Regulation of sphingolipid and glycosphingolipid metabolism in extrahepatic tissues by endotoxin

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Abstract **The host response to infection and inflammation is associated with multiple alterations in lipid metabolism. We have shown that endotoxin [lipopolysaccharide (LPS)] stimulates hepatic sphingolipid synthesis and increases ceramide and glucosylceramide (GlcCer) content in circulating lipoproteins in Syrian hamsters. LPS also increases the activity and mRNA levels of serine palmitoyltransferase (SPT) and GlcCer synthase, the committed enzymes in sphingolipid and glycosphingolipid (GSL) synthesis, respectively, in the liver. To determine whether sphingolipid and GSL metabolism are regulated in other tissues during the host response to infection, we examined the effect of LPS on the regulation of SPT and GlcCer synthase in extrahepatic tissues in Syrian hamsters. LPS significantly increased SPT activity in spleen and kidney after 16 h of treatment, but had no effect on SPT activity in lung and brain, suggesting that the effect of LPS on sphingolipid metabolism is tissue specific. LPS also increased SPT mRNA levels in spleen and kidney by ~3-fold, suggesting that the increase in SPT activity is due to an increase in SPT mRNA expression. LPS significantly increased GlcCer synthase activity in spleen and kidney, and produced 4- and 15-fold increases in GlcCer synthase mRNA levels in spleen and kidney, respectively. LPS treatment increased GlcCer content by 1.3-fold in spleen and by 6.2-fold in kidney. LPS also increased the content of ceramide trihexoside by 1.7-fold in spleen. These results suggest that LPS regulates sphingolipid and GSL metabolism in spleen and kidney. An increase in GSL metabolites in spleen and kidney during the host response to infection and inflammation may be required for modulation of immune responses and regulation of cell growth.** — Memon, R. A., W. M. Holleran, Y. Uchida, A. H. Moser, C. Grunfeld, and K. R. Feingold. **Regulation of sphingolipid and glycosphingolipid metabolism in extrahepatic tissues by endotoxin.** ***J. Lipid Res.*** **2001.** 42: **452–459.**

Supplementary key words serine palmitoyltransferase • glucosylceramide synthase • acute-phase response • spleen • kidney

Sphingolipids are important components of the cell membranes of all eukaryotic cells and exert a wide range of functions including regulation of cell growth, differentiation, and apoptosis (1, 2). Glycosphingolipids (GSL) are complex sphingolipids that contain a hydrophobic ceramide moiety and a hydrophilic oligosaccharide residue. These glycosylated sphingolipids are synthesized by the sequential addition of sugar residues to ceramide by linkage-specific glycosyltransferases (3). GSL are involved in a variety of important biological processes including cell recognition, proliferation, and differentiation, signal transduction, interaction with bacterial toxins, and modulation of immune responses (3, 4).

Several of the enzymes involved in the synthesis of sphingolipids and GSL have been identified and characterized. Serine palmitoyltransferase (SPT), the first and rate-limiting enzyme in sphingolipid biosynthesis, catalyzes the condensation of serine and palmitoyl-CoA to form 3-ketosphinganine (1). Glucosylceramide (GlcCer) synthase is the first committed enzyme in the GSL synthesis pathway and catalyzes the transfer of glucose from UDP-glucose to ceramide to form GlcCer. GlcCer is the precursor of all neutral GSL as well as sialic acid-containing acidic GSL or gangliosides (3). The cDNAs encoding SPT and GlcCer synthase were cloned and shown to have ubiquitous expression in eukaryotic systems (5, 6).

Previous studies by our laboratory and others have shown that the acute-phase response (APR) induced by infection or inflammation results in several alterations in lipid and lipoprotein metabolism, such as increased serum triglyceride and cholesterol levels, stimulation of fatty acid and cholesterol synthesis, enhanced lipolysis, and decreased ketogenesis (7–10). These metabolic alterations can be induced by endotoxin [lipopolysaccharide (LPS)] treatment, which mimicks gram-negative infections (11). More recently, we have shown that LPS stimulates hepatic

Abbreviations: APR, acute-phase response; CTH, ceramide trihexoside; GlcCer, glucosylceramide; GSL, glycosphingolipid(s); LacCer, lactosylceramide; LPS, lipopolysaccharide; SPT, serine palmitoyltransferase.

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sphingolipid synthesis in Syrian hamsters, and increases the activity and mRNA expression of SPT in liver (12). Moreover, LPS also increases the activity and mRNA expression and transcription of GlcCer synthase in the liver of Syrian hamsters and increases the content of GlcCer, ceramide trihexoside (CTH, also known as globotriosyl ceramide), and ganglioside GM_3 in the liver (13). Finally, circulating lipoproteins isolated from Syrian hamsters treated with LPS contain significantly higher levels of ceramide and GlcCer (12). These data suggest that an LPS-induced increase in the activity of SPT and GlcCer synthase stimulates the synthesis of sphingolipids and distal GSL in the liver, which may account for the higher ceramide and GlcCer content in circulating lipoproteins during the APR.

