Cysteinyl Leukotrienes and Their Receptors: Molecular and Functional Characteristics

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Key Words

Cysteinyl leukotrienes • Leukotriene receptors • Asthma • Leukotriene D₄ • Arachidonic acid pathway

Abstract

The cysteinyl leukotrienes (CysLTs) are a family of potent inflammatory lipid mediators synthesized from arachidonic acid by a variety of cells including mast cells, eosinophils, basophils and macrophages. The family includes leukotriene C₄ (LTC₄), leukotriene D₄ (LTD₄) and leukotriene E₄ (LTE₄), which are potent biological mediators in the pathophysiology of inflammatory diseases and trigger contractile and inflammatory processes through the specific interaction with cell surface receptors, belonging to the superfamily of G-protein-coupled receptor. Pharmacological characterizations have suggested the existence of at least 2 types of CysLT receptors based on potency of agonist and antagonist, designated as CysLT₁ and CysLT₂. The CysLT₁ receptors are mostly expressed in lung smooth muscle cells, interstitial lung macrophages and the spleen, and it has been studied a lot elucidating its role in the etiology of airway inflammation and asthma. On the other hand, CysLT₂ receptors are present in the heart, brain and adrenal glands. This review discusses the role of CysLTs and their receptor in the patho-

physiology of various inflammatory disorders. The understanding of CysLTs and their receptors in allergic airway disease is currently limited to CysLT₁-receptor-mediated effects, and the role of the CysLT₂ receptors is pharmacologically less well defined, as there is no specific antagonist available yet. Specific CysLT₂-receptor-selective antagonists would be very helpful to identify the precise role of CysLT and their receptors. Some recent evidence indicates the existence of additional receptor subtypes and requires further investigation for a better understanding of the role of the CysLT receptors. This review is an effort to summarize the localization, regulation and expression pattern along with the molecular and functional pharmacology of the CysLT receptors and to discuss their role in the pathophysiology of different diseases along with the recent update.

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Introduction

Cysteinyl leukotrienes (CysLTs), namely LTC₄, LTD₄, and LTE₄, are inflammatory lipid mediators derived from the lipoxygenase pathway of the arachidonic acid metabolism [1–8]. These are so designated because they were first identified in leukocytes and are characterized

Fig. 1. The arachidonic acid cascade and formation of leukotrienes. Arachidonic acid is catalyzed by the 5-LO enzyme. This reaction requires 5-LO-activating protein (FLAP) and results in the formation of LTA₄. 5-HPETE = 5-Hydroperoxyeicosatetraenoic acid. The unstable epoxide LTA₄ is conjugated with glutathione by LTC₄ synthase and yields LTC₄. LTC₄ is metabolized by γ -glutamyl transpeptidase to LTD₄, which is, in turn, metabolized by dipeptidase to LTE₄. LTA₄ is also metabolized to LTB₄ by LTA₄ hydrolase.

by the presence of cysteine and 3 conjugated double bonds in their chemical structure. The term CysLTs distinguishes itself from the non-cysteine-containing dihydroxyleukotriene leukotriene B₄ (LTB₄) [1, 3]. The studies on these compounds began with Feldberg and co-workers around 70 years ago. They first observed that a 'slow-reaction smooth-muscle-stimulating substance' was released from an isolated lung preparation, after perfusing with snake venom (a rich source of phospholipase A₂) or histamine, and was able to contract the isolated guinea pig ileum [9].

Biosynthesis of CysLTs

CysLTs are derived from the ubiquitous membrane constituent arachidonic acid and are members of a large family of molecules known as eicosanoids. These are named after the Greek word 'eicosa' meaning 20, as they are derived from 20-carbon polyunsaturated fatty acid [3, 10]. These biologically active lipids are rapidly generated at the sites of inflammation following a series of reactions initiated by cytosolic phospholipase A₂ which release the arachidonic acid from the phospholipids present at the nuclear envelope (fig. 1). The arachidonic acid reacts with the enzyme 5-lipoxygenase (5-LO) after binding to the

5-LO-activating protein and results in the formation of 5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid or LTA₄ [2, 11–14]. LTA₄ is converted to LTB₄ by the enzyme LTA₄ hydrolase, a cytosolic enzyme also exerting aminopeptidase activity and containing zinc at its catalytic center [15]. Inflammatory cells, such as eosinophils, basophils, mast cells and alveolar macrophages possessing the integral membrane protein LTC₄ synthase, synthesize CysLTs in response to biological and nonbiological stimuli [16–20]. LTC₄ synthase conjugates reduced glutathione at position C₆ of LTA₄ to form LTC₄, which is transferred to the extracellular space, where a γ -glutamyl transpeptidase cleaves the glutamic acid moiety to form LTD₄. This, in turn, is transformed into LTE₄ by the cleavage of the glycine moiety by dipeptidase enzyme [19]. The LTE₄ is the most stable CysLT and is a weak agonist for CysLT₁/CysLT₂ receptors [21, 22].

The loss of biological activity arises from catabolism through 3 major mechanisms: the formation of N-acetyl derivatives of LTE₄, the reaction with hypochlorous acid to form sulfoxide and LTB₄ and ω -oxidation and β -elimination to form shortened metabolites [23, 24].

