Lipopolysaccharide-Binding Protein Modulates Acetaminophen-Induced Liver Injury in Mice

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Acetaminophen toxicity is the most common cause of acute liver failure in the United States and Europe. Although much is known about the metabolism of acetaminophen, many questions remain regarding the pathogenesis of liver injury. In this study, we examined the role of lipopolysaccharide-binding protein (LBP), a protein important in mediating cellular response to lipopolysaccharides, by using LBP wild-type and knockout (KO) mice. We found that LBP KO mice were protected from acetaminophen-induced hepatotoxicity. At 350 mg/kg of acetaminophen, LBP KO mice had significantly less liver injury and necrosis than wild-type mice. Repletion studies in LBP KO mice using an LBP–adenoviral construct resulted in significantly more hepatic injury and necrosis after acetaminophen exposure compared with mice receiving the control adenoviral construct. In conclusion, LBP KO mice are protected from toxicity with a decrease in hepatic necrosis following acetaminophen challenge. This suggests a novel role for LBP in modulating acetaminophen-induced liver injury.

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A cetaminophen hepatotoxicity is the most common cause of acute liver failure in the United States and Europe, accounting for 20% to 75% of all cases of acute liver failure.1 Although a dose-dependent effect of acetaminophen hepatotoxicity has been seen, clinical variability in outcome to injury is also evident.2 Some of the variability in hepatotoxicity is due to differences in the metabolism of acetaminophen. An association between chronic alcohol use and acetaminophen hepatotoxicity is well recognized.3 This association may be due to the induction of CYP2E1 activity coupled with decreased glutathione (GSH) stores found after chronic alcohol use. Together, such changes lead to increased levels of N-acetyl-p-benzoquinone imine (NAPQI), the toxic metabolite of acetaminophen. However, this metabolite may not account for all the toxicities from the seemingly low doses of acetaminophen. Another potential determinant of susceptibility to liver injury from toxins such as acetaminophen is the innate immune response to liver injury. A pathogenic role for Kupffer cell activation after acetaminophen toxicity has been proposed.4 Pre-treatment with inhibitors of macrophage function such as gadolinium chloride or dextran sulfate has been shown to block acetaminophen-induced hepatic necrosis, supporting a role for Kupffer cells in acetaminophen-induced hepatotoxicity.5 In other forms of liver injury such as alcohol-induced injury, Kupffer cells mediate injury following activation by endogenous lipopolysaccharides (LPSs) and gut bacteria.6,7 Because LBP has been found to be an important mediator in the LPS activation of Kupffer cells,8,9 we sought to examine the role of LBP in acetaminophen-induced hepatotoxicity.

Materials and Methods

Animal Model. LBP knockout (KO) mice were a generous gift from Doug Golenbock.10 These animals had been backcrossed into the background C57Bl/6 strain at...
least 12 times before we acquired our colony. They were subsequently housed in a specific pathogen-free environment and were allowed to breed. Male LBP KO mice ranging from 12 to 16 weeks in age and appropriate age-matched male C57Bl/6 wild-type control mice (Jackson Laboratories, Bar Harbor, ME) were used for all of our experiments. The wild-type control mice were allowed to acclimate to their new surroundings for 1 week before being used in experiments. Experiments were performed in accordance with National Institutes of Health guidelines, and approval was obtained from the University of Michigan Animal Care and Use Committee. After a 16-hour fast, the mice were injected intraperitoneally with 350 mg/kg acetaminophen (Sigma, St. Louis, MO) dissolved in sterile saline or sterile saline alone as the vehicle control. After injection with acetaminophen, mice were fed ad libitum with standard chow.

**Plasma Aminotransferase Measurements.** Plasma or serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured at 37°C with a commercially available kit (Sigma Diagnostic).

**Histology.** Liver tissue was formalin fixed and paraffin embedded before sectioning. Liver sections were stained with hematoxylin-eosin. Quantitative analysis of the extent of tissue necrosis was performed after digitally imaging three high-power fields per slide in a random and blinded fashion. Areas of tissue necrosis or impending necrosis were identified according to the presence of decreased eosinophilia, loss of cell architecture, vacuolization, cell disruption, or karyolysis. For the sake of analysis, both areas of necrosis that had all the features and areas of impending necrosis that had most of the features without karyolysis were included in the quantitation of necrosis for the sake of analysis. The area of necrosis was highlighted and calculated using National Institutes of Health Image 1.61 software (National Institutes of Health, Bethesda, MD). The average area of necrosis from each experimental condition was calculated using National Institutes of Health Image 1.61 software.

