Endotoxin and Cytokines Increase Hepatic Sphingolipid Biosynthesis and Produce Lipoproteins Enriched in Ceramides and Sphingomyelin


Abstract—Alterations in triglyceride and cholesterol metabolism often accompany inflammatory diseases and infections. We studied the effects of endotoxin (lipopolysaccharide [LPS]) and cytokines on hepatic sphingolipid synthesis, activity of serine palmitoyltransferase (SPT), the first and rate-limiting enzyme in sphingolipid synthesis, and lipoprotein sphingolipid content in Syrian hamsters. Administration of LPS induced a 2-fold increase in hepatic SPT activity. The increase in activity first occurred at 16 hours, peaked at 24 hours, and was sustained for at least 48 hours. Low doses of LPS produced maximal increases in SPT activity, with half-maximal effect seen at \(0.3 \mu g\) LPS/100 g body weight. LPS increased hepatic SPT mRNA levels 2-fold, suggesting that the increase in SPT activity was due to an increase in SPT mRNA. LPS treatment also produced 75% and 2.5-fold increases in hepatic sphingomyelin and ceramide synthesis, respectively. Many of the metabolic effects of LPS are mediated by cytokines. Interleukin 1 (IL-1), but not tumor necrosis factor, increased both SPT activity and mRNA levels in the liver of intact animals, whereas both IL-1 and tumor necrosis factor increased SPT mRNA levels in HepG2 cells. IL-1 produced a 3-fold increase in SPT mRNA in HepG2 cells, and the half-maximal dose was 2 ng/mL. IL-1 also increased the secretion of sphingolipids into the medium. Analysis of serum lipoprotein fractions demonstrated that very low density lipoprotein, intermediate density lipoprotein, and low density lipoprotein isolated from animals treated with LPS contained significantly higher amounts of ceramide, glucosylceramide, and sphingomyelin. Taken together, these results indicate that LPS and cytokines stimulate hepatic sphingolipid synthesis, which results in an altered structure of circulating lipoproteins and may promote atherogenesis. (Arterioscler Thromb Vasc Biol. 1998;18:1257-1265.)

Key Words: acute-phase response ■ tumor necrosis factor ■ interleukin-1 ■ serine palmitoyltransferase ■ atherogenesis

Alterations in triglyceride and cholesterol metabolism often accompany inflammatory diseases and infections. These metabolic alterations can also be induced by administration of endotoxin (lipopolysaccharide [LPS]), which mimics Gram-negative infections. Low doses of LPS rapidly increase serum triglyceride levels, primarily by stimulating hepatic triglyceride production and VLDL secretion. Increased hepatic fatty acid synthesis, as well as increased adipose tissue lipolysis, provides the fatty acid substrate for increased triglyceride production. High doses of LPS do not stimulate hepatic VLDL secretion but inhibit the clearance of triglyceride-rich lipoproteins by decreasing lipoprotein lipase activity in heart, muscle, and adipose tissue and in postheparin plasma.

In rodents, LPS treatment increases serum cholesterol levels; however, this effect is delayed in onset compared with the increase in serum triglyceride levels and is primarily accounted for by an increase in LDL cholesterol. LPS increases hepatic cholesterol synthesis and the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis. Increased transcription of the HMG-CoA reductase gene leads to increased mRNA and protein levels, which account for the increase in hepatic HMG-CoA reductase activity. This effect of LPS on hepatic HMG-CoA reductase is specific, because mRNA levels of other important enzymes in cholesterol synthesis, such as HMG-CoA synthase, farnesyl pyrophosphate synthase, and squalene synthase, are not increased. In addition, LPS decreases the activity and mRNA levels of cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid synthesis. A decrease in bile acid synthesis would increase the availability of cholesterol for lipoprotein production. Finally, LPS has minimal or no effect on LDL receptor protein or mRNA levels in the liver, the organ primarily responsible for LDL clearance. These results suggest that the increased production of lipoproteins rather...
than a decrease in their clearance accounts for the increase in serum LDL cholesterol levels in rodents.