In addition to liver, the regulation of SPT and GlcCer synthase has been extensively studied in mammalian skin (14–16), where GlcCer plays an important role in cutaneous permeability barrier homeostasis (14, 15). Despite the ubiquitous expression of SPT and GlcCer synthase in tissues and the regulatory role of sphingolipid and GSL metabolites, relatively little is known about the factors that regulate the activity and expression of these enzymes in tissues other than liver and skin. The APR also occurs in extrahepatic tissues and previous studies from our laboratory have shown that several genes of lipoprotein metabolism are regulated in extrahepatic tissues (11). The present study was designed to determine whether LPS treatment regulates SPT and GlcCer synthase activity and mRNA expression in extrahepatic tissues. We have also examined whether LPS-induced alterations in SPT and GlcCer synthase activities are reflected in changes in tissue GSL content.

MATERIALS AND METHODS

Materials

[^{3}H]serine (30 Ci/mmol), [^{14}C]UDP-glucose (263 mCi/mmol), and [α-^{32}P]dCTP (3,000 Ci/mmol) were obtained from New England Nuclear (Boston, MA). LPS (*Escherichia coli* 055:B5) was purchased from Difco (Detroit, MI) and was freshly diluted to the desired concentrations in pyrogen-free 0.9% saline. The Multiprime DNA labeling system was purchased from Amersham International (Amersham, UK); minispin G-50 columns were from Worthington Biochemical (Freehold, NJ); oligo(dT) cellulose type 77F was from Pharmacia LKB Biotechnology (Uppsala, Sweden); and Nytran membranes were from Schleicher & Schuell (Keene, OH). XAR5 film (Eastman Kodak, Rochester, NY) was used for autoradiography. High performance thin-layer chromatography (HPTLC) plates (silica gel 60) were obtained from Merck (Rahway, NJ). Chromatography standards including ceramide, sphingomyelin, and GlcCer were purchased from Sigma (St. Louis, MO). CTH, lactosylceramide (LacCer), galactosylceramide (GalCer), and gangliosides were obtained from Matreya (Pleasant Gap, PA). The cDNA for SPT was provided by R. C. Dickson (University of Kentucky, Lexington, KY) (5), whereas that for GlcCer synthase was provided by S. Ichikawa and Y. Hirabayashi (Institute of Physical and Chemical Research, Hirosawa, Japan) (6).

Animal procedures

Male Syrian hamsters (140 to 160 g) were purchased from Charles River Laboratories (Wilmington, MA). The animals were maintained in a reverse light cycle room (3 AM to 3 PM dark, 3 PM to 3 AM light) and were provided with rodent chow and water ad libitum. Anesthesia was induced with halothane, and the animals were injected intraperitoneally with LPS (100 μg/100 g body weight) in 0.5 ml of 0.9% saline or with saline alone. Food was subsequently withdrawn from both control and treated animals because LPS induces anorexia (17). Animals were studied 2 to 24 h after LPS administration. At the indicated times, organs including spleen, kidney, lung, and brain were obtained, rapidly frozen in liquid nitrogen, and stored at −70°C for analysis. Our previous studies have shown that the dose of LPS used here (100 μg/100 g body weight) has significant effects on hepatic triglyceride, cholesterol, and sphingolipid metabolism in Syrian hamsters (8, 12, 13), but is far below the doses that cause death in rodents (50% lethal dose ~5 mg/100 g body weight). All animal studies were performed in accordance with the National Institutes of Health (NIH, Bethesda, MD) *Guidelines for the Care and Use of Laboratory Animals* and were approved by the institutional review board (Veterans Administration Medical Center, San Francisco, CA).

SPT activity assay

At the indicated times after LPS treatment, organs were isolated and homogenized in 0.25 M sucrose buffer containing 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 5 mM dithiothreitol, and 10 mM ethylenediaminetetraacetic acid. Microsomes were isolated by differential centrifugation. SPT activity was measured by the method of Williams, Wang, and Merrill (18) as described previously (12, 19). Briefly, the reaction mixture contained 50 μM pyridoxal phosphate, 150 μM palmitoyl-CoA, 1 mM L-[^{3}H]serine (specific activity, 50,000 dpm/nmol), and 0.1 mg of microsomal protein. The reaction was initiated by the simultaneous addition of palmitoyl-CoA and labeled serine, incubated at 37°C for 10 min, and terminated by the addition of 0.5 N ammonium hydroxide. The reaction product, 3-ketodihydrosphinganine, was isolated as described previously (19) and counted by liquid scintillation spectrometry. The enzyme activity is expressed as picomoles of 3-ketodihydrosphinganine formed per minute per milligram of microsomal protein.

