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Identification of GPR99 Protein as a Potential Third Cysteinyl Leukotriene Receptor with a Preference for Leukotriene E_4 Ligand*[S]

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Background: A most stable cysteinyl leukotriene, LTE_4, mediates vascular permeability in mice lacking the two known receptors.
Results: GPR99 deficiency abolishes LTE_4-induced vascular permeability in mice also lacking the two known receptors.
Conclusion: GPR99 is a potential third cysteinyl leukotriene receptor.
Significance: GPR99 may be a therapeutic target for inflammatory diseases.

The cysteinyl leukotrienes (cys-LTs), leukotriene C_4 (LTC_4), a conjugation product of glutathione and eicosatetraenoic acid, and its metabolites, LTD_4 and LTE_4, are lipid mediators of smooth muscle constriction and inflammation in asthma. LTD_4 is the most potent ligand for the type 1 cys-LT receptor ($CysLT_1R$), and LTC_4 and LTD_4 have similar lesser potency for $CysLT_2R$, whereas LTE_4 has little potency for either receptor. *Cysltr1/Cysltr2*$^{-/-}$ mice, lacking the two defined receptors, exhibited a comparable dose-dependent vascular leak to intradermal injection of LTC_4 or LTD_4 and an augmented response to LTE_4 as compared with WT mice. As LTE_4 retains a cysteine residue and might provide recognition via a dicarboxylic acid structure, we screened cDNAs within the P2Y nucleotide receptor family containing CysLTRs and dicarboxylic acid receptors with trans-activator reporter gene assays. GPR99, previously described as an oxoglutarate receptor (Oxgr1), showed both a functional and a binding response to LTE_4 in these transfectants. We generated *Gpr99*$^{-/-}$ and *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ mice for comparison with WT and *Cysltr1/Cysltr2*$^{-/-}$ mice. Strikingly, GPR99 deficiency in the *Cysltr1/Cysltr2*$^{-/-}$ mice virtually eliminated the vascular leak in response to the cys-LT ligands, indicating GPR99 as a potential $CysLT_3R$ active in the *Cysltr1/Cysltr2*$^{-/-}$ mice. Importantly, the *Gpr99*$^{-/-}$ mice showed a dose-dependent loss of LTE_4-mediated vascular permeability, but not to LTC_4 or LTD_4, revealing a preference of GPR99 for LTE_4 even when $CysLT_1R$ is present. As LTE_4 is the predominant cys-LT species in inflamed tissues, GPR99 may provide a new therapeutic target.

Leukotriene C_4 (LTC_4),[2] LTD_4, and LTE_4, collectively called cysteinyl leukotrienes (cys-LTs), are proinflammatory mediators derived from arachidonic acid through the 5-lipoxygenase/LTC_4 synthase pathway (1, 2). Intracellularly synthesized LTC_4 is exported via multidrug resistance-associated proteins and is rapidly metabolized in the extracellular space by cleavage removal of glutamic acid and then glycine to LTD_4 and LTE_4, respectively. The type 1 cys-LT receptor ($CysLT_1R$) is the high affinity receptor for LTD_4, whereas the type 2 receptor ($CysLT_2R$) can bind both LTC_4 and LTD_4 with one-log less affinity than that of $CysLT_1R$ for LTD_4 in transfected cells (3, 4). The cys-LTs are mediators of bronchial asthma on the basis of their potent bronchoconstrictive activity via the $CysLT_1R$ in pharmacologic studies and the clinical effectiveness of $CysLT_1R$ antagonists (5, 6).

LTE_4, the most abundant cys-LT at sites of inflammation due to its stability (7, 8), was only a weak agonist for the $CysLT_1R$ and $CysLT_2R$ in studies with transfectants (3, 4), suggesting that it might lack agonist activity. In contrast, pretreatment with LTE_4, but not LTC_4 or LTD_4, enhanced the response of guinea pig trachea (9) or human bronchial smooth muscle to histamine (10). Furthermore, in limited studies, LTE_4 was comparable with LTC_4 or LTD_4 in eliciting a vascular leak in skin in guinea pigs (11) or humans (12), and inhalation of LTE_4, but not LTD_4, by patients with asthma elicited an accumulation of eosinophils and basophils into the bronchial mucosa (13, 14). These observations strongly supported an agonist function for LTE_4, likely mediated by a receptor separate from those that are activated by its short-lived precursors (15).