Alternatively, CysLTs can also be produced through transcellular metabolism from neutrophil-derived LTA₄ by platelets and vascular endothelial cells. This transcellular biosynthesis is considered very important because it

could generate remarkably high concentrations of CysLTs in a local environment, ultimately affecting organ function [25–29]. Such transcellular biosynthesis of LTs has been reported also for mast cells [30], peripheral blood monocytes [31], human airway epithelial cells, alveolar macrophages [32], kidney-derived endothelial cells [33], keratinocytes [34] and chondrocytes [35].

CysLT Receptors

Nomenclature and Classification

CysLTs exert their effect through cell surface receptors [36]. Great efforts have been put into the characterization of these receptors ever since the discovery of these substances. According to the International Union of Pharmacology, the CysLT receptor nomenclature was originally based on the sensitivity to the so-called ‘classical’ antagonists, which include montelukast, zafirlukast, pranlukast, pobilukast and MK571 [3, 10, 37, 38].

Accordingly, CysLT receptors have been mainly divided into two classes: CysLT₁ that is sensitive to the classical antagonists and CysLT₂ which mediates several effects that are not inhibited by the classical antagonists. The only ‘dual antagonist’, i.e. BAYu9773, that has been reported to exhibit activity at both CysLT₁ and CysLT₂ receptors, is unfortunately neither very potent nor selective for the CysLT receptor classes, especially in human tissues [3, 39–41]. However, BAYu9773 has also been reported as a partial agonist for CysLT₂ receptor [42]. These pharmacological findings were confirmed by molecular biology studies that allowed the cloning of two genes for the human CysLT₁ and CysLT₂ receptors, which are located on the long arms of chromosomes X and 13 [43–45].

Molecular and Pharmacological Characterization of CysLT Receptors

CysLT₁ Receptor: Protein Structure, Location and Expression Pattern

The CysLT₁ receptor gene was localized to the X chromosome, Xq13–Xq21, and has been shown to consist of at least 3 exons, with the entire open reading frame in the last exon [44, 46, 47]. The human CysLT₁ receptor encodes a protein of 337 amino acids with a calculated molecular mass of 38 kDa, observed to migrate at a molecular weight of approximately 42 kDa as a monomeric form, but mostly present in dimeric and oligomeric forms, even

in the presence of denaturing agents [1, 3]. However, it has not been established whether the oligomerization of CysLT₁ receptor is of any importance in cell physiology [48]. Human CysLT₁ receptor has the highest homology (32% amino acid identity) with the purinoceptor P2Y₁ and the receptor for platelet-activating factor, whereas a lower homology (28% amino acid identity) with the other known leukotriene receptor, the BLT receptor. Human CysLT₁ receptor possesses 4 potential N-glycosylation sites, 1 in the extracellular N-tail, 2 in the second and 1 in the third extracellular loop, in addition to many potential protein kinase A and C phosphorylation sites, mostly located in the third intracellular loop and carboxyl terminal [1, 3, 10, 37, 46].

The CysLT₁ receptor mRNA was first identified in normal lung smooth muscle cells and interstitial macrophages by in situ hybridization of a CysLT₁ receptor antisense probe, with little or no expression in normal airway epithelial cells [44]. Expression of the CysLT₁ receptor mRNA and protein was also demonstrated in normal peripheral blood eosinophils, subsets of monocytes, macrophages and pregranulocytic CD34+ cells [49]. Human cord-blood-derived mast cells express the CysLT₁ receptor, but not the CysLT₂ receptor [37, 44, 47, 49, 50].

The hypothesis that the CysLT₁ receptor is expressed by a variety of airway mucosal inflammatory cells in asthma and that the numbers of cells expressing CysLT₁ receptor are increased in asthma, either stably or in association with an exacerbation, has been confirmed by results obtained from normal subjects which indicate that bronchial mucosal eosinophils, neutrophils, mast cells, macrophages, B lymphocytes and plasma cells, but not T lymphocytes, express the CysLT₁ receptor [51]. The study by Zhu et al. [51] further demonstrates that the numbers of CysLT₁ receptor mRNA and protein-positive inflammatory cells are significantly greater in stable asthmatic subjects and patients hospitalized for exacerbation of their asthma compared with controls, and that a strong positive correlation exists between this observation and the increased numbers of CD45+ progenitors [51]. In human nasal mucosa, CysLT₁ receptor has been localized at gene as well as protein level in blood vessels and in the interstitial cells such as vascular endothelial cells, eosinophils, mast cells, macrophages and neutrophils. Since subjects with aspirin-induced asthma have greater airway hyperresponsiveness to the effects of inhaled CysLTs than aspirin-tolerant asthmatics, Sousa et al. [52] hypothesized that this could be because of the elevated expression of CysLT₁ receptor on inflammatory cells.