Sphingolipids not only are important constituents of cell membranes but also have a wide range of functions, including mediation of several signal transduction pathways that regulate cell growth, differentiation, and apoptosis. Recent studies have shown that the liver synthesizes sphingolipids de novo and incorporates these newly synthesized sphingolipids into VLDLs, which are secreted. However, neither the addition of oleic acid, which accelerates hepatic VLDL secretion, nor choline deficiency, which inhibits hepatic VLDL secretion, alters the rate of hepatic sphingolipid synthesis, suggesting that de novo sphingolipid synthesis is associated with, but not required for, VLDL secretion. Studies have shown that palmitate, but not other fatty acids, stimulates sphingolipid synthesis. The relation between hepatic sphingolipid synthesis and the sphingolipid content of circulating lipoprotein particles has not been established.

The enzyme serine palmitoyltransferase (SPT) catalyzes the condensation of serine and palmitoyl CoA to form 3-ketosphinganine, the first and rate-limiting step in sphingolipid synthesis. A mammalian cDNA encoding the long-chain base 2 (LCB2) subunit of SPT was recently isolated. The factors that regulate sphingolipid synthesis and SPT activity in the liver have not been well characterized.

As described above, our previous studies have demonstrated that LPS and cytokines stimulate both hepatic fatty acid and cholesterol synthesis. Therefore, we hypothesized that LPS and cytokines may also stimulate hepatic sphingolipid synthesis. Hence, in this study, we examined the effects of LPS and cytokines on hepatic sphingolipid synthesis and SPT activity and mRNA levels in Syrian hamsters as well as in HepG2 cells. In addition, we measured the content of ceramides and sphingomyelin in lipoprotein fractions after LPS administration.

Methods

Materials

[α-32P]dCTP (3000 Ci/mmol) and [3H]serine (30 Ci/mmol) were purchased from New England Nuclear. LPS (Escherichia coli S58-B5) was purchased from Difco Laboratories and was freshly diluted to desired concentrations in pyrogen-free 0.9% saline (Kendall McGraw Laboratories, Inc). Human tumor necrosis factor (TNF)-α with a specific activity of 5 × 10^7 U/mg was provided by Genentech, Inc. Recombinant human interleukin (IL)-β with a specific activity of 1 × 10^7 U/mg was provided by Immunex. The cytokines were freshly diluted to desired concentrations in pyrogen-free 0.9% saline containing 0.1% human serum albumin. High-performance thin-layer chromatography (HPTLC) plates (silica gel 60) were obtained from Merck. Chromatography standards, including ceramide, glucosylceramide, and sphingomyelin, were purchased from Sigma Chemical Co. A multiprime DNA-labeling system was purchased from Amersham Chemical Co. A multiprime DNA-labeling system was purchased from Amersham International, minispin G-50 columns were from Pharmacia LKB Biotechnology AB, and Nytran membranes were from Schleicher & Schuell. Kodak XAR5 film was used for autoradiography. The cDNA for the LCB2 subunit of SPT was provided by Dr R. C. Dickson (University of Kentucky, Lexington).

Animal Procedures

Male Syrian hamsters (140 to 160 g) were purchased from Simonsen Laboratories (Gilroy, Calif). 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 either LPS, TNF, IL-1, or TNF plus IL-1 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 may induce anorexia. Animals were studied 4 to 48 hours after LPS administration or 16 hours after cytokine administration as indicated in the text. The doses of LPS used (0.1 to 100 μg/100 g bw) have significant effects on triglyceride and cholesterol metabolism in Syrian hamsters, but are far below doses that cause death in rodents (LD₅₀ ≈ 5 mg/100 g bw). Similarly, the doses of TNF and IL-1 (17 and 1 μg/100 g bw, respectively) were chosen because previous studies have demonstrated that these doses have marked effects on serum lipid and lipoprotein levels and reproduce many of the effects of LPS on lipid metabolism in Syrian hamsters.

