Blocking the Secretion of Hepatic Very Low Density Lipoproteins Renders the Liver More Susceptible to Toxin-induced Injury*

Received for publication, September 5, 2001, and in revised form, November 26, 2001
Published, JBC Papers in Press, December 5, 2001, DOI 10.1074/jbc.M108514200

Johan Björkergren‡§, Anne Beigneux‡§, Martin O. Bergo‡§, Jacquelyn J. Maher‡**,
and Stephen G. Young†§§

From the ‡Gladstone Institute of Cardiovascular Disease, §Cardiovascular Research Institute, and ¶Department of Medicine, University of California, San Francisco, and the Medical Science, San Francisco General Hospital, San Francisco, California 94110, and ¶¶Liver Center Laboratory, San Francisco General Hospital, University of California, San Francisco, California 94141-9100

Recently, we generated mice lacking microsomal triglyceride transfer protein (MTP) in the liver (MtpΔ/Δ) and demonstrated that very low density lipoprotein secretion from hepatocytes was almost completely blocked. The blockade in lipoprotein production was accompanied by mild to moderate hepatic steatosis, but the mice appeared healthy. Although hepatic MTP deficiency appeared to be innocuous, we hypothesized that a blockage in very low density lipoprotein secretion and the accompanying steatosis might increase the sensitivity of MtpΔ/Δ livers to additional hepatic insults. To address this issue, we compared the susceptibility of MtpΔ/Δ mice and MtpΔ/Δ mice controls to hepatic injury from Escherichia coli lipopolysaccharides, concanavalin A, and Pseudomonas aeruginosa exotoxin A. At baseline, neither the MtpΔ/Δ nor the MtpΔ/Δ mice had elevated serum parameters of injury (e.g. chemokine transcript levels and lipase peroxides) were disproportionately increased in the MtpΔ/Δ mice. Our results suggest that blocking lipoprotein secretion in the liver may increase the susceptibility of the liver to certain toxic challenges.

Microsomal triglyceride transfer protein (MTP) is critical for the assembly and secretion of apolipoprotein (apo) B-containing lipoproteins, both in the intestine and in the liver (1, 2). A genetic absence of MTP causes abetalipoproteinemia, a disease characterized by intestinal fat malabsorption, a virtual absence of chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins in the plasma, and strikingly low plasma levels of triglycerides and cholesterol. The fact that a deficiency in MTP reduces the plasma levels of atherogenic lipoproteins has attracted the attention of the pharmaceutical industry. Many companies have established MTP programs, with the goal of identifying MTP inhibitors suitable for treating humans with hyperlipidemias (3, 4). Thus far, however, the efficacy and safety of these compounds in humans has not been documented.

To investigate the role of MTP in lipoprotein assembly and secretion, we inactivated the MTP gene (Mtp) in mice (5). Heterozygous knockout mice (Mtp+/−) manifested slightly reduced levels of lipoprotein secretion, reduced levels of apoB100-containing lipoproteins in the plasma, and slightly increased levels of neutral lipids (triglycerides and cholesterol esters) in the liver. Homozygous knockout mice (Mtp−/−) died during embryonic development. Subsequently, we used Cre/LoxP recombination techniques to produce mice lacking Mtp expression in the liver but not in the intestine (6). Those mice, designated MtpΔ/Δ mice, exhibited strikingly reduced plasma levels of apoB100, sizable reductions in the plasma levels of cholesterol and triglycerides, and mild to moderate steatosis with increased levels of neutral lipids in the liver. The MtpΔ/Δ mice were healthy and grew normally; their plasma transaminase levels were normal, and their livers were free of inflammatory infiltrates (6).

The fact that it was possible to eliminate hepatic Mtp expression in a mammalian model without noticeable side effects supported the concept that it might be possible to develop MTP inhibitors to treat hyperlipidemias. Also encouraging were studies by Wetterau et al. (7) that showed that MTP inhibitors could reduce plasma lipoprotein levels in low density lipoprotein receptor-deficient rabbits without causing elevated transaminases or histologic evidence of liver inflammation.

In this study, we further investigated the notion that it might be possible, with impunity, to inhibit MTP and block hepatic lipoprotein production. We were suspicious, based on several observations, that MTP inhibition might not be as safe as our original studies and those of Wetterau et al. (7) had
implied. First, other human conditions associated with hepatic steatosis (e.g., diabetes mellitus, excessive consumption of ethanol, and obesity) increase the risk of developing hepatic inflammation and advanced liver disease (8–10). Second, severe liver disease has been reported in humans with abetalipoproteinemia (11, 12). Although treatment with short-chain triglycerides might have contributed to the liver disease in these cases, it is also possible that the inability of those livers to secrete lipoproteins caused them to be susceptible to steatohepatitis and advanced liver disease.