To seek an additional receptor for the cys-LTs, we generated mice lacking the now classical $CysLT_1R$ and $CysLT_2R$ described by Evans and colleagues (3, 4) for humans and then by others for mice (16–18). As compared with WT mice, the *Cysltr1/Cysltr2*$^{-/-}$ strain on a BALB/c background showed similar dose-dependent edema responses to intradermal injection of LTC_4 or LTD_4 into the ear, indicating the presence of another receptor. Unexpectedly, LTE_4 elicited more edema in the *Cysltr1/Cysltr2*$^{-/-}$ mice than LTD_4 or LTC_4 at the same dose and showed a 64-fold increase in potency in the *Cysltr1/Cysltr2*$^{-/-}$ mice as compared with WT mice, revealing a ligand preference for an unidentified receptor (19). Such a receptor could be important in asthma both because there is heterogeneity to the benefit of $CysLT_1R$ antagonists (20, 21) and because LTE_4, unlike transient LTC_4 and LTD_4, is sustained at levels that even

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[2] The abbreviations used are: LT, leukotriene; cys-LT, cysteinyl LT; $CysLT_1R$, type 1 cys-LT receptor; $CysLT_2R$, type 2 cys-LT receptor; CREB, cAMP-responsive element-binding protein; GPCR, G protein-coupled receptor; AERD, aspirin-exacerbated respiratory disease.

provide a urinary biomarker for the 5-lipoxygenase/LTC_4 synthase pathway (22).

We have now identified GPR99, previously recognized as a G protein-coupled receptor for oxoglutarate (Oxgr1) (23), as $CysLT_3R$, the LTE_4-selective receptor functioning in the *Cysltr1/Cysltr2*$^{-/-}$ strain. We identified GPR99 using a reporter gene expression assay to screen candidate cDNAs selected from the P2Y gene family that recognize dicarboxylic acids. We confirmed the nmol range binding of [^{3}H]LTE_4 onto microsome membranes from GPR99-transfected cells. We then generated triple-deficient *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ mice to show the loss of permeability-enhancing function to all three cys-LT ligands in contrast to their sustained function in the presence of GPR99 but absence of the classical receptors in the *Cysltr1/Cysltr2*$^{-/-}$ strain. Finally, a dose-dependent loss of edema responses to LTE_4, but not to LTC_4 or LTD_4, in *Gpr99*$^{-/-}$ mice as compared with WT mice shows a preference of GPR99 for LTE_4 even in the presence of the classic receptors.

EXPERIMENTAL PROCEDURES

Reporter Gene Assay—A trans-activator reporter gene assay system (Stratagene) was used to examine whether GPR91 or GPR99 can mediate LTE_4-specific signaling in transfectants. pFA-CMV-trans-activator plasmids contain a fusion cDNA of the DNA-binding domain of GAL4 with an activation domain derived from CREB or Elk-1. When co-transfected with a gene that activates adenylate cyclase or MAPK, the activation domain from CREB or Elk-1 is phosphorylated, respectively, and binds to the GAL4 DNA-binding elements. A co-transfected pFA-Luc reporter plasmid that contains GAL4-binding sites upstream of the firefly luciferase gene is then induced.

HeLa cells were transiently transfected with pCXN vector (a mammalian expression vector) control, pCXN-GPR91, or pCXN-GPR99 along with pFA-CMV-CREB or pFA-CMV-Elk-1 and pFA-Luc. After a 4-h incubation, the culture medium was replaced with serum-free DMEM. The next day, various concentrations of LTE_4, succinate (100 μM), or oxoglutarate (100 μM) were added to the medium, and the cells were further incubated for 16 h. Then, the cells were lysed, and the firefly luciferase activity was measured. A *Renilla* luciferase plasmid was used as an internal control to assess the transfection efficiency as described in the manufacturer's instructions (Promega).

