Cysteinyl Leukotrienes and Their Receptors; Emerging Concepts

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Cysteinyl leukotrienes (cys-LTs) are potent mediators of inflammation derived from arachidonic acid through the 5-lipoxygenase/leukotriene C synthsese pathway. The derivation of their chemical structures and identification of their pharmacologic properties predated the cloning of their classical receptors and the development of drugs that modify their synthesis and actions. Recent studies have revealed unanticipated insights into the regulation of cys-LT synthesis, the function of the cys-LTs in innate and adaptive immunity and human disease, and the identification of a new receptor for the cys-LTs. This review highlights these studies and summarizes their potential pathobiologic and therapeutic implications.

Key Words: Leukotrienes; 5-lipoxygenase; asthma; AERD

INTRODUCTION

Leukotrienes are lipid mediators generated from arachidonic acid through the 5-lipoxygenase (5-LO) pathway. They are named for their cells of origin (leukocytes) and the presence of three positionally conserved double bonds (triene). The 2 classes of leukotrienes in cysteinyl leukotrienes (cys-LTs) and leukotriene B4 (LTB4), have broad array of bioactivities and cellular targets. Both 5-LO inhibitors and cys-LT receptor antagonists are useful for the treatment of asthma and rhinitis.1-3 Recently studies using molecular approaches have demonstrated that cys-LTs possess multiple cell targets and immunologic functions, and act through a receptor system far more complex than previously anticipated. This review highlights these recent studies, and will consider their potential pathobiologic and therapeutic implications.

Regulation of leukotriene synthesis

Leukotriene synthesis is initiated during the activation of leukocytes, when arachidonic acid is liberated from the membrane phospholipids by a cytosolic phospholipase A2.4 5-LO activating protein presents arachidonic acid to 5-LO, which catalyzes the formation of 5-hydroperoxyeicosatetraenoic acid and then the unstable epoxide LTA4.5 In mast cells, macrophages, eosinophils, and basophils, LTA4, synthesize LTC4 (LTC4S) conjugates LTA4 to reduced glutathione, forming LTC4, the parent of the cys-LTs.6 Once formed, LTC4 is transported to extracellular space via the ATP-binding cassette (ABC) transporters-1 and-4 and then metabolized to LTD4 and LTE4 by γ-glutamyl transpeptidases and dipeptidases, respectively. The rapid extracellular metabolism of LTC4 and LTD4 results in short biologic half-lives relative to the stable mediator LTE4, which is abundant and readily detected in biologic fluids. In neutrophils, LTA4 is hydrolyzed by a cytosolic LTA4, hydrolase enzyme to form LTB4, a dihydroxy leukotriene that is a potent chemoattractant for neutrophils and monocytes.7

5-LO activity is substantially upregulated when granulocytes are exposed ex vivo to hematopoietic cytokines such as GM-CSF or (in the case of eosinophils) IL-5.8,9 In blood-derived human mast cells, IL-3 and IL-5 enhance the function of 5-LO by inducing its import from the cytosol to the nucleoplasm, whereas IL-4 potently induces expression and function of LTC4S.10 LTC4S enzymatic function can be inhibited by protein kinase C (PKC)-dependent phosphorylation, which can limit the generation of cys-LTs ex vivo.11 5-LO activity is suppressed by stimuli that induce cyclic adenosine monophosphate (cAMP) accumulation, leading to serine phosphorylation of 5-LO by cAMP-dependent protein kinase A (PKA).12,13 These in vitro studies suggest that LT production is tightly regulated by

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the microenvironment and intracellular phosphorylation events, with mechanisms that can respectively enhance and limit the expression and function of the critical metabolic enzymes dependent on context.

Cysteinyl leukotriene receptors

Early pharmacologic profiling studies predicted the existence of at least 2 cys-LT receptors in mammalian tissues.14 The molecular characterization of the classical G protein-coupled receptors (GPCRs) partially reconciled this pharmacology. The type 1 cys-LT receptor, CysLT1R, is a high-affinity receptor for LTD4 and the target of antagonists (Montelukast, Zafirlukast, and Pranlukast) that are used for the management of asthma. The cloned human CysLT1R gene encodes a GPCR of 339 amino acids.15 Human CysLT1R mRNA is expressed in bronchial smooth muscle and substantially in myeloid cells, such as macrophages and mast cells. The human CysLT1R is 38% identical to CysLT2R in amino acid sequence.16 CysLT1R binds LTC4 and LTD4 with equal affinity, and binds LTD4 with affinity one-log less than CysLT1R. CysLT2R is resistant to Montelukast, and is expressed both on cells that also express CysLT1R (e.g., myeloid cells, smooth muscle), as well as endothelial cells, cardiac Purkinje cells, adrenal medulla, and brain.16 The incompletely overlapping distribution of the 2 classical receptors for cys-LTs suggests that they have both complementary and distinct functions.