GlcCer synthase activity assay

The synthesis of GlcCer from exogenous ceramide was assayed as described (13, 15). Briefly, the reaction mixture contained 50 μM [^{14}C]UDP-glucose (70 mCi/mmol), 50 mM morpholinepropanesulfonic acid (pH 6.5), 5 mM $MnCl_2$, 2.5 mM $MgCl_2$, 1 mM NADPH, 5 mM 2-mercaptopropanol, and 1% (w/v) 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate. 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 0.2 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 phosphate-buffered saline (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 guanidinium thiocyanate method (20) as described earlier (8). Poly(A)$^+$ RNA was isolated with oligo(dT) cellulose and quantified by measuring the absorption at 260 nm. Gel electrophoresis, transfer, and Northern blotting were performed as described earlier (8). The uniformity of sample application was checked by ultraviolet visualization of the acridine orange-stained gels before transfer to Nytran membranes. The cDNA probe hybridization was per-

formed as described previously (8). The blots were exposed to X-ray films at −70°C for various time periods to ensure that measurements were done on the linear portion of curve and the bands were quantified by densitometry.

Analysis of sphingolipid and GSL content

The content of sphingolipid and GSL in spleen and kidney was quantified as described earlier (13). Briefly, the tissues (spleen and kidney) were cut into small pieces, homogenized with chloroform–methanol 2:1 (v/v), using a Polytron tissue homogenizer, and incubated at 40°C for 20 min. Total lipids were extracted consecutively with chloroform–methanol–water 1:2:0.1, 2:1:0.1, and 1:2:0.1 (v/v/v) followed by chloroform–methanol 1:1 (v/v). The resultant combined total lipids were then fractionated into neutral and acidic lipids, using a diethylaminoethyl-Sephadex A-25 (acetate form; Sigma) column (21). Desalting of the acidic lipid fraction was achieved by column chromatography (Sep-Pak C_{18}; Waters, Milford, MA). Approximately 0.5–1.5 mg of lipid from each sample was applied to HPTLC plates, along with individual standards including sphingomyelin, ceramide, GlcCer, LacCer, GalCer, CTH, and gangliosides GM_3, GM_1, and GD_{1a}. Ceramide and GlcCer were separated by development in chloroform–methanol–water 40:10:1 (v/v/v) to 2 cm and then to 5 cm, followed by chloroform–methanol–acetic acid 94:1:4 (v/v/v) to the top of the plate. Other neutral GSL were separated by chloroform–methanol–0.2% $CaCl_2$ 65:23:3 (v/v/v). Acidic GSL were developed in chloroform–methanol–0.02% $CaCl_2$ 55:40:10 (v/v/v), while sphingomyelin was separated using chloroform–methanol–acetic acid–water 50:30:8:4 (v/v/v/v). The plates were then sprayed with charring solution (cupric acetate reagent for ceramide and sphingomyelin), orcinol reagent (for neutral GSL), or resorcinol reagent (for acidic GSL), and heated. Individual lipids were quantified by scanning densitometry as previously described (19).

Statistics

Results are expressed as means ± SE. Statistical significance between two groups was determined by using the Student's *t*-test.

RESULTS

Our previous studies have shown that LPS significantly increases SPT activity and mRNA levels in Syrian hamster liver, with the maximal increase observed at 16 h (12). Therefore, we first examined the effect of 16-h LPS treatment (100 μg/100 g body weight) on SPT activity in extrahepatic tissues in Syrian hamsters. LPS increased SPT activity (**Fig. 1**) by 1.3-fold in spleen (control 44.4 ± 2.3, LPS 59.3 ± 1.2 pmol/min/mg protein; $P < 0.001$) and by 1.5-fold in kidney (control 50.8 ± 1.0, LPS 78.5 ± 2.5 pmol/min/mg protein; $P < 0.001$). On the other hand, LPS had no significant effect on SPT activity in lung (75.3 ± 5.6, LPS 85.6 ± 6.7 pmol/min/mg protein; P = NS) or brain (56.4 ± 2.5, LPS 60.9 ± 4.7 pmol/min/mg protein; P = NS), suggesting that the effect of LPS on SPT activity is tissue specific.

To determine the potential mechanism by which LPS increases SPT activity, we examined the effect of 16-h LPS treatment on SPT mRNA levels in spleen and kidney. **Figure 2A** presents a Northern blot of LPS effect on SPT mRNA expression in spleen and kidney, and the densitometric analysis of the blot is shown in Fig. 2B. The data show that LPS increased SPT mRNA levels by 2.8-fold in spleen and by 2.9-fold in kidney. These data suggest that the LPS-induced increase in SPT activity is due to an in-

