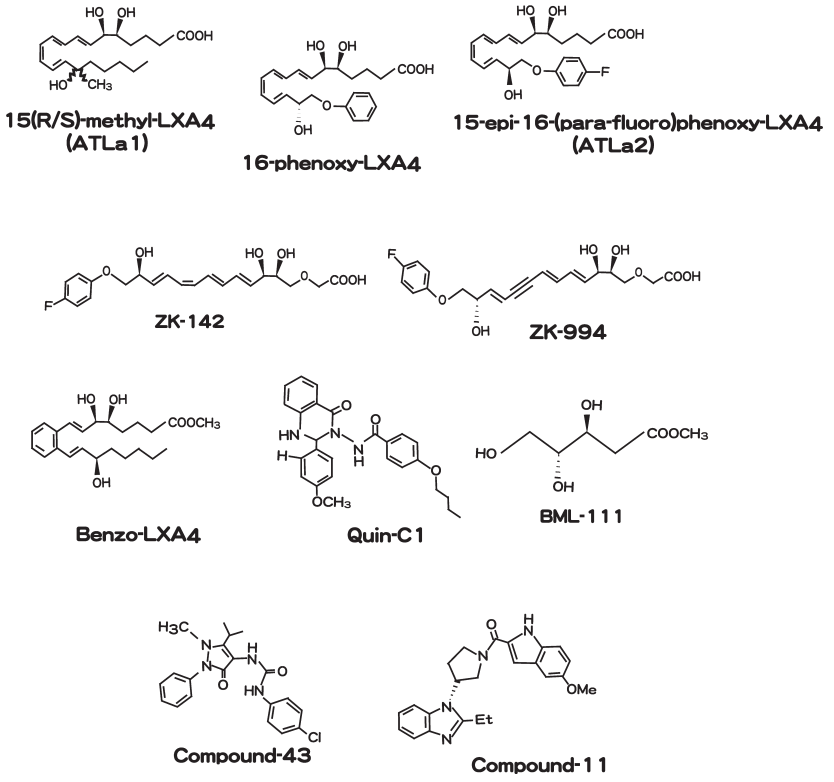

Figure 28. Chemical structures of stable LX analogs and synthetic ligands for ALX.

pouch and provides protection against ischemia−reperfusion-mediated injury to the myocardium in both murine and rat models.[783]

Intriguingly, Bae et al. identified a potent peptide, Trp-Arg-Trp-Trp-Trp-Trp-$CONH_2$ (WRWWWW, termed WRW^4), which inhibits the binding of WKYMVm to ALX in RBL-2H3 cells.[785] WRW^4 inhibits the activation of ALX by WKYMVm, resulting in the complete inhibition of the intracellular calcium increase, ERK1/2 activation, and chemotactic migration of cells toward WKYMVm. Furthermore, $A\beta_{42}$-peptide-induced superoxide generation and chemotactic migration of neutrophils are inhibited by WRW.[4] Thus, WRW^4 is the first specific ALX antagonist and is expected to be useful in the study of ALX signaling and in the development of drugs against ALX-related diseases.

6.4.3. Chemical Ligands. Nanamori et al. identified a substituted quinazolinone, named Quin-C1 {4-butoxy-*N*-[2-(4-methoxyphenyl)-4-oxo-1,4-dihydro-2*H*-quinazolin-3-yl]benzamide}, as a potent agonist for ALX (Figure 28).[786] This compound induces the chemotaxis of human neutrophils; maximal response is observed by 100 nM to 1 μM,[786] which elicits intracellular calcium increase in ALX-expressing rat basophilic leukemia (RBL) cells with an EC_{50} of 1.4 μM.[786] In degranulation assays, both MMK-1 and Quin-C1 exhibit lower efficacy and potency with EC_{50} values of 71.7 nM and 1.9 μM, respectively, compared with the EC_{50} for WKYMVm (22.9 nM). However, Quin-C1 does not induce neutrophil superoxide generation at up to 100 μM.[786]

BML-111 {5(*S*),6(*R*),7-trihydroxyheptanoic acid methyl ester} (Figure 28) is a C-7 truncated analog of LXA_4 that is equipotent as LXA_4 in the inhibition of LTB_4-induced PMN chemotaxis via ALX with an IC_{50} of 5 nM.[787] Currently, Zhang et al. demonstrated that BML-111 attenuates collagen-induced arthritis in part by negatively regulating the immune response.[788] In the murine model of carbon tetrachloride (CCl_4)-induced acute liver injury, in which ALX is upregulated after giving CCl_4, BML-111 effectively protects the liver, as evidenced by decreased serum aminotransferase (ALT, AST) levels and decreased histological damage.[789]

By a high-throughput screening and subsequent medicinal chemistry effort, Burli et al. recently found a potent agonistic compound, termed Compound-43 (Figure 28), which is pyrazolone derivatives.[790] Compound-43 exhibits intracellular calcium mobilization in human ALX-expressing CHO cells with an EC_{50} value of 44 nM and inhibits fMLP and IL-8 induced human neutrophil migration.[790] In the mouse ear inflammation model, the oral administration of this compound (50 mg/kg dose) reduces edema by 58%.[790] Compound-43 was further modified and resulted in the generation of Compound-11 (a series of benzimidazoles), which displays an EC_{50} value of 34 nM at ALX (Figure 28).[791] In chemotaxis assays of human PMNs, Compound-11 reduces migration triggered by fMLP and IL-8 with IC_{50} values of 220 nM and 1.7 μM, respectively.[791]

6.5. LXs and Cognate Receptors in Health and Diseases

Various findings highlight the roles of LXA_4 and ATL for promoting resolution of inflammation, because reduction in the numbers of PMNs, recruitment of monocytes to the sites of inflammation and injury, and clearance of apoptotic PMNs by macrophages are all the crucial events at the tissue level in wound healing and resolution. The physiological and pathophysiological roles of LXA_4 and ALX are summarized in Tables 11 and 12. Alterations in LXA_4 and ATL levels may be causally associated with the pathophysiology of several human diseases.[792] Namely, LX production appears to be deficient compared to LTs in many

Table 11. Key Biologic Actions of LXA_4 and ATL

cell types/tissues	bioactivities of LX and ATL
neutrophils	inhibition
	chemotaxis, adhesion, transmigration, L-selectin shedding, IL-1β production, MCP-1 production, CD11/CD18 expression
monocytes/macrophages	stimulation
	chemotaxis, adhesion, phagocytosis of apoptotic PMNs
	attenuation
	L-selectin shedding, CD11/CD18 expression
eosinophils	inhibition
	chemotais, PAF-stimulated chemotaxis, IL-5 and eotaxin secretions
endothelial cells	inhibition
	adhesiveness for PMNs, CD11/CD18 expression,
fibroblasts	inhibition
	IL-6 and IL-8 release, MMP-3 production, TIMP-1 and TIMP-2 expression
mesangial cells	inhibition
	proliferation, contractility and adherence for neutrophils
bronchial epithelium	inhibition
	bronchoconstriction
	stimulation
	calcium increase with resultant chloride release
dendritic cells	inhibition
	IL-12 production

Table 12. Related Diseases Ameliorated by LXA_4 and ATL

	related diseases and actions of LXA_4 and ATL
respiratory system	asthma
	• block airway hyper-responsiveness and pulmonary inflammation
	• reduction of eosinophil infiltration
	• nasal polyps and bronchial tissues)
	acute lung injury
	• protection (in ALX transgenic mice)
blood cells	myeloproliferative disorders
	• stimulation of bone marrow cells
	• enhancement of myeloid progenitor growth
kidney	glomerulonephritis
	• decreased neutrophil recruitment
	• reduction of pathogenic mediator expressions in renal parenchymal cells
	• inhibition of mesangial cell proliferation
	ischemia−reperfusion injury (I/R injury)
	• attenuation of hind-limb I/R-induced lung injury
	• detachment of adherent leukocytes in mesenteric I/R
	• protective in ischemic acute renal failure
skin	dermal inflammation
	• decreased neutrophil recruitment into ear skin
	• prevent vascular permeability
	• reduction of epidermal proliferation
colon	inflammatory bowel disease: colitis
	• attenuated pro-inflammatory gene expressions
	• reduced severity of colitis
blood vessel	angiogenesis
	• reduced endothelial cell proliferation and migration
	• reduced atherosclerotic plaque rupture
	atherosclerosis
	• protections in 15-LO transgenic rabbits
others	peritonitis
	• decreased PMN infiltration (in ALX transgenic mice)
	pleuritis
	• enhanced NO production

human diseases, e.g., cardiovascular,[793] asthma,[794] kidney inflammation,[795] cystic fibrosis,[796] and gastrointestinal[797] and periodontal disease.[798] To further examine the potential for functional links between LXs and ALX in vivo, transgenic mice overexpressing human ALX have been generated.[799] The ALX transgenic mice lead to decreased PMN infiltration into the peritoneum in zymosan A-initiated peritonitis, compared with their nontransgenic littermates. Metabolically stable analogs of LX and ATL are also useful tools in examining the roles and local actions of LXs in vivo.[800,753,773] Administration of LX stable analogs in animal models protects from tissue damage and inflammation and enhances resolution.[801] In the following parts, we summarize the current knowledge about the potential roles of LXs and ALX in several diseases.

6.5.1. Lung Injury. The involvement of LXs in asthma has been highlighted by the detection of LXA_4 in the BALF of patients with pulmonary diseases. The levels of LXA_4 are significantly higher in the sputum of mild asthmatic patients compared to those of normal subjects or severe asthmatics.[802] In addition, PMNs from mild asthmatics produce larger amounts of LXA_4 compared to normal individuals.[802] In a murine asthma model, allergen challenge initiates airway biosynthesis of LXA_4 and increases the expression of ALX. Administration of a stable analog of LXA_4 blocks both airway hyperresponsiveness (AHR) and pulmonary inflammation, as shown by decreased leukocytes and mediators, e.g., IL-5, IL-13, eotaxin, prostanoids, and cys-LTs. Moreover, transgenic expression of human ALX in mice leads to significant inhibition of pulmonary inflammation and eicosanoid-initiated eosinophil tissue infiltration.[794] Additionally, native LXA_4 given to human asthmatics inhibits LTC_4-stimulated AHR[803] and blocks LTD_4-initiated constriction of airway smooth muscle in vitro.[804] These findings suggest that LXs play a pivotal role in asthma, regulating airway hyperreactivity via effects on key proinflammatory pathways and antagonizing cys-LT-mediated actions on bronchial smooth muscle. In a spontaneously resolving model of acute lung injury (ALI), both selective pharmacologic inhibition and gene disruption of COX-2 block resolution of ALI.[805] Furthermore, the ALX transgenic mice also exhibit dramatic protection, indicating a

protective role of COX-2-derived mediators in ALI, e.g., ATL, in part via enhanced LX signaling.[805] Because the LXA_4—ALX pathway selectively regulates leukocyte functions, BLT1 transgenic mice, which show dramatically increased PMN trafficking to lungs after high limb ischemia—reperfusion, were used in the following analyses. Despite excessive recruitment of PMNs in BLT1 transgenic mice, intravenous injection of ATLa markedly impaired reperfusion-initiated PMN trafficking to the lungs, revealing the protective role of LXA_4 and ATL.[271] These results further provide compelling evidence for functional links between LXA_4 and ALX in vivo.[799]

6.5.2. Glomerulonephritis. LXs are potential therapeutics in ischemic acute renal failure (ARF), because these mediators can influence a variety of pathobiologic functions that are relevant to acute tubular necrosis (ATN), including vascular tone, epithelial cell injury and cytokine release, and leukocyte recruitment and clearance. In the concanavalin A—ferritin model of immune complex glomerulonephritis, treatment of rat neutrophils ex vivo with LXA_4 impairs their subsequent trafficking into inflamed glomeruli.[806] Furthermore, administration of ATL or LXA_4 analogue prior to ischemia leads to significant functional and morphologic protection and attenuation of chemokine and cytokine responses.[807,808] Deficient resolution was further observed in P-selectin-null mice.[809] P-selectin on the surfaces of activated endothelium and platelets mediates PMNs-endothelial cells and PMNs-platelets interactions in vivo, which are required for LXA_4 production. In an acute passive antiglomerular basement membrane antibody-induced experimental nephritis, being P-selectin-null results in the significant increase in glomerular PMNs and albuminuria, and LXA_4 formation and equilibrate neutrophil infiltration are restored by the administration of WT platelets into the null mice.[809] The protective actions of LXA_4 were also confirmed using genetically engineered animals with altered expression of 15-LO, an essential enzyme in one pathway for LXA_4 production. Intriguingly, by the transfection of human 15-LO mRNA into rat kidneys in vivo in glomerulonephritis, the glomerular functions (filtration and protein excretion) are preserved.[810] Hence, it may be possible to harness the LX network therapeutically in ARF and inflammatory glomerular disease.[795]

6.5.3. Dermal Inflammation. In a dermal inflammation model, stable LX analogs inhibit both PMN infiltration and vascular permeability changes when applied topically to mouse ears.[751,811] ATL inhibits PMN infiltration, edema, and epidermal proliferation in several in vivo inflammatory dermatoses.[812] In these cutaneous inflammatory models, when these effects are compared to other anti-inflammatory treatments, e.g., methylprednisolone and BLT1 antagonists, topical ATL shows equivalent efficacy on most end points measured.[812] These results extend the therapeutic potential of LXs, without the detrimental local and systemic side effects associated with currently used corticosteroids.

6.5.4. Other Diseases. In transgenic rabbits overexpressing 15-LO in a macrophage-specific manner, LXA_4 production is significantly enhanced.[813] In the models of microbe-associated inflammation and leukocyte-mediated bone destruction by initiating acute periodontitis, 15-LO transgenic rabbits show significantly reduced bone loss and local inflammation compared with WT rabbits.[813] Thus, the overproduction of LXA_4 is associated with dampened PMN-mediated tissue degradation and bone loss as well as the enhanced anti-inflammation status. Other studies using these transgenic rabbits further demonstrated protection from atherosclerosis,[814] suggesting that the mechanism for this protection is probably due to the anti-inflammatory action of LXA_4.[813] Recently, the expression of ALX was detected in mouse corneas. Moreover, topical treatment of LXA_4 increases the rate of re-epithelialization and attenuates the sequelae of thermal injury.[815] Interestingly, 15-LO-null mice exhibit a defect in corneal re-epithelialization that correlates with a reduction in endogenous LXA_4 formation, revealing a protective action of LXA_4 in wound healing. The efficacies of LXA_4 and its stable analogues were currently reported in other inflammatory diseases associated with PMN-mediated tissue injury, such as intestinal inflammation.[798,816,817]

6.6. Clinical Studies on LX Receptors Targeted Therapy

Aspirin is the most commonly administered nonsteroidal anti-inflammatory drug.[818,819] In addition to its antithrombotic and anti-inflammatory actions, low-dose aspirin has beneficial effects not only on the prevention and management of occlusive vascular diseases[820,821] but also on decreasing the incidence of cancer as well as possibly Alzheimer's disease.[822,823] As described in section 6.1, acetylation of vascular COX-2 by aspirin redirects the catalytic activity from generating intermediates of PGs and TXs to the production of intermediates of 15-epimeric LXA_4 formation, i.e., ATL.[736] ATL possesses protective activities in several target tissues and murine models of disease that include peritonitis, dermal inflammation, reperfusion injury, asthma, and angiogenesis.[824] Thus, a randomized clinical trial had been undertaken recently to elucidate whether ATL formation is aspirin-dependent in humans and its relationship to aspirin's antiplatelet activity.[825] This trial was conducted among 128 healthy subjects allocated to placebo or to 81, 325, or 650 mg daily doses of aspirin for 8 weeks, and plasma TXB_2, an indicator of platelet reactivity, and ATL were evaluated from blood at baseline and at 8 weeks. In this study, plasma ATL levels were significantly raised in the 81 mg aspirin group and moderately increased in the 325 mg group; however, there were no apparent changes in the 650 mg group. When ATL and TXB_2 were compared, low-dose aspirin initiated production of ATL opposite to the inhibition of TXs. Recently, Morris et al. reported the effects of low-dose aspirin in vivo using a model of skin blisters by topically applying cantharidin on the forearm of healthy male volunteers, causing an acute inflammatory response, including dermal edema formation and leukocyte trafficking.[826] Although not affecting blister fluid volume, low-dose aspirin reduces PMN and macrophage accumulations independent of NF-κB-regulated gene expression and inhibition of conventional prostanoids. Moreover, low-dose aspirin also enhances the ATL synthesis and ALX expression. Together, ATL acts as an anti-inflammatory mediator working through ALX, and mimicking its mode of action represents a new approach to treating inflammation-driven diseases.

7. CONCLUSIONS AND PERSPECTIVES

Pharmacological studies provided strong evidence for the presence of specific receptors that are activated by LTs and LXs and are now well-supported by the recombinant systems. However, there are several issues that should be addressed.

BLT1 and BLT2 differ significantly in their affinities and specificities for LTB_4, as well as in the distribution of their expressions. Furthermore, BLT2 is also activated by 12-HHT, which is produced from PGH_2 by thromboxane synthase in vivo, with a higher affinity than LTB_4. Experiments with BLT1-null mice suggest that BLT1 mediates the major activities of LTB_4 on

leukocytes. In contrast, the biological role of BLT2 still remains to be determined. Because this receptor is highly conserved across species, suggesting the functionally importance of BLT2, the extensive studies on BLT2-null mice will be needed to clarify the physiological roles of this receptor. Multiple receptors with different affinities and specificities have been identified for other chemoattractants and may allow for an increased ability to modulate responses to powerful inflammatory stimuli such as LTB_4. Therefore, further understanding of the different roles of multiple receptors for individual chemoattractants, such as BLT1 and BLT2, may give valuable insights into the complex regulation of inflammatory responses.

The relative potencies of LTC_4, LTD_4, and LTE_4 in various functional studies demonstrate that LTE_4 shows less potency and markedly less efficiency in activating $CysLT_1$ and $CysLT_2$. However, early studies indicate that LTE_4 causes skin swelling in human subjects as potently as LTC_4 and LTD_4, and airways of asthmatic subjects are selectively hyperresponsive to LTE_4. Recent studies have begun to uncover receptors selective for LTE_4: $P2Y_{12}$, an adenosine diphosphate receptor, and $CysLT_E$ receptor, which was observed functionally in the skin of mice lacking $CysLT_1$ and $CysLT_2$. These findings prompt a renewed focus on LTE_4 receptors as therapeutic targets. In addition, future studies on splice variants and heterodimerization of cys-LT receptors, which may be regulated at the cell- or tissue type-specific level, might lead to a better understanding of the signal transduction pathways via $CysLT_1$ and $CysLT_2$. Although one of the most promising aspects of the $CysLT_1$- and $CysLT_2$-null mice is the uncovering of new cys-LT receptors, the roles of each cys-LT receptor in the knockout animals remain to be elucidated. For example, in human airways, activation of $CysLT_1$ is responsible for the bronchoconstriction both in normal and asthmatic subjects. In contrast, there is no evidence for airway constriction in mice,[827,828] and the mouse $CysLT_1$ has only been implicated in constriction of the microvascular smooth muscles, leading to increased permeability and extravasation. Our recent findings concerning the pharmacological differences between human and mouse $CysLT_2$, together with the receptor distribution in different mouse strains, reveal the importance of the choice of the animal model and the difficulty of extrapolation to human inflammation.[490] $CysLT_2$ is also a candidate for asthma and atopy because $CysLT_2$ gene maps to a region of chromosome 13q14 that has been linked to asthma and atopy. Specific $CysLT_2$ antagonists have only been reported recently,[496,829] and thus, the pharmacological importance of $CysLT_2$ antagonism is yet to be proved. However, the development of drugs that target the $CysLT_2$ may be important not only for asthma, given that approximately 20% of patients fail to respond to $CysLT_1$ antagonists, but also for cardiovascular diseases.[830,831]

LTs are key inflammatory mediators, and new roles in the pathogenesis of a number of diseases are being discovered. Several LT receptor modifiers, e.g., selective antagonists for the cys-LT receptors, have already been used in clinical practice for asthma and are in trial for other inflammatory diseases. Despite such progress, the fundamental knowledge about the receptors for LTs is fairly limited and there would seem to be major opportunities for exploration of the cell biology, molecular biology, and pharmacology of LT receptors. Our understanding of the roles of LTs and their receptors in various diseases will evolve with further advances in understanding the regulation of the LT syntheses, the receptors that mediate the response to LTs, and the cellular and molecular events mediated through these receptors. Novel information in such areas would also be expected to create new targets for intervention with the LT pathways.

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Motonao Nakamura graduated from Hiroshima University in 1987. He received his Ph.D. in 1994 from The University of Tokyo, where he conducted his doctoral research on the cloning and characteristics of platelet-activating factor receptor under the direction of Prof. Takao Shimizu. After receiving his Ph.D. degree, he worked at JT Pharmaceutical Research Laboratory (Yokohama, Japan, 1987−2006) and at Tularik Inc. (South San Francisco, CA., Visiting Scientist, 1997−2000). He is now an Associate Professor of Takao Shimizu's lab. His research since 2006 has been almost entirely devoted to clarifying the lifetime of G protein-coupled receptors (GPCRs), such as transcriptional regulation, quality control in the ER, trafficking, modifications, signaling, and recycling/degradation control. He has also been studying the physiological roles of lipid GPCRs using knockout mice.