Normal human livers are required to face toxic insults. For example, intermittent lapses in the intestinal mucosal barrier can allow bacterial products to reach the liver (13). Normal livers from healthy individuals can cope with these challenges effectively, without inflammation or tissue injury. The livers of susceptible individuals, however, cannot effectively deal with these challenges, either because of genetic differences or metabolic derangements (14). This failure of normal protective mechanisms can lead to hepatic inflammation and, in some cases, to advanced liver disease.

We hypothesized that the blockade of hepatic lipoprotein production and resultant hepatic steatosis might render the liver more susceptible to toxic liver injury. To test this hypothesis, we compared the susceptibility of liver-specific MTP knockout mice and littermate controls to hepatic injury following challenges with exogenous toxins.

**EXPERIMENTAL PROCEDURES**

**Mttpfloxed/Mx1-Cre Mice**—A conditional Mttp allele, Mttpfloxed, in which exon 1 of Mttp is flanked by loxP sites, has been described previously (6). Mttpfloxed mice were bred with Mx1-Cre transgenic mice (15) to generate Mttpfloxed/Mx1-Cre mice. To excise exon 1 of Mttp and thus eliminate MTP expression in the liver, 21–22-day-old male Mttpfloxed/Mx1-Cre mice (16) were injected with polyinosinic-polycytidylic ribonucleic acid (PI-Pc; Sigma; 500 μg every other day for 8 days) (6). Littermate Mttpfloxed mice lacking the Cre transgene were also injected with PI-Pc. Excision of exon 1 was assessed by Southern blot analysis of SacI-digested genomic DNA using a 3′-flanking probe. The mice had a mixed genetic background (−50% 129SvJae and −50% C57BL/6). They were housed in a pathogen-free barrier facility with a 12-h light/dark cycle and were fed rodent chow containing 4.5% fat (Ralston Purina, St. Louis, MO). Genotypes were determined by Southern blots or by PCR with genomic DNA from tail biopsies.

**Measurement of Insulin and Glucose Levels**—Plasma glucose levels were measured with a glucose (Trinder) 100 kit from Sigma. Plasma insulin levels were measured with a 1-2-3 ultra-sensitive rat insulin enzyme-linked immunosorbent assay from Alpco (Windham, NH). Plasma glucose levels were determined with a glucose (Trinder) 100 kit from Sigma. Plasma insulin levels were measured with a 1-2-3 ultra-sensitive rat insulin enzyme-linked immunosorbent assay from Alpco (Windham, NH).

**DNA Microarray Experiments**—Murine 11K GeneChips (Affymetrix, Santa Clara, CA) were used to assess hepatic gene expression patterns. Total RNA was isolated from liver biopsies with TRIzol Reagent (Invitrogen) and purified further with a RNeasy Midi kit (Qiagen, Los Angeles, CA). cDNA was synthesized from the RNA with the SuperScript Choice System (Invitrogen) and T7-(dT)24 primers (Genset, La Jolla, CA). Biotin-labeled cRNA was transcribed from the cDNA in the presence of biotin-labeled nucleotides (RNA Transcription Labeling kit for GeneChip, Santa Clara, CA). The integrity of the total RNA and the cRNA was assessed by electrophoresis on a 1% agarose/formaldehyde gel. Fragmented cRNA was mixed with control enzyme-binding protein (SREBP)-1 and SREBP-2 were determined by Western blotting of whole-liver homogenates. Levels of sterol regulatory element-binding protein (SREBP)-1 and SREBP-2 were determined by Western blotting of nuclear extracts (19). To prepare the nuclear extracts, livers from four mice were pooled (~1.5 g) and homogenized in 30 ml buffer A (10 mM Hepes, pH 7.6, 25 mM KCl, 1 mM sodium EDTA, 2 mM succrose, 10% (v/v) glycerol, 150 μM spermine, 2 μM spermidine) and protease inhibitors (Complete-Mini, Roche Molecular Biochemicals). The homogenate was subjected to several strokes with a Teflon pestle and filtered through three layers of cheesecloth. To isolate the nuclear pellet, a 25-ml portion of the homogenate was then layered over 10 ml of buffer B and spun in an SW28 Ti rotor (Beckman Instruments, Palo Alto, CA) at 40,000 rpm for 1.5 h. Livers were homogenized in 1 ml of buffer (10 mM Hepes, pH 7.6, 100 mM KCl, 2 mM MgCl2, 1 mM sodium EDTA, 1 mM dithiothreitol, 10% glycerol), and protease inhibitors (Complete-Mini), 0.1 volume of 4 M (NH4)2SO4, pH 7.9, were added. The resuspended pellet was gently mixed and then centrifuged at 85,000 rpm in a TLA-100.2 rotor (Beckman Instruments) for 45 min at 4 °C. Aliquots of the supernatant containing the nuclear extracts (150 μg protein) were washed with whole-liver homogenates (100 μg protein) solution containing 8% polyacrylamide gels. Western blots were performed with rabbit antiserum against mouse SREBP-1 (20) and mouse SREBP-2 (21) and a rabbit antiserum against rat Scd1 (22). The binding of the primary antibodies was assessed by a horseradish peroxidase-labeled donkey anti-rabbit antibody and ECL Western blotting detection reagents (Amersham Biosciences).

