γ-Glutamyl Leukotrienase, a Novel Endothelial Membrane Protein, Is Specifically Responsible for Leukotriene D₄ Formation in Vivo

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The metabolism of cysteinyl leukotrienes in vivo and the pathophysiological effects of individual cysteinyl leukotrienes are primarily unknown. Recently we identified an additional member of the γ-glutamyl transpeptidase (GGT) family, γ-glutamyl leukotrienase (GGL), and developed mice deficient in this enzyme. Here we show that in vivo GGL, and not GGT as previously believed, is primarily responsible for conversion of leukotriene C₄ to leukotriene D₄, the most potent of the cysteinyl leukotrienes and the immediate precursor of leukotriene E₄. GGL is a glycoprotein consisting of two polypeptide chains encoded by one gene and is attached at the amino terminus of the heavy chain to endothelial cell membranes. In mice it localizes to capillaries and sinusoids in most organs and in lung to larger vessels as well. In contrast to wild-type and GGT-deficient mice, GGL-deficient mice do not form leukotriene D₄ and show leukotriene D₄ accumulation and significantly more airway hyperresponsiveness than wild-type mice in the experimental asthma, and induction of asthma results in increased LTD₄ protein levels and enzymatic activity. Thus, GGL plays an important role in leukotriene D₄ synthesis in vivo and in inflammatory processes. (Am J Pathol 2002, 161:481–490)

Cysteinyl leukotrienes (Cyst LTs) are important mediators of some inflammatory and immune disorders including anaphylaxis, Zymosan A-induced peritonitis, and asthma.1–4 The pathophysiological effects of Cyst LTs include stimulation of smooth muscle contraction leading to bronchoconstriction, edema formation, and mucus production. Synthesis of leukotriene C₄ (LTC₄), the parent Cyst LT, from leukotriene A₄ and glutathione is catalyzed by leukotriene C₄ synthase in macrophages, eosinophils, mast cells, and some leukemic cell lines.5–7 Conversion of LTC₄ to leukotriene D₄ (LTD₄) involves loss of a γ-glutamyl residue. Although γ-glutamyl transpeptidase (GGT) is known to catalyze LTD₄ formation in the test tube,8,9 the mechanism of LTD₄ formation in vivo is unknown. Because LTD₄ is more potent than its precursor and binds with much higher affinity to the cysteinyl LT1 receptor than LTC₄,10 understanding LTD₄ metabolism is important in clarifying its role in disease. In addition, clearance of Cyst LTs in the urine as leukotriene E₄ (LTE₄) requires LTD₄ formation because it is the immediate precursor of LTE₄.11–13

It is generally accepted that GGT is responsible for LTC₄/LTD₄ conversion in vivo.13,14 However, we recently identified and cloned a mouse GGT family member designated γ-glutamyl leukotrienase (GGL) because its primary natural substrate appears to be LTC₄.15,16 The enzyme also cleaves several other S-substituted glutathiones, but not GSH itself. The GGL gene is located ~3-kb upstream of GGT, and the two cDNAs share a 57% nucleotide identity.16 Because all known substrates for GGL are also substrates for GGT,16,17 it is not possible to assay for GGL in tissues from wild-type (WT) mice without also detecting GGT. To circumvent this problem we have used GGT-deficient mice for assays of GGL activity and developed antibodies specific for GGL.4,15–18 GGL is expressed at highest levels in the spleen and uterus.4,16 This observation is puzzling because it is not readily apparent what physiological function an enzyme such as GGL would have in tissues as different as spleen and uterus. Although the relative contributions of GGL and GGT to Cyst LT metabolism are unknown, our previous data indicate that no other enzymes in the mouse cleave LTC₄.4 Thus it is possible to take advantage of mice deficient in GGL, GGT, or both enzymes to evaluate their roles in Cyst LT metabolism in vivo and in pathophysiology.4,17

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Differences in organ distribution of the two enzymes suggest both different functions and different substrate specificities in vivo. For the most part, GGT is expressed on absorptive and secretory epithelium (the luminal surface of the proximal tubular cells of the kidney, the villous epithelium of the small intestine, the ductular epithelium of the pancreas, and the secretory epithelial cells of the seminal vesicle). These locations are consistent with the generally held idea that the major function of GGT is GSH cleavage. Although LTC₄ is a substrate for GGT in the test tube, the distribution of the enzyme contrasts with the known distribution and function of Cyst LTs. Using GGL-deficient mice, we have recently found that in Zymosan A-induced peritonitis most of the initial neutrophil accumulation is dependent on GGL activity and that GGL is responsible for most or all of the cleavage of endogenous LTC₄ that accumulates in the peritoneal cavity. These findings underscore the potential role of GGL in inflammation and provide evidence that it functions separately from GGT. Further studies of the characteristics of GGL and its roles in different pathophysiological conditions are essential for the understanding of its biological function.