Table 1. Summary of CysLT receptors

Receptors	Human CysLT ₁	Human CysLT ₂	Mouse CysLT ₁	Mouse CysLT ₂
Genebank Acc.	AH119711, AF133266	AF254664	AC021992	–
Chromosome	Xq13–q21	13q14.2	XD	14
Agonist	LTD ₄ >> LTC ₄ > LTE ₄	LTD ₄ = LTC ₄ > LTE ₄	LTD ₄ >> LTC ₄ > LTE ₄	LTD ₄ = LTC ₄ > LTE ₄
Antagonist	montelukast, zafirlukast, pranlukast, BAY u9773	BAYu9773	MK571	BAYu9773
Primary coupling	G _{αq}	G _{αq}	G _{αq}	G _{αq}
Primary expression	spleen, lung, smooth muscle	heart, pulmonary vein, adrenal medulla, placenta	skin, lung	spleen, thymus, adrenal gland, small intestine

The human CysLT₁ receptor has been shown to be most highly expressed in spleen, peripheral blood leukocytes and less strongly in lung, small intestine, pancreas and placenta and little or no expression in the liver, colon, kidney, skeletal muscle, thymus, ovary, testis, heart and brain [1, 3, 5, 10, 37, 44, 46, 50, 53]. The expression of a functional CysLT₁ receptor has been reported also in human saphenous veins, where it mediates contractile effects of CysLTs. In the gastrointestinal system, CysLT₁ receptor expression has been documented in small intestine and colon [37, 44, 47].

CysLT₂ Receptor: Protein Structure, Location and Expression Pattern

The gene encoding the human CysLT₂ receptor is on chromosome 13q14 [43, 54]. The CysLT₂ receptor has always been pharmacologically less defined than CysLT₁, mainly because of the lack of a selective antagonist. Human CysLT₁ and CysLT₂ receptors share only 38% amino acid identity with very low homology in the extreme carboxyl terminal. Gene cloning and characterization of human CysLT₂ receptor was first reported by Heise et al. [43], who used a rat EST clone with 40% homology to the human CysLT₁ receptor to find the human homolog by screening a brain cDNA library. The open reading frame of human CysLT₂ receptor encodes a protein of 346 amino acids, which appears to migrate at a molecular weight of 58 kDa in basophil lysate [43, 54]. Human CysLT₂ receptor possesses 4 potential N-glycosylation sites, 3 in the extracellular N-tail and 1 in the second extracellular loop, in addition to many potential protein kinase A and C phosphorylation sites mostly located in the third intracellular loop and carboxyl terminal [43].

The human CysLT₂ expression pattern is substantially different from that of human CysLT₁. It is highly expressed in the spleen and peripheral blood leukocytes,

but expression in the heart, adrenal gland and brain appears unique to this subtype. All the 4 chambers of the heart, the intraventricular septum, the apex and the pericardium have been shown to express human CysLT₂ by Northern blot and RT-PCR. Expression in the Purkinje fiber cells and coronary smooth muscles is also reported [43, 45, 54].

In the central nervous system, CysLT₂ receptor mRNA is highly expressed in several regions of the brain, with particular concentration in the hypothalamus, thalamus, putamen, pituitary and medulla [43]. Its expression has also been reported in the granulocytes of the brain parenchyma and in neuron and glial cells appearing in the late stage of traumatic injury and in areas surrounding the tumors [55]. CysLT₂ receptor expression is also widespread in most regions of the spinal cord [43, 54].

The adrenal gland may represent a novel tissue for future studies on the functions and role of CysLT₂ receptor in modulating the endocrine system because a very good level of expression was detected, particularly in medullary pheochromocytes [44].

In the immune system, moderate expression of CysLT₂ receptor mRNA has been reported in the spleen, lymph nodes and peripheral blood leukocytes, with very strong expression in eosinophils [43]. Eosinophils express higher levels of CysLT₂ than CysLT₁ as determined by competitive RT-PCR, suggesting unidentified roles for this receptor in these cells. Expression in the monocytes, neutrophils and T cells was also observed [56]. Compared with CysLT₁, CysLT₂ is only weakly expressed in lung smooth muscle cells, but elevated expression was detected in macrophages in close proximity to smooth muscle cells [43].

Signal Transduction Mechanism of CysLT Receptors

The characteristics of human and mouse CysLT receptors have been summarized in table 1. CysLT receptors

Fig. 2. Signal transduction mechanism and the biological effects mediated by CysLTs (LTC_4 , LTD_4 and LTE_4) and LTB_4 . Both CysLTs and LTB_4 act through GPCR coupled to G_q protein and thereby increase intracellular calcium. LTB_4 is a potent chemoattractant for the neutrophils, and CysLTs are potent bronchoconstrictors. The CysLTs and LTB_4 in common cause increased airway hyperreactivity (AHR), increased mucus secretion and increased capillary permeability. CysLTs also cause activation of eosinophils and its recruitment, and decrease eosinophil apoptosis. CysLTs are also involved in airway remodeling by myofibroblast accumulation and airway smooth muscle hyperplasia. PIP2 = Phosphatidylinositol biphosphate; IP3 = inositol triphosphate; DAG = diacylglycerol; ER = endoplasmic reticulum; PKC = protein kinase C.

have long been recognized as G-protein-coupled receptors (GPCRs), prior to their cloning, based on the fact that binding of the ligand to the receptor is enhanced by divalent cations, but inhibited by sodium ions and nonhydrolyzable GTP analogs [53, 57–59]. Hydrophobicity analysis of the deduced primary structure reveals that both CysLT_1 and CysLT_2 have 7 hydrophobic transmembrane domains linked by the 6 hydrophilic loops, signifying a typical character of GPCRs [10, 53].