[^{3}H]LTE_4 Binding Assay—[^{3}H]LTE_4 binding to the microsomal membranes from the CHO cell transfectants was assayed as described with modifications (16). [^{3}H]LTE_4 was prepared from [^{3}H]LTD_4 (PerkinElmer Life Sciences) by incubation with 10% FBS that contains dipeptidases, and the conversion was confirmed by reverse phase HPLC (24). 100 μg of membrane protein was incubated for 1 h at room temperature with varied concentrations of [^{3}H]LTE_4. The LTE_4-specific binding was determined by subtracting nonspecific binding, which was determined in the presence of 1 μM cold LTE_4, from total binding. For competition assays, various concentrations of LTC_4, LTD_4, LTE_4, MK-571, or oxoglutarate were added before incubation of the membrane protein with 2 nM [^{3}H]LTE_4. When LTC_4 and LTD_4 were used as competitors, 50 mM serine borate and 20 mM L-penicillamine were included, respectively, to prevent conversion of the peptide adduct.

Generation of Gpr99/Cysltr1/Cysltr2$^{-/-}$ *Mice—Cysltr1/Cysltr2*$^{-/-}$ mice were generated by crossbreeding of BALB/c *Cysltr1*$^{-/-}$ and *Cysltr2*$^{-/-}$ mice as described (19). C57BL/6 *Gpr99*$^{-/-}$ mice (the Knock-Out Mouse Project, University of California, Davis) were backcrossed on to the BALB/c background for four generations (N4). Because the *Cysltr1* (on chromosome X) and *Gpr99* (on chromosome 14) genes are on different chromosomes, BALB/c *Gpr99/Cysltr1*$^{-/-}$ mice were generated by crossbreeding as described for *Cysltr1/Cysltr2*$^{-/-}$ mice. However, the *Gpr99* and *Cysltr2* genes are both on chromosome 14, and they are ~47 megabase pairs apart; we first bred BALB/c *Gpr99*$^{-/-}$ mice with *Cysltr2*$^{-/-}$ mice to obtain *Gpr99*$^{+/-}$/*Cysltr2*$^{+/-}$ mice. We then bred *Gpr99*$^{+/-}$/*Cysltr2*$^{+/-}$ mice with BALB/c WT mice to obtain *Gpr99*$^{+/-}$/*Cysltr2*$^{+/-}$ (N5) mice with both mutant alleles on the same sister chromatid. These *Gpr99*$^{+/-}$/*Cysltr2*$^{+/-}$ mice were intercrossed to obtain *Gpr99/Cysltr2*$^{-/-}$ mice. Finally, *Gpr99/Cysltr1*$^{-/-}$ mice were crossbred with *Gpr99/Cysltr2*$^{-/-}$ mice to obtain the triple null (*Gpr99*/*Cysltr1/Cysltr2*$^{-/-}$) strain. *Gpr99*$^{-/-}$ and *Gpr99*/*Cysltr1/Cysltr2*$^{-/-}$ mice were viable and had no apparent abnormalities based on fertility, behavior, and gross findings at autopsy. 2–4-month-old, male *Gpr99*$^{-/-}$, *Cysltr1/Cysltr2*$^{-/-}$, and *Gpr99*/*Cysltr1/Cysltr2*$^{-/-}$ mice were used. WT littermates from the intercrossing of *Gpr99*$^{+/-}$ or *Gpr99*/*Cysltr1/Cysltr2*$^{+/-}$ mice were used as controls.

Pharmacologic Assessment of cys-LT-induced Ear Edema—Mice received intradermal injections of various concentrations of LTC_4, LTD_4, or LTE_4 in 25 μl of saline/dimethyl sulfoxide (DMSO) (vehicle) in the right ear and 25 μl of vehicle in the left ear. At 0, 30, 60, 120, and 240 min after the intradermal injection, ear thickness was measured with calipers (Dyer Co.). The results are presented as the difference of ear thickness between the right ear and left ear. All animal studies were approved by the Animal Care and Use Committee of the Dana-Farber Cancer Institute.