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REFERENCES

(1) Borgeat, P.; Hamberg, M.; Samuelsson, B. *J. Biol. Chem.* **1976**, *251*, 7816.
(2) Borgeat, P.; Samuelsson, B. *J. Biol. Chem.* **1979**, *254*, 2643.
(3) Borgeat, P.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1979**, *76*, 2148.
(4) Borgeat, P.; Samuelsson, B. *J. Biol. Chem.* **1979**, *254*, 7865.
(5) Yokomizo, T.; Izumi, T.; Shimizu, T. *Arch. Biochem. Biophys.* **2001**, *385*, 231.
(6) Shimizu, T. *Annu. Rev. Pharmacol. Toxicol.* **2009**, *49*, 123.
(7) Lee, T. H.; Mencia-Huerta, J. M.; Shih, C.; Corey, E. J.; Lewis, R. A.; Austen, K. F. *J. Clin. Invest.* **1984**, *74*, 1922.
(8) Terano, T.; Salmon, J. A.; Moncada, S. *Biochem. Pharmacol.* **1984**, *33*, 3071.
(9) Siddiqui, R. A.; Harvey, K. A.; Zaloga, G. P. *J. Nutr. Biochem.* **2008**, *19*, 417.
(10) Borgeat, P.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1979**, *76*, 3213.
(11) Murphy, R. C.; Hammarstrom, S.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1979**, *76*, 4275.
(12) Hammarstrom, S.; Murphy, R. C.; Samuelsson, B.; Clark, D. A.; Mioskowski, C.; Corey, E. J. *Biochem. Biophys. Res. Commun.* **1979**, *91*, 1266.
(13) Hammerstrom, S.; Samuelsson, B. *FEBS Lett.* **1980**, *122*, 83.
(14) Samuelsson, B.; Hammarstrom, S. *Prostaglandins* **1980**, *19*, 645.
(15) Samuelsson, B.; Dahlen, S. E.; Lindgren, J. A.; Rouzer, C. A.; Serhan, C. N. *Science* **1987**, *237*, 1171.
(16) Samuelsson, B.; Borgeat, P.; Hammarstrom, S.; Murphy, R. C. *Prostaglandins* **1979**, *17*, 785.
(17) Brink, C.; Dahlen, S. E.; Drazen, J.; Evans, J. F.; Hay, D. W.; Nicosia, S.; Serhan, C. N.; Shimizu, T.; Yokomizo, T. *Pharmacol. Rev.* **2003**, *55*, 195.
(18) Gronert, K.; Clish, C. B.; Romano, M.; Serhan, C. N. *Methods Mol. Biol.* **1999**, *120*, 119.
(19) Samuelsson, B. *Science* **1983**, *220*, 568.
(20) Penrose, J. F.; Austen, K. F. *Proc. Assoc. Am. Physicians* **1999**, *111*, 537.
(21) Robbiani, D. F.; Finch, R. A.; Jager, D.; Muller, W. A.; Sartorelli, A. C.; Randolph, G. J. *Cell* **2000**, *103*, 757.
(22) Werz, O.; Steinhilber, D. *Biochem. Pharmacol.* **2005**, *70*, 327.
(23) Lands, W. E. *J. Biol. Chem.* **1958**, *231*, 883.
(24) Lands, W. E. *J. Biol. Chem.* **1960**, *235*, 2233.
(25) Waku, K.; Lands, W. E. *J. Biol. Chem.* **1968**, *243*, 2654.
(26) Okuyama, H.; Lands, W. E. *J. Biol. Chem.* **1972**, *247*, 1414.
(27) Shindou, H.; Shimizu, T. *J. Biol. Chem.* **2009**, *284*, 1.
(28) Lewin, T. M.; Wang, P.; Coleman, R. A. *Biochemistry* **1999**, *38*, 5764.
(29) Hanasaki, K.; Arita, H. *Prostaglandins Other Lipid Mediators* **2002**, *68−69*, 71.
(30) Ni, Z.; Okeley, N. M.; Smart, B. P.; Gelb, M. H. *J. Biol. Chem.* **2006**, *281*, 16245.
(31) Kramer, R. M.; Roberts, E. F.; Manetta, J.; Putnam, J. E. *J. Biol. Chem.* **1991**, *266*, 5268.
(32) Clark, J. D.; Schievella, A. R.; Nalefski, E. A.; Lin, L. L. *J. Lipid Mediators Cell Signalling* **1995**, *12*, 83.
(33) Turk, J.; Ramanadham, S. *Can. J. Physiol. Pharmacol.* **2004**, *82*, 824.
(34) Balsinde, J.; Balboa, M. A. *Cell Signalling* **2005**, *17*, 1052.
(35) Tjoelker, L. W.; Eberhardt, C.; Unger, J.; Trong, H. L.; Zimmerman, G. A.; McIntyre, T. M.; Stafforini, D. M.; Prescott, S. M.; Gray, P. W. *J. Biol. Chem.* **1995**, *270*, 25481.
(36) Hattori, M.; Adachi, H.; Aoki, J.; Tsujimoto, M.; Arai, H.; Inoue, K. *J. Biol. Chem.* **1995**, *270*, 31345.
(37) Karasawa, K.; Kuge, O.; Kawasaki, K.; Nishijima, M.; Nakano, Y.; Tomita, M.; Yokoyama, K.; Setaka, M.; Nojima, S. *J. Biochem.* **1996**, *120*, 838.
(38) Bonventre, J. V.; Sapirstein, A. *Adv. Exp. Med. Biol.* **2002**, *507*, 25.
(39) Ohto, T.; Uozumi, N.; Hirabayashi, T.; Shimizu, T. *J. Biol. Chem.* **2005**, *280*, 24576.
(40) Ghosh, M.; Loper, R.; Gelb, M. H.; Leslie, C. C. *J. Biol. Chem.* **2006**, *281*, 16615.
(41) Bonventre, J. V.; Huang, Z.; Taheri, M. R.; O'Leary, E.; Li, E.; Moskowitz, M. A.; Sapirstein, A. *Nature* **1997**, *390*, 622.
(42) Uozumi, N.; Kume, K.; Nagase, T.; Nakatani, N.; Ishii, S.; Tashiro, F.; Komagata, Y.; Maki, K.; Ikuta, K.; Ouchi, Y.; Miyazaki, J.; Shimizu, T. *Nature* **1997**, *390*, 618.
(43) Nagase, T.; Uozumi, N.; Ishii, S.; Kume, K.; Izumi, T.; Ouchi, Y.; Shimizu, T. *Nat. Immunol.* **2000**, *1*, 42.
(44) Sharp, J. D.; White, D. L. *J. Lipid Mediators* **1993**, *8*, 183.
(45) Glover, S.; de Carvalho, M. S.; Bayburt, T.; Jonas, M.; Chi, E.; Leslie, C. C.; Gelb, M. H. *J. Biol. Chem.* **1995**, *270*, 15359.
(46) Schievella, A. R.; Regier, M. K.; Smith, W. L.; Lin, L. L. *J. Biol. Chem.* **1995**, *270*, 30749.
(47) Peters-Golden, M.; McNish, R. W. *Biochem. Biophys. Res. Commun.* **1993**, *196*, 147.
(48) Kramer, R. M.; Sharp, J. D. *FEBS Lett.* **1997**, *410*, 49.
(49) Kramer, R. M.; Roberts, E. F.; Manetta, J. V.; Hyslop, P. A.; Jakubowski, J. A. *J. Biol. Chem.* **1993**, *268*, 26796.
(50) Kita, Y.; Takahashi, T.; Uozumi, N.; Shimizu, T. *Anal. Biochem.* **2005**, *342*, 134.
(51) Kita, Y.; Takahashi, T.; Uozumi, N.; Nallan, L.; Gelb, M. H.; Shimizu, T. *Biochem. Biophys. Res. Commun.* **2005**, *330*, 898.
(52) Yoshikawa, K.; Kita, Y.; Kishimoto, K.; Shimizu, T. *J. Biol. Chem.* **2006**, *281*, 14663.
(53) Kihara, Y.; Matsushita, T.; Kita, Y.; Uematsu, S.; Akira, S.; Kira, J.; Ishii, S.; Shimizu, T. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 21807.
(54) Fredriksson, R.; Schioth, H. B. *Mol. Pharmacol.* **2005**, *67*, 1414.
(55) Bjarnadottir, T. K.; Gloriam, D. E.; Hellstrand, S. H.; Kristiansson, H.; Fredriksson, R.; Schioth, H. B. *Genomics* **2006**, *88*, 263.
(56) Honda, Z.; Nakamura, M.; Miki, I.; Minami, M.; Watanabe, T.; Seyama, Y.; Okado, H.; Toh, H.; Ito, K.; Miyamoto, T.; Shimizu, T. *Nature* **1991**, *349*, 342.
(57) Nakamura, M.; Honda, Z.; Izumi, T.; Sakanaka, C.; Mutoh, H.; Minami, M.; Bito, H.; Seyama, Y.; Matsumoto, T.; Noma, M.; Shimizu, T. *J. Biol. Chem.* **1991**, *266*, 20400.
(58) Jacoby, E.; Bouhelal, R.; Gerspacher, M.; Seuwen, K. *ChemMedChem* **2006**, *1*, 761.
(59) Ford-Hutchinson, A. W.; Bray, M. A.; Doig, M. V.; Shipley, M. E.; Smith, M. J. *Nature* **1980**, *286*, 264.
(60) Goetzl, E. J.; Pickett, W. C. *J. Exp. Med.* **1981**, *153*, 482.

(61) Shimizu, T.; Wolfe, L. S. *J. Neurochem.* **1990**, *55*, 1.
(62) Serhan, C. N.; Haeggstrom, J. Z.; Leslie, C. C. *FASEB J.* **1996**, *10*, 1147.
(63) Matsumoto, T.; Funk, C. D.; Radmark, O.; Hoog, J. O.; Jornvall, H.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 26.
(64) Radmark, O.; Werz, O.; Steinhilber, D.; Samuelsson, B. *Trends Biochem. Sci.* **2007**, *32*, 332.
(65) Minami, M.; Ohno, S.; Kawasaki, H.; Radmark, O.; Samuelsson, B.; Jornvall, H.; Shimizu, T.; Seyama, Y.; Suzuki, K. *J. Biol. Chem.* **1987**, *262*, 13873.
(66) Funk, C. D.; Radmark, O.; Fu, J. Y.; Matsumoto, T.; Jornvall, H.; Shimizu, T.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84*, 6677.
(67) Shimizu, T.; Radmark, O.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1984**, *81*, 689.
(68) Rouzer, C. A.; Matsumoto, T.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 857.
(69) Ueda, N.; Kaneko, S.; Yoshimoto, T.; Yamamoto, S. *J. Biol. Chem.* **1986**, *261*, 7982.
(70) Shimizu, T.; Izumi, T.; Seyama, Y.; Tadokoro, K.; Radmark, O.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 4175.
(71) Miller, D. K.; Gillard, J. W.; Vickers, P. J.; Sadowski, S.; Leveille, C.; Mancini, J. A.; Charleson, P.; Dixon, R. A.; Ford-Hutchinson, A. W.; Fortin, R.; Gauthier, J. Y.; Rodkey, J.; Rosen, R.; Rouzer, C.; Sigal, I. S.; Strader, C. D.; Evans, J. F. *Nature* **1990**, *343*, 278.
(72) Dixon, R. A.; Diehl, R. E.; Opas, E.; Rands, E.; Vickers, P. J.; Evans, J. F.; Gillard, J. W.; Miller, D. K. *Nature* **1990**, *343*, 282.
(73) Ferguson, A. D.; McKeever, B. M.; Xu, S.; Wisniewski, D.; Miller, D. K.; Yamin, T. T.; Spencer, R. H.; Chu, L.; Ujjainwalla, F.; Cunningham, B. R.; Evans, J. F.; Becker, J. W. *Science* **2007**, *317*, 510.
(74) Bain, G.; King, C. D.; Rewolinski, M.; Schaab, K.; Santini, A. M.; Shapiro, D.; Moran, M.; van de Wetering de Rooij, S.; Roffel, A. F.; Schuilenga-Hut, P.; Milne, G. L.; Lorrain, D. S.; Li, Y.; Arruda, J. M.; Hutchinson, J. H.; Prasit, P.; Evans, J. F. *Clin. Pharmacol. Ther.* **2010**, *87*, 437.
(75) Welsch, D. J.; Creely, D. P.; Hauser, S. D.; Mathis, K. J.; Krivi, G. G.; Isakson, P. C. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 9745.
(76) Lam, B. K.; Penrose, J. F.; Freeman, G. J.; Austen, K. F. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 7663.
(77) Jakobsson, P. J.; Mancini, J. A.; Riendeau, D.; Ford-Hutchinson, A. W. *J. Biol. Chem.* **1997**, *272*, 22934.
(78) Tanioka, T.; Nakatani, Y.; Semmyo, N.; Murakami, M.; Kudo, I. *J. Biol. Chem.* **2000**, *275*, 32775.
(79) Murakami, M.; Naraba, H.; Tanioka, T.; Semmyo, N.; Nakatani, Y.; Kojima, F.; Ikeda, T.; Fueki, M.; Ueno, A.; Oh, S.; Kudo, I. *J. Biol. Chem.* **2000**, *275*, 32783.
(80) Jakobsson, P. J.; Thoren, S.; Morgenstern, R.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 7220.
(81) Jakobsson, P. J.; Morgenstern, R.; Mancini, J.; Ford-Hutchinson, A.; Persson, B. *Am. J. Respir. Crit. Care Med.* **2000**, *161*, S20.
(82) Spanbroek, R.; Stark, H. J.; Janssen-Timmen, U.; Kraft, S.; Hildner, M.; Andl, T.; Bosch, F. X.; Fusenig, N. E.; Bieber, T.; Radmark, O.; Samuelsson, B.; Habenicht, A. J. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 663.
(83) Furstenberger, G.; Krieg, P.; Muller-Decker, K.; Habenicht, A. J. *Int. J. Cancer* **2006**, *119*, 2247.
(84) Werz, O.; Steinhilber, D. *Pharmacol. Ther.* **2006**, *112*, 701.
(85) Rouzer, C. A.; Shimizu, T.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1985**, *82*, 7505.
(86) Brock, T. G.; McNish, R. W.; Bailie, M. B.; Peters-Golden, M. *J. Biol. Chem.* **1997**, *272*, 8276.
(87) Hanaka, H.; Shimizu, T.; Izumi, T. *Biochem. J.* **2002**, *361*, 505.
(88) Bozza, P. T.; Yu, W.; Penrose, J. F.; Morgan, E. S.; Dvorak, A. M.; Weller, P. F. *J. Exp. Med.* **1997**, *186*, 909.
(89) Woods, J. W.; Coffey, M. J.; Brock, T. G.; Singer, I. I.; Peters-Golden, M. *J. Clin. Invest.* **1995**, *95*, 2035.
(90) Covin, R. B.; Brock, T. G.; Bailie, M. B.; Peters-Golden, M. *Am. J. Physiol.* **1998**, *275*, L303.
(91) Newcomer, M. E.; Gilbert, N. C. *J. Biol. Chem.* **2010**, *285*, 25109.
(92) Chen, X. S.; Zhang, Y. Y.; Funk, C. D. *J. Biol. Chem.* **1998**, *273*, 31237.
(93) Healy, A. M.; Peters-Golden, M.; Yao, J. P.; Brock, T. G. *J. Biol. Chem.* **1999**, *274*, 29812.
(94) Ohishi, N.; Minami, M.; Kobayashi, J.; Seyama, Y.; Hata, J.; Yotsumoto, H.; Takaku, F.; Shimizu, T. *J. Biol. Chem.* **1990**, *265*, 7520.
(95) Sala, A.; Bolla, M.; Zarini, S.; Muller-Peddinghaus, R.; Folco, G. *J. Biol. Chem.* **1996**, *271*, 17944.
(96) Hammarberg, T.; Radmark, O. *Biochemistry* **1999**, *38*, 4441.
(97) Provost, P.; Samuelsson, B.; Radmark, O. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 1881.
(98) Boyington, J. C.; Gaffney, B. J.; Amzel, L. M. *Science* **1993**, *260*, 1482.
(99) Youn, B.; Sellhorn, G. E.; Mirchel, R. J.; Gaffney, B. J.; Grimes, H. D.; Kang, C. *Proteins* **2006**, *65*, 1008.
(100) Oldham, M. L.; Brash, A. R.; Newcomer, M. E. *J. Biol. Chem.* **2005**, *280*, 39545.
(101) Neau, D. B.; Gilbert, N. C.; Bartlett, S. G.; Boeglin, W.; Brash, A. R.; Newcomer, M. E. *Biochemistry* **2009**, *48*, 7906.
(102) Gillmor, S. A.; Villasenor, A.; Fletterick, R.; Sigal, E.; Browner, M. F. *Nat. Struct. Biol.* **1997**, *4*, 1003.
(103) Gilbert, N. C.; Bartlett, S. G.; Waight, M. T.; Neau, D. B.; Boeglin, W. E.; Brash, A. R.; Newcomer, M. E. *Science* **2011**, *331*, 217.
(104) Ago, H.; Kanaoka, Y.; Irikura, D.; Lam, B. K.; Shimamura, T.; Austen, K. F.; Miyano, M. *Nature* **2007**, *448*, 609.
(105) Martinez Molina, D.; Wetterholm, A.; Kohl, A.; McCarthy, A. A.; Niegowski, D.; Ohlson, E.; Hammarberg, T.; Eshaghi, S.; Haeggstrom, J. Z.; Nordlund, P. *Nature* **2007**, *448*, 613.
(106) Fabre, J. E.; Goulet, J. L.; Riche, E.; Nguyen, M.; Coggins, K.; Offenbacher, S.; Koller, B. H. *J. Clin. Invest.* **2002**, *109*, 1373.
(107) Radmark, O.; Haeggstrom, J. *Adv. Prostaglandin, Thromboxane, Leukotriene Res.* **1990**, *20*, 35.
(108) Haeggstrom, J. Z.; Wetterholm, A.; Medina, J. F.; Samuelsson, B. *Adv. Prostaglandin, Thromboxane, Leukotriene Res.* **1994**, *22*, 3.
(109) Haeggstrom, J. Z.; Wetterholm, A.; Vallee, B. L.; Samuelsson, B. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 431.
(110) Minami, M.; Ohishi, N.; Mutoh, H.; Izumi, T.; Bito, H.; Wada, H.; Seyama, Y.; Toh, H.; Shimizu, T. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 620.
(111) Orning, L.; Krivi, G.; Fitzpatrick, F. A. *J. Biol. Chem.* **1991**, *266*, 1375.
(112) McGee, J.; Fitzpatrick, F. *J. Biol. Chem.* **1985**, *260*, 12832.
(113) Ohishi, N.; Izumi, T.; Minami, M.; Kitamura, S.; Seyama, Y.; Ohkawa, S.; Terao, S.; Yotsumoto, H.; Takaku, F.; Shimizu, T. *J. Biol. Chem.* **1987**, *262*, 10200.
(114) Mueller, M. J.; Andberg, M. B.; Samuelsson, B.; Haeggstrom, J. Z. *J. Biol. Chem.* **1996**, *271*, 24345.
(115) Rybina, I. V.; Liu, H.; Gor, Y.; Feinmark, S. J. *J. Biol. Chem.* **1997**, *272*, 31865.
(116) Orning, L.; Gierse, J.; Duffin, K.; Bild, G.; Krivi, G.; Fitzpatrick, F. A. *J. Biol. Chem.* **1992**, *267*, 22733.
(117) Izumi, T.; Minami, M.; Ohishi, N.; Bito, H.; Shimizu, T. *J. Lipid Mediators* **1993**, *6*, 53.
(118) Kull, F.; Ohlson, E.; Haeggstrom, J. Z. *J. Biol. Chem.* **1999**, *274*, 34683.
(119) Thunnissen, M. M.; Nordlund, P.; Haeggstrom, J. Z. *Nat. Struct. Biol.* **2001**, *8*, 131.
(120) Snelgrove, R. J.; Jackson, P. L.; Hardison, M. T.; Noerager, B. D.; Kinloch, A.; Gaggar, A.; Shastry, S.; Rowe, S. M.; Shim, Y. M.; Hussell, T.; Blalock, J. E. *Science* **2010**, *330*, 90.
(121) Hansson, G.; Lindgren, J. A.; Dahlen, S. E.; Hedqvist, P.; Samuelsson, B. *FEBS Lett.* **1981**, *130*, 107.
(122) Soberman, R. J.; Harper, T. W.; Murphy, R. C.; Austen, K. F. *Proc. Natl. Acad. Sci. U. S. A.* **1985**, *82*, 2292.
(123) Kikuta, Y.; Kusunose, E.; Endo, K.; Yamamoto, S.; Sogawa, K.; Fujii-Kuriyama, Y.; Kusunose, M. *J. Biol. Chem.* **1993**, *268*, 9376.