High-affinity ligand binding results in membrane-bound receptor conformational changes resulting in activation of G-protein GDP-GTP exchange, hydrolysis of GTP and intracellular signaling events as shown in figure 2. The LTD_4 -induced calcium mobilization in CysLT_1 -cRNA-injected *Xenopus laevis* oocytes or CysLT_1 -transfected HEK293 cells or CysLT_2 -transfected HEK293 cells is not inhibited by pertussis toxin [44, 47, 60], suggesting that the G protein involved is only of the G_q subunit. How-

ever, in differentiated U937 cells, and human monocytic leukemia THP1 cells, coupling to both the G_q and $\text{G}_{i/o}$ family has been observed [61–64]. So, CysLT signaling pathways are dependent on the cell type and the availability of trimeric G protein [65]. However, intracellular calcium mobilization was used to measure the receptor function in vitro in all the reported cloning studies. In the cells transfected with CysLT_1 cDNA, CysLT could increase the Ca^{2+} concentration in the rank order potency of $\text{LTD}_4 > \text{LTC}_4 \geq \text{LTE}_4$ [44, 47, 66], suggesting some sort of ligand selectivity at these receptors. However, in the cells transfected with CysLT_2 cDNA, the rank order potency was found to be $\text{LTC}_4 = \text{LTD}_4 > \text{LTE}_4$ [43, 45, 54]. The variations could come from the intrinsic nature of receptors, the signaling pathways coupled to the receptors in the different cell types or the experimental conditions. The expression level of the receptors could also affect the agonist efficacy. There are evidences suggesting that the receptors

associated with different physiological responses are not activated by all the native ligand [67, 68]. It has also been demonstrated that various polar isomers of the CysLTs, with slight alterations in the structures of the native ligand, affected both binding and potency of the agonists. In some cells, CysLT receptor activation leads to the release of various mediators, which are responsible for the biological actions of the CysLTs. For example, it has been reported that LTD₄ increases the release of arachidonic acid in isolated cells via a receptor-driven mechanism involving RNA and protein synthesis, leading to the formation of various metabolites [69]. Some of the effects of CysLTs in both respiratory and cardiovascular systems have been shown to be mediated via release of products of the cyclooxygenase pathway of arachidonic acid metabolism [70, 71]. In addition, CysLT receptors on endothelial cells have also been reported to be linked to the formation of nitric oxide [72, 73]. The release of such mediators may interfere with functional studies of CysLT receptors and since some studies have been performed in the presence of the cyclooxygenase inhibitor indomethacin, whereas others have been performed in the absence of cyclooxygenase inhibition, it may not always be possible to compare results of different studies.

Evidence for Further CysLT Receptor Subtypes

According to the current nomenclature of CysLT receptors, a CysLT₁ receptor is inhibited by the CysLT₁ receptor antagonists, whereas BAYu9773 is expected to inhibit both CysLT₁ and CysLT₂ receptors [74, 75]. Many investigators have reported data suggesting the presence of additional CysLT receptor subtypes in human tissues, based on either the observation that one ligand (i.e. LTE₄) failed to activate a CysLT receptor [76, 77] or that the dual antagonist BAYu9773 failed to antagonize all CysLT functional responses [74, 75], hence suggesting the existence of further CysLT receptor subtypes.

In endothelial intact porcine pulmonary arteries, LTC₄ is somewhat more potent than LTD₄, and the contractions to both agonists are only slightly inhibited by either the CysLT₁ receptor antagonist or the dual CysLT₁/CysLT₂ receptor antagonist [70]. Moreover, after endothelium denudation, LTC₄ and LTD₄ are equipotent, and in addition, the LTC₄-induced contractions are resistant to both CysLT₁ receptor antagonist and the dual CysLT₁/CysLT₂ receptor antagonist [70]. Moreover, the contractile response of human pulmonary artery to CysLTs was resistant to the CysLT₁ receptor antagonist MK571 and to the dual CysLT₁/CysLT₂ receptor antagonist activity. This suggests that the receptor in the human pulmonary

artery is different from the existing CysLT₁ and CysLT₂ receptors [77, 78]. These functional observations are further supported by the results obtained from ligand binding studies in human tissues [57, 79–81].

In silico study had predicted that LTE₄ might be a surrogate ligand for P2Y₁₂ receptor [82]. It has been shown that purinergic (P2Y₁₂) receptor is required for LTE₄-mediated pulmonary inflammation [21, 83]. Austen et al. [21] have shown that LTE₄ can induce the activation of extracellular signal-regulated kinase (ERK) in Chinese hamster ovary cells stably transfected with human P2Y₁₂ receptors exceeding the potency of LTD₄, and this effect of LTE₄ is inhibited by a P2Y₁₂-receptor-selective antagonist, clopidogrel. This signaling event was sensitive to pertussis toxin but resistant to MK571. Although P2Y₁₂ did not bind LTE₄ directly, knockdown of P2Y₁₂ receptors by RNA interference blocked LTE₄-mediated macrophage inflammatory protein 1β generation and prostaglandin D₂ production by LAD2 cells without significantly altering their responses to LTD₄ [21].