**Lipid Analyses—Liver levels of triacylglycerols (~100 mg) were homogenized with a Polytron, Ultra-Turbax T8 (VWR, San Francisco, CA), and lipids were extracted with chloroform/methanol, 2:1 (v/v). Plasma lipids were extracted with hexane/isopropyl alcohol, 3:2 (v/v). Before the lipid extraction, known amounts of tri- and pentadecanoic acid (Sigma) were added as internal standards (23). Triglycerides, phospholipids, and fatty acids were identified by thin-layer chromatography, transesterified with methanolic HCl (Aldrich), and quantified by gas chromatography (23).

**Lipid Peroxidation Assay—**Liver levels of mRNAs for a variety of cytokines were quantified by RNase protection assays (24) with a multiprobe cDNA template kit (mCK1b, mCK3, mCK5; PharMingen, San Diego, CA). Briefly, cRNA probes were transcribed with [α-32P]UTP (>800 Ci/mmol, Amersham Biosciences). Radiolabeled cRNA (5 × 10⁶ Cerenkov cpm) was combined with 20 μg of liver RNA in 10 μl of hybridization buffer. The mixture was incubated at 55 °C for 16 h, and then hybridization buffer was added. The RNA digestion was terminated with proteinase K and SDS, and the RNA-RNA hybrids were purified by phenol/chloroform extraction and ethanol precipitation. The double-stranded RNA was resuspended in electrophoresis buffer, denatured at 100 °C, and separated through 5% polyacrylamide/urea gels. RNA bands were visualized by autoradiography, and band intensity was quantified by densitometry (Hoefer Scientific Instruments, San Francisco, CA). Signal intensity was normalized to control RNAs (L32 or glyceraldehyde-3-phosphate dehydrogenase).

**Lipid Peroxidation Assay—**Thioarbituric acid-reactive substances (TBARS), frequently used to estimate levels of lipid peroxides (25, 26), were determined with 50-ng liver fragments. To prevent the peroxidation of lipids during the procedure, liver fragments were homogenized in a 1:1.5% KCl solution containing 50 mM desferoxamine (Sigma).
RESULTS

Phenotypic Analyses of Liver-specific MTP Knockout Mice—To generate mice lacking MTP in the liver (i.e., Mttp<sup>fl/fl</sup> mice), Cre expression in Mttp<sup>fl/fl</sup>Mx1-Cre mice was induced with pI-pC. Consistent with previous studies (6), the plasma triglyceride levels were lower in Mttp<sup>fl/fl</sup> mice than in Mttp<sup>fl/fl</sup> mice (Table I). The reduction in plasma triglyceride levels in Mttp<sup>fl/fl</sup> mice was accompanied by an increase in hepatic lipid stores, which was evident both from the gross appearance of the liver (Fig. 1, A and B) and from histology (Fig. 1, C–F). Biochemical studies revealed that the liver triglyceride stores were 3-fold higher in Mttp<sup>fl/fl</sup> mice than in littermate Mttp<sup>fl/fl</sup> mice (Table I). The amount of lipid accumulation in this model was modest in comparison to some other genetic models of lipid accumulation. For example, the livers of mice expressing a truncated SREBP-1a synthesize high levels of fatty acids and have a 21-fold increase in liver triglyceride stores (20).