Here we demonstrate that GGL is a glycoprotein composed of two polypeptide chains and is located on the surface of endothelial cells. GGL converts exogenously administered LTC₄ in the circulation, as well as formed in surface of endothelial cells. GGL converts exogenously presented of two polypeptide chains and is located on the surface of endothelial cells. LTC₄ is a substrate for GGT in the test tube, the distribution of the enzyme contrasts with the known distribution and function of Cyst LTs. Using GGL-deficient mice, we have recently found that in Zymosan A-induced peritonitis most of the initial neutrophil accumulation is dependent on GGL activity and that GGL is responsible for most or all of the cleavage of endogenous LTC₄ that accumulates in the peritoneal cavity. These findings underscore the potential role of GGL in inflammation and provide evidence that it functions separately from GGT. Further studies of the characteristics of GGL and its roles in different pathophysiological conditions are essential for the understanding of its biological function.

**Materials and Methods**

**Chemicals**

LTC₄, LTD₄, and LTE₄ were from Cayman Chemical Company (Ann Arbor, MI). Papain and N-glycosidase F were purchased from Sigma Chemical Co. (St. Louis, MO). Endoglycosidase H was from Boehringer Mannheim Co. (Indianapolis, IN). Anti-mouse CD31 monoclonal antibody was from Pharmingen (San Diego, CA). Texas Red-conjugated anti-rat antibody and Oregon Green conjugated anti-rabbit antibody were from Molecular Probes (Eugene, OR). The immunohistochemistry kit was from BioGenes (San Ramon, CA).

**Animals**

All GGT-, GGL-, and GGT/GGL-deficient mice were developed in our laboratory. All mice including WT controls were on a C57BL/129SvEv background and were used between 6 to 8 weeks of age. They were supplied with N-acetyl cysteine in the drinking water.

**Generation of Anti-GGL Polyclonal Antibody**

Rabbit anti-mouse GGL was generated by using an N-terminus truncated recombinant GGL protein. GGL cDNA fragment (nucleotides 426 to 1721) was expressed in-frame in an expression vector, pET-30a (+) (Novagen, Madison, WI) in *Escherichia coli*. The truncated protein with 100 amino acids at the N-terminus removed was then purified by using the Xpress System Protein Purification kit (Invitrogen, Carlsbad, CA). New Zealand White rabbits were injected with 200 μg of the protein in complete Freund’s adjuvant and boosted with 100 μg of protein in incomplete Freund’s adjuvant every 2 weeks. Rabbits made high-titer-specific anti-GGL antibodies as judged by Western blot analysis.

**Induction of Experimental Asthma**

*A. fumigatus* culture filtrate allergen (CF) (lot no. DC980809) was prepared and used as previously described. Mice were challenged as previously described; briefly, 50 μl of CF or saline control was administered intranasally to mice anesthetized with Metofane (Janssen, Toronto, Canada). Mice were challenged five times with 4 days between each challenge. Fifteen hours after the final CF challenge, airway resistance was measured and AHR determined by C200, and bronchoalveolar lavage fluid (BALF), serum, and lung tissue were collected. BALF total and differential cell counts, BALF mucine, and lung histology were evaluated as described. Data are representative of two independent experiments with seven to eight mice in each group.

**Western Blot Analysis**

Tissue homogenates were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the presence or absence of 0.1 mol/L of dithiothreitol and electrophoretically transferred to nitrocellulose membrane. The anti-serum was used at 1:20,000 dilution. The detecting system was a Phototope-HRP Detection Kit (New England BioLabs, Beverly MA).

For deglycosylation of GGL with endoglycosidases, tissue homogenates from spleen and uterus were subjected to endoglycosidase H (Boehringer Mannheim Co.) or N-glycosidase F (Sigma Chemical Co.) treatment as previously described. The reactions were performed at 37°C for 18 hours followed by Western blot analysis.