SPT Activity

At various time points after LPS treatment, livers were isolated and homogenized in 50 mmol/L HEPES, pH 7.4, containing 10 mmol/L EDTA, 5 mmol/L DTT, and 0.25 mol/L sucrose. Microsomes were isolated by differential centrifugation. Our assay for SPT activity was modified from the method of Williams et al as described earlier. In brief, the assay buffer contained 100 mmol/L HEPES, pH 8.3, 5.0 mmol/L DTT, and 2.5 mmol/L EDTA; the reaction mixture contained 50 μmol/L pyridoxal phosphate, 150 μmol/L palmitoyl-CoA, 1.0 mmol/L [3H]-serine (specific activity, 45 to 50 000 dpm/nmol), and 50 to 100 μg of microsomal protein in 0.1 mL of total assay volume. The assay mixture (protein, buffer, and pyridoxal phosphate) was preincubated for 10 minutes (37°C), and the assay was initiated by simultaneous addition of palmitoyl-CoA and [3H]-serine, incubated at 37°C for 10 minutes, and terminated by the addition of 0.2 mL of 0.5N NH₄OH. The reaction product, 3-ketodihydrosphinganine, was isolated as described previously and quantified by liquid scintillation spectrometry. Enzyme specific activity was expressed as picomoles of 3-ketodihydrosphinganine formed per minute per milligram of microsomal protein. Protein was determined by the Bradford procedure using BSA as the standard.

Isolation of RNA and Northern Blotting

Total RNA was isolated by a variation of the guanidinium thiocyanate method as described earlier. Poly A+ RNA from liver was isolated using oligo(dT) cellulose. Poly A+ RNA was quantified by measuring absorption at 260 nm. Equal amounts of poly A+ RNA were loaded on 1% agarose-formaldehyde gels and electrophoresed. The uniformity of sample applications was checked by UV visualization of the acridine orange–stained gels before transfer to Nytran membranes. We and others have found that LPS increases actin mRNA levels in liver by 2-fold to 5-fold in rodents. Similarly, TIM and TNF and IL-1 produce a 2-fold increase in actin mRNA levels. LPS also produced a 2-fold increase in hepatic mRNA levels for glyceraldehyde 3-phosphate dehydrogenase (G-3PD) and a 2.6-fold increase in cyclophilin mRNA. Thus, the mRNA levels of actin, G-3PD, and cyclophilin were chosen because previous studies have demonstrated that these mRNAs are upregulated in response to LPS and cytokines.

The relation between SPT activity and mRNA levels in Syrian hamsters is not well characterized. Hence, in this study, we examined the effects of LPS and cytokines on hepatic sphingolipid synthesis and SPT activity and mRNA levels in Syrian hamsters as well as in HepG2 cells. In addition, we measured the content of ceramides and sphingomyelin in lipoprotein fractions after LPS administration.
Sphingolipid Synthesis

Twenty-four hours after LPS administration, the animals were killed and the liver removed. Slices (0.5 mm thick) were prepared with a McIlwain tissue slicer, and 200 mg of tissue was placed in the outer well of a 25-mL flask containing 2 mL of Krebs-Ringer phosphate buffer and [3H]serine (1 mCi, Dupont-NEN). The flasks were gassed with 95% O2/5% CO2 for 15 seconds, stoppered with serum caps, and incubated for 1 hour at 37°C in a Dubnoff metabolic shaker at 100 oscillations/min. At the end of the incubation, Bligh-Dyer solution was added and total lipid extracts obtained.20 The lipid components were separated by HPTLC as described earlier49 using the following solvent sequence: (1) chloroform:methanol:water 90:10:1 (by volume), (2) petroleum ether:diethylether:acetic acid 70:50:1 (by volume), and (3) chloroform:methanol:water:acetic acid 60:35:4:5.0 (by volume) to ~15 cm. Lipids were visualized by Woods light fluorescence after staining with 8-anilino-1-naphthalene sulfonic acid and identified by cochromatography against known standards. The lipid spots corresponding to ceramides and sphingomyelin were scraped into scintillation vials and counted by liquid scintillation spectrometry.

HepG2 Cell Culture and Cytokine Treatment

HepG2 cells were obtained from the American Type Culture Collection (Rockville, Md) and maintained in minimum essential medium (Mediatech, Inc) supplemented with 10% FBS 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 and magnesium-free PBS, and the experimental medium (Dulbecco’s minimum essential medium plus 0.1% BSA) containing EDTA (1.5 mg/mL) and plasma was isolated. Lipoprotein fractions were isolated using the fast protein liquid chromatography system (Pharmacia Biotech, Inc) equipped with 2 Superose 6 HR 10/30 columns connected in series.21 In brief, plasma was centrifuged at 12,000 g at 12°C for 1 hour at 37°C in a Dubnoff metabolic shaker at 100 oscillations/min. The peaks of lipoprotein fractions. VLDL, IDL, LDL, and HDL were passed through the columns before the next sample was loaded. Each fraction of lipoprotein fractions was eluted, 50 fractions of 0.5 mL were collected. A total of 80 mL was passed through the columns before the next sample was loaded. Cells were incubated at 37°C for the indicated time. RNA purification and Northern blotting were performed according to previously described methods.4,5