Statistics—Results were expressed as mean ± S.E. Student's *t* test was used for the statistical analysis. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Identification of a Candidate cDNA Encoding a Receptor for LTE_4 Activation—Based on their amino acid sequence, $CysLT_1R$ and $CysLT_2R$ belong to the nucleotide P2Y G protein-coupled receptor family (25). Within the $P2Y_1$ subgroup, GPR91 and GPR99 do not bind to nucleotides but do bind to the glucose metabolism intermediates, succinate and oxoglutarate (α-ketoglutarate), respectively (23). Because both succinate and oxoglutarate are dicarboxylic acids, and two carboxylic acid groups are also present in LTE_4, we used a trans-activator reporter gene assay system to examine whether GPR91 or GPR99 can mediate LTE_4-specific signaling in transfectants. Cells transfected with pCXN-GPR91 or a control vector alone did not respond to LTE_4 in either assay. In contrast, cells transfected with pCXN-GPR99 responded to LTE_4 in a dose-dependent manner in both assays beginning at a submicromolar concentration (Fig. 1*A*). As expected, oxoglutarate

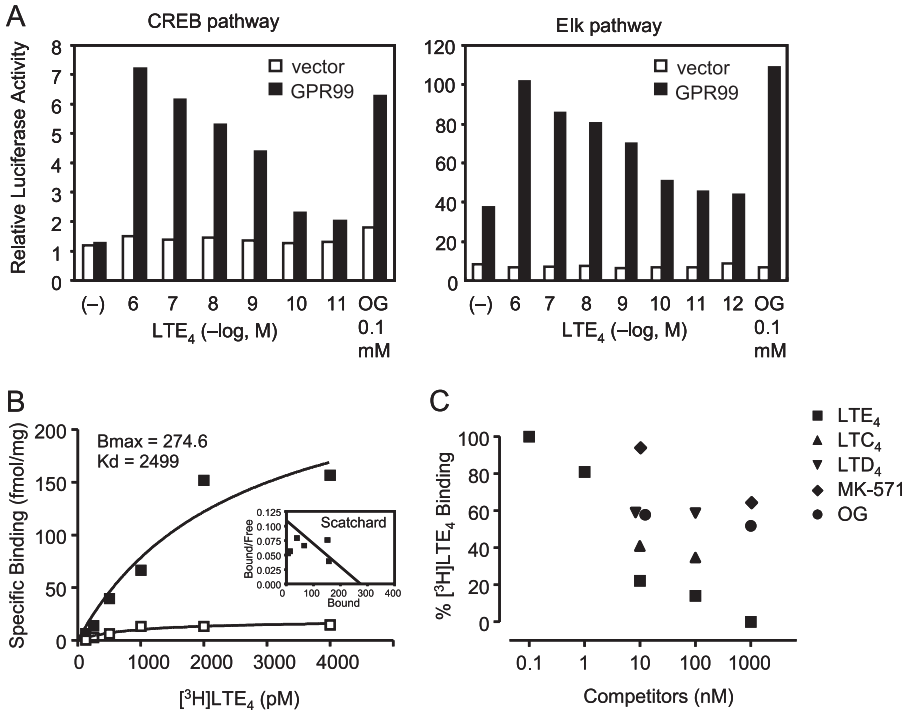

FIGURE 1. **LTE_4-elicited luciferase reporter gene and [^{3}H]LTE_4 binding assays.** *A*, LTE_4-elicited, CREB- or Elk-1-mediated luciferase expression in HeLa cells transfected with GPR99 (*black columns*) and vector (*white columns*). *OG*, oxoglutarate. *B*, specific [^{3}H]LTE_4 binding to 100 μg of microsomal membrane proteins from GPR99 (*closed squares*) and vector control (*open squares*) transfectants. *Inset*, Scatchard plot analysis for GPR99 transfectants is shown. *C*, competition for [^{3}H]LTE_4 binding (2 nM) by LTE_4 (*squares*), oxoglutarate (*circles*), LTC_4 (*triangles*), LTD_4 (*inverted triangles*), and MK-571 (*diamonds*). Data are representative of two independent experiments.

(α-ketoglutarate) at a concentration of 0.1 mM also activated cells transfected with GPR99.

