γ-Glutamyl Leukotriene Transpeptidase Gene Family Member, Is Expressed Primarily in Spleen*

(Received for publication, June 5, 1998, and in revised form, August 5, 1998)

Bing Z. Carter‡, Zheng-Zheng Shi§, Roberto Barrios§, and Michael W. Lieberman¶¶

From the Departments of ‡Pathology, and §Cell Biology, Baylor College of Medicine, Houston, Texas 77030

We have recently identified a mouse enzyme termed γ-glutamyl leukotriene transpeptidase (GGL) that converts leukotriene C4 (LTC4) to leukotriene D4 (LTD4). It also cleaves some other glutathione (GSH) conjugates, but not GSH itself (Carter, B. Z., Wiseman, A. L., Orkiszewski, R., Ballard, K. D., Ou, C.-N., and Lieberman, M. W. (1997) J. Biol. Chem. 272, 12305–12310). We have now cloned a full-length mouse cDNA coding for GGL activity and the corresponding gene. GGL and γ-glutamyl transpeptidase constitute a small gene family. The two cDNAs share a 57% nucleotide identity and 41% predicted amino acid sequence identity. Their corresponding genes have a similar intron-exon organization and are located 3 kilobases apart. A search of Genbank and reverse transcription-polymerase chain reaction analysis failed to identify additional family members. Mapping of the GGL transcription start site revealed that the GGL promoter is TATA-less but contains an initiator, a control element for transcription initiation. Northern blots for GGL expression were negative. As judged by ribonuclease protection, in situ hybridization, and measurement of enzyme activity, spleen had the highest level of GGL expression. GGL is also expressed in thymic lymphocytes, bronchiolar epithelial cells, pulmonary interstitial cells, cerebral, and brain stem neurons but not in glial cells. GGL is widely distributed in mice, suggesting an important role for this enzyme.

Glutathione conjugates play a central role in normal physiology and in responses to injury (1–6). Among the more important GSH derivatives are conjugates of eicosanoids, xenobiotics, and carcinogens. At least three types of eicosanoid-GSH conjugates have been identified, including derivatives of leukotriene A4, prostaglandins, and heparin. The LTA4-GSH conjugate (LTC4) and its cleavage products, LTD4 and LTE4, are powerful mediators of bronchoconstriction, vasoconstriction, and other effects. As a result, they are important mediators of asthma, coronary artery spasm, and nephropa-thies (7–15). Prostaglandins play diverse biological roles and are involved in the development of the inflammatory response, inhibition of platelet aggregation, and regulation of immune responses (6). Prostaglandins are inactivated and cleared by conjugation to GSH (3, 6). Hepoxilin A3 forms a GSH conjugate, heoxilin A3-C, that is known to be a potent regulator of hippocampal neurons (4). In addition, several neurotransmitters, including serotonin and dopamine, form GSH conjugates, suggesting an additional role for this pathway in the central nervous system (16, 17). GSH conjugation along with glucuronide formation is the major pathway by which toxins, drugs, and carcinogens such as CH3Hg, acetaminophen, and aflatoxin are detoxified and excreted (5, 18–21).

Until recently, it was thought that the sequential cleavage of GSH conjugates and their derivative cysteinyl-glycine conjugates was catalyzed by two enzymes. GGT was known to cleave a γ-glutamyl group from GSH conjugates, and a dipeptidase, often identified as membrane-bound dipeptidase, was believed to cleave the Cys-Gly bond of conjugates to yield Cys derivatives (2). We have recently developed mice deficient in GGT and have shown that tissues from these mice will not cleave GSH but retain the ability to metabolize LTC4 to LTD4, its Cys-Gly derivative (22, 23). We have partially purified this activity and termed it γ-glutamyl leukotriene (GGL) to reflect this fact (23); however, the enzyme will also cleave S-decylic GSH, other alkyl GSH derivatives, and S-nitrosyl GSH (23, 24). A human cDNA coding for an enzyme termed GGT-rel shares at least some properties with GGL (25). While we were preparing this manuscript for publication, a report identifying the rat homologue of human GGT-rel appeared (26).

In other experiments, we have produced membrane-bound dipeptidase-deficient mice and found that tissues from these mice are capable of metabolizing LTD4 to LTE4, the Cys derivative (27). Thus, like the cleavage of GSH conjugates, more than one enzyme is capable of catalyzing the second step of this pathway.