For dissociation of GGL from cell membranes, homogenates of WT uterus were centrifuged at 43,000 × g for 30 minutes at 4°C, and the membrane fraction was subjected either to dithiothreitol reduction or to papain digestion. The samples were reduced with 0.1 mol/L of dithiothreitol in the presence of 0.02% SDS at 60°C for 1 hour or incubated with papain at a final concentration of 1 mg of papain/1.5 g membrane protein at 25°C for 30 minutes. The reactants were centrifuged at 43,000 × g at 4°C for 10 minutes, and both supernatant and pellet were analyzed by Western blot. For assaying GGL protein level in the lungs after asthma induction, lung homogenates were directly analyzed by Western blot and differences were quantified by scanning densitometry.
**LTC₄/LTD₄ Conversion Assay**

LTC₄ conversion activity was assayed by high performance liquid chromatography (HPLC) as described previously. Specific activity was expressed as nmol of LTC₄ converted/mg protein/hour by measuring the formation of LTD₄ and LTE₂; the latter is formed by the action of membrane-bound dipeptidase on LTD₄.

To assay LTC₄/LTD₄ conversion activity in phosphate-buffered saline (PBS)-treated and A. fumigatus-treated lungs, organs were homogenized in 0.1 mol/L of Tris-HCl, pH 8.0, and the homogenates were directly incubated with LTC₄ for activity assay.

To assay the activity in different compartments of the spleen, we prepared spleen fractions. The organs were teased apart with fine forceps in PBS to release free cells. The cell suspension and the initial residues were separated by allowing the preparation to settle on ice for 5 minutes. The cells were collected by centrifugation at 1000 × g for 5 minutes. The supernatant was combined with the initial residues and this was designated the residual stroma. Both the cell suspension and the residual stroma were homogenized for the assay.

To examine the intravascular metabolism of exogenous LTC₄, LTC₄ at 100 µg/ml in ethanol was diluted in normal saline to 40 µg/ml, and 50 µl was injected intravenously through the tail vein (2 µg/mouse). Three mice from each of the WT, GGT-, GGL-, and GGT/GL-deficient group were treated. Ten minutes after the injection, blood samples were harvested by heart puncture and plasma was collected using Brand Serum Separators (Becton Dickinson, Franklin Lakes, NJ). Plasma from control mice (no LTC₄ administration) was used as a negative control. For inhibition of LTD₄/LTE₂ conversion, d-penicillamine (Sigma Chemical Co.) was injected intraperitoneally 5 minutes before the administration of LTC₄ at 5 mmol/kg body weight. Cyst LTs in plasma were analyzed by HPLC as described above.

**LTC₄ and LTE₂ Measurement in BALF**

BALF was collected in PBS and centrifuged. Supernatants were collected and stored at −80°C until assayed. BALF was first extracted and Cyst LTs concentrated using Sep-Park cartridges (Waters Corp., Milford, MA). They were assayed for LTC₄ and LTE₂ using leukotriene C₄ and leukotriene E₂ EIA kits (Cayman Chemical Company). To rule out the cross-reactivity among Cyst LTs, in some experiments samples were further fractionated by HPLC based on the retention times established with standards after extraction by Sep-Park cartridges and assayed for LTC₄ and LTE₂ by enzyme-linked immunosorbent assay. Data are representative of two independent experiments with seven to eight mice in each group.

