Regulation of Glycosphingolipid Metabolism in Liver during the Acute Phase Response*

Riaz A. Memon, Walter M. Holleran, Yoshikazu Uchida, Arthur H. Moser, Shinichi Ichikawa‡, Yoshio Hirabayashi‡, Carl Grunfeld, and Kenneth R. Feingold§

From the Departments of Medicine and Dermatology, University of California San Francisco, Metabolism Section, Medical Service and Dermatology Service, Department of Veterans Affairs Medical Center, San Francisco, California 94121 and the Laboratory of Cellular Glycobiology, Frontier Research Program, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Saitama 351-0198, Japan

The host response to infection is associated with multiple alterations in lipid and lipoprotein metabolism. We have shown recently that endotoxin (lipopolysaccharide (LPS)) and cytokines enhance hepatic sphingolipid synthesis, increase the activity and mRNA levels of serine palmitoyltransferase, the first committed step in sphingolipid synthesis, and increase the content of sphingomyelin, ceramide, and glucosylceramide (GlcCer) in circulating lipoproteins in Syrian hamsters. Since the LPS-induced increase in GlcCer content of lipoproteins was far greater than that of ceramide or sphingomyelin, we have now examined the effect of LPS and cytokines on glycosphingolipid metabolism. LPS markedly increased the mRNA level of hepatic GlcCer synthase, the enzyme that catalyzes the first glycosylation step of glycosphingolipid synthesis. The LPS-induced increase in GlcCer synthase mRNA levels was seen within 2 h, sustained for 8 h, and declined to base line by 24 h. LPS-induced increase in GlcCer synthase mRNA was partly accounted for by an increase in its transcription rate. LPS produced a 3- to 4-fold increase in hepatic GlcCer synthase activity and significantly increased the content of GlcCer (the immediate product of GlcCer synthase reaction) as well as ceramide trihexoside and ganglioside GM3 (products distal to the GlcCer synthase step) in the liver. Moreover, both tumor necrosis factor-α and interleukin-1β, cytokines that mediate many of the metabolic effects of LPS, increased hepatic GlcCer synthase mRNA levels in vivo as well as in HepG2 cells in vitro, suggesting that these cytokines can directly stimulate glycosphingolipid metabolism. These results indicate that LPS and cytokines up-regulate glycosphingolipid metabolism in vivo and in vitro. An increase in GlcCer synthase mRNA levels and activity leads to the increase in hepatic GlcCer content and may account for the increased GlcCer content in circulating lipoproteins during the acute phase response.

Glycosphingolipids (GSLs)1 are a diverse group of complex lipids that contain the hydrophobic ceramide moiety and a hydrophilic oligosaccharide residue (1). GSLs are synthesized by the sequential addition of sugar residues to ceramide by glycosyltransferases that are specific to each glycosidic linkage (1). GSLs are present in the plasma membrane of all eukaryotic cells and are involved in a variety of important biological processes, including cell recognition, proliferation and differentiation, regulation of cell growth, signal transduction, interaction with bacterial toxins, and modulation of immune responses (reviewed in Refs. 2–4).

The acute phase response represents an early and highly complex reaction of the host to infection, inflammation, or trauma and is accompanied by changes in hepatic synthesis of several acute phase proteins such as increases in C-reactive protein and serum amyloid A (5). The acute phase response is also accompanied by several changes in lipid and lipoprotein metabolism that include stimulation of fatty acid and cholesterol synthesis and a marked increase in serum triglyceride and cholesterol levels (6). These metabolic alterations can be induced by endotoxin (lipopolysaccharide (LPS)) treatment, which mimics Gram-negative infections (7). The effects of LPS are in turn mediated by cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and it has been shown that many of the metabolic effects of infection, inflammation, and trauma can be induced by these cytokines (reviewed in Ref. 7).