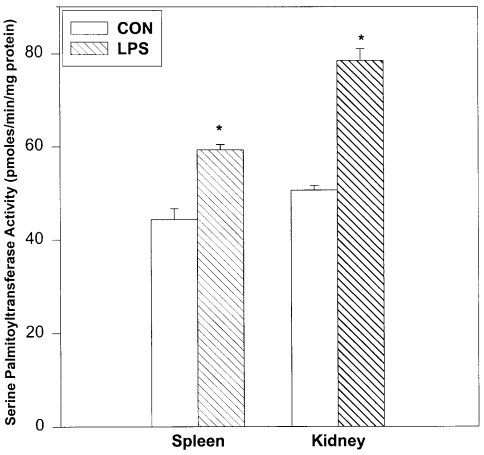

Fig. 1. Effect of LPS on SPT activity in spleen and kidney. Animals were injected intraperitoneally with either saline or LPS (100 μg/100 g body weight). Sixteen hours later the animals were killed, microsomes were isolated, and SPT activity was determined as described in Materials and Methods. Data are presented as means ± SEM, n = 5 for each group. CON, control. * $P < 0.001$.

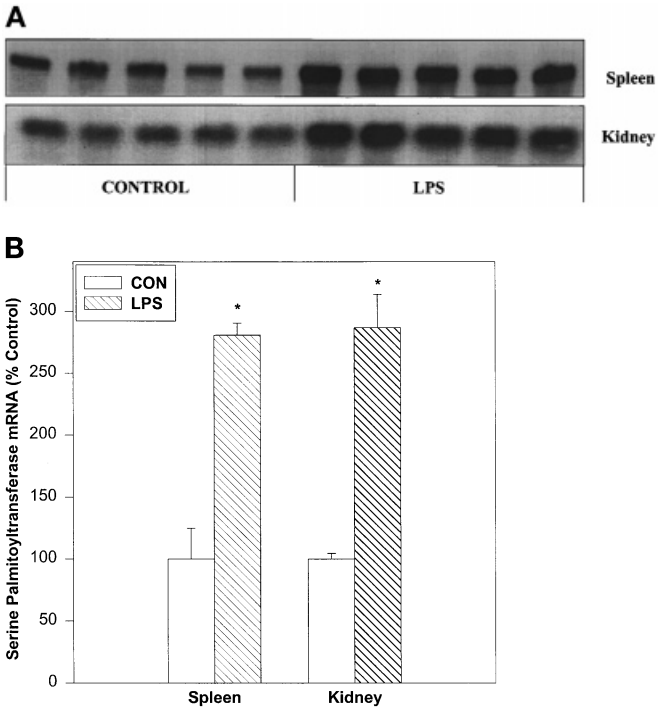

Fig. 2. Effect of LPS on SPT mRNA in spleen and kidney. Animals were injected intraperitoneally with either saline or LPS (100 μg/100 g body weight). Sixteen hours later the animals were killed, tissues were obtained, and poly(A)$^+$ RNA was isolated. Northern blots were prepared and probed with SPT cDNA as described in Materials and Methods. A: Northern blot of LPS effect on SPT mRNA in spleen and kidney. B: Data are presented as the percentage of control values as quantified by densitometry (mean ± SEM), n = 5 for each group. CON, control. * $P < 0.001$.

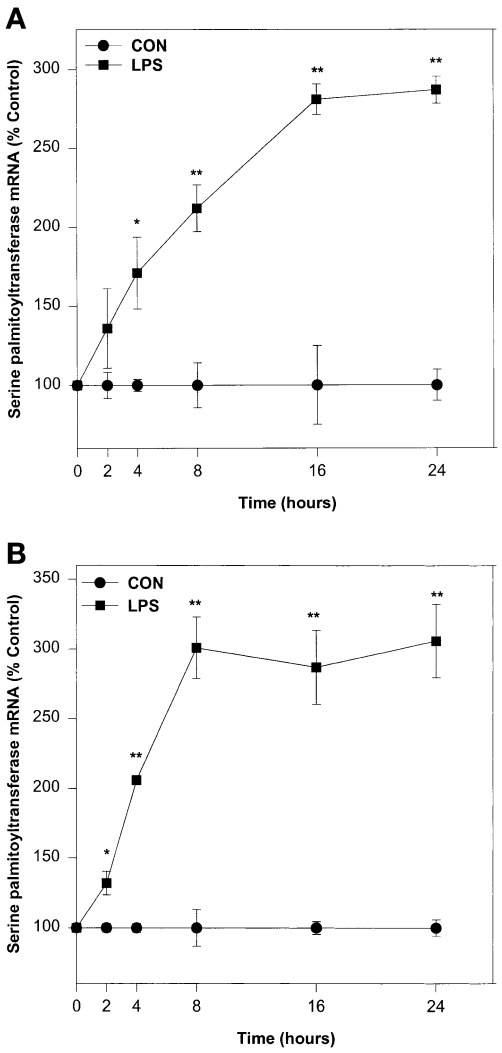

Fig. 3. Time course of LPS effect on SPT mRNA levels in spleen (A) and kidney (B). Syrian hamsters were injected intraperitoneally with either saline or LPS (100 μg/100 g body weight). Animals were killed at various time points and tissues were obtained for poly(A)$^+$ RNA isolation. Northern blots were probed with SPT cDNA as described in Materials and Methods. Data are presented as the percentage of control values as quantified by densitometry (mean ± SEM), n = 4 or 5 for each group at each time point. When error bars are not visible (B), they are within the marker that denotes the mean. CON, control. A: * $P < 0.02$, ** $P < 0.001$; B: * $P < 0.01$, ** $P < 0.001$.