A new picture is emerging in which a variety of enzymes participate in the biotransformation of GSH conjugates. At present, it is unknown how many enzymes are involved in each step and what role each plays in the regulation of these important biological molecules. To begin to examine this pathway in more detail, we have cloned a full-length mouse GGL cDNA and the corresponding gene and examined its expression in mouse tissues.

EXPERIMENTAL PROCEDURES

Materials—GTT-deficient mice were developed in our laboratory (22). All restriction enzymes were purchased from Boehringer Mannheim, and [α-32P]UTP and [γ-32P]ATP were from NEN Life Science Products. Primers and Dullbecco`s modified Eagle`s medium were from Life Technologies, Inc. Fetal bovine serum, gelatin, LTC4, LTD4, LTE4, and other chemicals were purchased from Sigma unless otherwise indicated.

RNA Isolation—Total RNAs were isolated from various GGT-deficient mouse tissues following the acid guanidinium thiocyanate proce-
Receptor. 100 μg of RNA from tissues of GGT-deficient mice (spine, small intestine, kidney, liver, lung, and brain) and 2.0 × 10^5 cpm of GGL probe were used for each assay following the manufacturer's instruction. Hybridization was carried out at 42 °C for 18 h followed by a rinse in 2× SSC at 57 °C for 1 h with a 100-fold dilution of RNAse R solution in the kit.

In Situ Hybridization—A GGL cDNA fragment stretching from base 28 to base 254 (Fig. 1) was cloned into the pT7Blue/R/T-Vector, and sequenced. The fragment shared 80% nucleotide sequence identity with the corresponding human GGT-rel sequence. To obtain the GGL cDNA sequence further downstream of cDNA I, spleen poly(A)^+ RNA from GGT-deficient mice was reverse transcribed using a lock-docking cDNA synthesis primer obtained from the Marathon cDNA amplification kit (CLONTech). 3'-RACE fragments were obtained by amplification of the cDNA using primers API and GGL1 (Fig. 1). The products were then cloned into pT7Blue/R/T-Vector and sequenced. The cDNA was further amplified by PCR using primers GGL9 (5'ACAGGGGAAGTTGGGATCC3') corresponding to bases 642–662 of human GGT-rel cDNA and GGL10 (5'GTCTCTCCAACGTTGGTGATC3') corresponding to bases 1295–1315 of human GGT-rel cDNA. A cDNA fragment of 680 bp (cDNA I) was obtained, cloned into pT7Blue/R/T-Vector, and sequenced. The fragment shared 80% nucleotide sequence identity with the corresponding human GGT-rel sequence.

To obtain the GGL cDNA sequence further upstream of cDNA I, spleen poly(A)^+ RNA from GGT-deficient mice was reverse transcribed using a Marathon cDNA amplification kit (CLONTech). The resulting cDNA was further amplified by PCR using primers GGL2 and GGL3 (Fig. 1) to obtain GGL cDNA II. To obtain the GGL cDNA sequence further upstream of cDNA I, spleen poly(A)^+ RNA from GGT-deficient mice was reverse transcribed using primer GGL4 (Fig. 1). 5'-RACE fragments were amplified using API from the Marathon cDNA amplification kit and GGL4. The products were then cloned and sequenced as above. The clone with the longest 5' end was chosen and further amplified by PCR using primers GGL5 (Fig. 1) and GGL4 to obtain GGL cDNA III. To obtain a full-length GGL cDNA, GGL cDNA II and cDNA III were fused and amplified by PCR using primers GGL5 and GGL3 (Marathon cDNA amplification kit as described by the manufacturer). A full-length GGL cDNA was cloned into pT7Blue/R/T-Vector, and six clones were sequenced. The sequence of GGL cDNA was chosen by comparing six GGL cDNA sequences (derived from PCR cloning) and the exon sequence of the gene (isolated from genomic library, see below). All sequencing was carried out by an automated Applied Biosystems 373 DNA sequencer using a Dye Terminators Cycle Sequencing Ready Reaction kit (ABI-Perkin Elmer). Comparison and alignment of GGL cDNA with other cDNAs and homology analyses of DNA and amino acid sequences were accomplished with the IntelliGenetics (IG) Suite (IntelliGenetics Inc., Palo Alto, CA).