Sphingolipids are important constituents of lipoproteins (8, 9). We have shown recently that LPS and cytokines up-regulate hepatic sphingolipid synthesis in Syrian hamsters (10). LPS induced a 75% and a 2.5-fold increase in hepatic sphingomyelin and ceramide synthesis, respectively, as well as a 2-fold increase in the activity of hepatic serine palmitoyltransferase (SPT), the first and rate-limiting enzyme in sphingolipid synthesis (10). LPS also increased SPT mRNA levels, suggesting that the increase in SPT activity was due to an increase in its mRNA. Finally, lipoproteins isolated from Syrian hamsters treated with LPS contained significantly higher levels of ceramide, sphingomyelin, and glucosylceramide (GlcCer) (10). It is of note that the increases in GlcCer levels (19-fold in VLDL and 7.3-fold in LDL) were greater than the increases in ceramide (3.7- and 2.2-fold in VLDL and LDL, respectively) and sphingomyelin (no change in VLDL and 84% increase in LDL), suggesting that GlcCer synthesis may be regulated by the acute phase response.

‡ To whom correspondence should be addressed: Metabolism Section (111F), Dept. of Veterans Affairs Medical Center, 4150 Clement St., San Francisco, CA 94121. Tel.: 415-750-2005; Fax: 415-750-6977; E-mail: kfgld@itsa.ucsf.edu.

§ To whom correspondence should be addressed: Metabolism Section (111F), Dept. of Veterans Affairs Medical Center, 4150 Clement St., San Francisco, CA 94121. Tel.: 415-750-2005; Fax: 415-750-6977; E-mail: kfgld@itsa.ucsf.edu.

1 The abbreviations used are: GSL(s), glycosphingolipid(s); LPS, lipopolysaccharide; TNF, tumor necrosis factor; IL, interleukin; SPT, serine palmitoyltransferase; GlcCer, glucosylceramide; BW, body weight; LDL, low density lipoprotein; VLDL, very low density lipoprotein; MOPS, 4-morpholinepropanesulfonic acid; CHAPS, 3-(3-cholamidopropyl)dimethyl ammonio)-1-propanesulfonic acid; PBS, phosphate-buffered saline; GM1, Galβ1,3GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc-Cer; GM3, NeuAcα2,3Galβ1,4Glc-Cer; GD1α, NeuAcα2,3Galβ1,3GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc-Cer;

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GlcCer is the precursor of all neutral GSLs as well as the sialic acid-containing acidic GSLs or gangliosides (11) and is synthesized by the enzyme GlcCer synthase (UDP-glucose:N-acetylglucosamine β-glucosyltransferase or GlcT-1; EC 2.4.1.80) that catalyzes the transfer of glucose from UDP-glucose to ceramide (11). The cDNA for human GlcCer synthase was recently cloned and was shown to be expressed ubiquitously (12). As the first enzyme in the GSL synthetic pathway, it is likely that the regulation of GlcCer synthase will play an important role in determining the rate of formation of GSLs. The metabolism of GlcCer and the regulation of GlcCer synthase has been extensively studied in mammalian skin (13–18). However, very little is known about the factors that regulate GlcCer synthase activity and expression in tissues other than the epidermis despite its ubiquitous expression (19, 20).

Because of the striking increase in GlcCer content of circulating lipoproteins following LPS treatment (10), we postulated that LPS and cytokines might increase GlcCer synthesis in the liver. The present study was designed to determine whether LPS and cytokines regulate GlcCer synthase mRNA and activity both in the liver of intact animals and in HepG2 cells (a human hepatoma cell line) in vitro. We have also examined the effect of LPS on the content of several GSLs in the liver.

EXPERIMENTAL PROCEDURES

Materials—[14C]UDP-glucose (263 mCi/mmol) and [α-32P]dCTP (3,000 Ci/mmol) were obtained from NEN Life Science Products. Multiprime DNA labeling system was purchased from Amersham Pharmacia Biotech (Amersham, United Kingdom); minispin G-50 columns were from Worthington; oligo(dt)-cellulose type 77F was from Amersham Pharmacia Biotech (Upsala, Sweden); and Nytran membranes were from Schleicher & Schuell. Kodak XAR5 film was used for autoradiography. High performance TLC plates (silica gel 60) were obtained from Merck. Chromatography standards, including ceramide, sphingomyelin, and GlcCer, were purchased from Sigma. Ceramide trihexoside and gangliosides were obtained from Matreya (Pleasant Gap, PA). LPS (Escherichia coli 55:B5) was purchased from Difco and was freshly diluted to desired concentrations in pyrogen-free 0.9% saline (Kendall McGraw Laboratories, Inc.). Human TNF-α with a specific activity of 5 × 10⁸ units/mg was provided by Genentech, Inc. Recombinant human IL-1β with a specific activity of 1 × 10⁶ units/mg was provided by Immunex. Human IL-6 was provided by Walters Fiers (University of Ghent, Ghent, Belgium). The cytokines were freshly diluted to desired concentrations in pyrogen-free 0.9% saline containing 0.1% human serum albumin.