Isolation of Lipoproteins

Twenty-four hours after LPS treatment, blood was collected in tubes containing EDTA (1.5 mg/mL) and plasma was isolated. Lipoprotein fractions were isolated using the fast protein liquid chromatography system (Pharmacia Biotech, Inc) equipped with 2 Superose 6 HR 10/30 columns connected in series.21 In brief, plasma was centrifuged at 12,000 g at 12°C for 1 hour at 37°C in a Dubnoff metabolic shaker at 100 oscillations/min. The peaks of lipoprotein fractions. VLDL, IDL, LDL, and HDL were passed through the columns before the next sample was loaded. Each fraction of lipoprotein fractions was eluted, 50 fractions of 0.5 mL were collected. A total of 80 mL was passed through the columns before the next sample was loaded. Cells were incubated at 37°C for the indicated time. RNA purification and Northern blotting were performed according to previously described methods.4,5

Analysis of Ceramides and Sphingomyelin in Lipoproteins

Total lipids were extracted from pooled lipoprotein fractions by the Bligh-Dyer technique20 and dried. The dried lipid extracts were redissolved in chloroform:methanol (4:1), and an aliquot of each sample was dried in an aluminum dish on a heater (60°C) and weighed. Approximately 50 µg of lipid from each sample was applied to HPTLC plates, along with standards to separate ceramide, glucosylceramide, and sphingomyelin. Ceramide and glucosylceramide were separated using the following solvent sequence: (1) chloroform:methanol:aceton (70:20:4 by volume) to 2 cm (from the applied position), (2) chloroform:methanol:acetone (80:10:10 by volume) to 6 cm, and (3) chloroform:diethylether:ethyl acetate:methanol (76:6:20:4 by volume) to the top of the plate. Sphingomyelin was separated by using chloroform:methanol:acetic acid:water (50:30:8:4 by volume) twice to the top. After development, the plates were dried (60°C), sprayed with a charting solution (contain-

ing 1.5% copper sulfate, 5% glacial acetic acid, 1% sulfuric acid, and 1% ortho-phosphoric acid), and charred in an oven (180°C) for 15 minutes. The charred plates were scanned with a TLC scanner (CAMAG Scientific, Inc), and the bands were quantified against the standards as previously described.22

Statistics

Results are expressed as mean±SEM. Statistical significance between 2 groups was determined by using the Student t test. Comparisons among several groups were done by ANOVA. When SDs were unequal, a nonparametric test (Mann-Whitney) was used to calculate the level of significance.

Results

Endotoxin Increases Hepatic SPT Activity and mRNA Levels

Our initial experiments determined the effect of LPS administration on SPT activity in the liver of Syrian hamsters. As shown in Figure 1A, SPT activity increases 2-fold 16 hours after LPS treatment. The increase in SPT activity after LPS administration peaks at 24 hours (3-fold increase) and is sustained for at least 48 hours. We next determined the dose-response curve for the effect of LPS on hepatic SPT activity 16 hours after LPS administration (Figure 1B). Relatively low doses of LPS cause a maximal increase in SPT activity (1 µg/100 g bw), and the half-maximal effect is seen with ~0.3 µg/100 g bw, indicating that the increase in SPT activity is a very sensitive host response to LPS.

To determine the potential mechanism by which LPS increases SPT activity, we determined the effect of 16-hour LPS treatment on hepatic SPT mRNA levels in Syrian hamsters. LPS treatment produced a 2-fold increase in mRNA levels of the LCB2 subunit of SPT in liver (Figure 2). These data suggest that the increase in hepatic SPT activity is due to an increase in SPT mRNA levels.