We then sought to show that GPR99 directly bound [^{3}H]LTE_4 using microsomal membranes from CHO cells transiently expressing GPR99. The [^{3}H]LTE_4 binding in the absence and presence of cold LTE_4 revealed a specific dose-dependent binding to GPR99 with a K_d of 2,499 pM and a B_{max} of 274.6 fmol/mg of protein (Fig. 1*B*). Essentially no specific binding was obtained with vector-only transfected cells. By competition assays with 2 nM [^{3}H]LTE_4, cold LTE_4 provided a dose-dependent inhibition (Fig. 1*C*). LTE_4 showed 80% inhibition at 10 nM, whereas inhibition at this concentration was 60% for LTC_4, 40% for LTD_4 and for oxoglutarate, and negligible for MK-571, a $CysLT_1R$ antagonist. Oxoglutarate completely inhibited the binding at 100 μM (not shown), whereas LTE_4 did so at 1 μM. These results suggest that GPR99 might have a preference for LTE_4 and qualify as a candidate for the elusive LTE_4 receptor, but they need to be extended with more comprehensive investigations of binding and signaling mechanisms. To determine whether this candidate LTE_4 receptor mediates the vascular permeability responses to cys-LTs in mice lacking the classical $CysLT_1R$ and $CysLT_2R$ receptors was the critical next step.

GPR99 Mediates LTE_4-induced Ear Edema in the Cysltr1/Cysltr2$^{-/-}$ and in WT Mice—The *Cysltr1/Cysltr2*$^{-/-}$ strain exhibits a markedly augmented swelling response to intracutaneous administration of LTE_4 and an essentially intact response to LTC_4 and LTD_4 (19). Thus, we sought to verify that GPR99 is a *bona fide* third receptor with a preference for LTE_4 by deleting it from this strain and studying skin swelling responses in the resultant triple-null mice. LTE_4 (0.5 nmol/site) was injected intradermally into the ear of BALB/c WT, *Gpr99*$^{-/-}$, *Cysltr1/Cysltr2*$^{-/-}$, and *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ mice, and tissue edema was assessed by measuring ear thickness at 0, 30, 60, 120, 180, and 240 min after the injection. Net ear swelling after correction for the left vehicle-injected ear peaked in WT mice at 30 min, and in *Cysltr1/Cysltr2*$^{-/-}$ mice, there was an enhanced peak response at 30 min (Fig. 2*A*) as reported previously (19). *Gpr99*$^{-/-}$ mice showed essentially the same response as WT mice, indicating an absence of a GPR99 role at this ligand dose. In contrast, *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ mice had a more than 90% loss of the peak response at 30 min as compared with WT, *Gpr99*$^{-/-}$, and *Cysltr1/Cysltr2*$^{-/-}$ mice ($p < 0.01$), indicating that the enhanced LTE_4-induced ear edema in *Cysltr1/Cysltr2*$^{-/-}$ mice is mediated through GPR99.

To assess the dose dependence of the GPR99 response in the absence and presence of the classical receptors, we reduced the dose of LTE_4 by 64-fold (0.008 nmol/site). The peak edema at 30 min with this minimal dose in WT mice was reduced by about one-half and again was exceeded by that in the *Cysltr1/Cysltr2*$^{-/-}$ mice (Fig. 2*B*). As expected, the *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ mice showed essentially no edema response at any time point due to the absence of all three receptors. Of note, at this dose, *Gpr99*$^{-/-}$ mice had a markedly reduced peak response ($p < 0.05$), suggesting that GPR99 is the major LTE_4 receptor when the ligand dose is below the threshold needed to activate $CysLT_1R$ or $CysLT_2R$ *in vivo*. To obtain further evidence supporting this suggestion, an intermediate dose of 0.0625 nmol of LTE_4 was used to elicit the vascular leak. The enhanced edema relative to WT mice in the *Cysltr1/Cysltr2*$^{-/-}$ strain was again virtually abolished in the *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ strain (Fig.