Animal Procedures—Male Syrian hamsters (140–160 g) were purchased from Charles River Laboratories (Wilmington, MA). The animals were maintained in a reverse-light-cycle room (3 a.m. to 3 p.m. dark, 3 p.m. to 3 a.m. light) and were provided with rodent chow and water ad libitum. Anesthesia was induced with halothane, and the animals were injected intraperitoneally with LPS, TNF-α, or IL-1β at the indicated doses in 0.5 ml of 0.9% saline or with saline alone. Food was subsequently withdrawn from both control and treated animals, because LPS and cytokines induce anorexia (21). Animals were studied 2–24 h after LPS administration or 8 h after cytokine administration as indicated in the text. The doses of LPS used (0.1 to 100 µg/100 g body weight (BW)) have significant effects on triglyceride, cholesterol, and sphingolipid metabolism in Syrian hamsters (10, 22, 23) but are far below doses that cause death in rodents (LD₅₀ ~5 mg/100 g BW). Similarly, the doses of TNF-α and IL-1β (17 and 1 µg/g BW, respectively) were chosen, because previous studies have demonstrated that these doses have marked effects on serum lipid and lipoprotein levels, reproducing many of the effects of LPS on lipid metabolism in Syrian hamster (10, 24).

GlcCer Synthase Activity Assay—The synthesis of GlcCer from exogenous ceramide was assayed as described (15, 18). Briefly, the total assay volume of 110 µl contained 50 µM UDP-[U-14C]glucose (70 mCi/ mmol), 50 mM MOPS (pH 6.5), 5 mM MnCl₂, 2.5 mM MgCl₂, 1 mM NADPH, 5 mM dimerocaprotopanol, and 1% v/v CHAPS. The solid substrate was prepared by adsorbing 20 µg of ceramide (Type IV; Sigma) onto 1 mg of silica gel, and the reaction was initiated with the addition of 0.1–0.2 mg of microsomal protein. The incubation was carried out at 37 °C for 30 min, and the reaction was terminated by the addition of ice-cold PBS. Pellets were washed four times by resuspension in PBS (4 °C) and centrifugation. The final pellets were resuspended in PBS, and the radioactivity was counted by liquid scintillation spectrometry.

Isolation of RNA and Northern Blotting—Total RNA was isolated by a variation of the guanidium thiocyanate method (25) as described earlier (22). Poly(A)⁺ RNA was isolated using oligo(dt)-cellulose and was quantitated by measuring absorption at 280 nm. Gel electrophoresis, transfer, and Northern blotting were performed as described previously (22). The uniformity of sample applications was checked by UV visualization of the acridine orange-stained gels before transfer to Nytran membranes. The cDNA probe hybridization was performed as described earlier (22). The blots were exposed to x-ray films for various time periods to ensure that measurements were done on the linear portion of the curve, and the bands were quantified by densitometry. We and other (22, 26) have found that LPS increases actin mRNA levels in liver by 2–5-fold in rodents. TNF-α and IL-1β produce a 2-fold increase in actin mRNA levels. LPS also produced a 2.6-fold increase in cyclophilin mRNA in liver (27). Thus, the mRNA levels of actin, and cyclophilin, which are widely used for normalizing data, cannot be used to study LPS-induced or cytokine-induced regulation of proteins in liver. However, the differing direction of the changes in mRNA levels for specific proteins after LPS or cytokine administration, the magnitude of the alterations, and the relatively small standard error of the mean make it unlikely that the changes observed were due to unequal loading of mRNA.