In Vivo Transfection of Murine NIH/3T3 Cells with Full-length GGL cDNA—Murine NIH/3T3 cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Full-length GGL cDNA was cloned into pT7Blue/R/T-Vector and six clones were sequenced. The sequence of GGL cDNA was chosen by comparing six GGL cDNA sequences (derived from PCR cloning) and the exon sequence of the gene (isolated from genomic library, see below). All sequencing was carried out by an automated Applied Biosystems 373 DNA sequencer using a Dye Terminators Cycle Sequencing Ready Reaction kit (ABI-Perkin Elmer). Comparison and alignment of GGL cDNA with other cDNAs and homology analyses of DNA and amino acid sequences were accomplished with the IntelliGenetics (IG) Suite (IntelliGenetics Inc., Palo Alto, CA).

**RESULTS**

Isolation and Analysis of Mouse GGL cDNA—As an initial step in examining the pathway(s) involved in the biotransformation of GSH conjugates, we cloned a full-length GGL cDNA by in situ hybridization in GGT-deficient and wild-type mice. In all cases, hybridizations with the sense strand were negative.

**Expression of Mice to Oxygen—GGT-deficient mice (22) and their wild-type littermates were exposed to 80% oxygen continuously for 120 h as described previously with minor modifications (33). They were sacrificed by metofane inhalation, then cervical dislocation. RNA was isolated from lungs of these mice for Northern blotting (31). Lung homogenates from oxygen-exposed and air-breathing control GGT-deficient mice were used to assay GGL activity (23).

GGG Gene Structure Identification—GGG genomic clones were isolated from a mouse A Fixil genomic library (Stratagene) screened with GGL cDNA containing the full-length coding region as described previously (30, 31). The intron-exon organization of the gene was determined by the same procedures as used for the mouse GGT gene (30) and mouse GSH synthetase gene (31).

**Primer Extension Analysis—26 μg of mouse spleen poly(A)^+ RNA was used for primer extension analysis. The primer was GGL5 (5'-CTGGACAGAGGATGGACGACGCCGCTTACAGATGAAG). The reaction was essentially as described previously (32).**

Detection of GGL RNA Expression in Mouse Tissues—Expression of GGL RNA in various mouse tissues was detected by Northern blot (31) and ribonuclease protection assay (Ambion). To obtain the probe for ribonuclease protection, a GGL cDNA fragment stretching from base 28 to base 173 (Fig. 1) was cloned into the pT7Blue/R/T plasmid. The plasmid was linearized with BamHI and transcribed by T7 RNA polymerase in the presence of [α-32P]UTP using an in vitro transcription kit (Stratagene). 100 μg of RNA from tissues of GGT-deficient mice (spine, small intestine, kidney, liver, lung, and brain) and 2.0 × 10^5 cpm of GGL probe were used for each assay following the manufacturer's instruction. Hybridization was carried out at 42 °C for 18 h followed by a rinse in 2× SSC at 57 °C for 1 h with a 100-fold dilution of RNAse R solution in the kit.

**Isolation and Analysis of Mouse GGL cDNA—As an initial step in examining the pathway(s) involved in the biotransformation of GSH conjugates, we cloned a full-length GGL cDNA by in situ hybridization in GGT-deficient and wild-type mice. In all cases, hybridizations with the sense strand were negative.**
human GGT-rel sequence also occurred in the rat GGT-rel cDNA sequence, which predicts 572 amino acids (26). From the putative structure of the rat protein, the histidine at position 371 of mouse GGL is deleted in rat GGT-rel (Fig. 2 and Ref. 26). GGL has six putative N-glycosylation sites, all located in the putative heavy chain (Fig. 1).

We also found that GGL and mouse GGT share an overall 57% cDNA sequence identity and 41% predicted amino acid sequence identity (Fig. 2). The predicted amino acid identity of mouse GGL and GGT sequence is highest in three regions (amino acids 43–111 of GGL and 39–107 of GGT; amino acids 140–176 of GGL and 133–169 of GGT; and amino acids 388–428 of GGL and 379–419 of GGT).