Endotoxin Increases Incorporation of [3H]Serine in Hepatic Sphingolipids

We next examined whether LPS administration also increases hepatic sphingolipid synthesis as measured by the incorporation of [3H]serine into hepatic ceramides and sphingomyelin. Syrian hamsters were treated with LPS (100 µg/100 g bw), and 24 hours later the incorporation of [3H]serine into hepatic sphingolipids was measured in liver slices. LPS produced a 2.5-fold increase in [3H]serine incorporation into hepatic ceramides and a 75% increase in incorporation into hepatic sphingomyelin (Figure 3). Thus, the increases in hepatic SPT activity and mRNA levels induced by LPS are reflected in increased sphingolipid synthesis in liver.

IL-1 Increases Hepatic SPT Activity and mRNA Levels

Many of the metabolic effects of LPS are mediated by cytokines; therefore, we next examined the effect of TNF, IL-1, and the combination of TNF and IL-1 on hepatic SPT activity and mRNA levels in Syrian hamsters. As shown in Figure 4A, administration of IL-1 produces a 50% increase in SPT activity (compared with saline-treated controls), whereas the combination of TNF and IL-1 results in an 80% increase in SPT activity. In contrast, TNF alone did not alter hepatic SPT activity. With regard to hepatic SPT mRNA levels, IL-1
produced a 3.3-fold increase, whereas treatment with IL-1 plus TNF produced a 2.6-fold increase in SPT mRNA levels (Figure 4B). TNF alone had no effect on SPT mRNA levels. Although the magnitude of the IL-1–induced increases in SPT activity and mRNA shows a trend toward being different from that seen with the combination of TNF and IL-1, these differences are not statistically significant. These results indicate that IL-1 regulates SPT at the level of both activity and mRNA, whereas TNF has no effect in hamsters in vivo.

IL-1 Increases SPT mRNA Levels and Sphingolipid Secretion in Cultured HepG2 Cells

To determine whether cytokines directly affect hepatocyte SPT mRNA levels, we next examined the effect of TNF and IL-1 in HepG2 cells, a human hepatoma cell line. As shown in Figure 5, TNF and IL-1 increase SPT mRNA levels in HepG2 cells by 2-fold and 3-fold, respectively. IL-6, another cytokine known to affect lipid metabolism, also produced a 2.7-fold increase in SPT mRNA in HepG2 cells (data not shown). Because IL-1 had a greater effect than TNF and also induced changes in vivo, we examined the effect of IL-1 on HepG2 SPT mRNA levels in more detail. As shown in Figure 6A, IL-1 significantly increased SPT mRNA levels after 8 hours of treatment, and this increase was sustained for at least 48 hours. The dose response of 48-hour treatment with IL-1 on SPT mRNA in HepG2 cells is shown in Figure 6B. The data show that low doses of IL-1 increase SPT mRNA levels in HepG2 cells. The maximal effect was seen at 10 to 100 ng/mL, whereas the half-maximal effect was observed at ~2 ng/mL.

We next determined the effect of IL-1 on the secretion of newly synthesized sphingolipids from HepG2 cells into the

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** Time course (A) and dose response (B) for effect of LPS on SPT activity in liver. Syrian hamsters were injected intraperitoneally with either saline or LPS (100 μg/100 g bw) (A) or with LPS at doses indicated on the x axis (B). Animals were killed at various time points (A) or 16 hours after LPS administration (B), liver microsomes were isolated, and SPT activity was determined as described in Methods. SPT activity in the control group was 23.64 ± 1.33 pmol·min⁻¹·mg⁻¹ protein. Data are mean ± SEM; n=5 for each group. A, *P<0.001. B, **P<0.05, ***P<0.002, and ****P<0.001. CON indicates control.

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** Effect of LPS on hepatic SPT mRNA. Animals were injected intraperitoneally with either saline or LPS (100 μg/100 g bw). Sixteen hours later, the animals were killed, livers were obtained, and poly A⁺ RNA was isolated. Northern blots were probed with SPT cDNA as described in Methods. Data are presented as the percentage of control values as quantified by densitometry (mean ± SEM); n=5 for the control group and 4 for the LPS group. *P<0.05.