Measurement of Transcription—Nuclei were isolated from hamster liver using the homogenization procedure described by Clarke et al. (28). The rate of transcription in hamster liver nuclei was measured using the nuclear run-on assay as described earlier (23). Radioactive RNA bound to nylon filters was quantified by liquid scintillation counting.

Analysis of Sphingolipid and Glycosphingolipid Content in Liver—Whole livers were cut into small pieces, homogenized with chloroform/methanol (2:1) using a Polytron tissue homogenizer, and incubated at 40 °C for 1 h. The lipids were then extracted with chloroform/methanol/acetate acid (1:2, 2:1, 1:2, and 1:1; v/v). The resultant combined total lipids were then fractionated into neutral and acidic lipids using DEAE-Sephadex A-25 (acetate form, Sigma) column as described earlier (29). Desalting of acidic lipid fraction was achieved by column chromatography (Sep-Pak C₁₅, Waters, Milford, MA). Approximately 0.5–1.5 mg of lipid from each sample was applied to high performance TLC plates, along with individual standards including ceramide, GlcCer, and sphingomyelin, ceramide trihexoside (globotriosyl ceramide or Glb3), and gangliosides GM3, GM1, and GD1a. Ceramide and GlcCer were separated by development in chloroform/methanol/water (40:10:1, v/v) to 2 cm and then to 5 cm, followed by chloroform/methanol/acidic acid (94:1:4, v/v) to the top of the plate. Other neutral GSLs were separated by chloroform/methanol/0.2% CaCl₂ (65:25:3, v/v). Acidic GSLs were developed in chloroform/methanol/0.02% CaCl₂ (55:40:10, v/v), while sphingomyelin was separated using chloroform/methanol/acetic acid water (50:30:8:4, v/v). The plates were then sprayed with either charring solution (cupric acetate reagent for ceramide and sphingomyelin), orcinol reagent (for neutral GSLs) or resorcinol reagent (for acidic GSLs), and heated. Individual lipids were quantified by scanning densitometry as described previously (30).

HepG2 Cell Culture and Cytokine Treatment—HepG2 cells (a human hepatoma cell line) were obtained from the American Type Culture Collection (Manassa, VA) and maintained in minimum essential medium (Mediatech, Inc.) supplemented with 10% fetal bovine serum under standard culture conditions (5% CO₂, 37 °C). Cells were seeded into 100-mm culture dishes and allowed to grow to 80% confluence. Immediately before the experiment, cells were washed with calcium-free PBS, and the experimental medium (Dulbecco’s minimum essential medium plus 0.1% bovine serum albumin) containing TNF-α, IL-1β, or IL-6 at the indicated concentrations was added. Cells were incubated at 37 °C for the indicated times. RNA purification and Northern blotting were performed according to previously described methods (22).

Statistics—Results are expressed as mean ± S.E. Statistical significance between two groups was determined by using the Student’s t test. Comparison among several groups was performed by analysis of variance, and significance was calculated by using Bonferroni’s post hoc test.
RESULTS

We first examined the effect of LPS treatment (100 μg/100 g BW) on GlcCer synthase mRNA levels in the liver of Syrian hamsters. As shown in Fig. 1A, hepatic GlcCer synthase mRNA levels increased nearly 20-fold within 2 h following LPS administration. The LPS-induced increase in GlcCer synthase mRNA levels is sustained for 8 h, returning to base line by 24 h. The dose-response curve for LPS effect on GlcCer synthase mRNA levels was performed at 8 h after administration. The data presented demonstrate that the LPS-induced increase in hepatic GlcCer synthase mRNA levels is a very sensitive response, with the half-maximal increase seen with -0.3 μg/100 g BW LPS and a maximal response at 1 μg/100 g BW (Fig. 1B). Thus, very low doses of LPS stimulate a rapid and marked increase in GlcCer synthase mRNA levels in liver.