We predicted that the microarray experiments might uncover many perturbations in the expression of genes affecting lipid metabolism. To address this issue, we compared hepatic gene expression in Mttp<sup>fl/fl</sup> and Mttp<sup>fl/fl</sup> mice with Affymetrix GeneChips. Remarkably, most genes involved in lipid metabolism were unchanged (e.g., acetyl-CoA carboxylase, acyl-Coenzyme A acyltransferase, apoE, ATP-citrate lyase, cholesterol 7-α-hydroxylase, fatty-acid synthase, fatty acid transport protein, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, 3-hydroxy-3-methylglutaryl-coenzyme A synthase, low density lipoprotein receptor, lipoprotein lipase, and peroxisome proliferator-activated receptor-α) (definition of unchanged: fold change <30%, p > 0.15). However, there were two noteworthy exceptions. First, Mttp expression was undetectable in the livers of Mttp<sup>fl/fl</sup> mice (n = 5), whereas Mttp expression in Mttp<sup>fl/fl</sup> mice was 6-fold higher than the threshold detection level (n = 7) (p = 0.00000002). Second, Scd1 expression in the livers of Mttp<sup>fl/fl</sup> mice was reduced by 69% compared with the livers of Mttp<sup>fl/fl</sup> mice (p < 0.0005). Northern blots and Western blots confirmed the reduction in Scd1 expression by SREBP-1 (27), so we hypothesized that the levels of mature SREBP-1<sub>c</sub> should be reduced in livers of Mttp<sup>fl/fl</sup> mice. Indeed, this was the case. SREBP-1<sub>c</sub> and SREBP-2<sub>c</sub> levels were reduced by ~50% in the livers of Mttp<sup>fl/fl</sup> mice (Fig. 2C).

SREBP-1c expression is reduced by low levels of insulin and induced by insulin replacement (28, 29). To determine whether the inactivation of Mttp affected glucose or insulin levels, plasma triglycerides, glucose, and insulin levels were measured in Mttp<sup>fl/fl</sup> mice (n = 20), Mttp<sup>fl/fl</sup> mice treated with subcutaneous injections of water (n = 10), and Mttp<sup>fl/fl</sup> mice (i.e., Mttp<sup>fl/fl</sup> mice treated with subcutaneous injections of pI-pC; n = 10). Consistent with the results in Table I, plasma triglyceride levels were significantly reduced in Mttp<sup>fl/fl</sup> mice (p < 0.001). Plasma glucose levels were reduced by ~20% in Mttp<sup>fl/fl</sup> mice (14.06 ± 0.88 mmol/liter in Mttp<sup>fl/fl</sup> mice versus 11.44 ± 0.50 in Mttp<sup>fl/fl</sup> mice; p < 0.05). Plasma insulin levels were reduced by ~45% in Mttp<sup>fl/fl</sup> mice (0.39 ± 0.30 ng/ml in Mttp<sup>fl/fl</sup> mice versus 0.21 ± 0.06 in Mttp<sup>fl/fl</sup> mice; p < 0.001). Thus, the lower plasma insulin levels in Mttp<sup>fl/fl</sup> mice might well contribute to the lower SREBP-1 levels.

Scd1 expression is also down-regulated by polyunsaturated fatty acids (30–32), so we sought to determine whether levels of polyunsaturated fatty acids were increased in Mttp<sup>fl/fl</sup> mice. Interestingly, the predominant polyunsaturated fatty acid, linoleic acid, was increased significantly in the livers of Mttp<sup>fl/fl</sup> mice. The amount of linoleic acid (as a percentage of the total fatty acids) in liver triglycerides was 34.2 ± 4.2 in Mttp<sup>fl/fl</sup> mice (n = 8) and 40.8 ± 2.7 in Mttp<sup>fl/fl</sup> mice (n = 7) (p = 0.0037); the percentage of linoleic acid in liver free fatty acids was 15.8 ± 2.7 in Mttp<sup>fl/fl</sup> mice and 22.7 ± 2.7 in Mttp<sup>fl/fl</sup> mice (p = 0.0003). These differences could not be accounted for by differences in the fatty acid composition of the plasma. The amount of linoleic acid (as a percentage of the total fatty acids) in plasma triglycerides was 27.2 ± 5.9 in Mttp<sup>fl/fl</sup> mice (n = 8) and 24.2 ± 16.3 in Mttp<sup>fl/fl</sup> mice (n = 7) (p = 0.62).