![Figure 3](http://atvb.ahajournals.org/)

**Figure 3.** Effect of LPS on hepatic sphingolipid synthesis. Animals were injected intraperitoneally with either saline or LPS (100 μg/100 g bw). Twenty-four hours later, the animals were killed, liver slices were prepared, and the incorporation of [H]serine into ceramides and sphingomyelin was measured as described in Methods. Data are mean ± SEM; n=5 for each group. *P<0.1 and **P<0.02. CON indicates control.
medium. The data in Figure 7 show that IL-1 significantly increased the secretion of labeled sphingolipids into the medium in a time-dependent manner (40% and 3.8-fold increases at 4 and 8 hours, respectively). Thus, IL-1 is capable of regulating SPT mRNA levels as well as sphingolipid secretion in HepG2 cells.

LPS Increases the Content of Ceramides and Sphingomyelin in Lipoprotein Fractions

Our previous studies have shown that LPS increases serum cholesterol and triglyceride levels in Syrian hamsters and alters the composition of lipoproteins, resulting in triglyceride-rich and cholesterol-rich particles. In this study, we isolated the lipoproteins by FPLC and measured the content of cholesterol and triglycerides in each FPLC fraction to characterize the peaks for different lipoprotein fractions. Both cholesterol and triglyceride content of the VLDL, IDL, LDL, and HDL fractions were increased in plasma samples from LPS-treated animals (data not shown).

FPLC fractions were pooled, on the basis of peaks for VLDL, IDL, LDL, and HDL, for LPS-treated and control samples, and total lipids were extracted. LPS significantly increased the amount of total extractable lipid (in mg/mL plasma) in the VLDL (control 2.09±0.18, LPS 3.5±0.31, P<0.003), IDL (control 0.85±0.04, LPS 1.5±0.08, P<0.001), and LDL (control 1.14±0.05, LPS 2.54±0.12, P<0.001) fractions. However, the effect of LPS on total extractable lipid in the HDL fraction was not significant (control 2.92±0.08, LPS 3.33±0.16).

The data in Figure 8 demonstrate the effect of 24-hour LPS treatment on the levels of ceramide, glucosylceramide, and sphingomyelin in different lipoprotein fractions. Because LPS also increased total extractable lipid in different lipoprotein fractions, we analyzed the data on ceramide, glucosylceramide, and sphingomyelin content in various lipoprotein fractions per milligram of total lipid to account for the LPS-induced increase in total lipid content. Consistent with previous findings, ceramide and glucosylceramide levels were low in all lipoprotein fractions from control animals (Figure 8A and 8B). LPS treatment significantly increased the levels of ceramide (Figure 8A) and glucosylceramide (Figure 8B) in each lipoprotein fraction. The most marked increase was seen in the VLDL fraction (3.7-fold and 18.9-fold increases in ceramide and glucosylceramide content, respectively). There was also a 2.2-fold increase in ceramide content and a 7.3-fold increase in glucosylceramide content in the LDL fraction from LPS-treated animals. The basal level of sphingomyelin was higher than the basal levels of ceramide and glucosylceramide in the VLDL, LDL, and HDL fractions (Figure 8B). LPS significantly increased sphingomyelin content in the IDL and LDL fractions (Figure 8C) but had no effect on sphingomyelin content in the VLDL and HDL fractions.

Discussion

Sphingolipids are important components of the plasma membranes of all eukaryotic cells and have a wide range of functions. However, relatively little is known about the
factors that regulate de novo sphingolipid synthesis or the activity of SPT, the first and rate-limiting enzyme in sphingolipid biosynthesis. In this study, we demonstrated that LPS stimulates hepatic sphingomyelin and ceramide synthesis in vivo. In addition, LPS produces a marked increase in SPT activity in the liver. This increase in SPT activity occurs at very low doses of LPS (1 mg/100 g bw) and is first observed 16 hours after LPS treatment. Moreover, these changes in SPT activity are accompanied by an increase in SPT mRNA levels in the liver. These results indicate that LPS increases hepatic sphingolipid synthesis by increasing SPT activity and mRNA levels.