In contrast to their affinities for LTC4 and LTD4, the cloned CysLT1R and CysLT2R receptors display trivial binding affinity for the stable metabolite LTE4. Nonetheless, studies of human tracheal explants and guinea pig tracheal rings had predicted the existence of a third cys-LT receptor with a preference for LTE4.14,17 LTE4 also was equipotent to its precursors for inducing weal and flare responses when injected intradermally into humans.18 Recently GPR17, previously reported as an oxyglutarate receptor,19 was identified as a potential LTE4 receptor.20 LTE4 binds and activates GPR17 at low nM range concentrations in transfected cells, and resists blockade by MK571, a prototype CysLT1R antagonist. The ability of LTE4 to induce cutaneous vascular permeability in mice depends largely on the presence of GPR99. GPR99 mRNA is expressed strongly by kidney and large quantities during acute inflammatory responses,20 signaling through the cognate P2Y2 receptors may limit potentially deleterious effects of CysLT1R signaling in cells that express both classes of receptors (Figure). Moreover, the overlap of the cytokines (IL-4) and protein kinases (PKA, PKC) that respectively enhance and suppress the functions of the synthetic and receptor systems suggest that cys-LT production may be regulated in parallel with end-organ responsiveness.

CysLT1R functions can also be regulated by direct physical interactions with other GPCRs. CysLT1R and CysLT2R heterodimerize in cultured human mast cells.20 The presence of CysLT1R limits the levels of membrane expression of CysLT2R, and dampens the capacity of CysLT1R to induce phosphorylation of extracellular signal regulated kinase and proliferation in this cell type. GPR17, a GPCR homologous to CysLT1R and CysLT2R,30 was originally “deorphanized” as a dual-specific receptor for cys-LTs and uracil nucleotides.31 However, we and others could not reproduce GPR17 activation by either ligand type in various assay systems.30,32,33 Instead, GPR17 functions as a negative regulator of LTD4-mediated CysLT1R activation, and markedly reduces binding of LTD4 when the two receptors are co-expressed in cell lines.32 Accordingly, mice lacking GPR17 (Gpr17−/− mice) showed markedly enhanced CysLT1R-dependent tissue-induced CysLT1R expression by human bronchial smooth muscle cells.21 CysLT1R can be inducibly expressed by mouse T cells stimulated through the T cell receptor.22 CysLT1R signaling is also controlled by PKA25 or PKC26-dependent phosphorylation and desensitization. PKC mediates ligand-induced internalization of CysLT1R following stimulation with LTD4.27 PKC activation by members of the purergic (P2Y) family of GPCRs, which are homologous to the cys-LT receptors, can induce heterologous, PKC-dependent phosphorylation and desensitization of CysLT1R without causing its internalization.28 Since nucleotides, the natural ligands for P2Y receptors, are released in large quantities during acute inflammatory responses,29 signaling through the cognate P2Y2 receptors may limit potentially deleterious effects of CysLT1R signaling in cells that express both classes of receptors (Figure). Moreover, the overlap of the cytokines (IL-4) and protein kinases (PKA, PKC) that respectively enhance and suppress the functions of the synthetic and receptor systems suggest that cys-LT production may be regulated in parallel with end-organ responsiveness.

Regulation of cysteinyl leukotriene receptor function

As is the case for the cys-LT synthesis, cellular responsiveness to cys-LTs can be modulated both by exogenous stimuli and intracellular phosphorylation events. IL-4 and IL-13 upregulate the expression and function of CysLT1R by human peripheral blood monocytes and monocyte-derived macrophages,21 IL-13, but not IL-4, upregulates CysLT1R expression as well in human monocytes.22 IL-13 and transforming growth factor beta