Expression of GGL cDNA in Murine NIH/3T3 Cells—

**Fig. 1.** Nucleotide sequence of mouse GGL cDNA and its deduced amino acid sequence. The numbers of nucleotides and amino acids are indicated to the right of the sequences. The *underlined sequences* are primers with their names and orientations (*horizontal arrows*) above them. The triangle above the sequence at amino acid 389 indicates the putative N terminus of the light chain; the *bold sequence* indicates the putative polyadenylation signal. *Asterisks* show the possible N-linked glycosylation sites. The *vertical arrows* (↑) mark the splice junctions.
confirm that the cDNA we cloned from GGT-deficient mouse spleen codes for GGL activity, we transfected the cloned full-length cDNA under the regulation of the cytomegalovirus promoter into murine NIH/3T3 cells. Normally, these cells do not express either GGL or GGT. We isolated three clones with enzyme activity, thus demonstrating that this cDNA codes for GGL (Fig. 3). The specific activities of the three clones for conversion of LTC4 to LTD4 were 1.93, 3.48, and 4.10 μmol/g of protein/h, respectively. In addition to LTD4, we detected LTE4 in our reactions (Fig. 3). The appearance of this leukotriene results from the cleavage of LTD4 to LTE4 by dipeptidase(s) in NIH/3T3 cells (23, 34). We were unable to demonstrate cleavage of GSH or γ-glutamyl-p-nitroanilide (a substrate used to assay GGT) by transfected 3T3 cells (data not shown); this result confirms our earlier findings with partially purified enzyme preparations from GGT-deficient mice (23).

**Genomic Structure of Mouse GGL**—A Southern blot of mouse DNA digested with EcoRI, HindIII, both EcoRI and HindIII, and BamHI and probed with a 0.37-kb GGL cDNA fragment yielded single bands (data not shown), indicating that GGL is a single copy gene. Subsequently, from 5 × 10⁸ phages of a mouse genomic λ FixII library, we isolated seven individual clones that hybridized with the cDNA probe. These were mapped with restriction endonucleases and subcloned. Intron-exon boundaries were identified by sequencing the subclones that hybridized to the cDNA probe and comparing their genomic sequences with the GGL cDNA sequence. As a result, we found that GGL has 12 exons spanning approximately 26 kb (Fig. 4). The average length of exons is 190 bp, whereas that of introns is about 2.2 kb. All the intron-exon boundaries follow the GT/AG rule of splice site consensus (35). Interestingly, the intron-exon organization of GGL is almost identical to that of GGT with all the splice sites of GGL being consistent with those of GGT except for intron 9, which is spliced 6 bp (2 amino acids) upstream of the site corresponding to that of GGT (Fig. 4).

**Analysis of GGL RNA**—To determine if GGL and GGT are similar in their 5' UTR, we examined the 5' UTR of mouse GGL cDNA by the RACE technique. We examined 46 clones containing the 5' UTR: 30 from kidney, 6 from liver, and 10 from spleen, and found only one 5' UTR that is part of first coding exon (data not shown). In contrast, the mouse GGT gene contains at least nine 5'-noncoding exons that generate seven types of
RNA with different 5' UTRs (32, 36–38). These findings suggest that regulation of mouse GGL transcription is different from that of mouse GGT (39).

We also found a shorter GGL RNA in spleen, small intestine, kidney, liver, and lung. Subsequent sequence analysis showed that its cDNA lacks exon 10 (data not shown), a deletion that should produce a shift in the reading frame and termination at amino acid 456. This shorter RNA could only be detected after two rounds of PCR; therefore, at most, it represents a very small portion of total GGL RNA and probably lacks physiological significance.

Sequence of the 5'-Flanking Region and Determination of Transcription Start Site—The DNA sequence containing part of GGL 5'-untranslated exon and 499 bases of the 5'-flanking region is presented in Fig. 5A. The transcription start site of GGL was determined by primer extension and ribonuclease protection analyses. For primer extension, mouse spleen poly(A)1 RNA was used with yeast tRNA as a negative control. The primer GGL6 for reverse transcription is located within the 5'-UTR of the GGL gene (Fig. 5A, bases 111 to 150 corresponding to bases 2180 to 2219 in Fig. 1). A product, 150 nucleotides in length, was found (Fig. 5B), indicating the 5'-UTR of GGL consists of 329 nucleotides (150 + 179). The result was confirmed by ribonuclease protection assay (Fig. 5C). The conditions for the reaction were the same as for the ribonuclease protection assay for detecting GGL RNA expression (see "Experimental Procedures") except that a 50-fold dilution of RNase R solution was used. The protected fragment (Fig. 5C) seems slightly longer than the expected 150 nucleotides. This appa-
ent extra length is the result of comparing the mobility of RNA with DNA markers. The transcription start site is marked as +1 (Fig. 5A). A TATA box was not found in GGL promoter region. However, an AT-rich sequence is located 14–26 bp upstream of the transcription start site (Fig. 5A). In addition, AP2 and CCAAT boxes and three tandem copies of SP1 binding sites are present in the putative GGL promoter region (Fig. 5A).