Expression of Inflammation-related Genes in Mttp<sup>fl/fl</sup> Mice—Because hepatic steatosis in some mouse models leads to hepatic inflammation (26), we suspected that the accumulation of lipids in Mttp<sup>fl/fl</sup> mice might affect the expression of many genes, including those involved in inflammatory responses. However, the microarray experiments did not uncover evidence for an active inflammatory response in Mttp<sup>fl/fl</sup> livers. Expression levels for inflammation-related genes (e.g., macrophage inflammatory protein (MIP)-1α, MIP-1β, MIP-2, interleukin (IL)-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, IL-18, interferon-α, interferon-β, interferon-γ, and TNF-α) and apoptosis-related genes (bax, bcl-2, caspases 1, 2, 3 and 7, c-jun, c-myc, cytochrome c, and fas) were either equally low in Mttp<sup>fl/fl</sup> and Mttp<sup>fl/fl</sup> livers or below the threshold of detection.

Susceptibility of Livers to Toxins—To determine whether Mttp<sup>fl/fl</sup> mice were particularly sensitive to hepatic injury, Mttp<sup>fl/fl</sup> and Mttp<sup>fl/fl</sup> mice were challenged with three toxins
known to cause acute liver inflammation (LPS, ConA, and PEA). The inflammatory response triggered by these toxins is characterized by the release of pro-inflammatory cytokines (e.g., TNF-α, interferon-γ, IL-2, and IL-6), which leads to hepatocyte injury and increased plasma levels of AST, ALT, and LDH (33–35). LPS stimulates monocytes and macrophages (33), whereas ConA primarily stimulates T lymphocytes (34). PEA inhibits protein synthesis, particularly in the liver, and also is a weak T-cell mitogen (35).

Baseline plasma levels of ALT, AST, and LDH were normal in Mttp<sup>ΔΔ</sup> mice and Mttp<sup>fl/lox</sup> mice (Fig. 3). At 4 and 24 h after intraperitoneal injections of E. coli LPS (1.0 μg/g), the plasma ALT, AST, and LDH levels were higher in Mttp<sup>ΔΔ</sup> mice than in littermate Mttp<sup>fl/lox</sup> mice (Fig. 3). The increased transaminase levels in Mttp<sup>ΔΔ</sup> mice were associated with an infiltration of polymorphonuclear leukocytes into the liver parenchyma and with occasional foci of hepatocellular necrosis (Fig. 4). The results were similar after challenges with ConA and PEA (Fig. 5). Again, AST, ALT, and LDH were significantly higher in Mttp<sup>ΔΔ</sup> mice than in Mttp<sup>fl/lox</sup> controls (Fig. 5).

We considered the possibility that the increased susceptibility of Mttp<sup>ΔΔ</sup> mice to toxin-mediated injury was not due to the blockade in lipoprotein secretion but instead was a spurious and unanticipated effect of the Mx1-Cre transgene (carried by the Mttp<sup>ΔΔ</sup> mice but not the Mttp<sup>fl/lox</sup> controls). To test this possibility, groups of Mttp<sup>fl/lox</sup> Mx1-Cre mice and littermate Mttp<sup>fl/lox</sup> mice were given injections of normal saline rather than pI-pC and then challenged with LPS (1.0 μg/g). The Mttp<sup>fl/lox</sup> Mx1-Cre mice did not exhibit an increased susceptibility to liver injury (Fig. 6), indicating that the enhanced sensitivity of Mttp<sup>ΔΔ</sup> mice to LPS was caused by the elimination of hepatic Mttp expression and the resultant blockade in lipoprotein secretion.

Expression of Cytokines in the Liver after the LPS Challenge—To determine the mechanism of enhanced liver injury in Mttp<sup>ΔΔ</sup> mice, we compared the expression of several cytokines in the plasma and liver of both groups of mice following LPS challenge. We first investigated TNF-α because it is known to be induced by LPS and because it is a mediator of tissue injury and inflammation (36). TNF-α was not detectable in the plasma of either Mttp<sup>ΔΔ</sup> mice or controls before the LPS challenge but was easily detectable within 4 h after LPS administration (Fig. 7). Of note, the post-challenge TNF-α levels were no different in Mttp<sup>ΔΔ</sup> and Mttp<sup>fl/lox</sup> mice (Fig. 7). Consistent with the plasma data, hepatic TNF-α mRNA levels were similar in Mttp<sup>ΔΔ</sup> and Mttp<sup>fl/lox</sup> mice (Fig. 8A).