**Immunohistochemistry and Immunofluorescence Analysis**

Formalin-fixed, paraffin-embedded tissue sections were deparaffinized and rehydrated using a standard protocol. The endogenous peroxidase was quenched by incubating the slides in 3% H₂O₂ in methanol for 5 minutes followed by two 5-minute PBS washes. The slides were incubated with trypsin (Trypsin 3-Pack; BioGenex, San Ramon, CA) for 20 minutes at 37°C, blocked with 1.5% normal goat serum in PBS at room temperature for 30 minutes, and incubated with protein A-purified anti-GGL polyclonal antibody at 10 µg/ml at 25°C for 1 hour. Peroxidase staining was achieved using BioGenex’s Super-sensitive detection kit and liquid diaminobenzidine. The sections were counterstained with hematoxylin. For immunofluorescence co-localization of mouse CD31 and GGL, we used zinc-fixed sections because the rat anti-mouse CD31 does not react well with the formalin-fixed antigen. The zinc fixative solution was 0.1 mol/L Tris buffer at pH 7.4, containing 0.05% calcium acetate, 0.5% zinc acetate, and 0.5% zinc chloride. Both rabbit anti-GGL and rat anti-CD31 (Pharmingen, San Diego, CA) antibodies were used at a concentration of 10 µg/ml. The fluorescence-labeled secondary antibodies were Texas Red-conjugated goat anti-rat IgG and Oregon Green conjugated goat anti-rabbit IgG (Molecular Probes). The fluorescence was detected with a fluorescence microscope (Nikon Optiphot-2; Nikon Inc., Nivelvile, NY) with a 568-nM filter for Texas Red and a 488-nM filter for Oregon Green.

**Statistical Analysis**

Statistical analyses were performed using Student’s t-test. Data are presented as mean ± SEM.

**Results**

**Characteristics of GGL Protein**

Because the mature GGT protein consists two disulfide-linked peptide chains generated from a single precursor polypeptide and GGL is a membrane protein with similarities to GGT, we examined the structure of GGL protein in tissue homogenates from spleen and uterus. Using SDS-PAGE and Western blotting with anti-GGL antibodies, we found that GGL protein consists of two polypeptides with molecular weights of 57 kd and 20 kd under reducing conditions and runs as a single band of 75 kd under nonreducing conditions (Figure 1A). These bands were not detected in homogenates of spleen and uterus from GGL-deficient mice, confirming the specificity of our antibodies. These results indicate that, like GGT, GGL consists of two polypeptides chains bonded through one or more S-S linkages.

We found that GGL is an N-linked glycoprotein, glycosylated on the heavy chain. Because the apparent molecular mass of 75 kd (Figure 1A) is greater than the predicted molecular weight of ~63 kd (573 amino acids) for the heavy chain, we examined protein glycosylation. For this purpose, tissue homogenates were incubated with either of two endoglycosidases, N-glycosidase F and endoglycosidase H. N-glycosidase F hydrolyzes all N-linked carbohydrate chains from glycoproteins...
A

Figure 1. Characterization on GGL protein by SDS-PAGE and Western blotting. A: Structural analysis on GGL. Lane 1 shows the truncated recombinant GGL protein used for immunization (reduced). Samples in lanes 2 to 5 were run under reducing conditions and those in lanes 6 to 9 were run under nonreducing conditions. Lanes 2 and 6 were samples from WT spleen, lanes 3 and 7 from GGL−/− spleen. Lanes 4 and 8 were samples from WT uterus, lanes 5 and 9 from GGL−/− uterus. B: Deglycosylation of GGL with endoglycosidase H and N-glycosidase F. Samples in lanes 1 to 4 were WT spleen homogenates and those in lanes 5 to 8 were WT uterus homogenates. Lanes 1 and 5 show untreated samples. Lanes 2 and 6 show samples incubated with the buffer in the absence of enzymes. Lanes 3 and 7 show samples treated with endoglycosidase H, and lanes 4 and 8 show samples treated with N-glycosidase F. C: Dissociation of GGL from the cell membrane. The left panel shows the result of releasing GGL protein from a uterus membrane preparation with dithiothreitol. Lane 1 is untreated sample, lane 2 is the supernatant after dithiothreitol treatment, and lane 3 is the pellet after dithiothreitol treatment. The right panel shows the result of limited papain digestion on a uterus membrane preparation. Lane 1 is the untreated sample, lane 2 is the supernatant from a papain-digested sample, lane 3 is the pellet from a papain-digested sample, lane 4 is the supernatant of the sample incubated with buffer in the absence of papain, and lane 5 is the pellet. The numbers at the left side of the figures indicate the molecular weights in kd. Twenty μg of total protein from spleen homogenates and 10 μg of total protein from uterus homogenates were analyzed in all of the experiments.