(124) Chen, L.; Hardwick, J. P. *Arch. Biochem. Biophys.* **1993**, *300*, 18.
(125) Kalsotra, A.; Strobel, H. W. *Pharmacol. Ther.* **2006**, *112*, 589.
(126) Kikuta, Y.; Kusunose, E.; Kondo, T.; Yamamoto, S.; Kinoshita, H.; Kusunose, M. *FEBS Lett.* **1994**, *348*, 70.
(127) Christmas, P.; Ursino, S. R.; Fox, J. W.; Soberman, R. J. *J. Biol. Chem.* **1999**, *274*, 21191.
(128) Wang, S.; Gustafson, E.; Pang, L.; Qiao, X.; Behan, J.; Maguire, M.; Bayne, M.; Laz, T. *J. Biol. Chem.* **2000**, *275*, 40686.
(129) Soberman, R. J.; Sutyak, J. P.; Okita, R. T.; Wendelborn, D. F.; Roberts, L. J., 2nd; Austen, K. F. *J. Biol. Chem.* **1988**, *263*, 7996.
(130) Baumert, T.; Huber, M.; Mayer, D.; Keppler, D. *Eur. J. Biochem.* **1989**, *182*, 223.
(131) Palosaari, P. M.; Vihinen, M.; Mantsala, P. I.; Alexson, S. E.; Pihlajaniemi, T.; Hiltunen, J. K. *J. Biol. Chem.* **1991**, *266*, 10750.
(132) Yokomizo, T.; Izumi, T.; Takahashi, T.; Kasama, T.; Kobayashi, Y.; Sato, F.; Taketani, Y.; Shimizu, T. *J. Biol. Chem.* **1993**, *268*, 18128.
(133) Yokomizo, T.; Ogawa, Y.; Uozumi, N.; Kume, K.; Izumi, T.; Shimizu, T. *J. Biol. Chem.* **1996**, *271*, 2844.
(134) Ensor, C. M.; Zhang, H.; Tai, H. H. *Biochem. J.* **1998**, *330* (Pt 1), 103.
(135) Powell, W. S.; Gravelle, F. *J. Biol. Chem.* **1989**, *264*, 5364.
(136) Primiano, T.; Li, Y.; Kensler, T. W.; Trush, M. A.; Sutter, T. R. *Carcinogenesis* **1998**, *19*, 999.
(137) Hori, T.; Yokomizo, T.; Ago, H.; Sugahara, M.; Ueno, G.; Yamamoto, M.; Kumasaka, T.; Shimizu, T.; Miyano, M. *J. Biol. Chem.* **2004**, *279*, 22615.
(138) Wheelan, P.; Zirrolli, J. A.; Morelli, J. G.; Murphy, R. C. *J. Biol. Chem.* **1993**, *268*, 25439.
(139) Wheelan, P.; Murphy, R. C. *J. Biol. Chem.* **1995**, *270*, 19845.
(140) Wheelan, P.; Hankin, J. A.; Bilir, B.; Guenette, D.; Murphy, R. C. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 326.
(141) Turner, S. R.; Tainer, J. A.; Lynn, W. S. *Nature* **1975**, *257*, 680.
(142) Goetzl, E. J.; Pickett, W. C. *J. Immunol.* **1980**, *125*, 1789.
(143) Powell, W. S.; Rokach, J.; Khanapure, S. P.; Manna, S.; Hashefi, M.; Gravel, S.; Macleod, R. J.; Falck, J. R.; Bhatt, R. K. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 728.
(144) Yokomizo, T.; Izumi, T.; Chang, K.; Takuwa, Y.; Shimizu, T. *Nature* **1997**, *387*, 620.
(145) Yokomizo, T.; Kato, K.; Terawaki, K.; Izumi, T.; Shimizu, T. *J. Exp. Med.* **2000**, *192*, 421.
(146) Miki, I.; Watanabe, T.; Nakamura, M.; Seyama, Y.; Ui, M.; Sato, F.; Shimizu, T. *Biochem. Biophys. Res. Commun.* **1990**, *166*, 342.
(147) Igarashi, T.; Yokomizo, T.; Tsutsumi, O.; Taketani, Y.; Shimizu, T.; Izumi, T. *Eur. J. Biochem.* **1999**, *259*, 419.
(148) Slipetz, D. M.; Scoggan, K. A.; Nicholson, D. W.; Metters, K. M. *Eur. J. Pharmacol.* **1993**, *244*, 161.
(149) Harvey, J. P.; Koo, C. H.; Boggs, J. M.; Young, R. N.; Goetzl, E. J. *Immunology* **1992**, *76*, 122.
(150) Hirai, H.; Tanaka, K.; Yoshie, O.; Ogawa, K.; Kenmotsu, K.; Takamori, Y.; Ichimasa, M.; Sugamura, K.; Nakamura, M.; Takano, S.; Nagata, K. *J. Exp. Med.* **2001**, *193*, 255.
(151) Ohnishi, H.; Miyahara, N.; Gelfand, E. W. *Allergol. Int.* **2008**, *57*, 291.
(152) Huang, W. W.; Garcia-Zepeda, E. A.; Sauty, A.; Oettgen, H. C.; Rothenberg, M. E.; Luster, A. D. *J. Exp. Med.* **1998**, *188*, 1063.
(153) Peres, C. M.; Aronoff, D. M.; Serezani, C. H.; Flamand, N.; Faccioli, L. H.; Peters-Golden, M. *J. Immunol.* **2007**, *179*, 5454.
(154) Pettersson, A.; Richter, J.; Owman, C. *Int. Immunopharmacol.* **2003**, *3*, 1467.
(155) Goodarzi, K.; Goodarzi, M.; Tager, A. M.; Luster, A. D.; von Andrian, U. H. *Nat. Immunol.* **2003**, *4*, 965.
(156) Ott, V. L.; Cambier, J. C.; Kappler, J.; Marrack, P.; Swanson, B. J. *Nat. Immunol.* **2003**, *4*, 974.
(157) Tager, A. M.; Bromley, S. K.; Medoff, B. D.; Islam, S. A.; Bercury, S. D.; Friedrich, E. B.; Carafone, A. D.; Gerszten, R. E.; Luster, A. D. *Nat. Immunol.* **2003**, *4*, 982.
(158) Toda, A.; Yokomizo, T.; Masuda, K.; Nakao, A.; Izumi, T.; Shimizu, T. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 806.
(159) Masuda, K.; Yokomizo, T.; Izumi, T.; Shimizu, T. *Biochem. J.* **1999**, *342* (Pt 1), 79.
(160) Fiore, S.; Romano, M.; Reardon, E. M.; Serhan, C. N. *Blood* **1993**, *81*, 3395.
(161) Goldman, D. W.; Goetzl, E. J. *J. Immunol.* **1982**, *129*, 1600.
(162) Goldman, D. W.; Gifford, L. A.; Olson, D. M.; Goetzl, E. J. *J. Immunol.* **1985**, *135*, 525.
(163) Gorman, R. R.; Ruppel, P. L.; Lin, A. H. *Adv. Prostaglandin, Thromboxane, Leukotriene Res.* **1985**, *15*, 661.
(164) Maghni, K.; de Brum-Fernandes, A. J.; Foldes-Filep, E.; Gaudry, M.; Borgeat, P.; Sirois, P. *J. Pharmacol. Exp. Ther.* **1991**, *258*, 784.
(165) Simon, M. I.; Strathmann, M. P.; Gautam, N. *Science* **1991**, *252*, 802.
(166) Gaudreau, R.; Le Gouill, C.; Metaoui, S.; Lemire, S.; Stankova, J.; Rola-Pleszczynski, M. *Biochem. J.* **1998**, *335* (Pt 1), 15.
(167) Nieves, D.; Moreno, J. J. *Biochem. Pharmacol.* **2006**, *72*, 1022.
(168) Watanabe, S.; Yamasaki, A.; Hashimoto, K.; Shigeoka, Y.; Chikumi, H.; Hasegawa, Y.; Sumikawa, T.; Takata, M.; Okazaki, R.; Watanabe, M.; Yokogawa, T.; Yamamura, M.; Hayabuchi, T.; Gerthoffer, W. T.; Halayko, A. J.; Shimizu, E. *J. Allergy Clin. Immunol.* **2009**, *124*, 59.
(169) Heller, E. A.; Liu, E.; Tager, A. M.; Sinha, S.; Roberts, J. D.; Koehn, S. L.; Libby, P.; Aikawa, E. R.; Chen, J. Q.; Huang, P.; Freeman, M. W.; Moore, K. J.; Luster, A. D.; Gerszten, R. E. *Circulation* **2005**, *112*, 578.
(170) Petrin, D.; Turcotte, S.; Gilbert, A. K.; Rola-Pleszczynski, M.; Stankova, J. *Cell Signalling* **2006**, *18*, 479.
(171) Haribabu, B.; Zhelev, D. V.; Pridgen, B. C.; Richardson, R. M.; Ali, H.; Snyderman, R. *J. Biol. Chem.* **1999**, *274*, 37087.
(172) Sabirsh, A.; Bristulf, J.; Owman, C. *Eur. J. Pharmacol.* **2004**, *499*, 53.
(173) Dryden, P.; Duronio, V.; Martin, L.; Hudson, A. T.; Salari, H. *Br. J. Pharmacol.* **1992**, *106*, 656.
(174) Okamoto, F.; Saeki, K.; Sumimoto, H.; Yamasaki, S.; Yokomizo, T. *J. Biol. Chem.* **2010**, *285*, 41113.
(175) Brach, M. A.; de Vos, S.; Arnold, C.; Gruss, H. J.; Mertelsmann, R.; Herrmann, F. *Eur. J. Immunol.* **1992**, *22*, 2705.
(176) Huang, L.; Zhao, A.; Wong, F.; Ayala, J. M.; Struthers, M.; Ujjainwalla, F.; Wright, S. D.; Springer, M. S.; Evans, J.; Cui, J. *Arterioscler., Thromb., Vasc. Biol.* **2004**, *24*, 1783.
(177) Gagnon, L.; Filion, L. G.; Dubois, C.; Rola-Pleszczynski, M. *Agents Actions* **1989**, *26*, 141.
(178) Rola-Pleszczynski, M.; Stankova, J. *Blood* **1992**, *80*, 1004.
(179) Kato, K.; Yokomizo, T.; Izumi, T.; Shimizu, T. *J. Exp. Med.* **2000**, *192*, 413.
(180) Bylund, J.; Harder, A. G.; Maier, K. G.; Roman, R. J.; Harder, D. R. *Arch. Biochem. Biophys.* **2003**, *412*, 34.
(181) Stankova, J.; Turcotte, S.; Harris, J.; Rola-Pleszczynski, M. *J. Immunol.* **2002**, *168*, 3570.
(182) Obinata, H.; Yokomizo, T.; Shimizu, T.; Izumi, T. *Biochem. Biophys. Res. Commun.* **2003**, *309*, 114.
(183) Hashidate, T.; Murakami, N.; Nakagawa, M.; Ichikawa, M.; Kurokawa, M.; Shimizu, T.; Nakamura, M. *FASEB J.* **2010**, *24*, 3500.
(184) Ichikawa, M.; Asai, T.; Saito, T.; Seo, S.; Yamazaki, I.; Yamagata, T.; Mitani, K.; Chiba, S.; Ogawa, S.; Kurokawa, M.; Hirai, H. *Nat. Med.* **2004**, *10*, 299.
(185) Egawa, T.; Tillman, R. E.; Naoe, Y.; Taniuchi, I.; Littman, D. R. *J. Exp. Med.* **2007**, *204*, 1945.
(186) Sabirsh, A.; Bywater, R. P.; Bristulf, J.; Owman, C.; Haeggstrom, J. Z. *Biochemistry* **2006**, *45*, 5733.
(187) Basu, S.; Jala, V. R.; Mathis, S.; Rajagopal, S. T.; Del Prete, A.; Maturu, P.; Trent, J. O.; Haribabu, B. *J. Biol. Chem.* **2007**, *282*, 10005.
(188) Gaudreau, R.; Le Gouill, C.; Venne, M. H.; Stankova, J.; Rola-Pleszczynski, M. *J. Biol. Chem.* **2002**, *277*, 31567.

(189) Mollerup, J.; Eriksen, H. N.; Albertsen, J.; Wulff, T.; Lambert, I. H.; Hoffmann, E. K. *Cell Physiol. Biochem.* **2007**, *20*, 143.
(190) Ferguson, S. S.; Downey, W. E., 3rd; Colapietro, A. M.; Barak, L. S.; Menard, L.; Caron, M. G. *Science* **1996**, *271*, 363.
(191) Goodman, O. B., Jr.; Krupnick, J. G.; Santini, F.; Gurevich, V. V.; Penn, R. B.; Gagnon, A. W.; Keen, J. H.; Benovic, J. L. *Nature* **1996**, *383*, 447.
(192) Jala, V. R.; Shao, W. H.; Haribabu, B. *J. Biol. Chem.* **2005**, *280*, 4880.
(193) Negishi, M.; Namba, T.; Sugimoto, Y.; Irie, A.; Katada, T.; Narumiya, S.; Ichikawa, A. *J. Biol. Chem.* **1993**, *268*, 26067.
(194) Shi, L. C.; Wang, H. Y.; Horwitz, J.; Friedman, E. *J. Neurochem.* **1996**, *67*, 1478.
(195) Ishii, S.; Shimizu, T. *Prog. Lipid Res.* **2000**, *39*, 41.
(196) Sugimoto, N.; Takuwa, N.; Okamoto, H.; Sakurada, S.; Takuwa, Y. *Mol. Cell. Biol.* **2003**, *23*, 1534.
(197) Okuno, T.; Ago, H.; Terawaki, K.; Miyano, M.; Shimizu, T.; Yokomizo, T. *J. Biol. Chem.* **2003**, *278*, 41500.
(198) Gaudreau, R.; Beaulieu, M. E.; Chen, Z.; Le Gouill, C.; Lavigne, P.; Stankova, J.; Rola-Pleszczynski, M. *J. Biol. Chem.* **2004**, *279*, 10338.
(199) Kuniyeda, K.; Okuno, T.; Terawaki, K.; Miyano, M.; Yokomizo, T.; Shimizu, T. *J. Biol. Chem.* **2007**, *282*, 3998.
(200) Murakami, M.; Kouyama, T. *Nature* **2008**, *453*, 363.
(201) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. *Science* **2000**, *289*, 739.
(202) Baneres, J. L.; Martin, A.; Hullot, P.; Girard, J. P.; Rossi, J. C.; Parello, J. *J. Mol. Biol.* **2003**, *329*, 801.
(203) Poudrel, J. M.; Hullot, P.; Vidal, J. P.; Girard, J. P.; Rossi, J. C.; Muller, A.; Bonne, C.; Bezuglov, V.; Serkov, I.; Renard, P.; Pfeiffer, B. *J. Med. Chem.* **1999**, *42*, 5289.
(204) Hori, T.; Sato, Y.; Takahashi, N.; Takio, K.; Yokomizo, T.; Nakamura, M.; Shimizu, T.; Miyano, M. *Protein Expr. Purif.* **2010**, *72*, 66.
(205) Kamohara, M.; Takasaki, J.; Matsumoto, M.; Saito, T.; Ohishi, T.; Ishii, H.; Furuichi, K. *J. Biol. Chem.* **2000**, *275*, 27000.
(206) Tryselius, Y.; Nilsson, N. E.; Kotarsky, K.; Olde, B.; Owman, C. *Biochem. Biophys. Res. Commun.* **2000**, *274*, 377.
(207) Gether, U.; Kobilka, B. K. *J. Biol. Chem.* **1998**, *273*, 17979.
(208) Iizuka, Y.; Yokomizo, T.; Terawaki, K.; Komine, M.; Tamaki, K.; Shimizu, T. *J. Biol. Chem.* **2005**, *280*, 24816.
(209) Showell, H. J.; Conklyn, M. J.; Alpert, R.; Hingorani, G. P.; Wright, K. F.; Smith, M. A.; Stam, E.; Salter, E. D.; Scampoli, D. N.; Meltzer, S.; Reiter, L. A.; Koch, K.; Piscopio, A. D.; Cortina, S. R.; Lopez-Anaya, A.; Pettipher, E. R.; Milici, A. J.; Griffiths, R. J. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 946.
(210) Yokomizo, T.; Kato, K.; Hagiya, H.; Izumi, T.; Shimizu, T. *J. Biol. Chem.* **2001**, *276*, 12454.
(211) Okuno, T.; Iizuka, Y.; Okazaki, H.; Yokomizo, T.; Taguchi, R.; Shimizu, T. *J. Exp. Med.* **2008**, *205*, 759.
(212) Woo, C. H.; You, H. J.; Cho, S. H.; Eom, Y. W.; Chun, J. S.; Yoo, Y. J.; Kim, J. H. *J. Biol. Chem.* **2002**, *277*, 8572.
(213) Mathis, S. P.; Jala, V. R.; Lee, D. M.; Haribabu, B. *J. Immunol.* **2010**, *185*, 3049.
(214) Bockaert, J.; Pin, J. P. *EMBO J.* **1999**, *18*, 1723.
(215) Lagerstrom, M. C.; Schioth, H. B. *Nat. Rev. Drug Discovery* **2008**, *7*, 339.
(216) Hirota, N.; Yasuda, D.; Hashidate, T.; Yamamoto, T.; Yamaguchi, S.; Nagamune, T.; Nagase, T.; Shimizu, T.; Nakamura, M. *J. Biol. Chem.* **2010**, *285*, 5931.
(217) Conner, A. C.; Hay, D. L.; Simms, J.; Howitt, S. G.; Schindler, M.; Smith, D. M.; Wheatley, M.; Poyner, D. R. *Mol. Pharmacol.* **2005**, *67*, 20.
(218) Eilers, M.; Hornak, V.; Smith, S. O.; Konopka, J. B. *Biochemistry* **2005**, *44*, 8959.
(219) Yasuda, D.; Okuno, T.; Yokomizo, T.; Hori, T.; Hirota, N.; Hashidate, T.; Miyano, M.; Shimizu, T.; Nakamura, M. *FASEB J.* **2009**, *23*, 1470.
(220) Warne, T.; Serrano-Vega, M. J.; Baker, J. G.; Moukhametzianov, R.; Edwards, P. C.; Henderson, R.; Leslie, A. G.; Tate, C. G.; Schertler, G. F. *Nature* **2008**, *454*, 486.
(221) Jaakola, V. P.; Griffith, M. T.; Hanson, M. A.; Cherezov, V.; Chien, E. Y.; Lane, J. R.; Ijzerman, A. P.; Stevens, R. C. *Science* **2008**, *322*, 1211.
(222) Nakamura, M.; Yasuda, D.; Hirota, N.; Shimizu, T. *IUBMB Life* **2010**, *62*, 453.
(223) VanLeeuwen, D.; Steffey, M. E.; Donahue, C.; Ho, G.; MacKenzie, R. G. *J. Biol. Chem.* **2003**, *278*, 15935.
(224) Birke, F. W.; Meade, C. J.; Anderskewitz, R.; Speck, G. A.; Jennewein, H. M. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 458.
(225) Diaz-Gonzalez, F.; Alten, R. H.; Bensen, W. G.; Brown, J. P.; Sibley, J. T.; Dougados, M.; Bombardieri, S.; Durez, P.; Ortiz, P.; de-Miquel, G.; Staab, A.; Sigmund, R.; Salin, L.; Leledy, C.; Polmar, S. H. *Ann. Rheum. Dis.* **2007**, *66*, 628.
(226) Back, M.; Bu, D. X.; Branstrom, R.; Sheikine, Y.; Yan, Z. Q.; Hansson, G. K. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 17501.
(227) Marder, P.; Sawyer, J. S.; Froelich, L. L.; Mann, L. L.; Spaethe, S. M. *Biochem. Pharmacol.* **1995**, *49*, 1683.
(228) Perkins, E. J.; Cramer, J. W.; Farid, N. A.; Gadberry, M. G.; Jackson, D. A.; Mattiuz, E. L.; O'Bannon, D. D.; Weiss, H. J.; Wheeler, W. J.; Wood, P. G.; Cassidy, K. C. *Drug Metab. Dispos.* **2003**, *31*, 1382.
(229) Jackson, W. T.; Froelich, L. L.; Boyd, R. J.; Schrementi, J. P.; Saussy, D. L., Jr.; Schultz, R. M.; Sawyer, J. S.; Sofia, M. J.; Herron, D. K.; Goodson, T., Jr.; Snyder, D. W.; Pechous, P. A.; Spaethe, S. M.; Roman, C. R.; Fleisch, J. H. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 286.
(230) Kuwabara, K.; Yasui, K.; Jyoyama, H.; Maruyama, T.; Fleisch, J. H.; Hori, Y. *Eur. J. Pharmacol.* **2000**, *402*, 275.
(231) Raychaudhuri, A.; Kotyuk, B.; Pellas, T. C.; Pastor, G.; Fryer, L. R.; Morrissey, M.; Main, A. J. *Inflammation Res.* **1995**, *44* (Suppl 2), S141.
(232) Tobin, M. J. *Am. J. Respir. Crit. Care Med.* **2002**, *165*, 598.
(233) Tsai, B. S.; Villani-Price, D.; Keith, R. H.; Zemaitis, J. M.; Bauer, R. F.; Leonard, R.; Djuric, S. W.; Shone, R. L. *Prostaglandins* **1989**, *38*, 655.
(234) Kemeny, L.; Ruzicka, T. *Agents Actions* **1991**, *32*, 339.
(235) Fretland, D. J.; Widomski, D.; Tsai, B. S.; Zemaitis, J. M.; Levin, S.; Djuric, S. W.; Shone, R. L.; Gaginella, T. S. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 572.
(236) Kishikawa, K.; Tateishi, N.; Maruyama, T.; Seo, R.; Toda, M.; Miyamoto, T. *Prostaglandins* **1992**, *44*, 261.
(237) Guo, X.; Ko, J. K.; Mei, Q. B.; Cho, C. H. *Digestion* **2001**, *63*, 180.
(238) Suda, T.; Ueno, Y.; Fujii, K.; Shinki, T. *J. Cell Biochem.* **2003**, *88*, 259.
(239) Meghji, S.; Sandy, J. R.; Scutt, A. M.; Harvey, W.; Harris, M. *Prostaglandins* **1988**, *36*, 139.
(240) Garcia, C.; Boyce, B. F.; Gilles, J.; Dallas, M.; Qiao, M.; Mundy, G. R.; Bonewald, L. F. *J. Bone Miner. Res.* **1996**, *11*, 1619.
(241) Hikiji, H.; Ishii, S.; Yokomizo, T.; Takato, T.; Shimizu, T. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 21294.
(242) Aharony, D.; Falcone, R. C.; Yee, Y. K.; Hesp, B.; Giles, R. E.; Krell, R. D. *Ann. N.Y. Acad. Sci.* **1988**, *524*, 162.
(243) Richards, I. M.; Griffin, R. L.; Oostveen, J. A.; Morris, J.; Wishka, D. G.; Dunn, C. J. *Am. Rev. Respir. Dis.* **1989**, *140*, 1712.
(244) Falcone, R. C.; Orzechowski, R. F.; Aharony, D. *Prostaglandins* **1990**, *39*, 241.
(245) Boie, Y.; Stocco, R.; Sawyer, N.; Greig, G. M.; Kargman, S.; Slipetz, D. M.; O'Neill, G. P.; Shimizu, T.; Yokomizo, T.; Metters, K. M.; Abramovitz, M. *Eur. J. Pharmacol.* **1999**, *380*, 203.
(246) Johansson, A. S.; Haeggstrom, J. Z.; Palmblad, J. *Prostaglandins, Leukotrienes, Essent. Fatty Acids* **2011**, *84*, 109.
(247) Taylor, B. M.; Crittenden, N. J.; Bruden, M. N.; Wishka, D. G.; Morris, J.; Richards, I. M.; Sun, F. F. *Prostaglandins* **1991**, *42*, 211.
(248) Richards, I. M.; Sun, F. F.; Taylor, B. M.; Shields, S. K.; Griffin, R. L.; Morris, J.; Wishka, D. G.; Smith, H. W.; Johnson, R. A.; Dunn, C. J. *Ann. N.Y. Acad. Sci.* **1991**, *629*, 274.