To determine whether the increased susceptibility of Mttp<sup>ΔΔ</sup> mice to toxins resulted from enhanced production of other inflammatory mediators within the liver, we examined the hepatic expression of multiple immunomodulatory cytokines 4 h after LPS challenge. The mRNA levels of the T-cell cytokine IL-2 were no different between Mttp<sup>ΔΔ</sup> and Mttp<sup>fl/lox</sup> mice nor were the mRNA levels for the anti-inflammatory cytokine IL-10 (Fig. 8B). Interestingly, however, hepatic expression levels for several chemokines (macrophage inflammatory protein (MIP) 1α, MIP-1β, and MIP-2) were induced to a greater extent in the Mttp<sup>ΔΔ</sup> mice than in Mttp<sup>fl/lox</sup> mice (Fig. 8C).

Lipid Peroxidation Products in Mttp<sup>ΔΔ</sup> Livers—LPS induces oxidant stress in the liver (37–40), which promotes lipid peroxidation, particularly in the presence of polysaturated fatty acids (41, 42). We therefore predicted that lipid peroxides would increase significantly in the livers of Mttp<sup>ΔΔ</sup> mice after an LPS challenge, due to their higher basal levels of polysaturated fatty acids. To test that possibility, we measured hepatic levels of TBARS as an indicator of tissue lipid peroxides.
in *Mttp* 

**DISCUSSION**

The inability of livers from *Mttp* 

in *Mttp* 

Mice were used to study the role of Mttp in the regulation of lipid metabolism and liver function. The study found that Mttp-mediated VLDL secretion and liver injury were associated with changes in plasma triglyceride levels and liver steatosis. The study also showed that the blockade in lipoprotein production did not cause widespread changes in the expression of the genes governing lipid metabolism. The serum transaminases were normal, and the animals exhibited normal vitality, growth, and fertility. Presumably, the ability of Mttp to regulate lipid metabolism and liver function is important in maintaining normal liver function and preventing liver injury.
believe that it is important to underscore several caveats regarding our experiments. First, our studies were conducted in Mttp^A/Δ mice, and the precise relevance of that animal model to humans is not yet established. Mttp^A/Δ mice lack MTP activity only in the liver. An MTP inhibitor drug would block MTP in both liver and intestine, and inhibiting intestinal lipoprotein production could actually limit the amount of lipid accumulation in the liver. Second, both hepatic MTP activity levels and apoB100 secretion rates were blocked by 95% in Mttp^A/Δ mice (6). The blockade would almost certainly be less profound in humans treated with MTP inhibitor drugs, because the drugs have been shown to partially block lipoprotein secretion at doses that only partially block MTP activity (3). A more modest level of MTP inhibition might be associated with less lipid accumulation in the liver and, correspondingly, less toxicity.

One might argue that the increased susceptibility of the Mttp^A/Δ mice to hepatic injury was due, at least in part, to their low plasma levels of triglyceride-rich lipoproteins. Triglyceriderich lipoproteins bind LPS and direct it away from macrophages in the liver (i.e., Kupffer cells). This bypass of the Kupffer cell population reduces LPS-mediated TNF-α release (43–45) and limits LPS-mediated organ injury. Interestingly, low plasma lipoprotein levels have been reported to enhance LPS hepatotoxicity (46). The low levels of triglyceride-rich lipoproteins in Mttp^A/Δ mice did not, however, appear to be the cause of their heightened sensitivity to LPS. In our experiments, LPS-induced increases in TNF-α and hepatic TNF-α transcripts were no different in Mttp^A/Δ mice and controls. Those findings suggest that the plasma lipoprotein levels did not significantly affect the access of Kupffer cells to the toxin. Furthermore, Mttp^A/Δ mice also exhibited exaggerated toxicity in response to ConA and PEA, neither of which bind lipoproteins.

Our findings with Mttp^A/Δ mice are reminiscent of the increased sensitivity to LPS in obese mice and rats. For example, Yang et al. (47) demonstrated that ob/ob mice and Zucker diabetic fatty rats (both of which have increased liver lipid stores) are more susceptible than nonobese controls to the development of steatohepatitis after an LPS challenge. These rodent models of obesity are clearly different from the Mttp^A/Δ mice in that they do not involve a blockade in VLDL secretion. They are also far more complex from a metabolic perspective. MTP deficiency in the liver simply prevents the assembly and secretion of VLDL, whereas a deficiency in leptin (as in the ob/ob mice) results in substantial changes in caloric intake, induces frank diabetes mellitus, and even changes the function of the immune system (48–51). Diabetes mellitus also produces complex metabolic changes in the liver as well as in multiple other tissues (52). Nevertheless, leptin deficiency, obesity, diabetes, and Mttp deficiency all share a common feature, hepatic steatosis. The current studies are important because they show that increased hepatic lipid stores from a blockade in lipoprotein secretion heighten the risk for toxin-mediated hepatic injury, and do so without the many metabolic derangements associated with leptin deficiency, obesity, and diabetes.