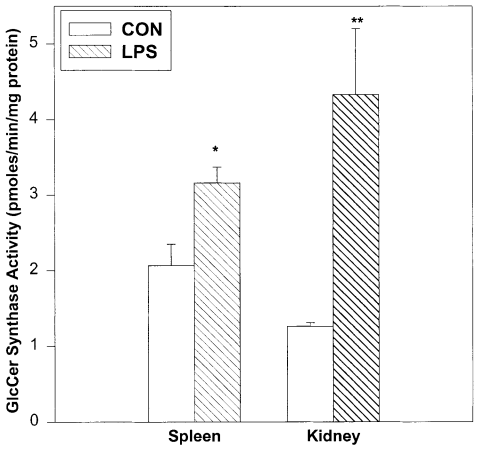

Fig. 4. Effect of LPS on GlcCer synthase activity in spleen and kidney. Animals were injected intraperitoneally with either saline or LPS (100 μg/100 g body weight). Sixteen hours later the animals were killed, microsomes were isolated, and GlcCer synthase activity was determined as described in Materials and Methods. Data are presented as means ± SEM, n = 5 for each group. CON, control. * $P < 0.02$, ** $P < 0.001$.

crease in SPT mRNA levels. Time-course studies showed that the LPS-induced increase in SPT mRNA levels in spleen (**Fig. 3A**) was evident by 2 h, reached a peak by 16 h, and was sustained for at least 24 h. Similarly, a small increase in SPT mRNA levels in kidney was first observed at 2 h after LPS treatment, became maximal at 8 h, and was sustained for at least 24 h (Fig. 3B).

To determine the effect of LPS on GSL metabolism in extrahepatic tissues, we first examined the effect of LPS on GlcCer synthase activity in spleen and kidney. The data presented in **Fig. 4** demonstrate that 16-h LPS treatment increased GlcCer synthase activity by 1.5-fold in spleen (control 2.07 ± 0.28, LPS 3.16 ± 0.21 pmol/min/mg protein; $P < 0.02$) and by 3.4-fold in kidney (control 1.26 ± 0.05, LPS 4.33 ± 0.87 pmol/min/mg protein; $P < 0.001$). We next examined the effect of LPS on GlcCer synthase mRNA levels in spleen and kidney. **Figure 5A** presents a Northern blot of LPS effect on GlcCer synthase mRNA expression in spleen and kidney, and the densitometric analysis of the blot is shown in Fig. 5B. LPS increased GlcCer synthase mRNA levels by 4.1-fold in spleen and by 15.4-fold in kidney (Fig. 5B). These data suggest that LPS increases GlcCer synthase activity by increasing its mRNA levels. We also examined the effect of LPS on GlcCer synthase mRNA expression in other tissues. LPS had no effect on GlcCer synthase mRNA levels in brain (control 100 ± 5, LPS 104 ± 4 densitometric units; P = NS) or small intestine (control 100 ± 21, LPS 109 ± 24 densitometric units; P = NS), but produced a 1.9-fold increase in GlcCer synthase mRNA levels in the lung (control 100 ± 13, LPS 195 ± 30 densitometric units; $P < 0.05$), suggesting that the effects of LPS on GlcCer synthase mRNA expression are tissue specific. We next examined the time course of LPS effect on GlcCer synthase mRNA expression in spleen and kidney. LPS acutely increased GlcCer synthase mRNA levels (maximal or near-maximal effect by 2 h) in both spleen (**Fig. 6A**) and kidney (Fig. 6B). The LPS-induced increase in GlcCer synthase mRNA levels in spleen was sustained for 24 h, whereas the increase in GlcCer synthase mRNA levels in kidney became maximal at 16 h after LPS administration and then slightly declined by 24 h.