(249) Silbaugh, S. A.; Stengel, P. W.; Cockerham, S. L.; Roman, C. R.; Saussy, D. L., Jr.; Spaethe, S. M.; Goodson, T., Jr.; Herron, D. K.; Fleisch, J. H. *Eur. J. Pharmacol.* **1992**, *223*, 57.
(250) Kim, E. Y.; Seo, J. M.; Kim, C.; Lee, J. E.; Lee, K. M.; Kim, J. H. *Free Radical Biol. Med.* **2010**, *49*, 1072.
(251) Cho, K. J.; Seo, J. M.; Lee, M. G.; Kim, J. H. *J. Immunol.* **2010**, *185*, 6329.
(252) Ryu, H. C.; Kim, C.; Kim, J. Y.; Chung, J. H.; Kim, J. H. *J. Invest. Dermatol.* **2010**, *130*, 1095.
(253) Hicks, A.; Goodnow, R., Jr.; Cavallo, G.; Tannu, S. A.; Ventre, J. D.; Lavelle, D.; Lora, J. M.; Satjawatcharaphong, J.; Brovarney, M.; Dabbagh, K.; Tare, N. S.; Oh, H.; Lamb, M.; Sidduri, A.; Dominique, R.; Qiao, Q.; Lou, J. P.; Gillespie, P.; Fotouhi, N.; Kowalczyk, A.; Kurylko, G.; Hamid, R.; Wright, M. B.; Pamidimukkala, A.; Egan, T.; Gubler, U.; Hoffman, A. F.; Wei, X.; Li, Y. L.; O'Neil, J.; Marcano, R.; Pozzani, K.; Molinaro, T.; Santiago, J.; Singer, L.; Hargaden, M.; Moore, D.; Catala, A. R.; Chao, L. C.; Benson, J.; March, T.; Venkat, R.; Mancebo, H.; Renzetti, L. M. *Prostaglandins Other Lipid Mediators* **2010**, *92*, 33.
(254) Showell, H. J.; Breslow, R.; Conklyn, M. J.; Hingorani, G. P.; Koch, K. *Br. J. Pharmacol.* **1996**, *117*, 1127.
(255) Falcone, R. C.; Aharony, D. *Eur. J. Pharmacol.* **1991**, *206*, 333.
(256) Sehmi, R.; Rossi, A. G.; Kay, A. B.; Cromwell, O. *Immunology* **1992**, *77*, 129.
(257) Surette, M. E.; Krump, E.; Picard, S.; Borgeat, P. *Mol. Pharmacol.* **1999**, *56*, 1055.
(258) Marcinkiewicz, J.; Grabowska, A.; Bryniarski, K.; Chain, B. M. *Immunology* **1997**, *91*, 369.
(259) Rola-Pleszczynski, M.; Bouvrette, L.; Gingras, D.; Girard, M. *J. Immunol.* **1987**, *139*, 513.
(260) Morita, H.; Takeda, K.; Yagita, H.; Okumura, K. *Biochem. Biophys. Res. Commun.* **1999**, *264*, 321.
(261) Griffiths, R. J.; Pettipher, E. R.; Koch, K.; Farrell, C. A.; Breslow, R.; Conklyn, M. J.; Smith, M. A.; Hackman, B. C.; Wimberly, D. J.; Milici, A. J.; Scampoli, D. N.; Cheng, J. B.; Pillar, J. S.; Pazoles, C. J.; Doherty, N. S.; Melvin, L. S.; Reiter, L. A.; Biggars, M. S.; Falkner, F. C.; Mitchell, D. Y.; Liston, T. E.; Showell, H. J. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 517.
(262) Dahlen, S. E.; Kumlin, M.; Bjorck, T.; Raud, J.; Wikstrom, E.; Hedqvist, P. *Ann. N.Y. Acad. Sci.* **1991**, *629*, 262.
(263) Wenzel, S. E. *Pharmacotherapy* **1997**, *17*, 3S.
(264) Chen, X. S.; Sheller, J. R.; Johnson, E. N.; Funk, C. D. *Nature* **1994**, *372*, 179.
(265) Goulet, J. L.; Snouwaert, J. N.; Latour, A. M.; Coffman, T. M.; Koller, B. H. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 12852.
(266) Griffiths, R. J.; Smith, M. A.; Roach, M. L.; Stock, J. L.; Stam, E. J.; Milici, A. J.; Scampoli, D. N.; Eskra, J. D.; Byrum, R. S.; Koller, B. H.; McNeish, J. D. *J. Exp. Med.* **1997**, *185*, 1123.
(267) Irvin, C. G.; Tu, Y. P.; Sheller, J. R.; Funk, C. D. *Am. J. Physiol.* **1997**, *272*, L1053.
(268) Byrum, R. S.; Goulet, J. L.; Snouwaert, J. N.; Griffiths, R. J.; Koller, B. H. *J. Immunol.* **1999**, *163*, 6810.
(269) Haribabu, B.; Verghese, M. W.; Steeber, D. A.; Sellars, D. D.; Bock, C. B.; Snyderman, R. *J. Exp. Med.* **2000**, *192*, 433.
(270) Tager, A. M.; Dufour, J. H.; Goodarzi, K.; Bercury, S. D.; von Andrian, U. H.; Luster, A. D. *J. Exp. Med.* **2000**, *192*, 439.
(271) Chiang, N.; Gronert, K.; Clish, C. B.; O'Brien, J. A.; Freeman, M. W.; Serhan, C. N. *J. Clin. Invest.* **1999**, *104*, 309.
(272) Goulet, J. L.; Griffiths, R. C.; Ruiz, P.; Spurney, R. F.; Pisetsky, D. S.; Koller, B. H.; Coffman, T. M. *J. Immunol.* **1999**, *163*, 359.
(273) Hallstrand, T. S.; Henderson, W. R., Jr. *Curr. Opin. Allergy Clin. Immunol.* **2010**, *10*, 60.
(274) Miyahara, N.; Takeda, K.; Miyahara, S.; Matsubara, S.; Koya, T.; Joetham, A.; Krishnan, E.; Dakhama, A.; Haribabu, B.; Gelfand, E. W. *Am. J. Respir. Crit. Care Med.* **2005**, *172*, 161.
(275) Miyahara, N.; Takeda, K.; Miyahara, S.; Taube, C.; Joetham, A.; Koya, T.; Matsubara, S.; Dakhama, A.; Tager, A. M.; Luster, A. D.; Gelfand, E. W. *J. Immunol.* **2005**, *174*, 4979.
(276) Terawaki, K.; Yokomizo, T.; Nagase, T.; Toda, A.; Taniguchi, M.; Hashizume, K.; Yagi, T.; Shimizu, T. *J. Immunol.* **2005**, *175*, 4217.
(277) Taube, C.; Miyahara, N.; Ott, V.; Swanson, B.; Takeda, K.; Loader, J.; Shultz, L. D.; Tager, A. M.; Luster, A. D.; Dakhama, A.; Gelfand, E. W. *J. Immunol.* **2006**, *176*, 3157.
(278) Angeli, V.; Faveeuw, C.; Roye, O.; Fontaine, J.; Teissier, E.; Capron, A.; Wolowczuk, I.; Capron, M.; Trottein, F. *J. Exp. Med.* **2001**, *193*, 1135.
(279) Kabashima, K.; Sakata, D.; Nagamachi, M.; Miyachi, Y.; Inaba, K.; Narumiya, S. *Nat. Med.* **2003**, *9*, 744.
(280) Kabashima, K.; Murata, T.; Tanaka, H.; Matsuoka, T.; Sakata, D.; Yoshida, N.; Katagiri, K.; Kinashi, T.; Tanaka, T.; Miyasaka, M.; Nagai, H.; Ushikubi, F.; Narumiya, S. *Nat. Immunol.* **2003**, *4*, 694.
(281) Machida, I.; Matsuse, H.; Kondo, Y.; Kawano, T.; Saeki, S.; Tomari, S.; Obase, Y.; Fukushima, C.; Kohno, S. *J. Immunol.* **2004**, *172*, 1833.
(282) Del Prete, A.; Shao, W. H.; Mitola, S.; Santoro, G.; Sozzani, S.; Haribabu, B. *Blood* **2007**, *109*, 626.
(283) Miyahara, N.; Ohnishi, H.; Matsuda, H.; Miyahara, S.; Takeda, K.; Koya, T.; Matsubara, S.; Okamoto, M.; Dakhama, A.; Haribabu, B.; Gelfand, E. W. *J. Immunol.* **2008**, *181*, 1170.
(284) Toda, A.; Terawaki, K.; Yamazaki, S.; Saeki, K.; Shimizu, T.; Yokomizo, T. *Biochimie* **2010**, *92*, 682.
(285) Cho, K. J.; Seo, J. M.; Shin, Y.; Yoo, M. H.; Park, C. S.; Lee, S. H.; Chang, Y. S.; Cho, S. H.; Kim, J. H. *Am. J. Respir. Cell Mol. Biol.* **2010**, *42*, 294.
(286) Mehrabian, M.; Allayee, H. *Curr. Opin. Lipidol.* **2003**, *14*, 447.
(287) Mehrabian, M.; Allayee, H.; Wong, J.; Shi, W.; Wang, X. P.; Shaposhnik, Z.; Funk, C. D.; Lusis, A. J. *Circ. Res.* **2002**, *91*, 120.
(288) Dwyer, J. H.; Allayee, H.; Dwyer, K. M.; Fan, J.; Wu, H.; Mar, R.; Lusis, A. J.; Mehrabian, M. *N. Engl. J. Med.* **2004**, *350*, 29.
(289) Dansky, H. M.; Shu, P.; Donavan, M.; Montagno, J.; Nagle, D. L.; Smutko, J. S.; Roy, N.; Whiteing, S.; Barrios, J.; McBride, T. J.; Smith, J. D.; Duyk, G.; Breslow, J. L.; Moore, K. J. *Genetics* **2002**, *160*, 1599.
(290) Spanbroek, R.; Grabner, R.; Lotzer, K.; Hildner, M.; Urbach, A.; Ruhling, K.; Moos, M. P.; Kaiser, B.; Cohnert, T. U.; Wahlers, T.; Zieske, A.; Plenz, G.; Robenek, H.; Salbach, P.; Kuhn, H.; Radmark, O.; Samuelsson, B.; Habenicht, A. J. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 1238.
(291) Sjostrom, M.; Johansson, A. S.; Schroder, O.; Qiu, H.; Palmblad, J.; Haeggstrom, J. Z. *Arterioscler., Thromb., Vasc. Biol.* **2003**, *23*, e37.
(292) Lotzer, K.; Spanbroek, R.; Hildner, M.; Urbach, A.; Heller, R.; Bretschneider, E.; Galczenski, H.; Evans, J. F.; Habenicht, A. J. *Arterioscler., Thromb., Vasc. Biol.* **2003**, *23*, e32.
(293) Aiello, R. J.; Bourassa, P. A.; Lindsey, S.; Weng, W.; Freeman, A.; Showell, H. J. *Arterioscler., Thromb., Vasc. Biol.* **2002**, *22*, 443.
(294) Subbarao, K.; Jala, V. R.; Mathis, S.; Suttles, J.; Zacharias, W.; Ahamed, J.; Ali, H.; Tseng, M. T.; Haribabu, B. *Arterioscler., Thromb., Vasc. Biol.* **2004**, *24*, 369.
(295) Davidson, E. M.; Rae, S. A.; Smith, M. J. *Ann. Rheum. Dis.* **1983**, *42*, 677.
(296) Elmgreen, J.; Nielsen, O. H.; Ahnfelt-Ronne, I. *Ann. Rheum. Dis.* **1987**, *46*, 501.
(297) Hashimoto, A.; Endo, H.; Hayashi, I.; Murakami, Y.; Kitasato, H.; Kono, S.; Matsui, T.; Tanaka, S.; Nishimura, A.; Urabe, K.; Itoman, M.; Kondo, H. *J. Rheumatol.* **2003**, *30*, 1712.
(298) Courtenay, J. S.; Dallman, M. J.; Dayan, A. D.; Martin, A.; Mosedale, B. *Nature* **1980**, *283*, 666.
(299) Hegen, M.; Sun, L.; Uozumi, N.; Kume, K.; Goad, M. E.; Nickerson-Nutter, C. L.; Shimizu, T.; Clark, J. D. *J. Exp. Med.* **2003**, *197*, 1297.
(300) Shao, W. H.; Del Prete, A.; Bock, C. B.; Haribabu, B. *J. Immunol.* **2006**, *176*, 6254.
(301) Kim, N. D.; Chou, R. C.; Seung, E.; Tager, A. M.; Luster, A. D. *J. Exp. Med.* **2006**, *203*, 829.

(302) Chou, R. C.; Kim, N. D.; Sadik, C. D.; Seung, E.; Lan, Y.; Byrne, M. H.; Haribabu, B.; Iwakura, Y.; Luster, A. D. *Immunity* **2010**, *33*, 266.
(303) Gadaleta, D.; Fantini, G. A.; Silane, M. F.; Davis, J. M. *Surgery* **1994**, *116*, 847.
(304) Houard, X.; Ollivier, V.; Louedec, L.; Michel, J. B.; Back, M. *FASEB J.* **2009**, *23*, 1376.
(305) Ahluwalia, N.; Lin, A. Y.; Tager, A. M.; Pruitt, I. E.; Anderson, T. J.; Kristo, F.; Shen, D.; Cruz, A. R.; Aikawa, M.; Luster, A. D.; Gerszten, R. E. *J. Immunol.* **2007**, *179*, 691.
(306) Kristo, F.; Hardy, G. J.; Anderson, T. J.; Sinha, S.; Ahluwalia, N.; Lin, A. Y.; Passeri, J.; Scherrer-Crosbie, M.; Gerszten, R. E. *Atherosclerosis* **2010**, *210*, 107.
(307) McFarland, H. F.; Martin, R. *Nat. Immunol.* **2007**, *8*, 913.
(308) Kihara, Y.; Yokomizo, T.; Kunita, A.; Morishita, Y.; Fukayama, M.; Ishii, S.; Shimizu, T. *Biochem. Biophys. Res. Commun.* **2010**, *394*, 673.
(309) Iizuka, Y.; Okuno, T.; Saeki, K.; Uozaki, H.; Okada, S.; Misaka, T.; Sato, T.; Toh, H.; Fukayama, M.; Takeda, N.; Kita, Y.; Shimizu, T.; Nakamura, M.; Yokomizo, T. *FASEB J.* **2010**, *24*, 4678.
(310) Kim, G. Y.; Lee, J. W.; Cho, S. H.; Seo, J. M.; Kim, J. H. *Arterioscler., Thromb., Vasc. Biol.* **2009**, *29*, 915.
(311) Owman, C.; Garzino-Demo, A.; Cocchi, F.; Popovic, M.; Sabirsh, A.; Gallo, R. C. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 9530.
(312) Martin, V.; Ronde, P.; Unett, D.; Wong, A.; Hoffman, T. L.; Edinger, A. L.; Doms, R. W.; Funk, C. D. *J. Biol. Chem.* **1999**, *274*, 8597.
(313) Devchand, P. R.; Keller, H.; Peters, J. M.; Vazquez, M.; Gonzalez, F. J.; Wahli, W. *Nature* **1996**, *384*, 39.
(314) Yu, K.; Bayona, W.; Kallen, C. B.; Harding, H. P.; Ravera, C. P.; McMahon, G.; Brown, M.; Lazar, M. A. *J. Biol. Chem.* **1995**, *270*, 23975.
(315) Forman, B. M.; Tontonoz, P.; Chen, J.; Brun, R. P.; Spiegelman, B. M.; Evans, R. M. *Cell* **1995**, *83*, 803.
(316) Kliewer, S. A.; Lenhard, J. M.; Willson, T. M.; Patel, I.; Morris, D. C.; Lehmann, J. M. *Cell* **1995**, *83*, 813.
(317) Narala, V. R.; Adapala, R. K.; Suresh, M. V.; Brock, T. G.; Peters-Golden, M.; Reddy, R. C. *J. Biol. Chem.* **2010**, *285*, 22067.
(318) Hwang, S. W.; Cho, H.; Kwak, J.; Lee, S. Y.; Kang, C. J.; Jung, J.; Cho, S.; Min, K. H.; Suh, Y. G.; Kim, D.; Oh, U. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 6155.
(319) Craib, S. J.; Ellington, H. C.; Pertwee, R. G.; Ross, R. A. *Br. J. Pharmacol.* **2001**, *134*, 30.
(320) Van Der Stelt, M.; Di Marzo, V. *Eur. J. Biochem.* **2004**, *271*, 1827.
(321) Szallasi, A.; Biro, T.; Szabo, T.; Modarres, S.; Petersen, M.; Klusch, A.; Blumberg, P. M.; Krause, J. E.; Sterner, O. *Br. J. Pharmacol.* **1999**, *126*, 1351.
(322) McHugh, D.; McMaster, R. S.; Pertwee, R. G.; Roy, S.; Mahadevan, A.; Razdan, R. K.; Ross, R. A. *J. Pharmacol. Exp. Ther.* **2006**, *316*, 955.
(323) Scow, D. T.; Luttermoser, G. K.; Dickerson, K. S. *Am. Fam. Physician* **2007**, *75*, 65.
(324) Berger, W.; De Chandt, M. T.; Cairns, C. B. *Int. J. Clin. Pract.* **2007**, *61*, 663.
(325) Hicks, A.; Monkarsh, S. P.; Hoffman, A. F.; Goodnow, R., Jr. *Expert. Opin. Invest. Drugs* **2007**, *16*, 1909.
(326) Alten, R.; Gromnica-Ihle, E.; Pohl, C.; Emmerich, J.; Steffgen, J.; Roscher, R.; Sigmund, R.; Schmolke, B.; Steinmann, G. *Ann. Rheum. Dis.* **2004**, *63*, 170.
(327) Evans, D. J.; Barnes, P. J.; Spaethe, S. M.; van Alstyne, E. L.; Mitchell, M. I.; O'Connor, B. J. *Thorax* **1996**, *51*, 1178.
(328) van Pelt, J. P.; de Jong, E. M.; van Erp, P. E.; Mitchell, M. I.; Marder, P.; Spaethe, S. M.; van Hooijdonk, C. A.; Kuijpers, A. L.; van de Kerkhof, P. C. *Biochem. Pharmacol.* **1997**, *53*, 1005.
(329) Mommers, J. M.; Van Rossum, M. M.; Kooijmans-Otero, M. E.; Parker, G. L.; van de Kerkhof, P. C. *Br. J. Dermatol.* **2000**, *142*, 259.
(330) Tong, W. G.; Ding, X. Z.; Hennig, R.; Witt, R. C.; Standop, J.; Pour, P. M.; Adrian, T. E. *Clin. Cancer Res.* **2002**, *8*, 3232.
(331) Hennig, R.; Ding, X. Z.; Tong, W. G.; Witt, R. C.; Jovanovic, B. D.; Adrian, T. E. *Cancer Lett.* **2004**, *210*, 41.
(332) Ding, X. Z.; Talamonti, M. S.; Bell, R. H., Jr.; Adrian, T. E. *Anticancer Drugs* **2005**, *16*, 467.
(333) Hennig, R.; Ventura, J.; Segersvard, R.; Ward, E.; Ding, X. Z.; Rao, S. M.; Jovanovic, B. D.; Iwamura, T.; Talamonti, M. S.; Bell, R. H., Jr.; Adrian, T. E. *Neoplasia* **2005**, *7*, 417.
(334) Saif, M. W.; Oettle, H.; Vervenne, W. L.; Thomas, J. P.; Spitzer, G.; Visseren-Grul, C.; Enas, N.; Richards, D. A. *Cancer J.* **2009**, *15*, 339.
(335) Gronke, L.; Beeh, K. M.; Cameron, R.; Kornmann, O.; Beier, J.; Shaw, M.; Holz, O.; Buhl, R.; Magnussen, H.; Jorres, R. A. *Pulm. Pharmacol. Ther.* **2008**, *21*, 409.
(336) Feldberg, W.; Kellaway, C. H. *J. Physiol.* **1938**, *94*, 187.
(337) Brocklehurst, W. E. *Prog. Allergy* **1962**, *6*, 539.
(338) Brocklehurst, W. E. *J. Physiol.* **1960**, *151*, 416.
(339) Augstein, J.; Farmer, J. B.; Lee, T. B.; Sheard, P.; Tattersall, M. L. *Nat. New Biol.* **1973**, *245*, 215.
(340) Strandberg, K.; Uvnas, B. *Acta. Physiol. Scand.* **1971**, *82*, 358.
(341) Jakschik, B. A.; Falkenhein, S.; Parker, C. W. *Proc. Natl. Acad. Sci. U. S. A.* **1977**, *74*, 4577.
(342) Orange, R. P.; Murphy, R. C.; Karnovsky, M. L.; Austen, K. F. *J. Immunol.* **1973**, *110*, 760.
(343) Morris, H. R.; Taylor, G. W.; Piper, P. J.; Sirois, P.; Tippins, J. R. *FEBS Lett.* **1978**, *87*, 203.
(344) Cheng, J. B.; Townley, R. G. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 949.
(345) Funk, C. D. *Science* **2001**, *294*, 1871.
(346) Christmas, P.; Weber, B. M.; McKee, M.; Brown, D.; Soberman, R. J. *J. Biol. Chem.* **2002**, *277*, 28902.
(347) Maclouf, J. A.; Murphy, R. C. *J. Biol. Chem.* **1988**, *263*, 174.
(348) Tornhamre, S.; Sjolinder, M.; Lindberg, A.; Ericsson, I.; Nasman-Glaser, B.; Griffiths, W. J.; Lindgren, J. A. *Eur. J. Biochem.* **1998**, *251*, 227.
(349) Kanaoka, Y.; Maekawa, A.; Penrose, J. F.; Austen, K. F.; Lam, B. K. *J. Biol. Chem.* **2001**, *276*, 22608.
(350) Lam, B. K. *Prostaglandins, Leukotrienes, Essent. Fatty Acids* **2003**, *69*, 111.
(351) Bresell, A.; Weinander, R.; Lundqvist, G.; Raza, H.; Shimoji, M.; Sun, T. H.; Balk, L.; Wiklund, R.; Eriksson, J.; Jansson, C.; Persson, B.; Jakobsson, P. J.; Morgenstern, R. *FEBS J.* **2005**, *272*, 1688.
(352) Martinez Molina, D.; Eshaghi, S.; Nordlund, P. *Curr. Opin. Struct. Biol.* **2008**, *18*, 442.
(353) Leier, I.; Jedlitschky, G.; Buchholz, U.; Cole, S. P.; Deeley, R. G.; Keppler, D. *J. Biol. Chem.* **1994**, *269*, 27807.
(354) Anderson, M. E.; Allison, R. D.; Meister, A. *Proc. Natl. Acad. Sci. U. S. A.* **1982**, *79*, 1088.
(355) Carter, B. Z.; Shi, Z. Z.; Barrios, R.; Lieberman, M. W. *J. Biol. Chem.* **1998**, *273*, 28277.
(356) Lee, C. W.; Lewis, R. A.; Corey, E. J.; Austen, K. F. *Immunology* **1983**, *48*, 27.
(357) Murakami, M.; Austen, K. F.; Bingham, C. O., 3rd; Friend, D. S.; Penrose, J. F.; Arm, J. P. *J. Biol. Chem.* **1995**, *270*, 22653.
(358) Sampson, A. P.; Siddiqui, S.; Buchanan, D.; Howarth, P. H.; Holgate, S. T.; Holloway, J. W.; Sayers, I. *Thorax* **2000**, *55* (Suppl 2), S28.
(359) Sayers, I.; Barton, S.; Rorke, S.; Beghe, B.; Hayward, B.; Van Eerdewegh, P.; Keith, T.; Clough, J. B.; Ye, S.; Holloway, J. W.; Sampson, A. P.; Holgate, S. T. *Thorax* **2003**, *58*, 417.
(360) Asano, K.; Shiomi, T.; Hasegawa, N.; Nakamura, H.; Kudo, H.; Matsuzaki, T.; Hakuno, H.; Fukunaga, K.; Suzuki, Y.; Kanazawa, M.; Yamaguchi, K. *Pharmacogenetics* **2002**, *12*, 565.
(361) Sanak, M.; Pierzchalska, M.; Bazan-Socha, S.; Szczeklik, A. *Am. J. Respir. Cell Mol. Biol.* **2000**, *23*, 290.
(362) Van Sambeek, R.; Stevenson, D. D.; Baldasaro, M.; Lam, B. K.; Zhao, J.; Yoshida, S.; Yandora, C.; Drazen, J. M.; Penrose, J. F. *J. Allergy Clin. Immunol.* **2000**, *106*, 72.
(363) Denis, D.; Charleson, S.; Rackham, A.; Jones, T. R.; Ford-Hutchinson, A. W.; Lord, A.; Cirino, M.; Girard, Y.; Larue, M.; Rokach, J. *Prostaglandins* **1982**, *24*, 801.