Figure. Cross-regulation of the cysteinyl leukotriene receptors. CysLT1R function is inhibited both by direct physical interactions with CysLT2R or GPR17, and by heterologous, PKC-dependent phosphorylation by P2Y receptors. The lack of both CysLT1R and CysLT2R amplifies cutaneous responses to LTE4, suggesting that both classical receptors cross-regulate GPR99. The requirement for P2Y2 receptors for the ability of LTE4 to amplify pulmonary eosinophilia could reflect an interaction with GPR99.
Cys-LTs are involved in both the upregulation of mast cell activation and LTD₄ production. Additionally, CysLT₁R and CysLT₄R localize to both hematopoietic and non-hematopoietic cell types in the naso-oral tract. "AERD" expression on hematopoietic cells decreases in patients with AERD or aspirin tolerant asthmatics relative to samples from aspirin tolerant controls. These findings strongly suggest that dysregulated cys-LT production in vivo is causally associated with peripheral blood granulocytes and eosinophils and is strongly associated with the levels of urinary LTE₄. The number and distribution of those leukocytes that are platelet-adherent correlated with the levels of urinary LTE₄. Mast cell activation accompanies the responses to aspirin challenge in AERD, and the administration of mast cell stabilizing cromone drugs blocks the rise in urinary LTE₄ that accompanies reactions. Collectively, these studies suggest that the dysregulation of cys-LT production in AERD reflects several cell types. Recently developed models of AERD in mice (see below) may more precisely define the cellular and molecular mechanisms responsible for dysregulated cys-LT production in AERD.

In addition to dysregulated cys-LT generation, subjects with AERD show enhanced end-organ reactivity to cys-LTs. Compared with aspirin tolerant asthmatics, individuals with AERD demonstrate bronchoconstriction in response to inhaled LTE₄ and LTD₄ at significantly lower doses. The numbers and percentages of CysLT₁R-positive mast cells, eosinophils, and monocytes in nasal biopsies from patients with AERD exceed those observed in the tissues of aspirin-tolerant asthmatic controls. CysLT₁R expression on hematopoietic cells decreases following desensitization to aspirin, a procedure that attenuates bronchial reactivity to LTE₄. The numbers and distributions of CysLT₁R-positive cells do not differ between aspirin tolerant asthmatics and subjects with AERD. Interestingly, bronchial reactivity to inhaled LTD₄ in AERD or aspirin tolerant
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Understanding functions of the cys-LTs and their receptors in mice

The development of mice lacking LTC4S (Ltc4s−/−), CysLT,R (Cysltr1−/−), CysLT,R (Cysltr2−/−), and both receptors (Cysltr1/Cysltr2−/−) has permitted in-depth studies of the role of cys-LTs in immune and inflammatory responses. These studies have revealed complex and, in some instances, unanticipated functions for cys-LTs and their receptors in a variety of biologic responses detailed below.

Vascular leak

In a mast cell and IgE-dependent model of passive cutaneous anaphylaxis, Ltc4s−/− mice displayed reductions in ear skin swelling of ~50% compared to wild-type (WT) mice. Intrapерitoneal injections of zymosan, a yeast cell wall glucan that elicits LTC4 generation from macrophages, induced vascular leak that was reduced in both the Ltc4s−/− and Cysltr1−/− strains by ~50% compared with WT controls. The responses of Cysltr2−/− mice were equivalent to those of WT controls. Thus, CysLT,R plays a key role in mediating vascular leak in models where cys-LTs are generated in response to antigen- or pathogen-dependent stimuli.

To determine whether additional cys-LT receptors participated in vascular leak, we subjected Cysltr1/Cysltr2−/− mice to direct intracutaneous challenges with cys-LTs. Surprisingly, LTC4 and LTD4 induced tissue edema in Cysltr1/Cysltr2−/− mice that was comparable to WT mice, and LTE4 induced marked tissue edema in this strain, with 64-fold enhanced sensitivity to LTE4 over the WT controls. This enhanced response to LTE4 was inhibited by pretreatment of the mice with pertussis toxin and a Rho kinase inhibitor, suggesting that it was mediated by a previously unrecognized G protein-coupled cys-LT receptor with a preference to LTE4. Given that GPR99 bound LTE4 in transfected cells, we generated Gpr99−/− and Gpr99/Cysltr1/Cysltr2−/− mice for comparison with WT and Cysltr1/Cysltr2−/− mice. GPR99 deletion from the Cysltr1/Cysltr2−/− mice eliminated the vascular leak in response to the cys-LT ligands, indicating that GPR99 is likely to be a true cys-LT receptor. Furthermore, the Gpr99−/− mice showed a dose-dependent loss of LTE4-mediated vascular permeability, but not to LTC4, or LTD4, suggesting a preference of GPR99 for LTE4.