We next determined whether the increases in SPT and GlcCer synthase activities were reflected in corresponding changes in sphingolipid and GSL content in the spleen and kidney. The major GSL detected in Syrian hamster spleen were GlcCer, LacCer, CTH, and gangliosides GM_3 and GD_{1a}. LPS significantly increased the content of GlcCer (the immediate product of the GlcCer synthase reaction) and CTH (a distal product of GlcCer synthase) by

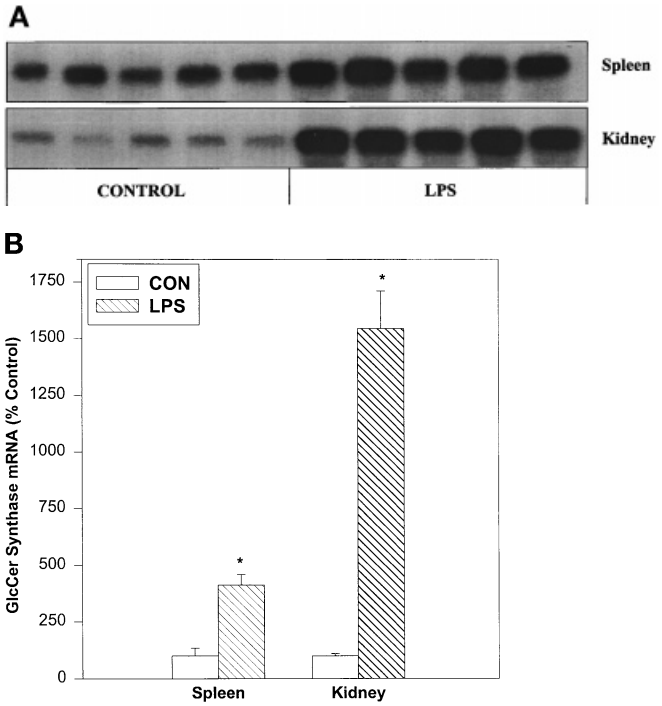

Fig. 5. Effect of LPS on GlcCer synthase mRNA in spleen and kidney. Animals were injected intraperitoneally with either saline or LPS (100 μg/100 g body weight). Sixteen hours later the animals were killed, tissues were obtained, and poly(A)$^+$ RNA was isolated. Northern blots were prepared and probed with GlcCer synthase cDNA as described in Materials and Methods. A: Northern blot of LPS effect on GlcCer synthase mRNA in spleen and kidney. B: Data are presented as the percentage of control values as quantified by densitometry (mean ± SEM); n = 5 for each group. CON, control. * $P < 0.001$.

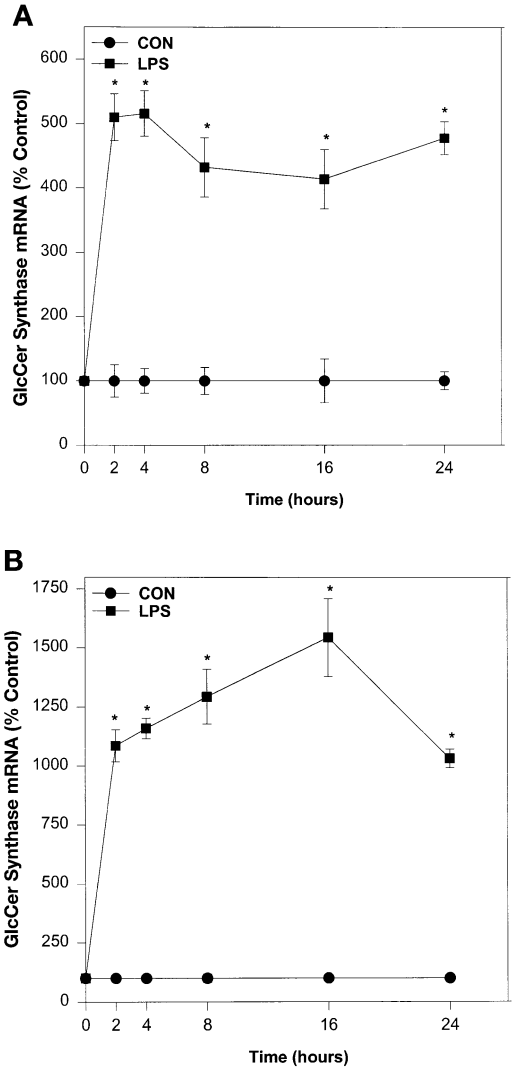

Fig. 6. Time course of LPS effect on GlcCer synthase mRNA levels in spleen (A) and kidney (B). Syrian hamsters were injected intraperitoneally with either saline or LPS (100 μg/100 g body weight). Animals were killed at various time points and tissues were obtained for poly(A)$^+$ RNA isolation. Northern blots were probed with GlcCer synthase cDNA as described in Materials and Methods. Data are presented as the percentage of control values as quantified by densitometry (mean ± SEM), n = 4 or 5 for each group at each time point. When error bars are not visible (B), they are within the marker that denotes the mean. CON, control. A: * $P < 0.001$; B: * $P < 0.001$.