In order to examine the mechanism for the induction of GlcCer synthase mRNA, we next measured the rate of transcription in liver nuclei obtained from control and LPS-treated (100 μg/100 g BW, 4-h treatment) hamsters. The data presented in Fig. 2 demonstrate that the rate of GlcCer synthase transcription was 2.4-fold higher in liver nuclei from LPS-treated hamsters, suggesting that an increased rate of transcription partly accounts for the increase in GlcCer synthase mRNA levels after LPS treatment.

We then determined if the increase in GlcCer synthase mRNA levels results in a change in hepatic GlcCer synthase activity. LPS treatment produced a 3.3- and 4.2-fold increase in GlcCer synthase activity in the liver after 8 and 16 h of treatment, respectively (Fig. 3). To determine whether the increase in hepatic GlcCer synthase activity is reflected in changes in hepatic GSLs, we next measured their content in the liver after LPS treatment. The major GSLs detected in Syrian hamster liver were GlcCer, ceramide trihexoside, and ganglioside GM3. The data presented in Fig. 4A demonstrate that the content of ceramide (the immediate precursor of GlcCer) is decreased, whereas the content of GlcCer (the immediate product of GlcCer synthase reaction) is significantly increased in the livers of LPS-treated animals. Moreover, the levels of ceramide trihexoside (Fig. 4A) and ganglioside GM3 (Fig. 4B), both distal products of GlcCer synthase, are also increased in the liver. Finally, the content of sphingomyelin, the most abundant sphingolipid in the liver, was not altered by LPS treatment (control, 25.6 ± 2.33 versus LPS 26.3 ± 1.6 μg/mg neutral lipid, p = not significant). Thus, the LPS-induced increase in GlcCer synthase activity regulates the levels of specific precursors and downstream glycosphingolipid metabolites in the liver.

Since pro-inflammatory cytokines mediate many of the met-
GlcCer synthase, we determined the effect of TNF-α in the acute phase response, could directly regulate hepatic increase (Fig. 5). To determine whether cytokines, that mediate synthase mRNA levels was observed at 1 ng/ml IL-1β response studies showed that the maximal increase in GlcCer was sustained for at least 24 h (Fig. 7A). Furthermore, the dose-response studies showed that the maximal increase in GlcCer synthase mRNA levels was observed at 1 ng/ml IL-1β, and the half-maximal response occurred at ~0.03 ng/ml (Fig. 7B), suggesting that the increase in GlcCer synthase mRNA levels in HepG2 cells is a very sensitive response to IL-1β. Thus, similar to the in vivo results presented above, very low doses of IL-1β rapidly increase GlcCer synthase mRNA levels in HepG2 cells in vitro.

DISCUSSION

In the present study we demonstrate that hepatic GlcCer synthase is markedly up-regulated during the acute phase response. In Syrian hamsters, LPS administration results in a 12–20-fold increase in GlcCer synthase mRNA levels in the liver. This stimulation occurs rapidly (within 2 h), and is a very sensitive response to LPS (half-maximal response at 0.3 μg/100 g BW compared with a LD₅₀ of approximately 5 mg/100 g BW). Moreover, this increase in GlcCer synthase mRNA levels is accompanied by a 3–4-fold increase in hepatic GlcCer synthase activity. Preliminary studies from our laboratory also indicate that LPS acutely produces a marked increase in GlcCer synthase mRNA levels in HepG2 cells, whereas IL-6 had no effect (Fig. 6). Because IL-1β was most effective in inducing GlcCer synthase mRNA in HepG2 cells as well as in the livers of intact animals, we performed additional studies on the effect of IL-1β in HepG2 cells. The data presented in Fig. 7A show that IL-1β produced a 9-fold increase in GlcCer synthase mRNA levels, with the earliest increase observed after 2 h and a maximal effect at 8 h. This effect was sustained for at least 24 h (Fig. 7A). Furthermore, the dose-response studies showed that the maximal increase in GlcCer synthase mRNA levels was observed at 1 ng/ml IL-1β, and the half-maximal response occurred at ~0.03 ng/ml (Fig. 7B), suggesting that the increase in GlcCer synthase mRNA levels in HepG2 cells is a very sensitive response to IL-1β. Thus, similar to the in vivo results presented above, very low doses of IL-1β rapidly increase GlcCer synthase mRNA levels in HepG2 cells in vitro.