Although Mttp^A/Δ and ob/ob mice both displayed exaggerated sensitivity to LPS compared with their respective controls, their responses to ConA and PEA were quite different. Faggin et al. (53) reported that ob/ob mice are resistant to ConA- and PEA-mediated hepatotoxicity, whereas in the current study Mttp^A/Δ mice were more sensitive to toxicity from these compounds. The toxicities of ConA and PEA are thought to be mediated by T cells (34, 35, 53). Faggin et al. (53) speculated that the resistance of the ob/ob mice to hepatic injury from those agents might be related to the deficiency in T-cell-mediated immunity associated with leptin deficiency (50, 53). A
deficiency in MTP, which is expressed largely in hepatocytes and intestinal enterocytes, would not be expected to cause immunodeficiency, and thus it is logical that that Mttp<sup>−/−</sup> mice would exhibit similar sensitivities to the three different toxic challenges.

The concept that hepatic steatosis can heighten susceptibility to the development of inflammation and more advanced liver disease is supported by more than data from experimental animals. Humans with diabetes mellitus, obesity, and chronic exposure to ethanol have increased hepatic lipid stores and are at increased risk for developing steatohepatitis and cirrhosis (8, 54, 55). Bacterial products at increased risk for developing steatohepatitis and cirrhosis animals. Humans with diabetes mellitus, obesity, and chronic liver disease is supported by more than data from experimental models implicating as important cofactors in the pathogenesis of in-flammation arising in fatty livers (8, 14). These compounds can cause liver injury not only by inducing cytokines such as TNF-α but also by causing release of reactive oxygen species from Kupffer cells (39).

The toxicity of LPS is mediated in part through the induction of TNF-α (33) and in part by stimulating macrophage production of reactive oxygen species (56). TNF-α also induces the production of reactive oxygen species within cells (57–59), which in turn can cause cellular injury. Studies have shown that in the setting of fatty liver, the severity of oxidative injury depends upon the degree of unsaturation of cellular lipids (60, 61). High levels of unsaturation amplify oxidative insults, leading to enhanced lipid peroxidation and downstream consequences such as chemokine production (62–64). In our experiments, the substantial LPS-induced rise in TBARS that occurred in Mttp<sup>−/−</sup> mice relative to control mice is likely due to their disproportionate stores of linoleic acid, an essential polyunsaturated fatty acid. Despite comparable induction of TNF-α in both groups of mice, the Mttp<sup>−/−</sup> mice, with their increased hepatic stores of linoleic acid, displayed more severe tissue damage. This manifested not only in a significant increase in lipid peroxidation but also in enhanced induction of inflammatory mediators (e.g., several chemokines).

We are at a loss to explain the enrichment of liver triglycerides in Mttp<sup>−/−</sup> mice with linoleic acid, particularly because there was no change in dietary lipids and no difference between Mttp<sup>−/−</sup> and control mice in the composition of fatty acids in the plasma. Perhaps the inability to secrete VLDL changes the spectrum of fatty acids that undergo α-oxidation, causing linoleic acid to accumulate. Alternatively, one could speculate that VLDL serves a special role in exporting essential polyunsaturated fatty acids to peripheral tissues. If so, a blockade in VLDL production might cause polyunsaturated fatty acids to accumulate. Whatever the mechanism, the enrichment in linoleic acid could render the liver more susceptible to oxidant damage (60, 61). Also, as noted under “Results,” the accumulation of linoleic acid might explain, at least in part, the reduced levels of SREBP-1 (31, 32) and Scd1 (30).

It would be interesting to determine whether the increased sensitivity of Mttp<sup>−/−</sup> mice to liver injury would be mitigated by additional manipulations that limit lipid accumulation. One potential approach was suggested in a recent paper by Matsuda and co-workers (65). They produced a conditional allele for SREBP cleavage-activating protein and then used the inducible Mx1-Cre transgene to produce mice lacking that protein in the liver. On a chow diet, those mice manifested reduced expression of genes driven by SREBP-1 and SREBP-2, an 80% reduction in hepatic lipid biosynthesis, and a 65% reduction in liver triglyceride stores (65). It was interesting to determine whether a deficiency in hepatic SREBP cleavage-activating protein would completely block the hepatic lipid accumulation in Mttp<sup>−/−</sup> mice, and if so, whether those mice would be protected from hepatic injury in response to exogenous toxins.