**Immunoblotting.** Hepatic CYP2E1, CYP1A2, and CYP3A11 levels were measured via immunoblot analysis. Hepatic microsomes were isolated using the methods described by Schenkman and Cinti with slight modifications. Briefly, frozen liver tissue was homogenized in 0.25 mol/L sucrose/10 mmol/L Tris-Cl (pH 7.4) and serially centrifuged at 6000 g for 10 minutes, 6,500 g for 15 minutes, and 12,000g for 10 minutes at 4°C. Following careful decanting of the supernatant, the pellets were resuspended in a CaCl2 (8 mmol/L) solution. The pellets were then incubated in this solution on ice for 30 minutes before centrifugation at 18,000g for 30 minutes. Microsomal pellets were washed with 0.15 mol/L KCl before use. Total protein was measured using the BCA protein assay method (Pierce Chemical, Rockford, IL). For immunoblot analysis, 10 μg of total protein was loaded into each lane. Cell extracts were separated via sodium dodecyl sulfate–polyacrylamide gel electrophoresis using a 10% to 12.5% gel under reducing condition using the methods of Laemmli. Transfer was performed electrophoretically to nitrocellulose using the methods of Towbin et al. The membrane was probed with a primary antibody—rabbit anti–rat CYP2E1—or anti–rat CYP1A2 antibody (Chemicon International, Temecula, CA). Both of these antibodies cross-react with mouse CYP2E1 or CYP1A2, respectively. For the anti–mouse CYP3A11, the membrane was probed with a rabbit anti–rat CYP3A2 antibody (Daichi Pure Chemicals, Tokyo, Japan) that is known to cross-react with mouse CYP3A11. A horse-radish peroxidase–linked secondary antibody against rabbit immunoglobulin G was used. Detection was performed with an electrochemiluminescence Western blotting kit (Amersham, Buckinghamshire, United Kingdom). The molecular weight was determined using a concurrently run prestained standard (Biorad, Hercules, CA).

For detection of acetaminophen protein adducts, immunoblots were performed using a rabbit antiserum generously provided by Dr. Jack A. Hinson (University of Arkansas, Little Rock, AR) using methods described previously.

**Real-Time Reverse-Transcription Polymerase Chain Reaction.** Total RNA was isolated with TRIzol reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). Reverse transcription was performed as described previously. To determine the relative amount of cytokine messenger RNA, amplification of sample complementary DNA was monitored using the Smart Cycler (Cepheid, Sunnyvale, CA) and the DNA fluorescent dye SYBR Green (Molecular Probes, Eugene, OR). Primers were designed using reported sequences in GenBank. Primer sequences are as follows: interleukin (IL)-6: 5’ GAGGATAACCCTCCACACAGACC, 3’ AAGTTGATCATTATGGTTGTTCA; tumor necrosis factor (TNF): 5’ GGAGAATGACCTTGAGCCTG, 3’ CTCCATTTGGCAGAACTCAG; IL-10: 5’ GCCATATGCGAAAATGATCCA, 3’ TTTCACAGGGGAGAATCG; glyceraldehyde-3-phosphate dehydrogenase: 5’ GTCTTCACCACATGGAGAAG, 3’ CCACCTTCTTGATGTTCATC. Primers were designed to span at least one intron, if possible, to minimize risk of genomic amplification. The specificity of the primers was verified via analysis.
of polymerase chain reaction (PCR) product with ethidium bromide–stained agarose gel electrophoresis as well as direct sequencing of the PCR product. All real-time PCR runs included a melting curve analysis to assure the specificity of the product with each PCR reaction.

The validity of the semiquantitative method was confirmed through a consistent log linear correlation of \( r^2 \geq 0.95 \) between starting template RNA concentration and threshold cycle. All the values were obtained by converting the threshold cycle of the sample to relative RNA concentration based on the calibrator RNA (a random RNA sample generating a standard curve for that specific amplicon). All values are expressed as a ratio of RNA concentration of target amplicon to the RNA concentration of a housekeeping gene (glyceraldehyde-3-phosphate dehydrogenase) to equalize for RNA concentrations between samples.