1.3- and 1.7-fold, respectively, in spleen (**Table 1**). In addition, there was a trend toward an increase in the LacCer content in spleen from LPS-treated hamsters, while the total neutral lipids, sphingomyelin, and ceramide content were not significantly different. The content of total acidic lipids and gangliosides GM_3 and GD_{1a} also was not altered by LPS treatment in spleen (data not shown). The major GSL detected in Syrian hamster kidney were GlcCer, LacCer, GalCer, and ganglioside GM_3. LPS treatment produced a 6.2-fold increase in the content of GlcCer in kidney (**Table 2**), whereas the content of total neutral lipids, sphingomyelin, ceramide, LacCer, and GalCer was not significantly altered by LPS treatment. The content of total acidic lipids and ganglioside GM_3 was also not altered by LPS treatment in kidney (data not shown). These data suggest that LPS upregulates the mRNA expression and activity of key enzymes of sphingolipid and GSL metabolism, and increases the content of selected GSL metabolites in spleen and kidney.

DISCUSSION

GSL are important constituents of the plasma membranes in all cell types; however, relatively little is known about the factors that regulate either de novo GSL synthesis or the activity/mRNA expression of regulatory enzymes in GSL biosynthesis. The exact physiological functions of individual GSL are not well characterized. It has been postulated that GSL play a role in cell differentiation and proliferation (4). GSL have also been identified as binding sites on the cell surface for viruses, bacteria, and toxins (22). Finally, studies have suggested that specific GSL, such as α-GalCer, may play a role in modulating immune responses by activating natural killer T cells (23).

We have shown that in vivo administration of LPS, a component of the gram-negative bacterial cell wall, increased the activity of SPT and GlcCer synthase, the regu-

TABLE 1. Effect of LPS on sphingolipid and GSL content in the spleen in Syrian hamsters

Experimental Group	Neutral Lipids	Sphingomyelin	Ceramide	GlcCer	LacCer	CTH
	mg/g tissue	*μg/mg NL*	*μg/mg NL*	*μg/mg NL*	*μg/mg NL*	*μg/mg NL*
Control	42.5 ± 3.2	5.49 ± 0.57	1.62 ± 0.19	1.19 ± 0.05	1.51 ± 0.33	1.35 ± 0.18
LPS	39.5 ± 1.0	5.12 ± 0.25	1.58 ± 0.06	1.52 ± 0.12	2.15 ± 0.15	2.29 ± 0.20
P value	NS	NS	NS	<0.05	NS	<0.01

Syrian hamsters were injected with saline or LPS (100 μg/100 g body weight) and 16 h later animals were killed and tissues were obtained. The levels of individual sphingolipids and GSL were quantified by high performance thin-layer chromatography. Data represent means ± SEM; n = 5 animals in each group. NL, neutral lipids; NS, not significant.

latory enzymes in sphingolipid and GSL biosynthesis, respectively, in the liver of Syrian hamsters (12, 13). Moreover, LPS increased the content of GlcCer in the liver and circulating lipoproteins (13). We have now demonstrated that LPS administration increases the activity of SPT and GlcCer synthase in the spleen and kidney in Syrian hamsters. This increase in SPT and GlcCer synthase activity is preceded by an increase in their mRNA levels, suggesting that LPS induces the activity of these enzymes by increasing their gene expression. The results from the time-course studies demonstrate that the LPS-induced increase in GlcCer synthase mRNA expression occurs earlier than the increase seen in SPT mRNA expression. The maximal or near-maximal increase in GlcCer synthase mRNA levels is observed within 2 h of LPS administration in both spleen and kidney, whereas the maximum increase in SPT mRNA levels is seen by 16 h in spleen and by 8 h in kidney. These data raise the possibility that the LPS-induced increase in GlcCer production may be the primary event, which then may signal for more substrate, resulting in an induction of SPT and increased sphingolipid synthesis.

While LPS markedly increased the activity and mRNA expression of SPT and GlcCer synthase in liver (12, 13), spleen, and kidney (present study), it had no effect on SPT activity in brain or lung. LPS also had no effect on GlcCer synthase mRNA expression in brain or small intestine, and produced a small increase in lung, suggesting that the effects of LPS on sphingolipid and GSL metabolism may be tissue or cell specific. It is important to note that these studies were carried out in vivo and the organs studied here consisted of a heterogeneous population of cells. It is possible that LPS exerts its effect on a subset of cells in a given tissue and therefore a lack of response or a decrease in mRNA expression in other cell types in that tissue may mask the effect that would have been obtained if the studies were carried out in isolated or cultured cells. Several previous studies have shown that LPS and cytokines regulate GSL metabolism in endothelial cells and selectively increase the content of specific GSL (24–27). However, none of these studies examined the regulation of enzymes of GSL metabolism in endothelial cells. Our unpublished studies indicate that LPS induces both SPT and GlcCer synthase mRNA expression in RAW 264.7 cells, a murine macrophage cell line, suggesting that LPS regulates GSL metabolism in cells that are activated during the APR.