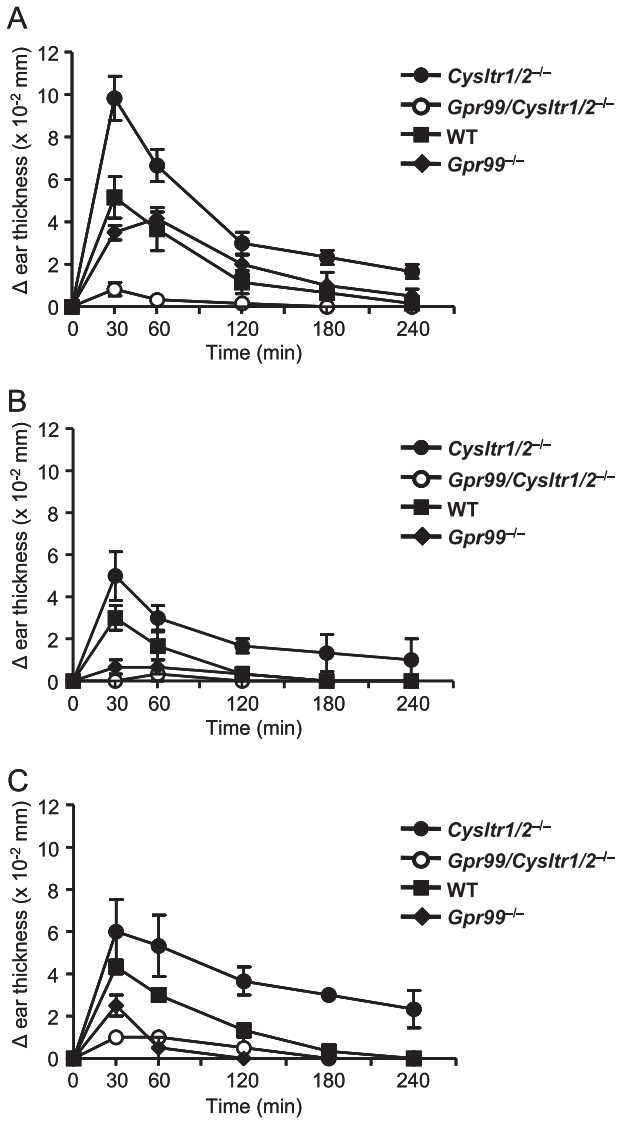

FIGURE 2. **GPR99 mediates LTE$_4$-induced ear edema in *Cysltr1/Cyslt2*$^{-/-}$ and WT mice.** WT (*squares*), *Gpr99*$^{-/-}$ (*diamonds*), *Cysltr1/Cysltr2*$^{-/-}$ (*closed circles*), and *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ (*open circles*) mice received intradermal injections of 0.5 (*A*), 0.008 (*B*), and 0.0625 nmol (*C*) of LTE$_4$ in the right ear and vehicle in the left ear. Ear thickness was measured at the indicated times after the injection. Results are expressed as the mean ± S.E. (3–6 mice per group) of the net differences in the thickness.

2*C*). The peak response in the *Gpr99*$^{-/-}$ strain at 30 min was also diminished to almost one-half that of the WT and absent beyond this time point. Taken together, the dose-response study with LTE$_4$ establishes GPR99 as the preferred LTE$_4$ receptor at one-eighth and one-sixty-fourth of the 0.5-nmol dose in the presence of the classical CysLTRs and at all doses when the classical receptors are absent.

GPR99 Mediates LTC$_4$- and LTD$_4$-induced Ear Edema in the Cysltr1/Cysltr2$^{-/-}$ *Mice*—To assess whether GPR99 mediates the permeability-enhancing action of the other cys-LT ligands, we injected LTC$_4$ and LTD$_4$ at 0.5 or 0.008 nmol/site intradermally into the ear of BALB/c WT, *Gpr99*$^{-/-}$, *Cysltr1/Cysltr2*$^{-/-}$, and *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ mice. With the high dose of LTC$_4$, WT mice had an ear edema response with peak at 30–60 min, and *Gpr99*$^{-/-}$ mice had a similar response (Fig. 3*A*). Although *Cysltr1/Cysltr2*$^{-/-}$ mice had a slightly reduced peak response to LTC$_4$ at 30–60 min as compared with WT mice, the *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ mice had a markedly reduced peak at 30 min, showing GPR99 to be a third cys-LT receptor. With the low dose of LTC$_4$, WT mice had a reduced ear edema response with peak at 30–60 min, and *Gpr99*$^{-/-}$ mice had a similar response (Fig. 3*B*). As expected, although *Cysltr1/Cysltr2*$^{-/-}$ and WT mice had a similar peak response at 30 min, the *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ mice had no response to the low dose LTC$_4$ at any time point. These results indicate that GPR99 can mediate LTC$_4$-induced edema responses in the absence of CysLT$_1$R and CysLT$_2$R.