Small intestine GlcCer synthase mRNA, suggesting that the effects of LPS on GSL metabolism are tissue-specific. The modest increase in LPS-induced GlcCer synthase transcription as compared with a profound increase in mRNA levels suggests that increased transcription only partly accounts for the increase in mRNA levels, and there could be additional regulatory mechanisms. It is also possible that GlcCer synthase mRNA degradation is altered during the acute phase response and is very difficult to evaluate in an in vivo animal model. The marked difference in the magnitude of increase in mRNA level versus enzyme activity also indicates additional regulatory mechanisms. It is also possible that GlcCer synthase protein may have a long half-life and therefore an acute increase in mRNA may not reflect a comparable increase in protein mass or activity. The half-life of GlcCer synthase protein in vivo is not known. Additional studies are required to address these issues.

LPS administration also produced significant increases in the content of several GSLs in the liver. Specifically, the levels of GlcCer, ceramide trihexoside, and ganglioside GM3 were increased by 1.8-, 2.1-, and 3.3-fold, respectively. In contrast, ceramide, the substrate of GlcCer synthase, decreased in the liver following LPS treatment. This LPS-induced decrease in ceramide content was observed as early as 1 h, suggesting that ceramide degradation may be increased during the acute phase response.

2 R. A. Memon, C. Grunfeld, and K. R. Feingold, unpublished observation.
ceramide content in the liver is likely due to the depletion of the ceramide pool secondary to enhanced synthesis of GlcCer and more distal GSLs during the acute phase response. A likely consequence of this increase in GlcCer synthesis in the liver is the increase in lipoprotein GlcCer content that we have reported previously (10).

The effects of LPS are mediated by its ability to stimulate a variety of immune cells that increase the synthesis and secretion of cytokines, peptides, and lipid mediators of inflammation (31). The interaction of immune cells with LPS is facilitated by a specific LPS-binding protein (32). LPS-binding protein binds with a high affinity to the lipid portion of LPS and then interacts with the monocyte differentiation antigen CD14 to up-regulate the synthesis of several cytokines including TNF-α and IL-1β (33). The stimulation of acute phase protein synthesis and the changes in lipid and lipoprotein metabolism during infection and inflammation are not direct actions of LPS on the liver; rather the hepatic effects are now known to be mediated by cytokines (34). We have shown previously that TNF-α and IL-1β increase serum triglyceride and cholesterol levels, stimulate hepatic lipogenesis, and enhance VLDL production (35, 36). Moreover, both TNF-α and IL-1β decrease fatty acid oxidation and ketone body production in the liver (37). We have also shown that anti-TNF antibodies or IL-1 receptor antagonist block the effects of LPS on triglyceride and cholesterol metabolism (38), indicating that these cytokines mediate the metabolic effects of LPS. In the present study, we demonstrate that like LPS, both TNF-α and IL-1β increase GlcCer synthase mRNA levels in vivo. Moreover, both TNF-α and IL-1β increased GlcCer synthase mRNA levels in HepG2 cells.

**Fig. 5.** Effect of cytokines on GlcCer synthase mRNA in liver. Animals were injected intraperitoneally with saline, TNF, or IL-1 at the doses indicated in the text. Eight hours later the animals were killed, livers were obtained, and poly(A)+ RNA was isolated. Northern blots were probed with GlcCer synthase cDNA as described under “Experimental Procedures.” Data are presented as the percentage of control values as quantified by densitometry (mean ± S.E.; n = 5 for each group. CON indicates saline control. *, p < 0.05 versus control; **, p < 0.001 versus control and TNF.

**Fig. 6.** Effect of cytokines on GlcCer synthase mRNA in HepG2 cells. TNF, IL-1, or IL-6 were added to HepG2 cells at a concentration of 100 ng/ml. Four hours later, poly(A)+ RNA was isolated, and Northern blots were probed with GlcCer synthase cDNA as described under “Experimental Procedures.” Data are presented as the percentage of control values as quantified by densitometry (mean ± S.E.; n = 5 for all groups. CON indicates control. *, p < 0.01 versus control; **, p < 0.001 versus control and < 0.01 versus TNF.