(364) Tate, S. S.; Meister, A. *Mol. Cell. Biochem.* **1981**, *39*, 357.
(365) Keillor, J. W.; Castonguay, R.; Lherbet, C. *Methods Enzymol.* **2005**, *401*, 449.
(366) Heisterkamp, N.; Rajpert-De Meyts, E.; Uribe, L.; Forman, H. J.; Groffen, J. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 6303.
(367) Carter, B. Z.; Wiseman, A. L.; Orkiszewski, R.; Ballard, K. D.; Ou, C. N.; Lieberman, M. W. *J. Biol. Chem.* **1997**, *272*, 12305.
(368) Shi, Z. Z.; Han, B.; Habib, G. M.; Matzuk, M. M.; Lieberman, M. W. *Mol. Cell. Biol.* **2001**, *21*, 5389.
(369) Han, B.; Luo, G.; Shi, Z. Z.; Barrios, R.; Atwood, D.; Liu, W.; Habib, G. M.; Sifers, R. N.; Corry, D. B.; Lieberman, M. W. *Am. J. Pathol.* **2002**, *161*, 481.
(370) Mayatepek, E.; Okun, J. G.; Meissner, T.; Assmann, B.; Hammond, J.; Zschocke, J.; Lehmann, W. D. *J. Lipid Res.* **2004**, *45*, 900.
(371) Sala, A.; Voelkel, N.; Maclouf, J.; Murphy, R. C. *J. Biol. Chem.* **1990**, *265*, 21771.
(372) Mayatepek, E.; Ferdinandusse, S.; Meissner, T.; Wanders, R. J. *Clin. Chim. Acta* **2004**, *345*, 89.
(373) Zaitsu, M.; Hamasaki, Y.; Tsuji, K.; Matsuo, M.; Fujita, I.; Aoki, Y.; Ishii, E.; Kohashi, O. *Eur. Respir. J.* **2003**, *22*, 35.
(374) Evans, J. F. *Prostaglandins Other Lipid Mediators* **2002**, *68−69*, 587.
(375) Sok, D. E.; Pai, J. K.; Atrache, V.; Sih, C. J. *Proc. Natl. Acad. Sci. U. S. A.* **1980**, *77*, 6481.
(376) Josch, C.; Klotz, L. O.; Sies, H. *Biol. Chem.* **2003**, *384*, 213.
(377) Raulf, M.; Stuning, M.; Konig, W. *Immunology* **1985**, *55*, 135.
(378) Nagaoka, I.; Yamashita, T. *Biochim. Biophys. Acta* **1987**, *922*, 8.
(379) Campbell, B. J.; Baker, S. F.; Shukla, S. D.; Forrester, L. J.; Zahler, W. L. *Biochim. Biophys. Acta* **1990**, *1042*, 107.
(380) Smyth, T. P.; Wall, J. G.; Nitanai, Y. *Bioorg. Med. Chem.* **2003**, *11*, 991.
(381) Adachi, H.; Kubota, I.; Okamura, N.; Iwata, H.; Tsujimoto, M.; Nakazato, H.; Nishihara, T.; Noguchi, T. *J. Biochem.* **1989**, *105*, 957.
(382) An, S.; Schmidt, F. J.; Campbell, B. J. *Biochim. Biophys. Acta* **1994**, *1226*, 337.
(383) Habib, G. M.; Shi, Z. Z.; Cuevas, A. A.; Guo, Q.; Matzuk, M. M.; Lieberman, M. W. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 4859.
(384) Birnbaum, J.; Kahan, F. M.; Kropp, H.; MacDonald, J. S. *Am. J. Med.* **1985**, *78*, 3.
(385) Habib, G. M.; Shi, Z. Z.; Cuevas, A. A.; Lieberman, M. W. *FASEB J.* **2003**, *17*, 1313.
(386) Jedlitschky, G.; Huber, M.; Volkl, A.; Muller, M.; Leier, I.; Muller, J.; Lehmann, W. D.; Fahimi, H. D.; Keppler, D. *J. Biol. Chem.* **1991**, *266*, 24763.
(387) Maclouf, J.; Antoine, C.; De Caterina, R.; Sicari, R.; Murphy, R. C.; Patrignani, P.; Loizzo, S.; Patrono, C. *Am. J. Physiol.* **1992**, *263*, H244.
(388) Drazen, J. M.; O'Brien, J.; Sparrow, D.; Weiss, S. T.; Martins, M. A.; Israel, E.; Fanta, C. H. *Am. Rev. Respir. Dis.* **1992**, *146*, 104.
(389) Lam, S.; Chan, H.; LeRiche, J. C.; Chan-Yeung, M.; Salari, H. *J. Allergy Clin. Immunol.* **1988**, *81*, 711.
(390) Csoma, Z.; Kharitonov, S. A.; Balint, B.; Bush, A.; Wilson, N. M.; Barnes, P. J. *Am. J. Respir. Crit. Care Med.* **2002**, *166*, 1345.
(391) Drazen, J. M.; Austen, K. F.; Lewis, R. A.; Clark, D. A.; Goto, G.; Marfat, A.; Corey, E. J. *Proc. Natl. Acad. Sci. U. S. A.* **1980**, *77*, 4354.
(392) Fleisch, J. H.; Rinkema, L. E.; Baker, S. R. *Life Sci.* **1982**, *31*, 577.
(393) Labat, C.; Ortiz, J. L.; Norel, X.; Gorenne, I.; Verley, J.; Abram, T. S.; Cuthbert, N. J.; Tudhope, S. R.; Norman, P.; Gardiner, P.; Brink, C. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 800.
(394) Secrest, R. J.; Olsen, E. J.; Chapnick, B. M. *Circ. Res.* **1985**, *57*, 323.
(395) Sakuma, I.; Gross, S. S.; Levi, R. *Prostaglandins* **1987**, *34*, 685.
(396) Ortiz, J. L.; Gorenne, I.; Cortijo, J.; Seller, A.; Labat, C.; Sarria, B.; Abram, T. S.; Gardiner, P. J.; Morcillo, E.; Brink, C. *Br. J. Pharmacol.* **1995**, *115*, 1382.
(397) Lynch, K. R.; O'Neill, G. P.; Liu, Q.; Im, D. S.; Sawyer, N.; Metters, K. M.; Coulombe, N.; Abramovitz, M.; Figueroa, D. J.; Zeng, Z.; Connolly, B. M.; Bai, C.; Austin, C. P.; Chateauneuf, A.; Stocco, R.; Greig, G. M.; Kargman, S.; Hooks, S. B.; Hosfield, E.; Williams, D. L., Jr.; Ford-Hutchinson, A. W.; Caskey, C. T.; Evans, J. F. *Nature* **1999**, *399*, 789.
(398) Heise, C. E.; O'Dowd, B. F.; Figueroa, D. J.; Sawyer, N.; Nguyen, T.; Im, D. S.; Stocco, R.; Bellefeuille, J. N.; Abramovitz, M.; Cheng, R.; Williams, D. L., Jr.; Zeng, Z.; Liu, Q.; Ma, L.; Clements, M. K.; Coulombe, N.; Liu, Y.; Austin, C. P.; George, S. R.; O'Neill, G. P.; Metters, K. M.; Lynch, K. R.; Evans, J. F. *J. Biol. Chem.* **2000**, *275*, 30531.
(399) Sarau, H. M.; Ames, R. S.; Chambers, J.; Ellis, C.; Elshourbagy, N.; Foley, J. J.; Schmidt, D. B.; Muccitelli, R. M.; Jenkins, O.; Murdock, P. R.; Herrity, N. C.; Halsey, W.; Sathe, G.; Muir, A. I.; Nuthulaganti, P.; Dytko, G. M.; Buckley, P. T.; Wilson, S.; Bergsma, D. J.; Hay, D. W. *Mol. Pharmacol.* **1999**, *56*, 657.
(400) Takasaki, J.; Kamohara, M.; Matsumoto, M.; Saito, T.; Sugimoto, T.; Ohishi, T.; Ishii, H.; Ota, T.; Nishikawa, T.; Kawai, Y.; Masuho, Y.; Isogai, T.; Suzuki, Y.; Sugano, S.; Furuichi, K. *Biochem. Biophys. Res. Commun.* **2000**, *274*, 316.
(401) Ravasi, S.; Capra, V.; Mezzetti, M.; Nicosia, S.; Rovati, G. E. *Mol. Pharmacol.* **2000**, *57*, 1182.
(402) Norman, P.; Abram, T. S.; Cuthbert, N. J.; Gardiner, P. J. *Eur. J. Pharmacol.* **1990**, *182*, 301.
(403) Mong, S.; Wu, H. L.; Scott, M. O.; Lewis, M. A.; Clark, M. A.; Weichman, B. M.; Kinzig, C. M.; Gleason, J. G.; Crooke, S. T. *J. Pharmacol. Exp. Ther.* **1985**, *234*, 316.
(404) Norman, P.; Abram, T. S.; Kluender, H. C.; Gardiner, P. J.; Cuthbert, N. J. *Eur. J. Pharmacol.* **1987**, *143*, 323.
(405) Weichman, B. M.; Tucker, S. S. *Prostaglandins* **1982**, *24*, 245.
(406) Levinson, S. L. *Prostaglandins* **1984**, *28*, 229.
(407) Pong, S. S.; DeHaven, R. N.; Kuehl, F. A., Jr.; Egan, R. W. *J. Biol. Chem.* **1983**, *258*, 9616.
(408) Metters, K. M.; Frey, E. A.; Ford-Hutchinson, A. W. *Eur. J. Pharmacol.* **1991**, *194*, 51.
(409) Norman, P.; Abram, T. S.; Cuthbert, N. J.; Tudhope, S. R.; Gardiner, P. J. *Eur. J. Pharmacol.* **1994**, *271*, 73.
(410) Gardiner, P. J.; Abram, T. S.; Tudhope, S. R.; Cuthbert, N. J.; Norman, P.; Brink, C. *Adv. Prostaglandin, Thromboxane, Leukotriene Res.* **1994**, *22*, 49.
(411) Sun, F. F.; Chau, L. Y.; Austen, K. F. *Fed. Proc.* **1987**, *46*, 204.
(412) Metters, K. M.; Sawyer, N.; Nicholson, D. W. *J. Biol. Chem.* **1994**, *269*, 12816.
(413) Bannenberg, G.; Dahlen, S. E.; Luijerink, M.; Lundqvist, G.; Morgenstern, R. *J. Biol. Chem.* **1999**, *274*, 1994.
(414) Lam, B. K.; Xu, K.; Atkins, M. B.; Austen, K. F. *Proc. Natl. Acad. Sci. U. S. A.* **1992**, *89*, 11598.
(415) Keppler, D.; Muller, M.; Klunemann, C.; Guhlmann, A.; Krauss, K.; Muller, J.; Berger, U.; Leier, I.; Mayatepek, E. *Adv. Enzyme Regul.* **1992**, *32*, 107.
(416) Keppler, D. *Rev. Physiol. Biochem. Pharmacol.* **1992**, *121*, 1.
(417) Qian, Y. M.; Qiu, W.; Gao, M.; Westlake, C. J.; Cole, S. P.; Deeley, R. G. *J. Biol. Chem.* **2001**, *276*, 38636.
(418) Schalling, M.; Neil, A.; Terenius, L.; Lindgren, J. A.; Miamoto, T.; Hokfelt, T.; Samuelsson, B. *Eur. J. Pharmacol.* **1986**, *122*, 251.
(419) Mayhan, W. G.; Sahagun, G.; Spector, R.; Heistad, D. D. *Am. J. Physiol.* **1986**, *251*, H471.
(420) Black, K. L.; Betz, A. L.; Ar, D. B. *Adv. Prostaglandin, Thromboxane, Leukotriene Res.* **1987**, *17A*, 508.
(421) Muller, A.; Ghiglieri-Bertez, C.; Modat, G.; Bonne, C. *Prostaglandins, Leukotrienes Med.* **1987**, *26*, 233.
(422) Ghiglieri-Bertez, C.; Cristol, J. P.; Bonne, C. *Biochim. Biophys. Acta* **1986**, *879*, 97.
(423) Baud, L.; Koo, C. H.; Goetzl, E. J. *Immunology* **1987**, *62*, 53.
(424) Cheng, J. B.; Lang, D.; Bewtra, A.; Townley, R. G. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 80.
(425) Krilis, S.; Lewis, R. A.; Corey, E. J.; Austen, K. F. *J. Clin. Invest.* **1983**, *72*, 1516.
(426) Hogaboom, G. K.; Mong, S.; Wu, H. L.; Crooke, S. T. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 1136.

(427) Cheng, J. B.; Townley, R. G. *Biochem. Biophys. Res. Commun.* **1984**, *119*, 612.
(428) Aharony, D.; Catanese, C. A.; Falcone, R. C. *J. Pharmacol. Exp. Ther.* **1989**, *248*, 581.
(429) Rovati, G. E.; Oliva, D.; Sautebin, L.; Folco, G. C.; Welton, A. F.; Nicosia, S. *Biochem. Pharmacol.* **1985**, *34*, 2831.
(430) Rovati, G. E.; Giovanazzi, S.; Mezzetti, M.; Nicosia, S. *Biochem. Pharmacol.* **1992**, *44*, 1411.
(431) Woszczek, G.; Pawliczak, R.; Qi, H. Y.; Nagineni, S.; Alsaaty, S.; Logun, C.; Shelhamer, J. H. *J. Immunol.* **2005**, *175*, 5152.
(432) Thivierge, M.; Doty, M.; Johnson, J.; Stankova, J.; Rola-Pleszczynski, M. *J. Immunol.* **2000**, *165*, 5221.
(433) Zhu, J.; Qiu, Y. S.; Figueroa, D. J.; Bandi, V.; Galczenski, H.; Hamada, K.; Guntupalli, K. K.; Evans, J. F.; Jeffery, P. K. *Am. J. Respir. Cell Mol. Biol.* **2005**, *33*, 531.
(434) Figueroa, D. J.; Breyer, R. M.; Defoe, S. K.; Kargman, S.; Daugherty, B. L.; Waldburger, K.; Liu, Q.; Clements, M.; Zeng, Z.; O'Neill, G. P.; Jones, T. R.; Lynch, K. R.; Austin, C. P.; Evans, J. F. *Am. J. Respir. Crit. Care Med.* **2001**, *163*, 226.
(435) Maekawa, A.; Kanaoka, Y.; Lam, B. K.; Austen, K. F. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 2256.
(436) Martin, V.; Sawyer, N.; Stocco, R.; Unett, D.; Lerner, M. R.; Abramovitz, M.; Funk, C. D. *Biochem. Pharmacol.* **2001**, *62*, 1193.
(437) Mollerup, J.; Jorgensen, S. T.; Hougaard, C.; Hoffmann, E. K. *Biochim. Biophys. Acta* **2001**, *1517*, 455.
(438) Frey, E. A.; Nicholson, D. W.; Metters, K. M. *Eur. J. Pharmacol.* **1993**, *244*, 239.
(439) Mong, S.; Miller, J.; Wu, H. L.; Crooke, S. T. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 508.
(440) Saussy, D. L., Jr.; Sarau, H. M.; Foley, J. J.; Mong, S.; Crooke, S. T. *J. Biol. Chem.* **1989**, *264*, 19845.
(441) Crooke, S. T.; Mattern, M.; Sarau, H. M.; Winkler, J. D.; Balcarek, J.; Wong, A.; Bennett, C. F. *Trends Pharmacol. Sci.* **1989**, *10*, 103.
(442) Watanabe, T.; Shimizu, T.; Miki, I.; Sakanaka, C.; Honda, Z.; Seyama, Y.; Teramoto, T.; Matsushima, T.; Ui, M.; Kurokawa, K. *J. Biol. Chem.* **1990**, *265*, 21237.
(443) Clark, M. A.; Cook, M.; Mong, S.; Crooke, S. T. *Eur. J. Pharmacol.* **1985**, *116*, 207.
(444) Baud, L.; Goetzl, E. J.; Koo, C. H. *J. Clin. Invest.* **1987**, *80*, 983.
(445) Capra, V.; Accomazzo, M. R.; Ravasi, S.; Parenti, M.; Macchia, M.; Nicosia, S.; Rovati, G. E. *Prostaglandins Other Lipid Mediators* **2003**, *71*, 235.
(446) Adolfsson, J. L.; Ohd, J. F.; Sjolander, A. *Biochem. Biophys. Res. Commun.* **1996**, *226*, 413.
(447) Hoshino, M.; Izumi, T.; Shimizu, T. *J. Biol. Chem.* **1998**, *273*, 4878.
(448) McMahon, B.; Stenson, C.; McPhillips, F.; Fanning, A.; Brady, H. R.; Godson, C. *J. Biol. Chem.* **2000**, *275*, 27566.
(449) Paruchuri, S.; Hallberg, B.; Juhas, M.; Larsson, C.; Sjolander, A. *J. Cell Sci.* **2002**, *115*, 1883.
(450) Capra, V.; Ravasi, S.; Accomazzo, M. R.; Parenti, M.; Rovati, G. E. *Biochem. Pharmacol.* **2004**, *67*, 1569.
(451) Ciccarelli, R.; D'Alimonte, I.; Santavenere, C.; D'Auro, M.; Ballerini, P.; Nargi, E.; Buccella, S.; Nicosia, S.; Folco, G.; Caciagli, F.; Di Iorio, P. *Eur. J. Neurosci.* **2004**, *20*, 1514.
(452) Parhamifar, L.; Jeppsson, B.; Sjolander, A. *Carcinogenesis* **2005**, *26*, 1988.
(453) Jiang, Y.; Kanaoka, Y.; Feng, C.; Nocka, K.; Rao, S.; Boyce, J. A. *J. Immunol.* **2006**, *177*, 2755.
(454) Ravasi, S.; Citro, S.; Viviani, B.; Capra, V.; Rovati, G. E. *Respir. Res.* **2006**, *7*, 42.
(455) Kim, M. H.; Lee, Y. J.; Kim, M. O.; Kim, J. S.; Han, H. J. *J. Cell Biochem.* 2010, *111*, 686.
(456) Capra, V.; Accomazzo, M. R.; Gardoni, F.; Barbieri, S.; Rovati, G. E. *J. Lipid Res.* **2010**, *51*, 1075.
(457) Saito, K.; Nagata, M.; Kikuchi, I.; Sakamoto, Y. *Ann. Allergy Asthma Immunol.* **2004**, *93*, 594.
(458) Meliton, A. Y.; Munoz, N. M.; Osan, C. M.; Meliton, L. N.; Leff, A. R. *Eur. Respir. J.* **2010**, *35*, 402.
(459) Boehmler, A. M.; Drost, A.; Jaggy, L.; Seitz, G.; Wiesner, T.; Denzlinger, C.; Kanz, L.; Mohle, R. *J. Immunol.* **2009**, *182*, 6789.
(460) Meliton, A. Y.; Munoz, N. M.; Leff, A. R. *J. Allergy Clin. Immunol.* **2007**, *120*, 1316.
(461) Nielsen, C. K.; Campbell, J. I.; Ohd, J. F.; Morgelin, M.; Riesbeck, K.; Landberg, G.; Sjolander, A. *Cancer Res.* **2005**, *65*, 732.
(462) Bhattacharya, M.; Peri, K. G.; Almazan, G.; Ribeiro-da-Silva, A.; Shichi, H.; Durocher, Y.; Abramovitz, M.; Hou, X.; Varma, D. R.; Chemtob, S. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 15792.
(463) Maekawa, A.; Austen, K. F.; Kanaoka, Y. *J. Biol. Chem.* **2002**, *277*, 20820.
(464) Thivierge, M.; Stankova, J.; Rola-Pleszczynski, M. *J. Immunol.* **2001**, *167*, 2855.
(465) Nelms, K.; Keegan, A. D.; Zamorano, J.; Ryan, J. J.; Paul, W. E. *Annu. Rev. Immunol.* **1999**, *17*, 701.
(466) Darnell, J. E., Jr. *Science* **1997**, *277*, 1630.
(467) Zhu, J.; Guo, L.; Watson, C. J.; Hu-Li, J.; Paul, W. E. *J. Immunol.* **2001**, *166*, 7276.
(468) Naik, S.; Billington, C. K.; Pascual, R. M.; Deshpande, D. A.; Stefano, F. P.; Kohout, T. A.; Eckman, D. M.; Benovic, J. L.; Penn, R. B. *J. Biol. Chem.* **2005**, *280*, 8722.
(469) Lefkowitz, R. J.; Cotecchia, S.; Samama, P.; Costa, T. *Trends Pharmacol. Sci.* **1993**, *14*, 303.
(470) Chidiac, P.; Hebert, T. E.; Valiquette, M.; Dennis, M.; Bouvier, M. *Mol. Pharmacol.* **1994**, *45*, 490.
(471) Hase, M.; Yokomizo, T.; Shimizu, T.; Nakamura, M. *J. Biol. Chem.* **2008**, *283*, 12747.
(472) Milligan, G.; Bond, R. A.; Lee, M. *Trends Pharmacol. Sci.* **1995**, *16*, 10.
(473) Adan, R. A.; Kas, M. J. *Trends Pharmacol. Sci.* **2003**, *24*, 315.
(474) Rana, B. K. *Mol. Pharmacol.* **2003**, *64*, 1.
(475) Seifert, R.; Wenzel-Seifert, K. *Life Sci.* **2003**, *73*, 2263.
(476) Dupre, D. J.; Le Gouill, C.; Gingras, D.; Rola-Pleszczynski, M.; Stankova, J. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 102.
(477) Kim, S. H.; Oh, J. M.; Kim, Y. S.; Palmer, L. J.; Suh, C. H.; Nahm, D. H.; Park, H. S. *Clin. Exp. Allergy* **2006**, *36*, 433.
(478) Kim, S. H.; Yang, E. M.; Park, H. J.; Ye, Y. M.; Lee, H. Y.; Park, H. S. *J. Clin. Immunol.* **2007**, *27*, 613.
(479) Ohd, J. F.; Nielsen, C. K.; Campbell, J.; Landberg, G.; Lofberg, H.; Sjolander, A. *Gastroenterology* **2003**, *124*, 57.
(480) Chibana, K.; Ishii, Y.; Asakura, T.; Fukuda, T. *J. Immunol.* **2003**, *170*, 4290.
(481) Duroudier, N. P.; Strachan, D. P.; Blakey, J. D.; Hall, I. P. *J. Allergy Clin. Immunol.* **2009**, *124*, 566.
(482) Thompson, M. D.; Storm van's Gravesande, K.; Galczenski, H.; Burnham, W. M.; Siminovitch, K. A.; Zamel, N.; Slutsky, A.; Drazen, J. M.; George, S. R.; Evans, J. F.; O'Dowd, B. F. *Pharmacogenetics* **2003**, *13*, 641.
(483) Thompson, M. D.; Capra, V.; Takasaki, J.; Maresca, G.; Rovati, G. E.; Slutsky, A. S.; Lilly, C.; Zamel, N.; McIntyre Burnham, W.; Cole, D. E.; Siminovitch, K. A. *Pharmacogenet. Genomics* **2007**, *17*, 539.
(484) Arriba-Mendez, S.; Sanz, C.; Isidoro-Garcia, M.; Davild, I.; Laffond, E.; Horeno, E.; Avila, C.; Lorente, F. *Pediatr. Allergy Immunol.* **2006**, *17*, 323.
(485) Hao, L.; Sayers, I.; Cakebread, J. A.; Barton, S. J.; Beghe, B.; Holgate, S. T.; Sampson, A. P.; Holloway, J. W. *Clin. Exp. Allergy* **2006**, *36*, 735.
(486) Zhang, J.; Migita, O.; Koga, M.; Shibasaki, M.; Arinami, T.; Noguchi, E. *Pediatr. Allergy Immunol.* **2006**, *17*, 242.
(487) Tudhope, S. R.; Cuthbert, N. J.; Abram, T. S.; Jennings, M. A.; Maxey, R. J.; Thompson, A. M.; Norman, P.; Gardiner, P. J. *Eur. J. Pharmacol.* **1994**, *264*, 317.
(488) Nothacker, H. P.; Wang, Z.; Zhu, Y.; Reinscheid, R. K.; Lin, S. H.; Civelli, O. *Mol. Pharmacol.* **2000**, *58*, 1601.