Our results also demonstrate that LPS administration specifically increases the content of GlcCer and CTH in spleen, and of GlcCer in kidney. Conversely, the content of major sphingolipids such as sphingomyelin and ceramide was not altered by LPS treatment in either spleen or kidney. Moreover, the content of several other neutral or acidic GSL was not altered by LPS treatment. These data suggest that the LPS-induced increase in SPT and GlcCer synthase serves to increase the synthesis of GlcCer in spleen and kidney, and of CTH (a downstream GSL metabolite) in spleen. The lack of an increase in ceramide content, despite an increase in SPT activity and mRNA expression, suggests that the newly synthesized ceramide may be utilized as a substrate for increasing GlcCer synthesis. It is also important to note that certain ceramide pools have rapid turnover rates (28) and, hence, an increase in SPT activity may not result in a net increase in

TABLE 2. Effect of LPS on sphingolipid and GSL content in the kidney in Syrian hamsters

Experimental Group	Neutral Lipids	Sphingomyelin	Ceramide	GlcCer	LacCer	GalCer
	mg/g tissue	*μg/mg NL*	*μg/mg NL*	*μg/mg NL*	*μg/mg NL*	*μg/mg NL*
Control	31.4 ± 1.9	113.6 ± 7.6	4.11 ± 0.56	0.18 ± 0.06	2.19 ± 0.17	5.58 ± 0.81
LPS	31.5 ± 1.0	111.2 ± 6.2	3.51 ± 0.25	1.12 ± 0.07	1.74 ± 0.16	4.62 ± 0.69
P value	NS	NS	NS	<0.001	NS	NS

Syrian hamsters were injected with saline or LPS (100 μg/100 g body weight) and 16 h later animals were killed and tissues were obtained. The levels of individual sphingolipids and GSL were quantified by high performance thin-layer chromatography. Data represent means ± SEM; n = 5 animals in each group. NL, Neutral lipids; NS, not significant.

tissue ceramide content during the APR in vivo. Our previous in vivo studies showed an increase in GlcCer and a decrease in ceramide content in liver after LPS (13). On the other hand, treatment with tumor necrosis factor and interleukin 1 (IL-1), cytokines that mediate most of the metabolic effects of LPS, increased both SPT mRNA levels and incorporation of [^{3}H]serine in ceramide in cultured hepatocytes (12), suggesting an increase in the rate of ceramide synthesis. Additional studies will be required to determine the local and systemic consequences for these tissue-specific alterations in ceramide content in vitro and in vivo.

The pathophysiological consequences of the induction of SPT and GlcCer synthase expression, and the subsequent increase in tissue GSL metabolites during the host response to infection and inflammation, remain to be established. Specific GSL such as α-GalCer and α-GlcCer are recognized as ligands by a T-cell receptor expressed on natural killer T lymphocytes (29). Gb3 has been characterized as a cell surface receptor for Shiga toxin on endothelial cells (30), whereas LacCer mediates tumor necrosis factor (TNF)-induced adhesion molecule expression in endothelial cells (31). More recently, Bovin et al. (32) reported that GalCer increased IL-1β, IL-6, and TNF mRNA levels in human mononuclear cells, whereas sulfatide decreased the secretion of IL-1β and IL-10 in LPS-activated mononuclear cells. These studies indicate that specific GSL are involved in distinct biological functions. GSL have also been implicated in the growth of lymphocytes and other cells. For example, Platt et al. (33) reduced GSL levels by 50–70% in the liver and lymphoid tissue in mice by using pharmacological doses of an inhibitor of GlcCer synthase. 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% because of a decrease in cell numbers (33), suggesting that GSL are required for proliferation of cells of the immune system. More recent studies have shown that GSL stimulate the proliferation of specific subsets of lymphocytes (23, 29, 34). It is important to note that spleen is primarily a lymphoid organ, and an increased production of GSL in spleen during the APR may play a role in regulating the immune response(s).

GlcCer has also been shown to stimulate the proliferation of cells in kidney, liver, and skin (35–37). Conversely, a mutant B16 melanoma cell line (GM-95), which is deficient in GlcCer synthase, has a slower growth rate and altered cell morphology as compared with the parental cells (38), suggesting that GlcCer may be required for normal cell growth. The kidneys of *cpk/cpk* mice, a model for polycystic kidney disease that is characterized by aberrant renal cell proliferation, have a high GlcCer content and GlcCer synthase activity, suggesting a role for GlcCer in renal cell proliferation (39). Moreover, inhibitors of GlcCer synthase have been shown to decrease the renal hypertrophy that occurs in streptozotocin diabetic rats (40). Finally, in vitro treatment with inhibitors of GlcCer synthase decreases the proliferation of renal cells as well as keratinocytes (41, 42). These studies suggest that GSL may play an important role in cellular proliferation. In the context of these data, it is tempting to speculate that the LPS-induced increase in tissue GSL may be required for regulating cellular proliferation during the APR.

In summary, the present study demonstrates that LPS upregulates SPT and GlcCer synthase in spleen and kidney, and this is accompanied by increases in specific GSL metabolites. An increase in GSL metabolites during the host response to infection and inflammation may be required for regulation of cell proliferation and modulation of immune responses.

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