B

C

Tissue Localization of GGL

We examined the distribution of GGL in spleen by separating it into a cell suspension and residual stroma. By light microscopy, the suspension contained red blood cells, lymphocytes, neutrophils, a small number of macrophages and fibroblasts, and a few endothelial cells, whereas the residual stroma contained predominantly sinusoidal and reticular material with attached endothelial cells, collagen fibers (with fibroblasts), macrophages, and a few lymphocytes. In both WT and GGT-deficient spleen, the residual stroma was ~10-fold enriched in LTC₄ cleavage activity (Figure 2). In GGL-deficient spleen, LTC₄ cleavage is approximately eightfold lower than in WT spleen and is associated with the suspended cells; this cleavage represents residual GGT activity.¹⁵,¹⁶ Thus in contrast to GGT, which is known to be expressed on lymphocytes in spleen (at relatively low levels),²⁸ GGL activity is associated with nonlymphoid cells in stroma.

Using immunohistochemistry, we found that GGL is expressed by the endothelial cells of sinusoids and capillaries in most organs. Sinusoid endothelial cells in WT spleen stained intensely (Figure 3B). As expected, GGL-
brane-bound dipeptidase,31 LTD4 was also detected in the Cyst LT profile in blood by HPLC.4,12 Ten minutes
GGT-, GGL-, and GGT/GGL-deficient mice and analyzed (Figure 3; G to L).

In mice, GGT and GGL are the only enzymes known to generate LTD4 from LTC4 in the test tube.4,15,16 The
endothelial location of GGL suggests that GGL may perform this function in vivo. To test this hypothesis, we
administered exogenous LTC4 intravenously to WT, GGT-, GGL-, and GGT/GGL-deficient mice and analyzed
the Cyst LT profile in blood by HPLC.4,12 Ten minutes after injection, 25 to 30% of the LTC4 had been
converted to LTE4 in both WT (Figure 4A) and GGT-deficient mice (Figure 4B), whereas little, if any, conversion was
detected in blood from GGL-deficient mice (Figure 4C). Similarly, LTE4 was also absent from the blood of GGT/
GGL-deficient mice (Figure 4D). When mice were pre-treated with δ-penicillamine, a specific inhibitor of
membrane-bound dipeptidase,31 LTD4 was also detected in the blood from WT and GGT-deficient mice (Figure 4, E
and F), indicating that the LTD4 is quickly converted to LTE4 in blood. These findings demonstrate that GGL is
responsible for LTD4 synthesis in blood.

The Role of GGL in Experimental Asthma

Because asthma is one of the most pressing medical problems associated with Cyst LT production, we examined
the role of GGL in CF-induced experimental asthma in mice. Although it is generally believed that GGT is
responsible for LTC4 to LTD4 conversion in asthma,3 our results on LTC4 metabolism in blood (Figure 4) and in
Zymosan A-induced peritonitis6 suggest that GGL might catalyze LTC4 to LTD4 conversion in asthma. To resolve
this discrepancy and to assess the relative roles of GGL and GGT, we induced asthma in mice using CF and studied
mice deficient in GGL, GGT, or both GGL and GGT. We found that the level of LTC4 in BALF increases
sixfold to eightfold in CF-treated GGL- and GGL/GGT-deficient mice compared to saline-treated controls
whereas little change is seen in WT mice and GGT-deficient mice (Figure 5A). These data indicate that during
the asthmatic response GGL is the principal enzyme that converts LTC4 to LTD4. Because the antibody used
to measure LTC4 has some cross reactivity with LTD4, we separated Cyst LTs in BALF by HPLC and verified that in
GGL- and GGL/GGT-deficient mice only LTC4 is present (data not shown). In parallel with this finding, we found
that there was a threefold to fivefold increase in LTE4 levels in CF-treated WT mice and GGT-deficient mice
(Figure 5B). We also detected what appear to be very low levels of LTE4 in all PBS-treated mice, including mice
deficient in GGL and both GGL and GGT. It is likely that this result stems from cross-reactivity with other arachi-
donic derivatives because LTE4 was not detectable in these samples after HPLC fractionation (data not shown).
Our data indicate that GGL deficiency prevents the generation of LTD4 and LTE4 in experimental asthma and are
further confirmation that GGL is the principle enzyme for LTC4 to LTD4 conversion in vivo.