(489) Hui, Y.; Yang, G.; Galczenski, H.; Figueroa, D. J.; Austin, C. P.; Copeland, N. G.; Gilbert, D. J.; Jenkins, N. A.; Funk, C. D. *J. Biol. Chem.* **2001**, *276*, 47489.
(490) Ogasawara, H.; Ishii, S.; Yokomizo, T.; Kakinuma, T.; Komine, M.; Tamaki, K.; Shimizu, T.; Izumi, T. *J. Biol. Chem.* **2002**, *277*, 18763.
(491) Ito, Y.; Hirano, M.; Umemoto, N.; Zang, L.; Wang, Z.; Oka, T.; Shimada, Y.; Nishimura, Y.; Kurokawa, I.; Mizutani, H.; Tanaka, T. *BMB Rep.* **2008**, *41*, 139.
(492) James, A. J.; Sampson, A. P. *Clin. Exp. Allergy* **2001**, *31*, 1660.
(493) Hishinuma, T.; Suzuki, N.; Aiba, S.; Tagami, H.; Mizugaki, M. *Br. J. Dermatol.* **2001**, *144*, 19.
(494) Goulet, J. L.; Byrum, R. S.; Key, M. L.; Nguyen, M.; Wagoner, V. A.; Koller, B. H. *J. Immunol.* **2000**, *164*, 4899.
(495) Phan, S. H.; McGarry, B. M.; Loeffler, K. M.; Kunkel, S. L. *Biochemistry* **1988**, *27*, 2846.
(496) Wunder, F.; Tinel, H.; Kast, R.; Geerts, A.; Becker, E. M.; Kolkhof, P.; Hutter, J.; Erguden, J.; Harter, M. *Br. J. Pharmacol.* **2010**, *160*, 399.
(497) Kamohara, M.; Takasaki, J.; Matsumoto, M.; Matsumoto, S.; Saito, T.; Soga, T.; Matsushime, H.; Furuichi, K. *Biochem. Biophys. Res. Commun.* **2001**, *287*, 1088.
(498) Daniels, S. E.; Bhattacharrya, S.; James, A.; Leaves, N. I.; Young, A.; Hill, M. R.; Faux, J. A.; Ryan, G. F.; le Souef, P. N.; Lathrop, G. M.; Musk, A. W.; Cookson, W. O. *Nature* **1996**, *383*, 247.
(499) Kimura, K.; Noguchi, E.; Shibasaki, M.; Arinami, T.; Yokouchi, Y.; Takeda, K.; Yamakawa-Kobayashi, K.; Matsui, A.; Hamaguchi, H. *Hum. Mol. Genet.* **1999**, *8*, 1487.
(500) Woszczek, G.; Chen, L. Y.; Nagineni, S.; Alsaaty, S.; Harry, A.; Logun, C.; Pawliczak, R.; Shelhamer, J. H. *J. Immunol.* **2007**, *178*, 5262.
(501) Copeland, N. G.; Jenkins, N. A. *Trends Genet.* **1991**, *7*, 113.
(502) Slutsky, A. S.; Zamel, N. *Clin. Exp. Allergy* **1995**, *25* (Suppl 2), 33.
(503) Zamel, N.; McClean, P. A.; Sandell, P. R.; Siminovitch, K. A.; Slutsky, A. S. *Am. J. Respir. Crit. Care Med.* **1996**, *153*, 1902.
(504) Slutsky, A. S.; Zamel, N. *Am. J. Respir. Crit. Care Med.* **1997**, *156*, S130.
(505) Upton, M. N.; McConnachie, A.; McSharry, C.; Hart, C. L.; Smith, G. D.; Gillis, C. R.; Watt, G. C. *BMJ [Br. Med. J.]* **2000**, *321*, 88.
(506) Milligan, G. *Mol. Pharmacol.* **2004**, *66*, 1.
(507) Maggio, R.; Novi, F.; Scarselli, M.; Corsini, G. U. *FEBS J.* **2005**, *272*, 2939.
(508) Bulenger, S.; Marullo, S.; Bouvier, M. *Trends Pharmacol. Sci.* **2005**, *26*, 131.
(509) Lohse, M. J. *Curr. Opin. Pharmacol.* **2010**, *10*, 53.
(510) Mellor, E. A.; Frank, N.; Soler, D.; Hodge, M. R.; Lora, J. M.; Austen, K. F.; Boyce, J. A. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 11589.
(511) Corrigan, C.; Mallett, K.; Ying, S.; Roberts, D.; Parikh, A.; Scadding, G.; Lee, T. *J. Allergy Clin. Immunol.* **2005**, *115*, 316.
(512) Jiang, Y.; Borrelli, L. A.; Kanaoka, Y.; Bacskai, B. J.; Boyce, J. A. *Blood* **2007**, *110*, 3263.
(513) Raport, C. J.; Schweickart, V. L.; Chantry, D.; Eddy, R. L., Jr.; Shows, T. B.; Godiska, R.; Gray, P. W. *J. Leukocyte Biol.* **1996**, *59*, 18.
(514) Blasius, R.; Weber, R. G.; Lichter, P.; Ogilvie, A. *J. Neurochem.* **1998**, *70*, 1357.
(515) Ciana, P.; Fumagalli, M.; Trincavelli, M. L.; Verderio, C.; Rosa, P.; Lecca, D.; Ferrario, S.; Parravicini, C.; Capra, V.; Gelosa, P.; Guerrini, U.; Belcredito, S.; Cimino, M.; Sironi, L.; Tremoli, E.; Rovati, G. E.; Martini, C.; Abbracchio, M. P. *EMBO J.* **2006**, *25*, 4615.
(516) Lecca, D.; Trincavelli, M. L.; Gelosa, P.; Sironi, L.; Ciana, P.; Fumagalli, M.; Villa, G.; Verderio, C.; Grumelli, C.; Guerrini, U.; Tremoli, E.; Rosa, P.; Cuboni, S.; Martini, C.; Buffo, A.; Cimino, M.; Abbracchio, M. P. *PLoS One* **2008**, *3*, e3579.
(517) Daniele, S.; Lecca, D.; Trincavelli, M. L.; Ciampi, O.; Abbracchio, M. P.; Martini, C. *Cell Signalling* **2010**, *22*, 697.
(518) Maekawa, A.; Balestrieri, B.; Austen, K. F.; Kanaoka, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 11685.
(519) Maekawa, A.; Xing, W.; Austen, K. F.; Kanaoka, Y. *J. Immunol.* **2010**, *185*, 1846.
(520) Bandeira-Melo, C.; Woods, L. J.; Phoofolo, M.; Weller, P. F. *J. Exp. Med.* **2002**, *196*, 841.
(521) Lu, D.; Yang, H.; Shaw, G.; Raizada, M. K. *Endocrinology* **1998**, *139*, 365.
(522) Lee, D. K.; Lanca, A. J.; Cheng, R.; Nguyen, T.; Ji, X. D.; Gobeil, F., Jr.; Chemtob, S.; George, S. R.; O'Dowd, B. F. *J. Biol. Chem.* **2004**, *279*, 7901.
(523) Boivin, B.; Vaniotis, G.; Allen, B. G.; Hebert, T. E. *J. Recept. Signal Transduction Res.* **2008**, *28*, 15.
(524) Gobeil, F., Jr.; Dumont, I.; Marrache, A. M.; Vazquez-Tello, A.; Bernier, S. G.; Abran, D.; Hou, X.; Beauchamp, M. H.; Quiniou, C.; Bouayad, A.; Choufani, S.; Bhattacharya, M.; Molotchnikoff, S.; Ribeiro-Da-Silva, A.; Varma, D. R.; Bkaily, G.; Chemtob, S. *Circ. Res.* **2002**, *90*, 682.
(525) Marrache, A. M.; Gobeil, F.; Zhu, T.; Chemtob, S. *Endothelium* **2005**, *12*, 63.
(526) Neves, J. S.; Radke, A. L.; Weller, P. F. *J. Allergy Clin. Immunol.* **2010**, *125*, 477.
(527) Jones, T. R.; Labelle, M.; Belley, M.; Champion, E.; Charette, L.; Evans, J.; Ford-Hutchinson, A. W.; Gauthier, J. Y.; Lord, A.; Masson, P.; McAuliffe, M.; McFarlane, C. S.; Metters, K. M.; Pickett, C.; Piechuta, H.; Rochette, C.; Rodger, I. W.; Sawyer, N.; Yoyng, R. N.; Zamboni, R.; Abraham, W. M. *Can. J. Physiol. Pharmacol.* **1995**, *73*, 191.
(528) Dekhuijzen, P. N.; Koopmans, P. P. *Clin. Pharmacokinet.* **2002**, *41*, 105.
(529) Spector, S. L.; Smith, L. J.; Glass, M. *Am. J. Respir. Crit. Care Med.* **1994**, *150*, 618.
(530) Aharony, D. *Am. J. Respir. Crit. Care Med.* **1998**, *157*, S214.
(531) Krell, R. D.; Aharony, D.; Buckner, C. K.; Keith, R. A.; Kusner, E. J.; Snyder, D. W.; Bernstein, P. R.; Matassa, V. G.; Yee, Y. K.; Brown, F. J. *Am. Rev. Respir. Dis.* **1990**, *141*, 978.
(532) Liu, Y. C.; Khawaja, A. M.; Rogers, D. F. *Br. J. Pharmacol.* **1998**, *124*, 563.
(533) Obata, T.; Okada, Y.; Motoishi, M.; Nakagawa, N.; Terawaki, T.; Aishita, H. *Jpn. J. Pharmacol.* **1992**, *60*, 227.
(534) Tamaoki, J.; Kondo, M.; Sakai, N.; Nakata, J.; Takemura, H.; Nagai, A.; Takizawa, T.; Konno, K. *Am. J. Respir. Crit. Care Med.* **1997**, *155*, 1235.
(535) Hay, D. W. *Chest* **1997**, *111*, 35S.
(536) Nakai, H.; Konno, M.; Kosuge, S.; Sakuyama, S.; Toda, M.; Arai, Y.; Obata, T.; Katsube, N.; Miyamoto, T.; Okegawa, T.; Kawasaki, A. *J. Med. Chem.* **1988**, *31*, 84.
(537) Barnes, N. C.; de Jong, B.; Miyamoto, T. *Chest.* **1997**, *111*, 52S.
(538) Ishii, A.; Nakagawa, T.; Nambu, F.; Motoishi, M.; Miyamoto, T. *Int. Arch. Allergy Appl. Immunol.* **1990**, *92*, 404.
(539) Obata, T.; Kobayashi, T.; Okada, Y.; Nakagawa, N.; Terawaki, T.; Aishita, H. *Life Sci.* **1992**, *51*, 1577.
(540) Yu, G. L.; Wei, E. Q.; Wang, M. L.; Zhang, W. P.; Zhang, S. H.; Weng, J. Q.; Chu, L. S.; Fang, S. H.; Zhou, Y.; Chen, Z.; Zhang, Q.; Zhang, L. H. *Brain Res.* **2005**, *1053*, 116.
(541) Chu, L. S.; Wei, E. Q.; Yu, G. L.; Fang, S. H.; Zhou, Y.; Wang, M. L.; Zhang, W. P. *Acta Pharmacol. Sin.* **2006**, *27*, 282.
(542) Qian, X. D.; Wei, E. Q.; Zhang, L.; Sheng, W. W.; Wang, M. L.; Zhang, W. P.; Chen, Z. *Eur. J. Pharmacol.* **2006**, *549*, 35.
(543) Hay, D. W.; Muccitelli, R. M.; Tucker, S. S.; Vickery-Clark, L. M.; Wilson, K. A.; Gleason, J. G.; Hall, R. F.; Wasserman, M. A.; Torphy, T. J. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 474.
(544) Mong, S.; Wu, H. L.; Miller, J.; Hall, R. F.; Gleason, J. G.; Crooke, S. T. *Mol. Pharmacol.* **1987**, *32*, 223.
(545) Smith, E. F., 3rd; Kinter, L. B.; Jugus, M.; Wasserman, M. A.; Eckardt, R. D.; Newton, J. F. *Circ. Shock* **1988**, *25*, 21.
(546) Osborn, R. R.; Hay, D. W.; Wasserman, M. A.; Torphy, T. J. *Pulm. Pharmacol.* **1992**, *5*, 153.
(547) Jones, T. R.; Zamboni, R.; Belley, M.; Champion, E.; Charette, L.; Ford-Hutchinson, A. W.; Frenette, R.; Gauthier, J. Y.; Leger, S.;

Masson, P.; McFarlane, C. S.; Piechuta, H.; Rokach, J.; Williams, H.; Young, R. N.; DeHaven, R. N.; Pong, S. S. *Can. J. Physiol. Pharmacol.* **1989**, *67*, 17.
(548) Gaddy, J. N.; Margolskee, D. J.; Bush, R. K.; Williams, V. C.; Busse, W. W. *Am. Rev. Respir. Dis.* **1992**, *146*, 358.
(549) Mitra, P.; Oskeritzian, C. A.; Payne, S. G.; Beaven, M. A.; Milstien, S.; Spiegel, S. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 16394.
(550) Mamedova, L.; Capra, V.; Accomazzo, M. R.; Gao, Z. G.; Ferrario, S.; Fumagalli, M.; Abbracchio, M. P.; Rovati, G. E.; Jacobson, K. A. *Biochem. Pharmacol.* **2005**, *71*, 115.
(551) Jonsson, E. W. *Pulm. Pharmacol. Ther.* **1997**, *10*, 29.
(552) Back, M.; Jonsson, E. W.; Dahlen, S. E. *Eur. J. Pharmacol.* **1996**, *317*, 107.
(553) Wikstrom Jonsson, E.; Rosenqvist, U.; Dahlen, S. E. *Eur. J. Pharmacol.* **1998**, *357*, 203.
(554) Snyder, D. W.; Giles, R. E.; Keith, R. A.; Yee, Y. K.; Krell, R. D. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 548.
(555) Aharony, D.; Falcone, R. C.; Krell, R. D. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 921.
(556) Krell, R. D.; Giles, R. E.; Yee, Y. K.; Snyder, D. W. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 557.
(557) Krell, R. D.; Kusner, E. J.; Aharony, D.; Giles, R. E. *Eur. J. Pharmacol.* **1989**, *159*, 73.
(558) Ferrari, F.; Mennuni, L.; Caselli, G.; Zanelli, T.; Makovec, F. *Eur. J. Pharmacol.* **2004**, *504*, 223.
(559) Adams, G. K., 3rd; Lichtenstein, L. *J. Immunol.* **1979**, *122*, 555.
(560) Chand, N. *Lung* **1979**, *156*, 271.
(561) Mitchell, H. W.; Denborough, M. A. *Eur. J. Pharmacol.* **1979**, *54*, 69.
(562) Herman, A. G.; Laekeman, G. M.; van Beek, H. A. *Br. J. Pharmacol.* **1979**, *66*, 385.
(563) Welton, A. F.; Crowley, H. J.; Miller, D. A.; Yaremko, B. *Prostaglandins* **1981**, *21*, 287.
(564) Farmer, J. B.; Richards, I. M.; Sheard, P.; Woods, A. M. *Br. J. Pharmacol.* **1975**, *55*, 57.
(565) Wanner, A.; Zarzecki, S.; Hirsch, J.; Epstein, S. *J. Appl. Physiol.* **1975**, *39*, 950.
(566) Ahmed, T.; Greenblatt, D. W.; Birch, S.; Marchette, B.; Wanner, A. *Am. Rev. Respir. Dis.* **1981**, *124*, 110.
(567) Dahlen, S. E.; Bjork, J.; Hedqvist, P.; Arfors, K. E.; Hammarstrom, S.; Lindgren, J. A.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1981**, *78*, 3887.
(568) Coles, S. J.; Neill, K. H.; Reid, L. M.; Austen, K. F.; Nii, Y.; Corey, E. J.; Lewis, R. A. *Prostaglandins* **1983**, *25*, 155.
(569) Laitinen, L. A.; Laitinen, A.; Haahtela, T.; Vilkka, V.; Spur, B. W.; Lee, T. H. *Lancet* **1993**, *341*, 989.
(570) Panettieri, R. A., Jr. *Am. J. Respir. Crit. Care Med.* **1998**, *158*, S133.
(571) Nicosia, S.; Capra, V.; Rovati, G. E. *Pulm. Pharmacol. Ther.* **2001**, *14*, 3.
(572) Arm, J. P. *Allergy Asthma Proc.* **2004**, *25*, 37.
(573) Ravasi, S.; Citro, S.; Viviani, B.; Capra, V.; Rovati, G. E. *Respir. Res.* **2006**, *7*, 42.
(574) Krell, R. D.; Tsai, B. S.; Berdoulay, A.; Barone, M.; Giles, R. E. *Prostaglandins* **1983**, *25*, 171.
(575) Buckner, C. K.; Fedyna, J. S.; Robertson, J. L.; Will, J. A.; England, D. M.; Krell, R. D.; Saban, R. *J. Pharmacol. Exp. Ther.* **1990**, *252*, 77.
(576) Dahlen, S. E.; Hedqvist, P.; Hammarstrom, S.; Samuelsson, B. *Nature* **1980**, *288*, 484.
(577) Buckner, C. K.; Krell, R. D.; Laravuso, R. B.; Coursin, D. B.; Bernstein, P. R.; Will, J. A. *J. Pharmacol. Exp. Ther.* **1986**, *237*, 558.
(578) DeLegge, M.; Murthy, K. S.; Grider, J. R.; Makhlouf, G. M. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 857.
(579) Bautz, F.; Denzlinger, C.; Kanz, L.; Mohle, R. *Blood* **2001**, *97*, 3433.
(580) Mita, H.; Hasegawa, M.; Saito, H.; Akiyama, K. *Clin. Exp. Allergy* **2001**, *31*, 1714.
(581) Figueroa, D. J.; Borish, L.; Baramki, D.; Philip, G.; Austin, C. P.; Evans, J. F. *Clin. Exp. Allergy* **2003**, *33*, 1380.
(582) Sousa, A. R.; Parikh, A.; Scadding, G.; Corrigan, C. J.; Lee, T. H. *N. Engl. J. Med.* **2002**, *347*, 1493.
(583) Smith, L. J.; Glass, M.; Minkwitz, M. C. *Clin. Pharmacol. Ther.* **1993**, *54*, 430.
(584) Foster, A.; Chan, C. C. *Int. Arch. Allergy Appl. Immunol.* **1991**, *96*, 279.
(585) Underwood, D. C.; Osborn, R. R.; Newsholme, S. J.; Torphy, T. J.; Hay, D. W. *Am. J. Respir. Crit. Care Med.* **1996**, *154*, 850.
(586) Spada, C. S.; Nieves, A. L.; Krauss, A. H.; Woodward, D. F. *J. Leukocyte Biol.* **1994**, *55*, 183.
(587) Salmon, M.; Walsh, D. A.; Huang, T. J.; Barnes, P. J.; Leonard, T. B.; Hay, D. W.; Chung, K. F. *Br. J. Pharmacol.* **1999**, *127*, 1151.
(588) Turner, C. R.; Smith, W. B.; Andresen, C. J.; Swindell, A. C.; Watson, J. W. *Pulm. Pharmacol.* **1994**, *7*, 49.
(589) Fregonese, L.; Silvestri, M.; Sabatini, F.; Rossi, G. A. *Clin. Exp. Allergy* **2002**, *32*, 745.
(590) Wijnholds, J.; Evers, R.; van Leusden, M. R.; Mol, C. A.; Zaman, G. J.; Mayer, U.; Beijnen, J. H.; van der Valk, M.; Krimpenfort, P.; Borst, P. *Nat. Med.* **1997**, *3*, 1275.
(591) Mellor, E. A.; Maekawa, A.; Austen, K. F.; Boyce, J. A. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 7964.
(592) Mellor, E. A.; Austen, K. F.; Boyce, J. A. *J. Exp. Med.* **2002**, *195*, 583.
(593) Henderson, W. R., Jr.; Lewis, D. B.; Albert, R. K.; Zhang, Y.; Lamm, W. J.; Chiang, G. K.; Jones, F.; Eriksen, P.; Tien, Y. T.; Jonas, M.; Chi, E. Y. *J. Exp. Med.* **1996**, *184*, 1483.
(594) Espinosa, K.; Bosse, Y.; Stankova, J.; Rola-Pleszczynski, M. *J. Allergy Clin. Immunol.* **2003**, *111*, 1032.
(595) Panettieri, R. A.; Tan, E. M.; Ciocca, V.; Luttmann, M. A.; Leonard, T. B.; Hay, D. W. *Am. J. Respir. Cell Mol. Biol.* **1998**, *19*, 453.
(596) Evans, T. W.; Rogers, D. F.; Aursudkij, B.; Chung, K. F.; Barnes, P. J. *Clin. Sci. (London)* **1989**, *76*, 479.
(597) Bochnowicz, S.; Underwood, D. C. *Prostaglandins, Leukotrienes, Essent. Fatty Acids* **1995**, *52*, 403.
(598) Joris, I.; Majno, G.; Corey, E. J.; Lewis, R. A. *Am. J. Pathol.* **1987**, *126*, 19.
(599) Woodward, D. F.; Wasserman, M. A.; Weichman, B. M. *Eur. J. Pharmacol.* **1983**, *93*, 9.
(600) Nakagawa, N.; Obata, T.; Kobayashi, T.; Okada, Y.; Nambu, F.; Terawaki, T.; Aishita, H. *Jpn. J. Pharmacol.* **1992**, *60*, 217.
(601) Beller, T. C.; Maekawa, A.; Friend, D. S.; Austen, K. F.; Kanaoka, Y. *J. Biol. Chem.* **2004**, *279*, 46129.
(602) Moos, M. P.; Mewburn, J. D.; Kan, F. W.; Ishii, S.; Abe, M.; Sakimura, K.; Noguchi, K.; Shimizu, T.; Funk, C. D. *FASEB J.* **2008**, *22*, 4352.
(603) Nagase, T.; Uozumi, N.; Ishii, S.; Kita, Y.; Yamamoto, H.; Ohga, E.; Ouchi, Y.; Shimizu, T. *Nat. Med.* **2002**, *8*, 480.
(604) Peters-Golden, M.; Bailie, M.; Marshall, T.; Wilke, C.; Phan, S. H.; Toews, G. B.; Moore, B. B. *Am. J. Respir. Crit. Care Med.* **2002**, *165*, 229.
(605) Beller, T. C.; Friend, D. S.; Maekawa, A.; Lam, B. K.; Austen, K. F.; Kanaoka, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 3047.
(606) Levi, R.; Burke, J. A. *Eur. J. Pharmacol.* **1980**, *62*, 41.
(607) Michelassi, F.; Landa, L.; Hill, R. D.; Lowenstein, E.; Watkins, W. D.; Petkau, A. J.; Zapol, W. M. *Science* **1982**, *217*, 841.
(608) Fiedler, V. B.; Mardin, M.; Gardiner, P. J.; Abram, T. S. *Int. J. Cardiol.* **1985**, *8*, 451.
(609) Letts, L. G.; Piper, P. J. *Adv. Prostaglandin, Thromboxane, Leukotriene Res.* **1983**, *11*, 391.
(610) Roth, D. M.; Lefer, D. J.; Hock, C. E.; Lefer, A. M. *Am. J. Physiol.* **1985**, *249*, H477.
(611) Burke, J. A.; Levi, R.; Guo, Z. G.; Corey, E. J. *J. Pharmacol. Exp. Ther.* **1982**, *221*, 235.
(612) Bittl, J. A.; Pfeffer, M. A.; Lewis, R. A.; Mehrotra, M. M.; Corey, E. J.; Austen, K. F. *Cardiovasc. Res.* **1985**, *19*, 426.