Acknowledgments—We thank K. Feingold for advice; J. Horton for a probe for stearoyl-CoA desaturase 1 and antibodies to SREBP-1 and SREBP-2; J. Ozols for the antibodies against stearoyl-CoA desaturase; M. R. H. H. Kellerstein and R. A. Neese for assessment of the fatty acid content of hepatic lipids; and an anonymous reviewer for the suggestion to measure glucose and insulin levels in Mttp<sup>−/−</sup> mice. We also thank S. Orwod and G. Howard for comments on the manuscript.
VLDL Secretion and Liver Injury

42. Bondy, S. C., and Marwah, S. (1995) FEBS Lett. 375, 53–55
43. Harris, H. W., Grunfeld, C., Feingold, K. R., and Rapp, J. H. (1990) J. Clin. Invest. 86, 696–702
44. Harris, H. W., Grunfeld, C., Feingold, K. R., Read, T. E., Kane, J. P., Jones, A. L., Eichbaum, E. B., Bland, G. F., and Rapp, J. H. (1993) J. Clin. Invest. 91, 1028–1034
45. Vreugdenhil, A. C. E., Snoek, A. M. P., van’t Veer, C., Greve, J.-W. M., and Buurman, W. A. (2001) J. Clin. Invest. 107, 225–234
46. Feingold, K. R., Funk, J. L., Moser, A. H., Shigenaga, J. K., Rapp, J. H., and Grunfeld, C. (1995) Infect. Immun. 63, 2041–2046
47. Yang, S. Q., Lin, H. Z., Lane, M. D., Clemens, M., and Diehl, A. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2557–2562
48. Faggioni, R., Moser, A., Feingold, K. R., and Grunfeld, C. (2000) Am. J. Pathol. 156, 1781–1787
49. Flier, J. S. (1998) Nat. Med. 4, 1124–1125
50. Lord, G. M., Matarese, G., Howard, J. K., Baker, R. J., Bloom, S. R., and Lechler, R. I. (1998) Nature 394, 897–901
51. Matarese, G., Di Giacomo, A., Sanna, V., Lord, G. M., Howard, J. K., Tusco, A. D., Bloom, S. R., Lechler, R. I., Zappacosta, S., and Fontana, S. (2001) J. Immunol. 166, 5909–5916
52. Unger, R. H., and Orci, L. (2000) Int. J. Obes. Relat. Metab. Disord. 24, 28–42
53. Faggioni, R., Jones-Carson, J., Reed, D. A., Dinarello, C. A., Feingold, K. R., Grunfeld, C., and Fantuzzi, G. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 2367–2372
54. Reeves, H. L., Burt, A. D., Wood, S., and Day, C. P. (1996) J. Hepatol. 25, 677–683
55. Poonawala, A., Nair, S. P., and Thuluvath, P. J. (2000) Hepatology 32, 689–692
56. White, J. E., and Tzan, M.-F. (2001) Am. J. Respir. Cell Mol. Biol. 24, 164–169
57. Adamson, G. M., and Billings, R. E. (1992) Arch. Biochem. Biophys. 294, 223–229
58. Schulze-Osthoff, K., Bakker, A. C., Vanhaesebroeck, B., Beyaert, R., Jacobs, W. A., and Fiers, W. (1992) J. Biol. Chem. 267, 5317–5323
59. Gossens, V., Grooten, J., De Vos, K., and Fiers, W. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8115–8119
60. Nanji, A. A., Sadrzadeh, S. M. H., and Dammenberg, A. J. (1994) Alcohol. Clin. Exp. Res. 18, 1024–1028
61. Nanji, A. A., Zhao, S., Sadrzadeh, S. M. H., Dannenberg, A. J., Tahan, S. R., and Waxman, D. J. (1994) Alcohol. Clin. Exp. Res. 18, 1280–1285
62. DeForge, L. E., Preston, A. M., Takeuchi, E., Kenney, J., Boxer, L. A., and Remick, D. G. (1993) J. Biol. Chem. 268, 23568–23576
63. Lee, J. S., Kahlon, S. S., Culbrett, R., and Cooper, J. A. D., Jr. (1999) J. Interferon Cytokine Res. 19, 761–767
64. Casola, A., Burger, N., Liu, T., Jamaluddin, M., Brasier, A. R., and Garoff, R. P. (2000) J. Biol. Chem. 276, 19715–19722
65. Matsuda, M., Korn, B. S., Hammer, R. E., Moon, Y.-A., Komuro, R., Horton, J. D., Goldstein, J. L., Brown, M. S., and Shimomura, I. (2001) Genes Dev. 15, 1206–1216