It has been shown that administration of LTE₄ to the airways of sensitized mice potentiates eosinophilia and goblet cell metaplasia in response to low-dose aerosolized allergen. These responses persist in mice lacking both CysLT₁ receptor and CysLT₂ receptor, but not in mice lacking P2Y₁₂ receptors. The effects of LTE₄ on P2Y₁₂ in the airway were abrogated by platelet depletion [84]. Thus, the P2Y₁₂ receptor is required for proinflammatory actions of the stable abundant mediator LTE₄ and is a novel potential therapeutic target for asthma.

Out of all the CysLTs, LTE₄ binds poorly to the classical CysLT₁ and CysLT₂ receptors and is much less active on normal airways [21, 22]. However, earlier studies have reported that LTE₄ caused skin swelling in human subjects with similar potency as other CysLTs and the airways of asthmatic subjects (particularly those that were aspirin sensitive) were hyperresponsive to LTE₄. Austen et al. [21] have examined the dose-dependent ear edema elicited by CysLTs in a mouse strain deficient in both CysLT₁ and CysLT₂ receptor. The dose-dependent ear edema produced by means of injection of LTD₄ and LTC₄ in a strain deficient in both CysLT₁ and CysLT₂ receptor was equivalent to that in the wild-type control animals, indicating the presence of a novel receptor. Moreover, they were sensitive to LTE₄, exhibiting the same extent of ear swelling in a much lower dose as compared to wild-type control animals [21]. The LTE₄-mediated vascular leak was markedly inhibited by pretreatment with pertussis toxin or rho kinase inhibitor in the mice deficient in both CysLT₁ and CysLT₂ receptor, suggesting involve-

ment of a GPCR linked to $G\alpha_i$ proteins and rho kinase [21, 83]. Additionally, the response to LTE_4 was blocked by approximately 30% by means of treatment of the mice with indomethacin. This particular sensitivity of an unknown receptor to LTE_4 raises the possibility of a novel receptor, designated as CysLT_E receptor [21, 83].

The existence of a functional crosstalk between the nucleotide and the CysLT systems has been documented in mediating inflammatory responses [85]. Both types of mediators accumulate at the sites of inflammation, and inflammatory cells often coexpress both P2Y and CysLT receptors. In human monocyte/macrophage-like cells, CysLT₁ receptor function is regulated by extracellular nucleotides via heterologous desensitization, and the CysLT₁ receptor antagonists montelukast and pranlukast functionally interact with P2Y receptor signaling pathways in the same cells. There are close structural and phylogenetic relationships between P2Y and CysLT receptors, which cluster together into the 'purine receptor cluster' of the rhodopsin family of GPCRs [86]. Mellor et al. [75] proposed that both CysLT₁ and a yet unidentified LTC₄-preferring receptor (probably CysLT₃ receptor) were upregulated in human mast cells by treatment with the proinflammatory cytokine IL-4 and mediated dual responses to both CysLTs and UDP.

It has been reported that GPR17, an orphan receptor present at an intermediate phylogenetic position between P2Y and CysLT receptor families, may represent the yet unidentified elusive receptor responding to both nucleotides and CysLTs [86, 87].

Ciana et al. [86] have shown that the GPR17 receptors overexpressed in a variety of different cell lines responded to both, nucleotides and CysLTs in a specific and concentration-dependent manner. Based on this, they have also suggested the role of GPR17 in the P2Y and CysLT receptor signaling systems and their interaction with each other. Moreover, GPR17 has been found to be highly expressed in organs that can typically undergo ischemic injury (brain, heart and kidney). Different studies have shown that in vivo knockdown of GPR17 by either CysLT/P2Y receptor antagonists or by antisense technology markedly prevented evolution of ischemic brain damage [86] and also reduced tissue damage and related histological and motor deficits during spinal cord injury [87], suggesting GPR17 as a common molecular target mediating the inflammatory effects induced in vivo by nucleotides and CysLTs [86]. Thus, GPR17 may represent the common molecular target of CysLTs and nucleotides that sensitizes neurons to ischemic damage, thereby contributing to injury propagation after ischemia.

Taken together, all these evidences support the existence of additional CysLT receptor subtypes, which needs further characterization.

Regulation of CysLTs and Their Receptors

The CysLT receptors are regulated immunologically. Several studies have shown the upregulation of CysLT receptors by cytokines. A study by Thivierge et al. [88] has reported that human CysLT₁ receptor expression could be upregulated because of augmented transcriptional activity by priming cells with IL-5 in eosinophil-differentiated HL-60 cells. It is possible that the IL-5 secreted by the HL-60 cells stimulated CysLT receptor production in an autocrine pathway [53]. IL-4 and IL-13 but not IFN- γ were also able to induce CysLT₁ receptor expression in human monocytes and monocyte-derived macrophages [89]. However, IFN- γ was able to induce both, the CysLT₁ and CysLT₂ receptor expression in airway smooth muscle cells [90]. The upregulation by IL-1 β and IFN- γ seems interesting because they are generally considered counter-regulatory cytokines, inhibiting both the production and activities of the proallergic Th2-like cytokines. IFN- γ is indeed one of the theoretical approaches to obtain IgE immunomodulation in asthma, albeit adverse effects make its use limited [91]. The regulation of human CysLT₁ receptor expression by IL-13 has also been reported in the literature for lung fibroblasts and airway smooth muscle cells [92, 93].