Th2 Immunity

Lung Th2 immunity to the house dust mite Dermatophagoides farinae (Df) requires stimulation of the myeloid C-type lectin receptor, Dectin-2. Based on a protocol of sensitization of naive WT mice by means of adoptive transfer of Df-pulsed dendritic cells (DCs), Th2 responses to Df require the expressions of LTC4S and CysLT,R by DCs. Interestingly, both Cysltr2−/− mice and Gpr17−/− mice showed markedly augmented eosinophilic pulmonary inflammation, serum IgE, and Th2 cytokine generation in response to Df sensitization and challenge compared to WT controls. DLt4-pulsed DCs derived from Cysltr2−/− mice and Gpr17−/− mice induce an enhanced pulmonary eosinophilic and Th2 immune response in WT mice when compared with WT DCs. The enhanced response induced by Gpr17−/− DCs was eliminated by introduction of the Cysltr1−/− allele, whereas the introduction of the Ltc4s−/− allele eliminated the potentiation of Th2 immunity induced by transfer of Cysltr2−/− DCs. Thus, constitutive downregulation of CysLT,R function by GPR17 and CysLT,R may be critical to maintain homeostasis during the induction of Th2 immunity, at least to allergens (and potentially microbes) that bear ligands for Dectin-2.

Activation of innate lymphoid cells

Type 2 innate lymphoid cells (ILC2) are innate lymphocytes that release large quantities of IL-5 and IL-13 when activated by cytokines, such as IL-33, IL-25, or thymic stromal lymphopoietin (TSLP), derived from epithelial cells. A recent study implicated the cys-LTs in the activation of ILC2 cells. Intrapulmonary challenge of mice with an extract from the mold Alternaria alternata strongly induced the generation of cys-LTs in the lung, and the recruitment and activation of ILC2. ILC2 expressed CysLT,R and responded to stimulation in vitro and in vivo with LTD4, by proliferating and releasing cytokines. Interestingly, while both LTD4 and IL-33 caused lung ILC2 to generate IL-5 and IL-13, only LTD4 caused them to generate IL-4. Ex vivo stimulation of lung ILC2 with either LTD4 or LTE4 caused the production of IL-5. While the IL-5 production in response to LTD4 could be blocked by Montelukast, LTE4-induced IL-5 production was resistant to Montelukast. This study suggests that cys-LTs can contribute to Th2 immunity through direct actions at ILC2. These effects reflect cys-LT actions both classical and nonclassical receptors that can induce effector cytokine production.

Platelet-dependent pulmonary eosinophilia

Platelets are essential for the development of pulmonary eosinophilia and airway remodeling in mouse models of ovalbumin (OVA) sensitization and challenge. Activated platelets express P-selectin, which permits their adherence to leukocytes and primes leukocytes for directed migration via integrins. Mouse and human platelets express both CysLT,R and CysLT,R, as well as the P2Y1 receptor, a homologue of the cys-LT receptors that binds ADP. Stimulation of mouse platelets with LTC4 strongly induces their expression of P-selectin in an entirely CysLT,R-dependent manner, while LTD4 or LTE4 are inactive. Intratracheal administration of LTC4, but not LTD4, mark-
edly amplifies the recruitment of eosinophils to the airways of sensitized mice challenged with low-dose OVA. This amplifica-
tion requires platelets, and is attenuated in Cysltr2–/– mice, sug-
suggesting a direct stimulatory effect of LTC4 on platelet-associ-
cated CysLT1R in the lung vasculature.

Although LTC4 fails to directly activate mouse or human plate-
lets in vitro, intratracheal administration of LTE4, like that of
LTC4, potentiates OVA-induced eosinophilia in a platelet-depen-
dent manner in WT mice. In this model, LTE4 is fully ac-
tive in Cysltr1/Cysltr2–/– mice, suggesting that it acts at a non-
classical cyst-LT receptor. Both the effects of LTE4 (in vivo) and
of LTC4 (in vivo and in vitro) depend exclusively on the P2Y12 re-
cptor. A computer modeling study predicted that P2Y12 recep-
tors might recognize LTE4 as a surrogate ligand, and LTE4 elicits calcium flux and phosphorylation of extracellular signal
regulated kinase in transfected cells over-expressing human
P2Y12 receptors. Nonetheless, radiolabeled LTE4 does not di-
rectly bind to microsomal membranes from P2Y12 receptor-ex-
pressing transfectants. It is presently unknown whether the in-
volvement of P2Y12 in LTE4-dependent signaling responses and
airway inflammation reflects a direct interaction between P2Y12
receptors and a bona fide LTE4 receptor, such as GPR99. The
therapeutic potential of drugs that block P2Y12 receptors in
asthma or AERD is unexplored.