The host response to infection and inflammation is accompanied by changes in hepatic synthesis of acute-phase proteins. During the acute-phase response, hepatic synthesis of several proteins, such as C-reactive protein, serum amyloid A, and HMG-CoA reductase, is increased, whereas the synthesis of several other proteins, such as albumin, transferrin, and apoE, is inhibited. These changes in hepatic acute-phase protein synthesis are primarily regulated at the level of gene transcription. The acute-phase response in rodents is also accompanied by stimulation of hepatic fatty acid and cholesterol synthesis and a marked increase in serum triglyceride and cholesterol levels. The LPS-induced increases in hepatic sphingolipid synthesis, SPT activity, and SPT mRNA levels can also be considered a part of the acute-phase response.

Many of the metabolic changes that occur during the host response to infection or inflammation are mediated by proinflammatory cytokines, such as TNF, IL-1, and IL-6 (reviewed in Hardardottir et al). We previously showed that these cytokines stimulate hepatic fatty acid and cholesterol synthesis and increase VLDL production and secretion. Like LPS, these cytokines also inhibit cholesterol 7α-hydroxylase in the liver and lipoprotein lipase activity in adipose tissue. In the present study, IL-1 administration increased both SPT activity (50% to 80%) and mRNA levels (2-fold to 3-fold) in the liver of Syrian hamsters; however, TNF had no such effect on SPT activity or mRNA levels in intact animals. Conversely, treatment of HepG2 cells, a human hepatoma cell line, with TNF, IL-1, or IL-6 increased SPT mRNA levels by 2-fold to 3-fold. Compared with TNF, IL-1 had a greater effect in HepG2 cells and also increased the secretion of sphingolipids into the medium. There are 2 potential explanations for the difference between the effects of TNF and IL-1 on sphingolipid metabolism in Syrian hamsters and HepG2 cells. First, it is possible that the results obtained are simply due to differences in species responses (ie, TNF stimulates sphingolipid metabolism in humans but not hamsters). Moreover, HepG2 cells are derived from a liver carcinoma cell line and thus may respond differently to various treatments than human or rodent hepatocytes. Second, these differences could be due to a dose or timing phenomenon (ie, at higher doses or different times, TNF...
might be effective in vivo). In either case, these studies demonstrate that cytokines, which mediate the acute-phase response, are capable of regulating hepatic sphingolipid metabolism.

It is now widely recognized that many of the actions of cytokines are mediated by the hydrolysis of sphingomyelin to ceramide by the activation of sphingomyelinases (reviewed by Chatterjee29). Ceramide is an important signal transduction molecule that acts as a second messenger in a variety of biological processes, such as regulation of cell proliferation and differentiation, apoptosis, and immune and inflammatory responses.26,31 Ceramide is also a precursor for other sphingolipid mediators, such as sphingosine-1-phosphate.32 It is therefore possible that the LPS-induced increase in hepatic sphingolipid synthesis provides additional substrate (ie, sphingomyelin and ceramide) for these cytokine signaling pathways. It is also possible that the increase in ceramide synthesis in liver may be involved in the hepatotoxic effects of LPS.

The primary function of the acute-phase response is to protect the organism from further injury and help in the repair response.22 However, if this response to infection or inflammatory stimuli is protracted or sustained, it may have deleterious consequences.33 Several epidemiological studies have suggested a link between chronic infections or the acute-phase response and atherosclerosis. There is an increased incidence of coronary heart disease in patients with Helicobacter pylori or Chlamydia pneumoniae infections, chronic dental infections, chronic bronchitis, and rheumatoid arthritis.34–38 C-reactive protein levels, a marker for the acute-phase response, are also elevated in patients with coronary heart disease, stroke, and unstable angina.37 Thus, it is possible that some of the changes produced during the acute-phase response may have effects that could promote atherogenesis.

We and others have shown that LPS-induced stimulation of hepatic fatty acid and cholesterol synthesis leads to changes in the composition of lipoproteins, resulting in cholesterol-rich and triglyceride-rich LDL particles that may be proatherogenic.4,40 Sphingolipids, such as sphingomyelin, ceramide, and glucosylceramide, are integral components of lipoproteins.8,41,42 In this study, we demonstrated that the VLDL, IDL, and LDL fractions contain markedly higher levels of ceramide, glucosylceramide, and sphingomyelin, whereas HDL contains higher amounts of ceramide and glucosylceramide. Recent studies by Merrill et al8 have shown that de novo sphingolipid synthesis is not essential for lipoprotein secretion and that dietary changes that alter the rate of VLDL secretion do not alter the rate of hepatic sphingolipid synthesis. However, an increase in hepatic sphingolipid synthesis during the host response to LPS, in conjunction with an LPS-induced increase in cholesterol and triglyceride synthesis, could alter the structure or functions of lipoproteins.