The transcript levels of CysLT₂ receptor appear to be upregulated by the Th2-like cytokine IL-4 in human umbilical vein endothelial cells [94]. However, this regulation is decreased by Th1-like cytokines (TNF- α and IL-1 β) in a rapid and partially reversible manner in these cells [94, 95]. Fujii et al. [96] postulated that CysLT₂ receptor might modulate exacerbations of asthma, because they observed that CysLT₂ receptor expression on eosinophils was increased, during asthma exacerbation, and that was upregulated by IFN- γ . These observations lead to the conclusion that Th1- and Th2-like cytokines can alter the CysLT receptor expression and could be important in the pathogenesis of asthma.

Human nasal mast cells release a variety of signals on activation, which target the bronchi vasculature and recruit other immune cells to the inflammatory site. Prominent among such signals are the CysLTs, comprising LTC₄, LTD₄ and LTE₄. LTC₄, the parent compound, is secreted from mast cells following Ca²⁺ influx through store-operated calcium-release-activated calcium (CRAC) channels [97, 98]. Di Capite et al. [97, 98] have shown that activated mast cells release a paracrine

signal that evokes Ca^{2+} signals in spatially separate resting mast cells. The paracrine signal was identified as CysLT and stimulated further CysLT production, triggering a positive feedback cascade. Acutely isolated mast cells from patients with allergic rhinitis exhibited store-operated Ca^{2+} influx through CRAC channels and responded to CysLTs. This suggests a positive-feedback cascade involving CRAC channels, and CysLTs constitute a novel mechanism for sustaining mast cell activation [97, 98].

Gender Differences in Generation of CysLTs

Pergola et al. [99] have provided interesting gender differences in leukotriene generation. Formation of leukotrienes and 5-hydroperoxyeicosatetraenoic acid in female blood stimulated with lipopolysaccharide plus N-formyl-methionyl-leucylphenylalanine or with Ca^{2+} ionophore A23187 was significantly higher than male blood. This gender difference in the capacities of lipopolysaccharide/N-formyl-methionyl-leucylphenylalanine-induced generation of leukotrienes and other 5-LO products may better reflect pathophysiological conditions in the body. However, 5-LO protein levels and 5-LO activity in homogenates of blood or isolated neutrophils were not different between genders. In neutrophils from females, 5-LO resided in the cytoplasm of resting cells and redistributed to the nucleus upon stimulation, whereas in neutrophils from males, 5-LO was detected in both the cytosol and the nuclear compartment of resting cells, and upon stimulation, the compartmentalization of 5-LO was not significantly altered. However, cytosolic phospholipase A_2 subcellular compartmentalization does not differ between genders. Treatment of female neutrophils with 5α -dihydrotestosterone caused translocation of 5-LO to the nuclear compartment in a rapid and concentration-dependent manner, whereas 17β -estradiol and progesterone had no effect [99]. The study by Pergola et al. [99] suggests that male neutrophils should have higher levels of ERK activity versus female cells. The basal phosphorylation status of ERK2 and of its substrate Elk1 was significantly higher in male neutrophils versus female cells. ERK2 expression and phosphorylation of p38 mitogen-activated protein kinase did not vary between genders. Finally, incubation of female neutrophils with male plasma caused activation of ERKs. Thus, the synthesis of leukotrienes and 5-hydroperoxyeicosatetraenoic acid and 5-LO subcellular compartmentalization in human neutrophils depend on gender, connected to a differential activation status of ERKs [99].

Pathophysiological Functions of CysLT Receptors

The pathophysiological role of CysLTs in several inflammatory conditions is well documented with a general emphasis on asthma, and during the last 25 years a considerable effort has been made to identify and develop receptor antagonists to improve asthma management, limit its morbidity and reduce the side effects of current medications. However, CysLTs and their receptor functions are not only confined to asthma etiology, the evidence for their potential involvement in allergy, vasculature and neurological disease as well as cancer has also been well explored.

Asthma

Asthma is a chronic inflammatory disease which includes the narrowing of the small airways of the lung upon exposure to certain 'triggers' resulting in the difficulty in breathing and increased responsiveness. It is one of the common diseases with an increasing prevalence among children (approx. 10%) as well as the adult (approx. 5%) population [2, 3, 38, 100].