To examine the pathophysiological role of GGL in CF-induced experimental asthma, we evaluated the cellular
composition in BALF and severity of AHR in WT and mutant mice by evaluating PC200 after CF challenge. The
results show that mice of all types challenged with CF developed a stereotypic asthma syndrome, character-
bized by an increase in the AHR as revealed by enhanced sensitivity to acetylcholine challenge (Figure 5C) and
increased eosinophil infiltration and goblet cell metaplasia with mucus overproduction (data not shown). All mice
exhibited similar total and differential cell counts and mucus levels in BALF at the time of BALF collection (data
not shown). These results indicate that CF can effectively induce asthma in mice whether or not LTD4 is present.
We found that in all groups of mice CF treatment resulted in a greater than twofold decrease in PC200 compared to
saline treatment (Figure 5C). This result is not surprising because both LTC4 and LTD4 are known to be involved in
asthma.2,3 It is interesting that the PC200 values in CF-
treated GGL- and GGL/GGT-deficient mice are signifi-
cantly lower (P < 0.01) than that for CF-treated WT mice; this finding indicates that deficiency in GGL further ex-
acerbates AHR. Although the difference between the PC200 for GGT-deficient mice and that for WT mice after CF treatment is not statistically significant (Figure 5C), it shows a trend in that direction. Whether this result indi-
cates involvement of GGT functions unrelated to Cyst LT metabolism in asthma or is not a real difference remains to be determined.

Response of GGL Enzymatic Activity and Protein to Asthma Induction

The observation that LTC4 does not accumulate in CF-
treated WT and GGT-deficient mice led us to examine GGL protein levels and LTC4/LTD4 conversion activity in lungs after CF-treatment. We used Western blot to deter-
mine GGL protein levels in lungs in WT and GGT-defi-
cient mice, and found that after CF treatment these mice exhibit a twofold increase in GGL protein as quantified by
scanning densitometry (Figure 6A). This result indicates that expression of GGL protein is increased in response to asthma development.

To evaluate the changes in LTC$_4$/LTD$_4$ conversion activity of GGL and GGT, we used HPLC to examine the enzyme activities in fresh lung homogenates from mice after saline or CF treatment. In agreement with previously reported data, in saline-treated mice LTC$_4$/LTD$_4$ conversion activity in lung homogenates is predominantly the result of GGT activity.$^{4,16}$ We found that compared to saline treatment, CF treatment resulted in a twofold increase in GGL activity in GGT-deficient mice (Figure 6B). (Recall that GGL enzyme activity can only be measured directly in GGT-deficient mice.) This increase is similar to the increase in GGL protein levels (Figure 6A). As expected, there was no detectable LTC$_4$ to LTD$_4$ conversion activity in GGL/GGT-deficient mice (data not shown).$^{16}$ With respect to GGT, we found a decrease in enzyme activity in CF-treated GGL-deficient mice (Figure 6B). Why GGT enzyme activity is reduced after CF treatment in our experiments remains to be determined. The total LTC$_4$/LTD$_4$ conversion activity in CF-treated mice was the same as that for saline-treated mice, reflecting the increase in GGL and decrease in GGT activity. These data provide further evidence that GGL is the enzyme responsible for the initiation of Cyst LT metabolism in asthmatic inflammation. They also raise the questions of the physiological role of GGT in lung.

**Discussion**

Our results demonstrate that GGL, like GGT, is processed into two S-S linked peptide chains and is anchored in the plasma membrane by the heavy chain through its amino terminus (Figure 1). In contrast to GGT, which is located primarily on the luminal surface of epithelial cells,$^{9,19}$ GGL is expressed on the surface of endothelial cells of capillaries and sinusoids in most organs.
and larger vessels in the lung (Figure 3 and data not shown). Our results do not rule out low-level GGL expression on other endothelial cells or even other cell types at levels below the sensitivity of our techniques, but it is likely that any such expression is quantitatively minor. Our data also indicate that GGL plays a key role in regulating conversion of intravascular LTC4 as well as those formed in CF-induced asthma, to LTD4 in mice (Figures 4 and 5).

Previously we found that spleen and uterus expressed the highest levels of GGL. Our current study provides an explanation for this phenomenon: the abundant sinusoidal endothelium in spleen and the rich capillary network in endometrium contribute to the high levels of GGL in these organs (Figure 3B and data not shown). The fact that endothelium of large vessels in most organs expresses little, if any, of GGL protein highlights variations in its expression among different types of endothelial cells and the physiological importance of GGL. Using in situ hybridization we have previously reported tissue distribution of GGL; these results show some differences with our current results. The reason for this discrepancy is not readily apparent, but because our chemical and immunohistochemistry results agree, we believe that the results reported here accurately reflect the distribution of GGL.