(613) Zukowska-Grojec, Z.; Pamnani, M.; Feuerstein, G. *Clin. Exp. Hypertens. A* **1984**, *6*, 1575.
(614) Tomoike, H.; Egashira, K.; Yamada, A.; Hayashi, Y.; Nakamura, M. *Circulation* **1987**, *76*, 480.
(615) McLeod, J. D.; Piper, P. J. *Prostaglandins* **1991**, *41*, 395.
(616) Egan, J. W.; Griswold, D. E.; Hillegass, L. M.; Newton, J. F.; Eckardt, R. D.; Slivjak, M. J.; Smith, E. F., 3rd *Prostaglandins* **1989**, *37*, 597.
(617) Marone, G.; Giordano, A.; Cirillo, R.; Triggiani, M.; Vigorito, C. *Ann. N.Y. Acad. Sci.* **1988**, *524*, 321.
(618) Albazzaz, M. K.; Patel, K. R.; Shakir, S.; Dargie, H. J.; Reid, J. M. *Am. Rev. Respir. Dis.* **1989**, *139*, 188.
(619) Marom, Z.; Shelhamer, J. H.; Bach, M. K.; Morton, D. R.; Kaliner, M. *Am. Rev. Respir. Dis.* **1982**, *126*, 449.
(620) Hoffstein, S. T.; Malo, P. E.; Bugelski, P.; Wheeldon, E. B. *Exp. Lung Res.* **1990**, *16*, 711.
(621) Hasegawa, S.; Ichiyama, T.; Hashimoto, K.; Suzuki, Y.; Hirano, R.; Fukano, R.; Furukawa, S. *Platelets* **2010**, *21*, 253.
(622) Wang, C. G.; Du, T.; Xu, L. J.; Martin, J. G. *Am. Rev. Respir. Dis.* **1993**, *148*, 413.
(623) Undem, B. J.; Weinreich, D. *J. Auton. Nerv. Syst.* **1993**, *44*, 17.
(624) Undem, B. J.; Hubbard, W.; Weinreich, D. *J. Auton. Nerv. Syst.* **1993**, *44*, 35.
(625) Bloomquist, E. I.; Kream, R. M. *Exp. Lung Res.* **1990**, *16*, 645.
(626) Ellis, J. L.; Undem, B. J. *J. Physiol.* **1991**, *436*, 469.
(627) Tsopanoglou, N. E.; Pipili-Synetos, E.; Maragoudakis, M. E. *Eur. J. Pharmacol.* **1994**, *258*, 151.
(628) Filep, J.; Rigter, B.; Frolich, J. C. *Am. J. Physiol.* **1985**, *249*, F739.
(629) Piper, P. J.; Stanton, A. W.; McLeod, L. J. *Prostaglandins* **1985**, *29*, 61.
(630) Petric, R.; Ford-Hutchinson, A. *J. Lipid Mediators Cell Signalling* **1995**, *11*, 231.
(631) Garcia, J. G.; Noonan, T. C.; Jubiz, W.; Malik, A. B. *Am. Rev. Respir. Dis.* **1987**, *136*, 161.
(632) Ohtaka, H.; Tsang, J. Y.; Foster, A.; Hogg, J. C.; Schellenberg, R. R. *J. Appl. Physiol.* **1987**, *63*, 582.
(633) Schreiber, M. D.; Heymann, M. A.; Soifer, S. J. *Pediatr. Res.* **1987**, *21*, 176.
(634) Pfeffer, M. A.; Pfeffer, J. M.; Lewis, R. A.; Braunwald, E.; Corey, E. J.; Austen, K. F. *Am. J. Physiol.* **1983**, *244*, H628.
(635) Bisgaard, H.; Olsson, P.; Bende, M. *Clin. Allergy* **1986**, *16*, 289.
(636) Chan, C. C.; Ford-Hutchinson, A. *J. Invest. Dermatol.* **1985**, *84*, 154.
(637) Hua, X. Y.; Dahlen, S. E.; Lundberg, J. M.; Hammarstrom, S.; Hedqvist, P. *Naunyn-Schmiedebergs Arch. Pharmakol.* **1985**, *330*, 136.
(638) Snyder, D. W.; Krell, R. D. *J. Pharmacol. Exp. Ther.* **1984**, *231*, 616.
(639) Gardiner, P. J.; Norman, P.; Cuthbert, N. J.; Tudhope, S. R.; Abram, T. S. *Eur. J. Pharmacol.* **1993**, *238*, 19.
(640) Sakata, K.; Back, M. *Eur. J. Pharmacol.* **2002**, *436*, 119.
(641) Back, M.; Norel, X.; Walch, L.; Gascard, J.; Mazmanian, G.; Dahlen, S.; Brink, C. *Eur. J. Pharmacol.* **2000**, *401*, 381.
(642) Back, M.; Norel, X.; Walch, L.; Gascard, J.; de Montpreville, V.; Dahlen, S.; Brink, C. *Eur. J. Pharmacol.* **2000**, *401*, 389.
(643) Capra, V.; Bolla, M.; Belloni, P. A.; Mezzetti, M.; Folco, G. C.; Nicosia, S.; Rovati, G. E. *Br. J. Pharmacol.* **1998**, *123*, 590.
(644) Ravasi, S.; Capra, V.; Panigalli, T.; Rovati, G. E.; Nicosia, S. *Biochem. Pharmacol.* **2002**, *63*, 1537.
(645) Maekawa, A.; Kanaoka, Y.; Xing, W.; Austen, K. F. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 16695.
(646) Paruchuri, S.; Jiang, Y.; Feng, C.; Francis, S. A.; Plutzky, J.; Boyce, J. A. *J. Biol. Chem.* **2008**, *283*, 16477.
(647) Paruchuri, S.; Tashimo, H.; Feng, C.; Maekawa, A.; Xing, W.; Jiang, Y.; Kanaoka, Y.; Conley, P.; Boyce, J. A. *J. Exp. Med.* **2009**, *206*, 2543.
(648) Gauvreau, G. M.; Parameswaran, K. N.; Watson, R. M.; O'Byrne, P. M. *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 1495.
(649) Lewis, R. A.; Austen, K. F.; Soberman, R. J. *N. Engl. J. Med.* **1990**, *323*, 645.
(650) Barnes, N. C.; Piper, P. J.; Costello, J. F. *Thorax* **1984**, *39*, 500.
(651) Adelroth, E.; Morris, M. M.; Hargreave, F. E.; O'Byrne, P. M. *N. Engl. J. Med.* **1986**, *315*, 480.
(652) Green, S. A.; Malice, M. P.; Tanaka, W.; Tozzi, C. A.; Reiss, T. F. *Thorax* **2004**, *59*, 100.
(653) Mastalerz, L.; Gawlewicz-Mroczka, A.; Nizankowska, E.; Cmiel, A.; Szczeklik, A. *Clin. Exp. Allergy* **2002**, *32*, 1360.
(654) Kumlin, M.; Dahlen, B. *Clin. Exp. Allergy* **2000**, *30*, 585.
(655) Mastalerz, L.; Setkowicz, M.; Sanak, M.; Szczeklik, A. *J. Allergy Clin. Immunol.* **2004**, *113*, 771.
(656) Lipworth, B. J. *Lancet* **1999**, *353*, 57.
(657) Drazen, J. M.; Israel, E.; O'Byrne, P. M. *N. Engl. J. Med.* **1999**, *340*, 197.
(658) Carucci, J. A.; Washenik, K.; Weinstein, A.; Shupack, J.; Cohen, D. E. *Arch. Dermatol.* **1998**, *134*, 785.
(659) Yanase, D. J.; David-Bajar, K. *J. Am. Acad. Dermatol.* **2001**, *44*, 89.
(660) Silverberg, N. B.; Paller, A. S. *J. Am. Acad. Dermatol.* **2004**, *50*, 485.
(661) Wei, C.; Zhu, P.; Shah, S. J.; Blair, I. A. *Mol. Pharmacol.* **2009**, *76*, 516.
(662) Serhan, C. N.; Yang, R.; Martinod, K.; Kasuga, K.; Pillai, P. S.; Porter, T. F.; Oh, S. F.; Spite, M. *J. Exp. Med.* **2009**, *206*, 15.
(663) O'Flaherty, J. T.; Jacobson, D.; Redman, J. *J. Immunol.* **1988**, *140*, 4323.
(664) Powell, W. S.; Gravel, S.; MacLeod, R. J.; Mills, E.; Hashefi, M. *J. Biol. Chem.* **1993**, *268*, 9280.
(665) Powell, W. S.; Rokach, J. *Prog. Lipid Res.* **2005**, *44*, 154.
(666) Powell, W. S.; Gravelle, F.; Gravel, S. *J. Biol. Chem.* **1992**, *267*, 19233.
(667) Zhang, Y.; Styhler, A.; Powell, W. S. *J. Leukocyte Biol.* **1996**, *59*, 847.
(668) Zimpfer, U.; Dichmann, S.; Termeer, C. C.; Simon, J. C.; Schroder, J. M.; Norgauer, J. *Inflammation Res.* **2000**, *49*, 633.
(669) Powell, W. S.; Gravel, S.; Khanapure, S. P.; Rokach, J. *Blood* **1999**, *93*, 1086.
(670) Erlemann, K. R.; Cossette, C.; Gravel, S.; Stamatiou, P. B.; Lee, G. J.; Rokach, J.; Powell, W. S. *Biochem. Biophys. Res. Commun.* **2006**, *350*, 151.
(671) Erlemann, K. R.; Cossette, C.; Gravel, S.; Lesimple, A.; Lee, G. J.; Saha, G.; Rokach, J.; Powell, W. S. *Free Radical Biol. Med.* **2007**, *42*, 654.
(672) Folco, G.; Murphy, R. C. *Pharmacol. Rev.* **2006**, *58*, 375.
(673) Powell, W. S.; MacLeod, R. J.; Gravel, S.; Gravelle, F.; Bhakar, A. *J. Immunol.* **1996**, *156*, 336.
(674) Powell, W. S.; Gravelle, F.; Gravel, S. *J. Biol. Chem.* **1994**, *269*, 25373.
(675) Erlemann, K. R.; Rokach, J.; Powell, W. S. *J. Biol. Chem.* **2004**, *279*, 40376.
(676) Cossette, C.; Patel, P.; Anumolu, J. R.; Sivendran, S.; Lee, G. J.; Gravel, S.; Graham, F. D.; Lesimple, A.; Mamer, O. A.; Rokach, J.; Powell, W. S. *J. Biol. Chem.* **2008**, *283*, 11234.
(677) Patel, P.; Cossette, C.; Anumolu, J. R.; Gravel, S.; Lesimple, A.; Mamer, O. A.; Rokach, J.; Powell, W. S. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 698.
(678) Powell, W. S.; Gravel, S.; Gravelle, F. *J. Lipid. Res.* **1995**, *36*, 2590.
(679) Hevko, J. M.; Bowers, R. C.; Murphy, R. C. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 293.
(680) Hevko, J. M.; Murphy, R. C. *J. Biol. Chem.* **2002**, *277*, 7037.
(681) Schwenk, U.; Schroder, J. M. *J. Biol. Chem.* **1995**, *270*, 15029.
(682) Erlemann, K. R.; Cossette, C.; Grant, G. E.; Lee, G. J.; Patel, P.; Rokach, J.; Powell, W. S. *Biochem. J.* **2007**, *403*, 157.
(683) Berhane, K.; Ray, A. A.; Khanapure, S. P.; Rokach, J.; Powell, W. S. *J. Biol. Chem.* **1998**, *273*, 20951.

(684) Bowers, R. C.; Hevko, J.; Henson, P. M.; Murphy, R. C. *J. Biol. Chem.* **2000**, *275*, 29931.
(685) Powell, W. S.; Gravel, S.; Halwani, F.; Hii, C. S.; Huang, Z. H.; Tan, A. M.; Ferrante, A. *J. Immunol.* **1997**, *159*, 2952.
(686) O'Flaherty, J. T.; Taylor, J. S.; Thomas, M. J. *J. Biol. Chem.* **1998**, *273*, 32535.
(687) Hosoi, T.; Koguchi, Y.; Sugikawa, E.; Chikada, A.; Ogawa, K.; Tsuda, N.; Suto, N.; Tsunoda, S.; Taniguchi, T.; Ohnuki, T. *J. Biol. Chem.* **2002**, *277*, 31459.
(688) Jones, C. E.; Holden, S.; Tenaillon, L.; Bhatia, U.; Seuwen, K.; Tranter, P.; Turner, J.; Kettle, R.; Bouhelal, R.; Charlton, S.; Nirmala, N. R.; Jarai, G.; Finan, P. *Mol. Pharmacol.* **2003**, *63*, 471.
(689) Koike, D.; Obinata, H.; Yamamoto, A.; Takeda, S.; Komori, H.; Nara, F.; Izumi, T.; Haga, T. *J. Biochem.* **2006**, *139*, 543.
(690) Brink, C.; Dahlen, S. E.; Drazen, J.; Evans, J. F.; Hay, D. W.; Rovati, G. E.; Serhan, C. N.; Shimizu, T.; Yokomizo, T. *Pharmacol. Rev.* **2004**, *56*, 149.
(691) Wise, A.; Foord, S. M.; Fraser, N. J.; Barnes, A. A.; Elshourbagy, N.; Eilert, M.; Ignar, D. M.; Murdock, P. R.; Steplewski, K.; Green, A.; Brown, A. J.; Dowell, S. J.; Szekeres, P. G.; Hassall, D. G.; Marshall, F. H.; Wilson, S.; Pike, N. B. *J. Biol. Chem.* **2003**, *278*, 9869.
(692) Cai, T. Q.; Ren, N.; Jin, L.; Cheng, K.; Kash, S.; Chen, R.; Wright, S. D.; Taggart, A. K.; Waters, M. G. *Biochem. Biophys. Res. Commun.* **2008**, *377*, 987.
(693) O'Flaherty, J. T.; Cordes, J. F.; Lee, S. L.; Samuel, M.; Thomas, M. J. *Biochim. Biophys. Acta* **1994**, *1201*, 505.
(694) O'Flaherty, J. T.; Cordes, J.; Redman, J.; Thomas, M. J. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 129.
(695) O'Flaherty, J. T.; Kuroki, M.; Nixon, A. B.; Wijkander, J.; Yee, E.; Lee, S. L.; Smitherman, P. K.; Wykle, R. L.; Daniel, L. W. *J. Biol. Chem.* **1996**, *271*, 17821.
(696) O'Flaherty, J. T.; Kuroki, M.; Nixon, A. B.; Wijkander, J.; Yee, E.; Lee, S. L.; Smitherman, P. K.; Wykle, R. L.; Daniel, L. W. *J. Immunol.* **1996**, *157*, 336.
(697) Norgauer, J.; Barbisch, M.; Czech, W.; Pareigis, J.; Schwenk, U.; Schroder, J. M. *Eur. J. Biochem.* **1996**, *236*, 1003.
(698) Hosoi, T.; Sugikawa, E.; Chikada, A.; Koguchi, Y.; Ohnuki, T. *Biochem. Biophys. Res. Commun.* **2005**, *334*, 987.
(699) Langlois, A.; Chouinard, F.; Flamand, N.; Ferland, C.; Rola-Pleszczynski, M.; Laviolette, M. *J. Leukocyte Biol.* **2009**, *85*, 656.
(700) Lin, L. L.; Wartmann, M.; Lin, A. Y.; Knopf, J. L.; Seth, A.; Davis, R. J. *Cell* **1993**, *72*, 269.
(701) Sundaram, S.; Ghosh, J. *Biochem. Biophys. Res. Commun.* **2006**, *339*, 93.
(702) Ghosh, J.; Myers, C. E. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 13182.
(703) Sturm, G. J.; Schuligoi, R.; Sturm, E. M.; Royer, J. F.; Lang-Loidolt, D.; Stammberger, H.; Amann, R.; Peskar, B. A.; Heinemann, A. *J. Allergy Clin. Immunol.* **2005**, *116*, 1014.
(704) Powell, W. S.; Chung, D.; Gravel, S. *J. Immunol.* **1995**, *154*, 4123.
(705) Leckie, M. J.; ten Brinke, A.; Khan, J.; Diamant, Z.; O'Connor, B. J.; Walls, C. M.; Mathur, A. K.; Cowley, H. C.; Chung, K. F.; Djukanovic, R.; Hansel, T. T.; Holgate, S. T.; Sterk, P. J.; Barnes, P. J. *Lancet* **2000**, *356*, 2144.
(706) Kay, A. B.; Phipps, S.; Robinson, D. S. *Trends Immunol.* **2004**, *25*, 477.
(707) Flood-Page, P. T.; Menzies-Gow, A. N.; Kay, A. B.; Robinson, D. S. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 199.
(708) Humbles, A. A.; Lloyd, C. M.; McMillan, S. J.; Friend, D. S.; Xanthou, G.; McKenna, E. E.; Ghiran, S.; Gerard, N. P.; Yu, C.; Orkin, S. H.; Gerard, C. *Science* **2004**, *305*, 1776.
(709) Radinger, M.; Lotvall, J. *Pharmacol. Ther.* **2009**, *121*, 174.
(710) Guilbert, M.; Ferland, C.; Bosse, M.; Flamand, N.; Lavigne, S.; Laviolette, M. *Am. J. Respir. Cell Mol. Biol.* **1999**, *21*, 97.
(711) Powell, W. S.; Ahmed, S.; Gravel, S.; Rokach, J. *J. Allergy Clin. Immunol.* **2001**, *107*, 272.
(712) Dallaire, M. J.; Ferland, C.; Page, N.; Lavigne, S.; Davoine, F.; Laviolette, M. *Eur. Respir. J.* **2003**, *21*, 918.
(713) Stamatiou, P. B.; Chan, C. C.; Monneret, G.; Ethier, D.; Rokach, J.; Powell, W. S. *J. Biol. Chem.* **2004**, *279*, 28159.
(714) Gibbs, B. F. *Clin. Exp. Med.* **2005**, *5*, 43.
(715) Monneret, G.; Boumiza, R.; Gravel, S.; Cossette, C.; Bienvenu, J.; Rokach, J.; Powell, W. S. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 627.
(716) Iikura, M.; Suzukawa, M.; Yamaguchi, M.; Sekiya, T.; Komiya, A.; Yoshimura-Uchiyama, C.; Nagase, H.; Matsushima, K.; Yamamoto, K.; Hirai, K. *J. Allergy Clin. Immunol.* **2005**, *116*, 578.
(717) Ghosh, J.; Myers, C. E. *Biochem. Biophys. Res. Commun.* **1997**, *235*, 418.
(718) Gupta, S.; Srivastava, M.; Ahmad, N.; Sakamoto, K.; Bostwick, D. G.; Mukhtar, H. *Cancer* **2001**, *91*, 737.
(719) Rioux, N.; Castonguay, A. *Carcinogenesis* **1998**, *19*, 1393.
(720) Moody, T. W.; Leyton, J.; Martinez, A.; Hong, S.; Malkinson, A.; Mulshine, J. L. *Exp. Lung Res.* **1998**, *24*, 617.
(721) Avis, I.; Hong, S. H.; Martinez, A.; Moody, T.; Choi, Y. H.; Trepel, J.; Das, R.; Jett, M.; Mulshine, J. L. *FASEB J.* **2001**, *15*, 2007.
(722) Edderkaoui, M.; Hong, P.; Vaquero, E. C.; Lee, J. K.; Fischer, L.; Friess, H.; Buchler, M. W.; Lerch, M. M.; Pandol, S. J.; Gukovskaya, A. S. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2005**, *289*, G1137.
(723) Hammamieh, R.; Sumaida, D.; Zhang, X.; Das, R.; Jett, M. *BMC Cancer* **2007**, *7*, 138.
(724) Ghosh, J. *Biochem. Biophys. Res. Commun.* **2004**, *315*, 624.
(725) O'Flaherty, J. T.; Rogers, L. C.; Paumi, C. M.; Hantgan, R. R.; Thomas, L. R.; Clay, C. E.; High, K.; Chen, Y. Q.; Willingham, M. C.; Smitherman, P. K.; Kute, T. E.; Rao, A.; Cramer, S. D.; Morrow, C. S. *Biochim. Biophys. Acta* **2005**, *1736*, 228.
(726) Helgadottir, A.; Manolescu, A.; Thorleifsson, G.; Gretarsdottir, S.; Jonsdottir, H.; Thorsteinsdottir, U.; Samani, N. J.; Gudmundsson, G.; Grant, S. F.; Thorgeirsson, G.; Sveinbjornsdottir, S.; Valdimarsson, E. M.; Matthiasson, S. E.; Johannsson, H.; Gudmundsdottir, O.; Gurney, M. E.; Sainz, J.; Thorhallsdottir, M.; Andresdottir, M.; Frigge, M. L.; Topol, E. J.; Kong, A.; Gudnason, V.; Hakonarson, H.; Gulcher, J. R.; Stefansson, K. *Nat. Genet.* **2004**, *36*, 233.
(727) Mestas, J.; Ley, K. *Trends Cardiovasc. Med.* **2008**, *18*, 228.
(728) Hoogeveen, R. C.; Morrison, A.; Boerwinkle, E.; Miles, J. S.; Rhodes, C. E.; Sharrett, A. R.; Ballantyne, C. M. *Atherosclerosis* **2005**, *183*, 301.
(729) Matsushima, K.; Morishita, K.; Yoshimura, T.; Lavu, S.; Kobayashi, Y.; Lew, W.; Appella, E.; Kung, H. F.; Leonard, E. J.; Oppenheim, J. J. *J. Exp. Med.* **1988**, *167*, 1883.
(730) Pease, J. E.; Williams, T. J. *Curr. Opin. Pharmacol.* **2001**, *1*, 248.
(731) Stamatiou, P.; Hamid, Q.; Taha, R.; Yu, W.; Issekutz, T. B.; Rokach, J.; Khanapure, S. P.; Powell, W. S. *J. Clin. Invest.* **1998**, *102*, 2165.
(732) Muro, S.; Hamid, Q.; Olivenstein, R.; Taha, R.; Rokach, J.; Powell, W. S. *J. Allergy Clin. Immunol.* **2003**, *112*, 768.
(733) Serhan, C. N.; Hamberg, M.; Samuelsson, B. *Proc. Natl. Acad. Sci. U. S. A.* **1984**, *81*, 5335.
(734) Chiang, N.; Arita, M.; Serhan, C. N. *Prostaglandins, Leukotrienes, Essent. Fatty Acids* **2005**, *73*, 163.
(735) Fierro, I. M.; Serhan, C. N. *Braz. J. Med. Biol. Res.* **2001**, *34*, 555.
(736) Claria, J.; Serhan, C. N. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 9475.
(737) Claria, J.; Lee, M. H.; Serhan, C. N. *Mol. Med.* **1996**, *2*, 583.
(738) Schwab, J. M.; Serhan, C. N. *Curr. Opin. Pharmacol.* **2006**, *6*, 414.
(739) Mukherjee, P. K.; Marcheselli, V. L.; Serhan, C. N.; Bazan, N. G. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 8491.
(740) Ariel, A.; Serhan, C. N. *Trends Immunol.* **2007**, *28*, 176.
(741) Schwab, J. M.; Chiang, N.; Arita, M.; Serhan, C. N. *Nature* **2007**, *447*, 869.
(742) Fiore, S.; Ryeom, S. W.; Weller, P. F.; Serhan, C. N. *J. Biol. Chem.* **1992**, *267*, 16168.
(743) Chiang, N.; Fierro, I. M.; Gronert, K.; Serhan, C. N. *J. Exp. Med.* **2000**, *191*, 1197.
(744) Maddox, J. F.; Colgan, S. P.; Clish, C. B.; Petasis, N. A.; Fokin, V. V.; Serhan, C. N. *FASEB J.* **1998**, *12*, 487.