The pathogenesis of asthma involves several different cells and mediators and varies from individual to individual depending on the stimulus [101]. However, CysLTs are thought to play an important role in the pathogenesis of acute and chronic asthma as they are the most potent bronchoconstrictors found in humans to date. The inherent tone of human airway is thought to be maintained by a balance between the effects of contractile mediators including CysLTs and LTB_4 , histamine and the relaxing elements such as prostaglandin E_2 [102, 103]. Thus, in chronic asthmatic conditions, the contractile elements are increased mainly due to enhanced CysLT production [104, 105]. The remarkable contractile activity of LTC_4 and LTD_4 on isolated human bronchi has been described around three decades ago and confirmed in vivo in healthy human subjects [105, 106]. Experimental data suggest that CysLTs are one of the important inflammatory components of asthma and their effects include increased microvascular permeability leading to pulmonary edema, increased mucus secretion and decreased clearance by impairing the ciliary activity and airway smooth muscle cell hyperplasia which leads to airway remodeling [107–109]. CysLTs are produced mainly by the eosinophils and also by the mast cells at the rapid onset of allergic asthma. The number and activity of the eosinophils are increased resulting in the increased concentration of CysLTs in bronchoalveolar lavage fluid and urine [104]. The eosinophils are thought to play a pivotal

role in the process of chronic inflammation associated with asthma, and CysLTs increase eosinophil survival in response to paracrine signals from mast cells and lymphocytes. Moreover, eosinophils also express CysLT₁ receptors, which mediate several autocrine actions of CysLTs. CysLTs promote leukocyte maturation and migration from the bone marrow into the circulatory system, and they are chemoattractant for eosinophils, increasing their cellular adhesion and transendothelial migration across the vessel wall into the airways [53].

Allergic Rhinitis

Allergic rhinitis affects around 20% of the population in developed countries and is associated with significant morbidity, reduced quality of life and productivity. Allergic rhinitis is closely related to asthma and is also a major risk factor for the development of asthma [110].

It affects the upper airways, and the predominant symptoms such as rhinorrhea, nasal obstruction, nasal itching and sneezing are the consequence of a complex pathophysiological response to nasal allergen exposure [3].

Most of the cells involved in the pathophysiology of allergic rhinitis produce and release CysLTs. In addition to the effects of CysLTs on other inflammatory mediators, various inflammatory mediators can also exert regulatory effects on CysLTs. Several studies have confirmed the ability of Th2 cytokines to augment the synthesis of CysLTs as well as the expression of the CysLT₁ receptor. Th2 cytokines also upregulate CysLT₁ receptors, a mechanism that, theoretically, can enhance CysLT actions [88, 89]. Some of non-Th2-type cytokines such as IFN- γ and IL-1 β stimulate CysLT₁ receptor expression on smooth muscle cells and endothelial cells [90, 93, 111]. IL-16 has been found to be increased in the patients during allergic rhinitis and to act as chemoattractant for eosinophils. IL-16 stimulates eosinophils to release eotaxin, which further activates C-C chemokine receptor 3 to release LTC₄ and IL-4 [112]. In human nasal mucosa, CysLT₁ receptor has been localized both at the gene and protein level in blood vessels and in the interstitial cells, namely vascular endothelial cells, eosinophils, mast cells, macrophages and neutrophils [90, 93, 110]. Since CysLT₁ seems to be a major causative factor for allergic rhinitis, CysLT₁ receptor antagonists used in the clinic have exhibited beneficial use in allergic rhinitis patients and are considered as a major therapeutic approach.

Cardiovascular Diseases

The actions of leukotrienes in the cardiovascular system are well established and suggest the existence of a

strong link between the leukotriene pathway and cardiovascular diseases [70, 72, 73, 77, 113]. CysLTs are characterized by their potent constrictor actions on the microvasculature, enhanced permeability of the postcapillary venules, reduced coronary blood flow through direct coronary constriction and reduced myocardial contractility and cardiac output without affecting the heart rate [114–116]. CysLTs also exert negative inotropic action on myocardium and decrease coronary blood flows with no effect on heart rate. CysLTs may also regulate vascular tone in specific vascular beds and can mediate contraction through the receptors present on the endothelium or smooth muscle cells, as well as relaxation, which is endothelium dependent [113]. The production of CysLTs is increased in ischemia-reperfusion injury in patients as well as in experimental animal models, and 5-LO-activating protein inhibitors were found to provide protection in a rabbit ischemia model [117, 118].

CysLT receptors are found on endothelial cells and vascular smooth muscle preparations. Study demonstrated that human saphenous veins were sensitive to LTC₄ and LTD₄, and these ligands were equipotent in producing a marked constriction, which was unaltered by inhibitors of nitric oxide. However, lower concentrations of LTC₄ and LTD₄ induced relaxations in human saphenous veins that were dependent on nitric oxide and prostanoid release [119]. Moreover, human artery smooth muscle cells have been shown to increase intracellular calcium by LTC₄, which was not inhibited by CysLT₁ receptor antagonist but inhibited by a calcium channel blocker, nicardipine, which is known to be a vasorelaxant [120]. This suggested a role for CysLT₂ receptor in mediating coronary vasoconstriction. CysLT₂ receptor has been found to be present in the entire heart, especially in the conduction system, as confirmed by the Northern blot analysis [43, 45, 47], but it has been unexplored till date due to unavailability of CysLT₂-receptor-specific antagonist.