With the 0.5 nmol/site of LTD$_4$, WT mice and *Gpr99*$^{-/-}$ mice showed peak edema responses at 30–60 min (Fig. 3*C*). The *Cysltr1/Cysltr2*$^{-/-}$ mice showed a reduced peak response at 30 min, whereas there was virtually no response to LTD$_4$ in the *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ mice. With the low dose of LTD$_4$, WT mice and *Gpr99*$^{-/-}$ mice showed reduced but similar peak edema responses at 30–60 min (Fig. 3*D*). Although *Cysltr1/Cysltr2*$^{-/-}$ and WT mice had a similar peak response at 30–60 min, the *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ mice had virtually no response. Thus, GPR99 can mediate LTD$_4$-induced edema responses in the absence of CysLT$_1$R and CysLT$_2$R.

The findings in the triple receptor-deficient mice indicate GPR99 as a potential CysLT$_3$R, a receptor capable of responding to each of the cys-LTs in the absence of the classical receptors, CysLT$_1$R and CysLT$_2$R. A preference or greater sensitivity of GPR99 for LTE$_4$ is shown by the enhanced response of the *Cysltr1/Cysltr2*$^{-/-}$ mice as compared with WT mice but not to LTC$_4$ or LTD$_4$. This is supported by the reduction of the permeability response to low and intermediate doses of LTE$_4$ in the *Gpr99*$^{-/-}$ strain. At a high dose of LTE$_4$, the GPR99-deficient strain is not impaired due to the dominance of the CysLT$_1$R and CysLT$_2$R over GPR99. The strength and sensitivity of the LTE$_4$-mediated vascular leak in the absence of the classical receptors implies their negative regulation for GPR99 function or sensitivity.

The human GPR99 gene was originally identified as an orphan G protein-coupled receptor (GPCR) with homology to a P2Y nucleotide receptor subfamily by searching the human genomic databases with known nucleotide receptors (26). Human GPR99 shares 36% identical amino acids both with the ATP receptor, P2Y$_1$, and with GPR91, which had been identified as another orphan GPCR at that time. Mouse GPR99 shares 85% identical amino acids with human GPR99. The motif Y*X*VTRPL, which is unique to the P2Y$_1$ subfamily, and the motif H*XX*(R/K), which is found in all nucleotide-binding GPCRs, are conserved between mouse and human GPR99. Mouse GPR99 is 33% identical to mouse GPR91, 32% identical to mouse CysLT$_2$R, and 27% identical to mouse CysLT$_1$R. The H*XX*(R/K) motif is conserved in GPR99, GPR91, CysLT$_2$R, and CysLT$_1$R, and the Y*X*VTRPL motif is conserved only in GPR99 and GPR91 (supplemental Fig. 1). The positively charged arginine residues in both motifs are required for succinate-elicited GPR91 activation as assessed by site-directed mutagenesis and functional reporter gene assays (23). Another arginine (position 95 in mouse GPR91 and position 99 in human GPR91) and a histidine (position 99 in mouse GPR91 and position 103 in human GPR91) are also required for succinate-elicited GPR91