**Fig. 7.** Time course (A) and dose response (B) for the effect of IL-1 on GlcCer synthase mRNA in HepG2 cells. HepG2 cells were incubated with 100 ng/ml IL-1 at the indicated times (A) or with the indicated concentrations of IL-1 (B) for 4 h. At the end of the incubations, poly(A)+ RNA was isolated, and Northern blots were probed with GlcCer synthase cDNA as described under “Experimental Procedures.” Data are mean ± S.E.; n = 4 for all groups. A: *, p < 0.002; **, p < 0.001. B: *, p < 0.001.
cells, suggesting that cytokines mediators of acute phase response can directly affect hepatocyte GlcCer synthase.

It is believed that changes in the production of specific proteins during the acute phase play an important homeostatic role in the host response to infection, inflammation, and trauma (39, 40). For example, increases in both C-reactive protein and complement 3 during the acute phase response may help in the opsonization of bacteria, immune complexes, and foreign particles (39, 40). Similarly, an increase in serum amyloid A during the acute phase response has been shown to redirect the metabolism of HDL from hepatocytes toward macrophages at the site of inflammation (41). We have postulated that the changes in lipid and lipoprotein metabolism that occur during the host response to infection and inflammation may also be beneficial (7, 42). For example, elevations in serum lipoprotein levels may enhance neutralization of LPS, lipoteichoic acid (a component of the cell wall of Gram-positive bacteria which is analogous to LPS), and viruses (7, 42, 43). Additionally, alterations in lipid metabolism in the liver and other tissues may allow for the redistribution of nutrients to support the increased energy needs of cells that are involved in host defense and tissue repair such as macrophages and lymphocytes (7, 42). The precise role that the increases in intracellular or lipoprotein GSLs (Ref. 10 and the present study) might have during the acute phase response is not clear at this time.

However, GSLs have been implicated in the growth of lymphocytes and other cells. For example, Platt et al. (44), using an inhibitor of GlcCer synthase, reduced GSL levels in mice by 50–70% in liver and lymphoid tissue. The GSL-depleted mice grew more slowly, but otherwise did not appear grossly abnormal. Examination of lymphoid tissue, spleen, and thymus revealed that these organs were reduced in size by 50% due to a decrease in cell numbers (44). More recent studies have shown that natural killer T lymphocytes express a T cell antigen receptor that recognizes GSLs as the ligand and GSLs stimulate the proliferation of natural killer T cells (45). Thus, it is possible that the increase in GlcCer synthase activity and the production of GSLs during the acute phase response plays a role in the immune response.

In addition to stimulating proliferation of T lymphocytes (45), GlcCer also stimulate the proliferation of other cell types and tissues (46–49). Conversely, a mutant B16 melanoma cell line (GM-95) that lacks GlcCer synthase activity has a slower growth rate and altered cell morphology as compared with the parental cells (50), suggesting that GlcCer may be required for normal cell growth. Inhibition of GlcCer synthase activity also has been shown to decrease the renal hypertrophy that occurs in diabetic animals (51) and to decrease renal cell proliferation in vitro (52). Finally, inhibition of GlcCer synthase activity decreases keratinocyte proliferation (53). Thus, the increase in tissue GSLs during the acute phase response reported here may play a role in regulating cellular proliferation.

Finally, the increase in hepatic GlcCer synthase activity and enhanced synthesis of GSLs in liver could result in the secretion of lipoproteins that are enriched in GSLs. We have shown recently that the content of GlcCer in increased in circulating lipoproteins during the acute phase response (10). The effect of increased glycosphingolipid content on lipoprotein function is not known. Since cell membrane GSLs are exploited as receptors by a number of microorganisms, including bacteria and viruses (54), it is possible that lipoproteins enriched in GSLs might play a protective role by either binding to microorganisms or by interfering with their binding to the cells.

In summary, the present study demonstrates that hepatic GlcCer synthase activity and mRNA levels are acutely increased during LPS and cytokine-induced acute phase re-
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