The study by Helgadottir et al. [121] reports that subjects with cardiovascular inflammation exhibited an increased expression of a version of the *ALOX5AP* gene and a neutrophil-enhanced release of leukotrienes. While 2 forms of this gene were observed, both of them were associated with a higher prevalence of myocardial infarction and stroke [121]. Since arterial wall inflammation is the hallmark of atherosclerosis, these results together with the earlier report suggest that atherosclerosis is intimately linked with overexpression of the CysLT receptors, augmented 5-LO activity and enhanced activity of the *ALOX5AP* gene [121, 122]. Thus, inhibitors of 5-LO might prove to be useful in cardiovascular inflammation.

Cancer

It has been reported that 5-LO enzyme is expressed by a wide variety of tumor cells and tissues, and thus 5-LO and its products might have a role in the process of carcinogenesis and tumor growth [123, 124]. The leukotrienes have also been shown to be released by the tissues derived from colon cancer patients [125]. CysLT₁ receptors were also detected in astrocytoma, ganglioglioma and metastatic adenocarcinoma [126, 127]. In addition, exposing intestinal epithelial cells in culture to LTD₄ for extended periods causes upregulation of proteins associated with colon carcinogenesis [122]. Sjolander et al. [64] have reported that LTD₄ regulates the survival and dissemination of colon cancer cells through the required activation of cytosolic phospholipase A₂, and that CysLT₁ receptor was highly expressed at the protein level in colorectal cancer patients. These findings suggest the pharmacological treatment of selected forms of cancer and encourage the pharmacological treatment of this disease with the antileukotrienes already marketed for asthma. In addition, further work is necessary to differentiate the CysLT receptor implicated in the cellular transformation and to establish the potential role for the CysLT in colon cancer.

Neuroendocrine Effects

The expression of CysLT₂ receptor in the brain as well as adrenal gland is quite significant, but little attention has been paid to the area of neuroendocrine modulation by the CysLTs. CysLT₂ receptor mRNA is highly expressed in numerous regions of the brain, with particular concentration in the hypothalamus, thalamus, putamen, pituitary and medulla [43]. There are extensive evidences which exist regarding the synthesis and actions of CysLTs in the brain of different animal species and humans [43]; however, much additional work will be required to obtain a clear understanding of their role in central nervous system pathophysiology. LTC₄ has been reported to enhance the release of luteinizing hormone from rat anterior pituitary [128] and luteinizing-hormone-releasing hormone from the median eminence, and the latter effect was not inhibited by CysLT₁ antagonist [129]. This suggests the existence of some subtype of the CysLT receptor. CysLT production has also been reported to increase in the cerebrospinal fluid in some disease states such as multiple sclerosis [130] and intracerebral hemorrhage which causes impairment of the blood-brain barrier and finally neuronal edema and death [131].

The adrenal gland comprised of both the cortex and the medulla has a very good level of expression of CysLT₂ receptors and represents a novel tissue for future studies on CysLT functions and CysLT₂ receptor role in modulating the neuronal and endocrine system, particularly in medullary pheochromocytes [44].

Discussion

The cloning of the CysLT receptors confirmed much of the earlier pharmacological characterization of agonists and antagonists for these receptors in different tissues and cells and has made it possible to investigate the genomic structure of these receptors and their polymorphisms, the regulation of expression of CysLT receptors in normal and diseased states, and their potential as new therapeutic targets. The cloning of these receptors will prompt more detailed investigations about their signal transduction systems and the regulation of their expression in normal and disease states. The CysLT₁ receptor is activated by the CysLTs and blocked by the classical CysLT₁ antagonists (high-affinity receptor), whereas the CysLT₂ receptor is also activated by these ligands and blocked by either the nonselective CysLT₁ or CysLT₂ antagonist. Most of our knowledge of the pathophysiological role of CysLTs in allergic airway disease is currently limited to CysLT₁-receptor-mediated effects, whereas the role of the CysLT₂ receptor still remains to be clarified. The development of an antagonist for the CysLT₂ receptors will be helpful in understanding their role in the pathophysiology of several inflammatory reactions.

CysLTs exert a range of the proinflammatory effects and have proved to be important mediators in asthma, allergic rhinitis and other inflammatory conditions such as cardiovascular diseases, cancer and certain central nervous system diseases such as multiple sclerosis. The CysLTs exert their effects through CysLT₁ and CysLT₂ receptors, which are the members of the GPCR superfamily. Unexpectedly high expression of the CysLT₁ receptor in the spleen and of the CysLT₂ receptor in the adrenal medulla need to be further explored. Further, the reason for the coexpression of the CysLT₁ and CysLT₂ receptors in eosinophils needs attention. Likely, in some disorders involving tissues and cells that coexpress both types of receptors, specific CysLT₁ receptor antagonists might be less efficacious than nonselective antagonists because of their inability to interfere with all the functions exerted by CysLTs.

It is worth noting that very recent evidence for the existence of additional receptor subtypes (such as CysLT₃ and CysLT_E receptors), the formation of homo/heterodimers, receptor crosstalk and CysLT receptor nuclear localization might be in part responsible for known CysLT functions while hiding new roles to be uncovered in the

near future. A better understanding of the precise roles of CysLTs and their receptors in the normal and disease state will lead to successful development of safer therapeutic agents than the currently available therapies for several inflammatory disorders.

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