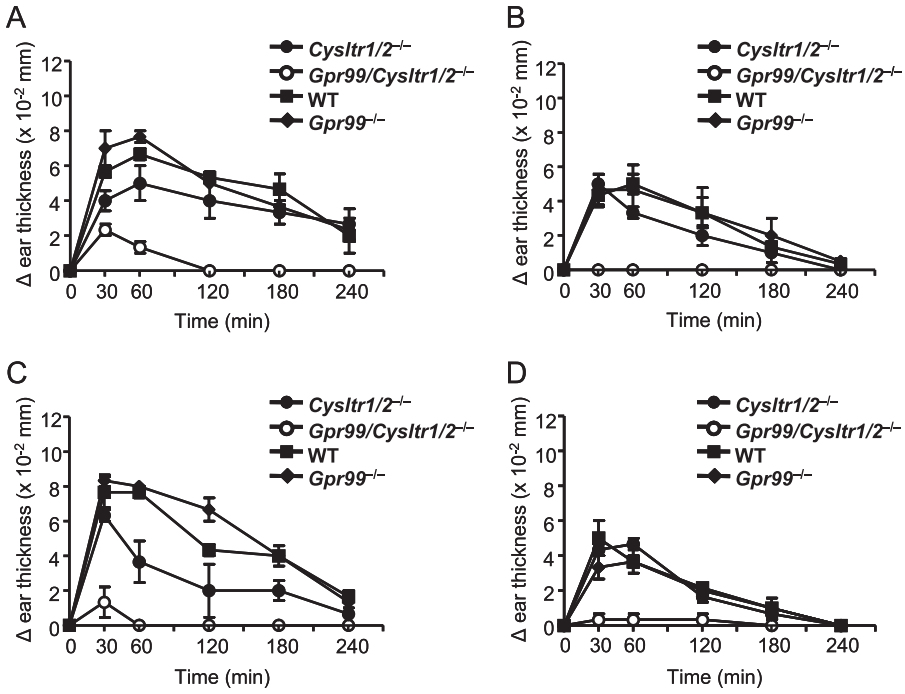

FIGURE 3. **GPR99 mediates LTC$_4$- and LTD$_4$-induced ear edema in *Cysltr1/Cyslt2*$^{-/-}$ mice.** WT (*squares*), *Gpr99*$^{-/-}$ (*diamonds*), *Cysltr1/Cysltr2*$^{-/-}$ (*closed circles*), and *Gpr99/Cysltr1/Cysltr2*$^{-/-}$ (*open circles*) mice received intradermal injections of 0.5 (*A*) and 0.008 nmol (*B*) of LTC$_4$ or 0.5 (*C*) and 0.008 nmol (*D*) of LTD$_4$ in the right ear and vehicle in the left ear. Ear thickness was measured at the indicated times after the injection. Results are expressed as the mean ± S.E. (3 mice per group) of the net differences in the thickness.

activation (supplemental Fig. 1). Because these four amino acids are conserved in GPR99, they may be important in binding dicarboxylic acid ligands. Particularly, the arginine in the *YX*VTRPL motif, which is lacking in CysLT$_2$R and CysLT$_1$R, might account for the preference of GPR99 for LTE$_4$.

Northern blot analysis with human tissues revealed that GPR99 mRNA is expressed in kidney and placenta (26). By a quantitative RT-PCR, human GPR99 mRNA is predominantly expressed in trachea, salivary glands, kidney, fetal brain, and lung and highly expressed in umbilical cord blood-derived mast cells (27). A quantitative RT-PCR analysis in the mouse revealed that GPR99 mRNA is highly expressed in kidney, testis, and smooth muscle (23). Given that CysLT$_1$R and CysLT$_2$R are also expressed on smooth muscle cells in airways and blood vessels (3, 4, 28, 29), expression and function of GPR99 on smooth muscle may be affected by the presence of CysLT$_1$R and CysLT$_2$R.

LTE$_4$ has a sufficient biologic half-life to be excreted into the urine (30), and urinary LTE$_4$ excretion is significantly increased in spontaneous acute asthma flares (22). Asthmatic individuals show LTE$_4$-induced bronchoconstriction as an aerosol in the same concentration range as LTD$_4$ and LTC$_4$ (31, 32), and LTE$_4$ potentiates airway hyperresponsiveness to histamine (33). Furthermore, patients with aspirin-exacerbated respiratory disease (AERD) have both persistently high basal urinary levels of LTE$_4$ and a marked (~10-fold) further increment in urinary LTE$_4$ excretion with aspirin challenge (34). Treatment with montelukast, a CysLT$_1$R antagonist, improves lung function and quality of life of patients with AERD (6), but its beneficial effect is limited and heterogeneous among patients (35). GPR99 may be involved in the chronicity of signs and symptoms, such as nasal polyps, in patients with AERD.

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