AERD-like models

Although several cellular abnormalities in eicosanoid synthe-
sis and receptor function have been described in AERD, the
lack of a valid model of the disease has restrained progress in
defining the key pathogenetic steps. Hirata et al. generated a
transgenic mouse strain over expressing LTC4s and examined
the phenotype in OVA-induced pulmonary inflammation with
or without treatment with a COX inhibitor, sulpyrine. OVA-
challenged LTC4s-transgenic mice, but not similarly treated WT
mice, demonstrated a significant increase in airway resistance
by sulphine treatment. This is associated with increases in LTC4
and LTB4 in bronchoalveolar lavage (BAL) fluid in sulpyrine-
treated OVA-challenged transgenic mice. Importantly, the in-
crease in airway resistance was inhibited by Pranlukast, a CysLT
R antagonist. This study demonstrates that the pathogeno-
monic feature of aspirin-induced bronchoconstriction can be
reproduced in a mouse model, and suggests that the overex-
pression of LTC4s described in tissues from patients with
AERD has a potentially causal role.

Prostaglandin E2 (PGE2) controls cyst-LT generation by activat-
ing PKA and inducing phosphorylation of 5-LO. Tissue inflam-
flammation is typically associated with increased PGE2 produc-
tion, reflecting the co-expression of 2 inducible enzymes; COX-
2 (a largely aspirin-resistant enzyme) and microsomal PGE2
synthase-1 (mPGES-1), which isomerizes COX-2-derived PGH2
to PGE2. Nasal polyps from subjects with AERD contain less
PGE2 than nasal polyps from aspirin tolerant controls, possi-
bly relating to epigenetic modifications of COX-2 and/or mP-
GES-1 expression. Mice lacking mPGES-1 (Ptges–/–) cannot
upregulate PGE2 production with inflammation, and display a
remarkably AERD-like phenotype when subjected to a model of
Df-induced pulmonary disease. Compared with WT controls,
Ptges–/– mice show increased eosinophilic inflammation and
levels of cyst-LTs in the BAL fluid. Challenge with inhaled
lysinic aspirin causes marked increases in airway resistance,
robust release of cyst-LTs, and pulmonary mast cell activation in
the Ptges–/– strain, but not in WT controls. Aspirin challenge
profoundly depletes lung PGE2 in the Ptges–/– mice, but not in
the WT controls, suggesting that the mPGES-1 is needed to
maintain PGE2 levels when COX-1 is inhibited. Ptges–/– mice
also show increased numbers of platelet-adherent granulocytes
in both the peripheral blood and lungs compared with WT
controls. Importantly, cyst-LT production, mast cell activation,
and the changes in airway resistance were blocked by depletion
of platelets or blockade of the TP receptor for thromboxane A2.
This model may be useful in defining the potential pathogenet-
ic role of GPR99, CysLT2R, and P2Y12 receptors in AERD, as well
as unraveling the complex interplay between cyst-LTs, platelets,
and mast cells that lead to the physiologic response to aspirin
challenges.

CONCLUSIONS

While the drugs capable of inhibiting cyst-LT formation and
blocking CysLT,R are useful, it is clear that the cyst-LT system is
far more complex than initially appreciated. The involvement
of the cyst-LTs in the induction of Th2 immunity and the effec-
tor phase of the immune response suggests additional potential
applications for currently available pharmacologic agents.
However, the recognition that cyst-LTs act through at least three
receptors and the resistance of 2 of these (CysLT1R and GPR99)
the blockade by currently available drugs presents both chal-
enges and opportunities for further therapeutic development.
The availability of a broad array of valid animal models should
facilitate progress in this area, while continuing to reveal unan-
ticipated biological functions for the cyst-LTs and their recep-
tors.

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REFERENCES

1. Israel E, Rubin P, Kemp JP, Grossman J, Pierson W, Siegel SC, Ting-


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1993;6:1468-73.