An increase in these sphingolipid levels in lipoproteins could have a number of consequences that may increase the atherogenicity of lipoprotein particles. For example, it was recently shown that increased ceramide levels in LDL facilitate LDL aggregation, which, in turn, enhances their uptake by macrophages, leading to foam cell formation.43 Moreover, LDL isolated from atherosclerotic lesions is either aggregated or has an increased tendency to aggregate and is enriched 10-fold to 50-fold in ceramides compared with plasma LDL.44 An increase in sphingomyelin in lipoproteins could also exert...
proatherogenic effects. Sphingomyelin is the substrate for the formation of ceramide by sphingomyelinases in the arterial wall and macrophages. Spiegel and colleagues have shown that sphingomyelin inhibits the activity of lecithin-cholesterol acyltransferase, which could decrease the reverse–cholesterol transport pathway, thereby increasing the risk of atherogenesis. Sphingomyelin also slows the clearance of triglyceride-rich lipoproteins, which could result in an accumulation of VLDL and chylomicron remnant particles that are atherogenic. Several earlier studies have shown that sphingomyelin accumulates in parallel with cholesterol within the arterial intima during experimental atherogenesis. More recently, it was reported that there is marked accumulation of glucosylceramide and lactosylceramide in human atherosclerotic plaques. The activity of SPT, the rate-limiting enzyme in sphingolipid synthesis, is also increased in rabbit aorta during experimental atherogenesis. Taken together, these findings support the concept that enhanced sphingolipid synthesis leading to the enrichment of lipoproteins with ceramide, glucosylceramide, and sphingomyelin may be an important determinant of their atherogenic potential.

In addition to the increased ceramide levels in LDL isolated from atherosclerotic lesions and the increased sphingolipid levels in lipoproteins isolated from LPS-treated animals reported here, several other changes are produced during the acute-phase response that could be proatherogenic. During the acute-phase response, marked changes occur in HDL-associated enzymes that result in the loss of protective function of HDL and convert it to a proinflammatory molecule (reviewed in Berliner'). These changes include marked decreases in HDL-associated apoA-I, paraoxonase, and platelet-activating factor (PAF)–acyethylhydrolase. ApoA-I has been shown to prevent aggregation of LDL, whereas paraoxonase and PAF-acyethylhydrolase normally protect LDL from oxidation by biologically active lipids. However, when these enzymes are depleted during the acute-phase response, there is a reciprocal increase in serum amyloid A, ceruloplasmin, and apoJ in the acute-phase HDL, which results in an increased susceptibility of LDL to oxidation. We and others have shown that LPS and cytokines produce similar changes in apoA-I, serum amyloid A, and apoJ protein and mRNA levels in rodents. LPS and cytokines also decrease the protein and mRNA levels of lecithin-cholesterol acyltransferase, another HDL-associated enzyme that plays a key role in reverse cholesterol transport. A decrease in reverse cholesterol transport could increase the risk of atherogenesis. Sphingomyelin also inhibits the activity of lecithin-cholesterol acyltransferase. Thus, the host response to infection and inflammatory stimuli is accompanied by several changes that could increase the atherogenicity of lipoprotein particles.

In summary, this study demonstrated that LPS increases the activity and mRNA levels of SPT, a key regulatory enzyme in sphingolipid synthesis, in liver. Cytokines, particularly IL-1, mimic the effects of LPS on SPT activity in vivo and also directly regulate SPT mRNA levels in HepG2 cells. LPS enhances hepatic sphingolipid synthesis, and the lipoproteins isolated from LPS-treated animals are enriched in sphingomyelin, ceramide, and glucosylceramide. These changes in lipoprotein composition may alter their structure and function and enhance their atherogenic potential.

Acknowledgments

This work was supported by grants from the Research Service of the Department of Veterans Affairs (C.G., K.R.F.) and the National Institutes of Health (C.G.) (DK 49448).

References


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Arterioscler Thromb Vasc Biol. 1998;18:1257-1265
doi: 10.1161/01.ATV.18.8.1257

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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