**GGL and GGT Are Members of the Same Gene Family**—Because of the similarity between GGL and GGT, we reasoned that one might be derived from the other by gene duplication and divergence, and they might be tightly linked. Therefore, we examined our mouse genomic GGT clones (30) and found that one of them (gta) contained the 5′ end of GGL about 3 kb 3′ of GGT.

We used two strategies to search for additional GGT/GGL family members. First, we used the peptide sequences of GGT and GGL to search Genbank (“BLAST P” version 2.0.4, National Center for Biotechnology Information) but found no significant sequence homology to these two proteins. We also tried an approach based on RT-PCR and degenerate primers (41). Briefly, three pairs of degenerate oligonucleotides corresponding to three conserved regions of the amino acid sequences (amino acids 68–73, 154–159, and 404–409 of GGL; see Fig. 2) were used to amplify cDNAs reverse transcribed from RNAs of liver and kidney of GGT-deficient mice. The PCR products were subsequently cloned and sequenced, but again, we failed to identify any additional family members.

**Detection of GGL RNA Expression in Mouse Tissues**—To determine the level of GGL expression in mouse tissues, we used Northern blot analysis and ribonuclease protection assays. Northern blot analysis failed to detect any GGL signal from spleen, small intestine, kidney, liver, and lung, suggesting that GGL is expressed at low levels (or not at all) in these organs (data not shown); these findings are in agreement with data on human and rat GGT-rel (25, 26). When more sensitive ribonuclease protection assays were performed, a protected fragment of the expected size was obtained from most organs (Fig. 6).

**Localization of GGL Expression by in Situ Hybridization**—To study the distribution of GGL more specifically, we used in situ hybridization to examine GGL expression in tissues of GGT-deficient mice (Fig. 7, Table I). Enzyme activity data as another measurement of GGL expression are also presented in Table I.

**To detect any additional family members.**

**Detection of GGL RNA Expression in Mouse Tissues**—To determine the level of GGL expression in mouse tissues, we used Northern blot analysis and ribonuclease protection assays. Northern blot analysis failed to detect any GGL signal from spleen, small intestine, kidney, liver, and lung, suggesting that GGL is expressed at low levels (or not at all) in these organs (data not shown); these findings are in agreement with data on human and rat GGT-rel (25, 26). When more sensitive ribonuclease protection assays were performed, a protected fragment of the expected size was obtained from most organs (Fig. 6).

**Localization of GGL Expression by in Situ Hybridization**—To study the distribution of GGL more specifically, we used in situ hybridization to examine GGL expression in tissues of GGT-deficient mice (Fig. 7, Table I). Enzyme activity data as another measurement of GGL expression are also presented in Table I.

Almost all lymphocytes present in the spleen were positive by in situ hybridization (Fig. 7A). Thymus was positive in the medulla and paracortical region (Table I). Although we did not determine the phenotype of these lymphocytes, the locations of GGL-positive cells and their abundance in these organs suggest that both T and B cells express GGL. Interestingly, thymus expressed much lower GGL enzyme activity than spleen (Table I), although both tissues stained strongly by in situ hybridization. These findings suggest that GGL expression may be regulated post-transcriptionally in different tissues.

In the lung, all columnar bronchial epithelial cells and numerous interstitial cells in the alveolar septae were positive (Fig. 7B and Table I), but it was not possible to determine, unambiguously, the lineage of these interstitial cells. Chondrocytes in bronchial cartilage were also positive. Some proximal pulmonary arteries in both GGT-deficient and wild-type mice contain cardiac myocytes (a normal finding in mice), which stained positively for GGL. Although a great number of cells were stained positively by in situ hybridization and a signal was detected by ribonuclease protection assay, lung expressed very low GGL activity (Table I).