Our data also clarify the relation between GGL and GGT function. GGT has long been thought to be the enzyme that catalyzes LTC4/LTD4 conversion in vivo. However, our results on LTC4 metabolism in the blood and in experimental asthma indicate that GGL performs much, if not all this function in vivo (Figures 4 and 5). In addition to its expression on secretory and absorptive epithelium, GGT has been reported to be expressed on capillary endothelial cells in the brain. GGT in brain capillaries is apparently involved in the transport of neutral amino acids across blood-brain barrier. Lymphocytes also express low levels of GGT. Thus it is theoretically possible that GGT could function to a limited extent in some brain capillaries or lymphocytes to convert circulating LTC4 to LTD4; however, our data argue against any significant participation of GGT in the intravascular metabolism of Cyst LTs in that we could not detect metabolism of LTC4 in GGL-deficient mice (which express GGT) (Figure 4). Because GGL has a more limited substrate spectrum than GGT and does not cleave GSH, its presence on endothelium allows synthesis of LTD4 without interfering with circulating GSH. In addition, the Km of GGL for LTC4 is 10-fold lower than that of GGT, further implicating the role of GGL in LTD4 synthesis in vivo. All of these observations argue for a central role for GGL in LTD4 synthesis and in the regulation of responses to injury.

In humans Cyst LTs are important agonists of AHR in asthma, and blocking the action of Cyst LTs by Cyst LT receptor antagonists is an important part of asthma therapy. Our findings elucidate the mechanism by which Cyst LTs are metabolized during the development of experimental asthma and demonstrate that GGL plays a key role in the conversion of LTC4 to LTD4 during asthmatic inflammation. This finding is significant because it clarifies the mechanism of how LTD4 is synthesized in vivo. In our study, AHR shows significant increase in GGL-deficient mice (Figure 4C). Because LTC4 formed is quickly converted to LTD4 by GGL and further to LTE4 by membrane-bound dipeptidase in WT and GGT-deficient mice (Figure 5, A and B), we reason that the increase in AHR in GGL- and GGL/GGT-deficient mice results from LTC4 accumulation. This result indicates that GGL not only converts LTC4 to more potent LTD4, but also serves as a critical enzyme to promote the chemical clearance of Cyst LTs, thus to limit potentially deleterious airway obstruction. This finding is pathophysiologically important because defect and insufficiency on GGL function caused by any pathological factors may lead to the development and/or exacerbation of asthma. The fact that GGL expression is increased during asthma underscores the importance of this enzyme in regulating the metabolism of Cyst LTs (Figure 6).
It seems unlikely that GGT functions in Cyst LT metabolism during asthma. GGT enzyme activity falls during asthma (Figure 5B), and there is no evidence of conversion of LTC4 to LTD4 (as measured by LTE4 accumulation. Figure 4B; in Figure 4B, GGT activity is assessed in GGL-deficient mice). We have previously reported that lung homogenates from untreated mice contain more GGT activity than GGL activity (see also Figure 6B).4,16 Although this finding may seem counterintuitive in terms of asthma, GGT probably functions primarily in GSH homeostasis, which is known to be critical for protection against oxidative damage in lung.3,37

Previous studies have shown that LTD4 can mediate eosinophil infiltration, mucus production, and bronchial constriction.3 Because GGL- and GGL/GGT-deficient mice do not synthesize LTD4 after CF challenge (Figure 5) and develop eosinophil infiltration and mucus production similar to those in CF-treated WT mice and GGT-deficient mice (data not shown), it follows that LTC4 plays a role similar to that of LTD4 in asthma. We previously observed a delayed neutrophil infiltration in Zymosan A-induced peritonitis in the GGL-deficient mice.4 In the current study, we found similar total and differential cell counts and mucin levels in BALF from WT, GGL-, GGT-, and GGL/GGT-deficient mice. The difference between these two studies may reflect differences in inducing agent, physiological location, or the inflammatory process.

In summary, GGL appears to have evolved from GGT to cleave a substrate related to inflammatory processes. Most of the structural features of GGT have been preserved in the structure of GGL, but its expression appears to be restricted to some endothelial cells. The importance of GGL in pathophysiological events is underscored by its role in regulating metabolism of Cyst LTs in vivo.

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