(745) Fiore, S.; Maddox, J. F.; Perez, H. D.; Serhan, C. N. *J. Exp. Med.* **1994**, *180*, 253.
(746) Murphy, P. M.; Ozcelik, T.; Kenney, R. T.; Tiffany, H. L.; McDermott, D.; Francke, U. *J. Biol. Chem.* **1992**, *267*, 7637.
(747) Bao, L.; Gerard, N. P.; Eddy, R. L., Jr.; Shows, T. B.; Gerard, C. *Genomics* **1992**, *13*, 437.
(748) Ye, R. D.; Cavanagh, S. L.; Quehenberger, O.; Prossnitz, E. R.; Cochrane, C. G. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 582.
(749) Perez, H. D.; Holmes, R.; Kelly, E.; McClary, J.; Andrews, W. H. *Gene* **1992**, *118*, 303.
(750) Nomura, H.; Nielsen, B. W.; Matsushima, K. *Int. Immunol.* **1993**, *5*, 1239.
(751) Takano, T.; Fiore, S.; Maddox, J. F.; Brady, H. R.; Petasis, N. A.; Serhan, C. N. *J. Exp. Med.* **1997**, *185*, 1693.
(752) Chiang, N.; Takano, T.; Arita, M.; Watanabe, S.; Serhan, C. N. *Br. J. Pharmacol.* **2003**, *139*, 89.
(753) Maddox, J. F.; Hachicha, M.; Takano, T.; Petasis, N. A.; Fokin, V. V.; Serhan, C. N. *J. Biol. Chem.* **1997**, *272*, 6972.
(754) Ariel, A.; Chiang, N.; Arita, M.; Petasis, N. A.; Serhan, C. N. *J. Immunol.* **2003**, *170*, 6266.
(755) Sodin-Semrl, S.; Taddeo, B.; Tseng, D.; Varga, J.; Fiore, S. *J. Immunol.* **2000**, *164*, 2660.
(756) Gronert, K.; Gewirtz, A.; Madara, J. L.; Serhan, C. N. *J. Exp. Med.* **1998**, *187*, 1285.
(757) Gao, J. L.; Murphy, P. M. *J. Biol. Chem.* **1993**, *268*, 25395.
(758) Gao, J. L.; Chen, H.; Filie, J. D.; Kozak, C. A.; Murphy, P. M. *Genomics* **1998**, *51*, 270.
(759) Hartt, J. K.; Barish, G.; Murphy, P. M.; Gao, J. L. *J. Exp. Med.* **1999**, *190*, 741.
(760) Fiore, S.; Serhan, C. N. *Biochemistry* **1995**, *34*, 16678.
(761) Rabiet, M. J.; Huet, E.; Boulay, F. *Eur. J. Immunol.* **2005**, *35*, 2486.
(762) Lee, T. H.; Horton, C. E.; Kyan-Aung, U.; Haskard, D.; Crea, A. E.; Spur, B. W. *Clin. Sci. (London)* **1989**, *77*, 195.
(763) Bandeira-Melo, C.; Bozza, P. T.; Diaz, B. L.; Cordeiro, R. S.; Jose, P. J.; Martins, M. A.; Serhan, C. N. *J. Immunol.* **2000**, *164*, 2267.
(764) Ramstedt, U.; Serhan, C. N.; Nicolaou, K. C.; Webber, S. E.; Wigzell, H.; Samuelsson, B. *J. Immunol.* **1987**, *138*, 266.
(765) Godson, C.; Mitchell, S.; Harvey, K.; Petasis, N. A.; Hogg, N.; Brady, H. R. *J. Immunol.* **2000**, *164*, 1663.
(766) Romano, M.; Maddox, J. F.; Serhan, C. N. *J. Immunol.* **1996**, *157*, 2149.
(767) Kenakin, T. *Nat. Rev. Drug. Discovery* **2002**, *1*, 103.
(768) Kenakin, T. *Trends Pharmacol. Sci.* **1997**, *18*, 456.
(769) Chiang, N.; Serhan, C. N.; Dahlen, S. E.; Drazen, J. M.; Hay, D. W.; Rovati, G. E.; Shimizu, T.; Yokomizo, T.; Brink, C. *Pharmacol. Rev.* **2006**, *58*, 463.
(770) Clish, C. B.; O'Brien, J. A.; Gronert, K.; Stahl, G. L.; Petasis, N. A.; Serhan, C. N. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 8247.
(771) Phillips, E. D.; Chang, H. F.; Holmquist, C. R.; McCauley, J. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3223.
(772) Wu, S. H.; Wu, X. H.; Liao, P. Y.; Dong, L. *Prostaglandins, Leukotrienes, Essent. Fatty Acids* **2007**, *76*, 173.
(773) Guilford, W. J.; Parkinson, J. F. *Prostaglandins, Leukotrienes, Essent. Fatty Acids* **2005**, *73*, 245.
(774) Petasis, N. A.; Keledjian, R.; Sun, Y. P.; Nagulapalli, K. C.; Tjonahen, E.; Yang, R.; Serhan, C. N. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1382.
(775) Baek, S. H.; Seo, J. K.; Chae, C. B.; Suh, P. G.; Ryu, S. H. *J. Biol. Chem.* **1996**, *271*, 8170.
(776) Le, Y.; Gong, W.; Li, B.; Dunlop, N. M.; Shen, W.; Su, S. B.; Ye, R. D.; Wang, J. M. *J. Immunol.* **1999**, *163*, 6777.
(777) Christophe, T.; Karlsson, A.; Dugave, C.; Rabiet, M. J.; Boulay, F.; Dahlgren, C. *J. Biol. Chem.* **2001**, *276*, 21585.
(778) Seo, J. K.; Choi, S. Y.; Kim, Y.; Baek, S. H.; Kim, K. T.; Chae, C. B.; Lambeth, J. D.; Suh, P. G.; Ryu, S. H. *J. Immunol.* **1997**, *158*, 1895.
(779) Klein, C.; Paul, J. I.; Sauve, K.; Schmidt, M. M.; Arcangeli, L.; Ransom, J.; Trueheart, J.; Manfredi, J. P.; Broach, J. R.; Murphy, A. J. *Nat. Biotechnol.* **1998**, *16*, 1334.
(780) Hu, J. Y.; Le, Y.; Gong, W.; Dunlop, N. M.; Gao, J. L.; Murphy, P. M.; Wang, J. M. *J. Leukocyte Biol.* **2001**, *70*, 155.
(781) Tsuruki, T.; Ito, A.; Takaha, K.; Yoshikawa, M. *J. Invest. Dermatol.* **2004**, *123*, 242.
(782) Tsuruki, T.; Yoshikawa, M. *Peptides* **2006**, *27*, 820.
(783) Hecht, I.; Rong, J.; Sampaio, A. L.; Hermesh, C.; Rutledge, C.; Shemesh, R.; Toporik, A.; Beiman, M.; Dassa, L.; Niv, H.; Cojocaru, G.; Zauberman, A.; Rotman, G.; Perretti, M.; Vinten-Johansen, J.; Cohen, Y. *J. Pharmacol. Exp. Ther.* **2009**, *328*, 426.
(784) Shemesh, R.; Toporik, A.; Levine, Z.; Hecht, I.; Rotman, G.; Wool, A.; Dahary, D.; Gofer, E.; Kliger, Y.; Soffer, M. A.; Rosenberg, A.; Eshel, D.; Cohen, Y. *J. Biol. Chem.* **2008**, *283*, 34643.
(785) Bae, Y. S.; Lee, H. Y.; Jo, E. J.; Kim, J. I.; Kang, H. K.; Ye, R. D.; Kwak, J. Y.; Ryu, S. H. *J. Immunol.* **2004**, *173*, 607.
(786) Nanamori, M.; Cheng, X.; Mei, J.; Sang, H.; Xuan, Y.; Zhou, C.; Wang, M. W.; Ye, R. D. *Mol. Pharmacol.* **2004**, *66*, 1213.
(787) Lee, T. H.; Lympany, P.; Crea, A. E.; Spur, B. W. *Biochem. Biophys. Res. Commun.* **1991**, *180*, 1416.
(788) Zhang, L.; Zhang, X.; Wu, P.; Li, H.; Jin, S.; Zhou, X.; Li, Y.; Ye, D.; Chen, B.; Wan, J. *Inflammation Res.* **2008**, *57*, 157.
(789) Zhang, L.; Wan, J.; Li, H.; Wu, P.; Jin, S.; Zhou, X.; Yuan, P.; Xiong, W.; Li, Y.; Ye, D. *Hepatol. Res.* **2007**, *37*, 948.
(790) Burli, R. W.; Xu, H.; Zou, X.; Muller, K.; Golden, J.; Frohn, M.; Adlam, M.; Plant, M. H.; Wong, M.; McElvain, M.; Regal, K.; Viswanadhan, V. N.; Tagari, P.; Hungate, R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3713.
(791) Frohn, M.; Xu, H.; Zou, X.; Chang, C.; McElvaine, M.; Plant, M. H.; Wong, M.; Tagari, P.; Hungate, R.; Burli, R. W. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6633.
(792) Serhan, C. N. *Annu. Rev. Immunol.* **2007**, *25*, 101.
(793) Brezinski, D. A.; Nesto, R. W.; Serhan, C. N. *Circulation* **1992**, *86*, 56.
(794) Levy, B. D.; De Sanctis, G. T.; Devchand, P. R.; Kim, E.; Ackerman, K.; Schmidt, B. A.; Szczeklik, W.; Drazen, J. M.; Serhan, C. N. *Nat. Med.* **2002**, *8*, 1018.
(795) Kieran, N. E.; Maderna, P.; Godson, C. *Kidney Int.* **2004**, *65*, 1145.
(796) Karp, C. L.; Flick, L. M.; Park, K. W.; Softic, S.; Greer, T. M.; Keledjian, R.; Yang, R.; Uddin, J.; Guggino, W. B.; Atabani, S. F.; Belkaid, Y.; Xu, Y.; Whitsett, J. A.; Accurso, F. J.; Wills-Karp, M.; Petasis, N. A. *Nat. Immunol.* **2004**, *5*, 388.
(797) Mangino, M. J.; Brounts, L.; Harms, B.; Heise, C. *Prostaglandins Other Lipid Mediators* **2006**, *79*, 84.
(798) Pouliot, M.; Clish, C. B.; Petasis, N. A.; Van Dyke, T. E.; Serhan, C. N. *Biochemistry* **2000**, *39*, 4761.
(799) Devchand, P. R.; Arita, M.; Hong, S.; Bannenberg, G.; Moussignac, R. L.; Gronert, K.; Serhan, C. N. *FASEB J.* **2003**, *17*, 652.
(800) Serhan, C. N.; Maddox, J. F.; Petasis, N. A.; Akritopoulou-Zanze, I.; Papayianni, A.; Brady, H. R.; Colgan, S. P.; Madara, J. L. *Biochemistry* **1995**, *34*, 14609.
(801) Bandeira-Melo, C.; Serra, M. F.; Diaz, B. L.; Cordeiro, R. S.; Silva, P. M.; Lenzi, H. L.; Bakhle, Y. S.; Serhan, C. N.; Martins, M. A. *J. Immunol.* **2000**, *164*, 1029.
(802) Bonnans, C.; Vachier, I.; Chavis, C.; Godard, P.; Bousquet, J.; Chanez, P. *Am. J. Respir. Crit. Care Med.* **2002**, *165*, 1531.
(803) Christie, P. E.; Spur, B. W.; Lee, T. H. *Am. Rev. Respir. Dis.* **1992**, *145*, 1281.
(804) Dahlen, S. E.; Franzen, L.; Raud, J.; Serhan, C. N.; Westlund, P.; Wikstrom, E.; Bjorck, T.; Matsuda, H.; Webber, S. E.; Veale, C. A.; Puustinen, T.; Haeggstrom, J.; Nicolaou, K. C.; Samuelsson, B. *Adv. Exp. Med. Biol.* **1988**, *229*, 107.
(805) Fukunaga, K.; Kohli, P.; Bonnans, C.; Fredenburgh, L. E.; Levy, B. D. *J. Immunol.* **2005**, *174*, 5033.
(806) Papayianni, A.; Serhan, C. N.; Phillips, M. L.; Rennke, H. G.; Brady, H. R. *Kidney Int.* **1995**, *47*, 1295.

(807) Leonard, M. O.; Hannan, K.; Burne, M. J.; Lappin, D. W.; Doran, P.; Coleman, P.; Stenson, C.; Taylor, C. T.; Daniels, F.; Godson, C.; Petasis, N. A.; Rabb, H.; Brady, H. R. *J. Am. Soc. Nephrol.* **2002**, *13*, 1657.
(808) Kieran, N. E.; Doran, P. P.; Connolly, S. B.; Greenan, M. C.; Higgins, D. F.; Leonard, M.; Godson, C.; Taylor, C. T.; Henger, A.; Kretzler, M.; Burne, M. J.; Rabb, H.; Brady, H. R. *Kidney Int.* **2003**, *64*, 480.
(809) Mayadas, T. N.; Mendrick, D. L.; Brady, H. R.; Tang, T.; Papayianni, A.; Assmann, K. J.; Wagner, D. D.; Hynes, R. O.; Cotran, R. S. *Kidney Int.* **1996**, *49*, 1342.
(810) Munger, K. A.; Montero, A.; Fukunaga, M.; Uda, S.; Yura, T.; Imai, E.; Kaneda, Y.; Valdivielso, J. M.; Badr, K. F. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 13375.
(811) Takano, T.; Clish, C. B.; Gronert, K.; Petasis, N.; Serhan, C. N. *J. Clin. Invest.* **1998**, *101*, 819.
(812) Schottelius, A. J.; Giesen, C.; Asadullah, K.; Fierro, I. M.; Colgan, S. P.; Bauman, J.; Guilford, W.; Perez, H. D.; Parkinson, J. F. *J. Immunol.* **2002**, *169*, 7063.
(813) Serhan, C. N.; Jain, A.; Marleau, S.; Clish, C.; Kantarci, A.; Behbehani, B.; Colgan, S. P.; Stahl, G. L.; Merched, A.; Petasis, N. A.; Chan, L.; Van Dyke, T. E. *J. Immunol.* **2003**, *171*, 6856.
(814) Shen, J.; Herderick, E.; Cornhill, J. F.; Zsigmond, E.; Kim, H. S.; Kuhn, H.; Guevara, N. V.; Chan, L. *J. Clin. Invest.* **1996**, *98*, 2201.
(815) Gronert, K.; Maheshwari, N.; Khan, N.; Hassan, I. R.; Dunn, M.; Laniado Schwartzman, M. *J. Biol. Chem.* **2005**, *280*, 15267.
(816) Kantarci, A.; Van Dyke, T. E. *Prostaglandins, Leukotrienes, Essent. Fatty Acids* **2005**, *73*, 289.
(817) Serhan, C. N. *Prostaglandins, Leukotrienes, Essent. Fatty Acids* **2005**, *73*, 141.
(818) Vane, J. R. *Nat. New. Biol.* **1971**, *231*, 232.
(819) Flower, R.; Gryglewski, R.; Herbaczynska-Cedro, K.; Vane, J. R. *Nat. New. Biol.* **1972**, *238*, 104.
(820) Marcus, A. J.; Broekman, M. J.; Pinsky, D. J. *N. Engl. J. Med.* **2002**, *347*, 1025.
(821) Hennekens, C. H.; Sechenova, O.; Hollar, D.; Serebruany, V. L. *J. Cardiovasc. Pharmacol. Ther.* **2006**, *11*, 170.
(822) Flower, R. *BMJ [Br. Med. J.]* **2003**, *327*, 572.
(823) Imperiale, T. F. *N. Engl. J. Med.* **2003**, *348*, 879.
(824) Serhan, C. N.; Chiang, N.; Van Dyke, T. E. *Nat. Rev. Immunol.* **2008**, *8*, 349.
(825) Chiang, N.; Bermudez, E. A.; Ridker, P. M.; Hurwitz, S.; Serhan, C. N. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 15178.
(826) Morris, T.; Stables, M.; Hobbs, A.; de Souza, P.; Colville-Nash, P.; Warner, T.; Newson, J.; Bellingan, G.; Gilroy, D. W. *J. Immunol.* **2009**, *183*, 2089.
(827) Martin, T. R.; Gerard, N. P.; Galli, S. J.; Drazen, J. M. *J. Appl. Physiol.* **1988**, *64*, 2318.
(828) Richter, M.; Sirois, P. *Eur. J. Pharmacol.* **2000**, *389*, 225.
(829) Huang, X. J.; Zhang, W. P.; Li, C. T.; Shi, W. Z.; Fang, S. H.; Lu, Y. B.; Chen, Z.; Wei, E. Q. *Glia* **2008**, *56*, 27.
(830) Moos, M. P.; Funk, C. D. *Trends Cardiovasc. Med.* **2008**, *18*, 268.
(831) Riccioni, G.; Capra, V.; D'Orazio, N.; Bucciarelli, T.; Bazzano, L. A. *J. Leukocyte Biol.* **2008**, *84*, 1374.