**In situ hybridization on kidney was positive in proximal and distal tubules as well as in some collecting ducts (Fig. 7C).** Other structures, such as glomeruli and blood vessels, were negative. The crypt cells of the small intestine (Fig. 7D) were positive, whereas villous cells showed only focally positive stain. The myocardium was positive (Table I). In the liver, a few isolated portoportic hepatocytes and lymphocytes in the perportal region were positive, whereas other hepatocytes and other cells were negative. It is likely that the small percentage of GGL-positive cells in liver is the explanation for our inability
**Fig. 7.** **In situ hybridization analysis of GGL expression.** A, section of spleen. Most lymphocytes in white and red pulp are positive. B, section of lung. A bronchiole (br) shows strong epithelial staining. All columnar epithelial cells are positive. Peripheral pulmonary parenchyma shows scattered positive cells in the interstitium. C, section of kidney shows weak staining of distal tubular (d) and strong staining of proximal tubular (p) cells. Some collecting ducts are positive, and glomeruli (g) are negative. D, section of small intestine. Epithelial cells of the crypts are positive (the intestinal lumen is labeled L). Other structures of the intestinal wall are negative. E, section of brain showing hippocampus. All neuronal elements are stained, but fibers and glial cells are negative. F, section of cerebellum. Purkinje cells (big arrows) are strongly positive, and granular neurons (small arrows) are weakly positive. Glial cells, white matter, and vessels are negative. All sections were also hybridized to digoxigenin-labeled sense probe; no reaction product was seen (all sections were photographed at 70× magnification and were not counterstained). Tissues were from GGT-deficient mice.
to detect expression by ribonuclease protection (Fig. 6) and low level of GGL activity by enzyme assay (Table I).

In the brain, all cortical neurons, including those of the hippocampus and basal ganglia, and brain stem neurons were positive (Table I). The signal was confined to neuronal bodies. In cerebellum, Purkinje cells were strongly positive, whereas granular neurons were weakly positive. In contrast, white matter and glial cells were negative (Fig. 7, E and F). Endothelial cells were also negative.

Expression of GGL in Mouse Lung after Oxygen Exposure—Increased levels of rat GGT-rel RNA have been reported in lung after chronic exposure to isobutyl nitrite and in tumors induced by this agent (26). To examine the effects of other agents on the induction of GGL expression, we exposed wild-type and GGT-deficient mice to 80% oxygen for 120 h and performed Northern analysis of RNA from lungs of these mice and air breathing controls. We were unable to detect any GGL RNA in either O2-exposed or control mice. Failure to find elevated levels of GGL RNA after oxygen exposure is not the result of generalized tissue damage since both wild-type and GGT-deficient mice are responsive to oxygen injury as judged by elevated levels of γ-glutamyl cysteine synthetase mRNA in exposed mice.2 We also examined GGL enzyme activity in GGT-deficient mice exposed to O2 and their air-breathing controls. No apparent difference of GGL activity in lung was found between O2-exposed GGT-deficient mice and their air-breathing controls (results not shown).

DISCUSSION

We have cloned a mouse cDNA (Fig. 1) that encodes GGL activity (Fig. 3). Its high sequence identity with human and rat GGT-rel (Fig. 2 and Ref. 26) suggests that GGT-rel is the human/rat homologue of GGL (23, 25, 26). As judged by experiments with ST3 cells carrying a full-length GGL cDNA, mouse GGL cleaves GSH conjugates (LTC4) (Fig. 3) but not GSH itself (or γ-glutamyl-p-nitroanilide) (see also Refs. 23 and 24). We emphasize this point because the function of GGL seems to be the cleavage of these conjugated GSH molecules and not the cleavage of GSH itself (25, 26).

Our data also demonstrate that the mouse GGL gene and the mouse GGT gene are related by nucleotide sequence and are located within a few kilobases of one another (Fig. 4). Thus, GGL and GGT constitute a small gene family in which one gene probably evolved from the other by gene duplication. It may be that GGL is descended from GGT as a need arose for more selective cleavage of conjugated glutathiones in higher organisms. A search for other family members using Genbank and PCR strategies did not yield additional candidates. At present, we cannot rule out the possibility that other family members exist since their sequences may be too divergent to be identified by our methods.

Although we have not investigated the regulation of GGL expression in detail, we have used the RACE technique to look for a variety of 5′ ends as evidence of different promoters or genes. The fact that all the GGL cDNAs we have identified to date in kidney (30 clones), liver (6 clones), and spleen (10 clones) have the same 5′ UTR suggests that GGL is expressed from one or a limited number of promoters. In contrast, the selective expression of GGT in different tissues in the mouse is governed in part by at least seven independent promoters (39). Hence, the two genes have adopted different regulatory strategies in the mouse.

GGL is expressed in spleen at high levels relative to other mouse organs (Figs. 6 and 7, Table I). We do not know what role, if any, GGL plays in immune function, but it is worth noting that relative to other organs, GGT expression in mouse spleen is relatively low (23). In fact, about 90% of the cleavage of GSH conjugates (as measured by LTC4 cleavage) is carried out by GGL in this organ (23).

Under normal circumstances, GGL is expressed at very high levels in most organs. The expression of GGL in bronchial epithelium and the interstitial cells of the lung is consistent with the role leukotrienes are believed to play in normal lung epithelial cell function and asthma; however, exposure to high levels of O2 for 5 days did not result in detectable increases in steady-state levels of GGL RNA or in increases in GGL enzyme activity in lung. In contrast, other investigators have found elevated levels of rat GGT-rel RNA in lungs from rats exposed for 2 years to isobutyl nitrite (26). GGL is expressed in the proximal tubules of the kidney, which is the site of highest GGT expression (Table I and Refs. 23 and 39). GSH cleavage is an important function of GGT in kidney; as mentioned above, GGL does not cleave GSH. In addition, GGT is able to cleave all known substrates for GGL (23, 24). These observations suggest that GGL may have an as yet unrecognized function in kidney. In the brain, GGL expression is confined to neurons. Virtually all cerebral, cerebellar, and brain stem neurons express GGL as judged by in situ hybridization, whereas glial cells, vascular elements, and endothelial cells are GGL-negative (Fig. 7, Table I). At present, the meaning of this observation remains obscure.

In summary, GGL is a member of the GGT gene family and cleaves a subset of molecules cleaved by GGT. Highest levels of expression are found in spleen. The wide distribution of GGL in many organs suggests an important function for this enzyme.

Acknowledgments—We thank Drs. Mark Majesky and Antonio Bal-dini for thoughtful discussion and Amy Wiseman and Andrew Bahler for the help with some experiments.

REFERENCES

1. Meister, A., and Larsson, A. (1995) The Metabolic Basis of Inherited Diseases (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) 7th ed., pp. 1461–1477, McGraw-Hill, New York.
2. Maycock, A. L., Pang, S.-S., Evans, J. F., and Miller, D. K. (1989) Leukotrienes and Lipocysteine: Chemical, Biochemical and Clinical Aspects (Rokach, J., ed) pp. 143–208, Elsevier, Amsterdam.
3. Ishikawa, T. (1992) Trends Biochem. Sci. 17, 463–468.
4. Ace-Sta ani, C. R., Laneuville, O., Stek, W.-G., Corey, E. J., Gurevich, N., Wu, P., and Carlen, P. L. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3037–3041.
5. Parkinson, A. (1996) Cassaret et Douil's Toxicology: The Basic Science of Poisons (Klaassen, C. D., ed) 5th ed., pp. 113–186, McGraw-Hill, New York.
6. Zilversmit, B. (1990) Textbook of Rheumatology (Kelley, W. N., Harris, E. D., Jr., Rudy, S., and Sledge, C. B., eds) pp. 201–212, Harcourt Brace Jovanovich, Inc., Philadelphia.
7. Piper, P. J. (1984) Physiol. Rev. 64, 744–761.
8. Samelson, K. (1984) Science 220, 586–575.
9. Lewis, R. A., and Austen, K. F. (1984) J. Clin. Invest. 73, 889–897.
10. Lewis, R. A., Austen, K. F., and Soberman, R. J. (1990) N. Engl. J. Med. 323, 643–655.
11. Henderson, W. R., Jr. (1994) Ann. Intern. Med. 121, 684–697.
12. Denzlinger, C., Rapp, S., Hageman, W., and Keppler, D. (1985) Science 220, 330–332.
13. Kato, T., Lianos, E. A., Fukunaga, M., Takahashi, K., and Barf, P. F. (1993) J. Clin. Invest. 91, 1507–1515.
14. Pietri, R., and Ford-Hutchinson, A. W. (1994) Kidney Int. 46, 1322–1329.
15. Nasser, G. M., and barf, P. F. (1995) Miner. Electrolyte Metab. 21, 262–270.
16. Abramovitz, M., Homma, H., Ishigaki, S., Tansey, F., Cammer, W., and Listowsky, I. (1988) J. Neurochem. 50, 50–57.
17. Cooper, A. J. L. (1992) The Molecular and Genetic Basis of Neurological Disease (Rosenberg, R. N., Prusiner, S. B., DiMauro, S., and Barchi R. L., eds) pp. 1195–1230, Butterworth-Heinemann, Boston.
18. Duteczak, W. J., and Ballatori, N. (1992) J. Pharmacol. Exp. Ther. 262, 619–623.
19. Duteczak, W. J., Clarkson, T. W., and Ballatori, N. (1991) Am. J. Physiol. 260, G873–G880.
20. Heinichen, C. A., and Ballatori, N. (1994) J. Toxicol. Environ. Health. 41, 387–409.
21. Eaton, D. L., and Gallagher, E. P. (1994) Annu. Rev. Pharmacol. Toxicol. 34, 1355–1372.
22. Lieberman, M. W., Wiseman, A. L., Shi, Z.-Z., Carter, B. Z., Barrios, R., Ou, C.-N., Chevez-Barrios, P., Wang, Y., Habib, G. M., Goodman, J. C., Huang, S. L., Lebovitz, R. M., and Matzuk, M. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 7923–7926.
23. Carter, B. Z., Wiseman, A. L., Orkiszewski, R., Ballard, K. D., Ou, C.-N., and Lieberman, M. W. (1997) J. Biol. Chem. 272, 12305–12310.
24. Lieberman, M. W., Shields, J. E., Will, Y., Reed, D. J., and Carter, B. Z. (1998) 2. R. Barrios, Z.-Z. Shi, and M. W. Lieberman, unpublished results.
Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Related Diseases 4 (Honn, K. V., Nigam, S., Marnett, L. J., and Dennis, E., eds) Plenum Publishing Corp., New York, in press

25. Heisterkamp, N., Rajpert-De Meyts, E., Uribe, L., Forman, H. J., and Groffen, J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6303–6307

26. Potdar, P. D., Andrews, K. L., Nettlesheim, P., and Ostrowski, L. E. (1997) Am. J. Physiol. 273, L1082–L1089

27. Habib, G. M., Shi, Z.-Z., Cuevas, A. A., Guo, Q.-X., Matzuk, M. M., and Lieberman, M. W. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 4859–4863

28. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159

29. Kingston, R. E. (1994) Current Protocols in Molecular Biology (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds) pp. 9.1.1–9.5.6, Wiley Interscience, New York

30. Shi, Z-Z., Habib, G. M., Lebovitz, R. M., and Lieberman, M. W. (1995) Gene (Amst.) 167, 233–237

31. Shi, Z.-Z., Carter, B. Z., Habib, G. M., He, X., Sazer, S., Lebovitz, R. M., and Lieberman, M. W. (1996) Arch. Biochem. Biophys. 331, 218–224

32. Sepulveda, A. R., Carter, B. Z., Habib, G. M., Lebovitz, R. M., and Lieberman, M. W. (1994) J. Biol. Chem. 269, 10699–10705

33. Welty, S. E., Rivera, J. L., and Wu, B. (1997) Free Radical Biol. Med. 23, 898–908

34. Habib, G. M., Barrios, R., Shi, Z.-Z., and Lieberman, W. M. (1996) J. Biol. Chem. 271, 16273–16280

35. Mount, S. M. (1982) Nucleic Acids Res. 10, 459–472

36. Rajagopalan, S., Wan, D.-F., Habib, G. M., Sepulveda, A. R., McLeod, M. R., Lebovitz, R. M., and Lieberman, M. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6179–6183

37. Carter, B. Z., Habib, G. M., Sepulveda, A. R., Barrios, R., Wan, D.-F., Lebovitz, R. M., and Lieberman, M. W. (1994) J. Biol. Chem. 269, 24581–24585

38. Habib, G. M., Carter, B. Z., Sepulveda, A. R., Shi, Z.-Z., Wan, D.-F., Lebovitz, R. M., and Lieberman, M. W. (1995) J. Biol. Chem. 270, 15711–15715

39. Lieberman, M. W., Barrios, R., Carter, B. Z., Habib, G. M., Lebovitz, R. M., Rajagopalan, S., Sepulveda, A. R., Shi, Z.-Z., and Wan, D.-F. (1995) Am. J. Pathol. 147, 1175–1185

40. Smale, S. T. (1997) Biochim. Biophys. Acta 1351, 73–88

41. Gavin, B. J., and McMahon, A. P. (1993) Methods Enzymol. 225, 653–663