**Enzyme-Linked Immunosorbent Assay for TNF-α.** TNF-α levels were measured with a sandwich enzyme-linked immunosorbent assay using antibodies from Pharmingen (San Diego, CA) according to the manufacturer’s instructions.

**Endotoxin Measurements.** Plasma endotoxin levels were measured using the QCL Chromogenic LAL Assay (Biowhittaker, Walkersville, MD) according to the manufacturer’s instructions. To minimize product inhibition, all samples were diluted 1:10 and heated to 70°C for 10 minutes prior to assay. Endotoxin-free containers and water were used for the assay.

**Hepatic GSH Levels.** Measurements of GSH were performed using the enzymatic recycling method described by Griffith.17 For liver tissue, the livers were perfused with warm saline before excision and quick-frozen in liquid nitrogen. The frozen tissue was then pulverized with a mortar and pestle before being placed in 1% picric acid. For GSH measurements, the acid extract supernatant was added to 700 \( \mu \)L 0.3 mmol/L reduced nicotinamide-adenine dinucleotide phosphate, 100 \( \mu \)L 6 mmol/L DTNB and metal-free water for a total volume of 1 mL. The sample was warmed in a 30°C water bath for 10 to 15 minutes; 10 \( \mu \)L of 50 U/mL GSH reductase was then added and the sample was monitored at a wavelength of 412 continuously until it exceeded 2.0 absorbance units.

**Statistical Analysis.** Analysis was performed using Statview software (SAS Institute, Cary, NC). Unless indicated otherwise, data are expressed as the mean ± SE. Analysis was performed using the Student t test and ANOVA when more than two variables were compared. A P value of less than .05 was considered statistically significant. Kaplan-Meier analysis with log rank testing was performed on the survival data.

**Results**

**LBP Knockout Animals Are Protected From Acetaminophen-Induced Liver Injury.** To evaluate the role of LBP in acetaminophen-induced toxicity, LBP KO and wild-type C57Bl/6 mice were given a single dose of acetaminophen at either 500 mg/kg or 350 mg/kg. At 500 mg/d, all the wild-type mice died by day 3, while 1 out of 6 LBP KO mice survived (Table 1). After 350 mg/kg, all the wild-type mice died by day 3, whereas all the LBP KO mice survived (Fig. 1). Because such dramatic and significant difference in survival was observed with the 350-mg/kg dose of acetaminophen, all subsequent studies were performed with this dose of acetaminophen to decipher the mechanisms through which the presence or absence of LBP can alter acetaminophen toxicity.

Because the main toxicity of acetaminophen occurs through its effect on the liver, we sought to examine liver injury by assessing plasma aminotransferases levels and liver histology. LBP KO and wild-type animals were sacrificed 6 and 24 hours after acetaminophen administration for the harvest of plasma and liver tissue. Another group of animals was given a single injection of saline and sacrificed after 24 hours. With saline alone, there was no significant difference in baseline plasma aminotransferase levels between the LBP KO and wild-type animals 24 hours after saline injection. In contrast, a significant elevation in plasma aminotransferases was found after acet-

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**Table 1. Survival at Day 3 After Acetaminophen Administration (either 500 mg/d or 350 mg/d) in Wild-type and LBP KO Mice (n = 6 Mice/Group)**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Acetaminophen 500 mg/kg</th>
<th>Acetaminophen 350 mg/kg</th>
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<tbody>
<tr>
<td>Wild-type</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>LBP KO</td>
<td>1/6</td>
<td>6/6*</td>
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*P < .05.

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Fig. 1. Percentage survival over time of LBP KO mice versus wild-type mice after acetaminophen administration (350 mg/kg). LBP KO, lipopolysaccharide-binding protein knockout.
aminophen administration. Peak ALT levels were noted in both groups of animals at 6 hours, with a significantly greater amount of plasma ALT in LBP wild-type mice compared with LBP KO mice (4417 ± 658.2 vs. 2275.7 ± 97.2 U/L; *P < .05) (Fig. 2A). Similarly, increased AST levels were noted in control wild-type mice compared with LBP KO mice (5265.9 ± 666.4 vs. 2373.6 ± 378.3 U/L; *P < .01) (Fig. 2B). Liver histology was examined for liver necrosis. Acetaminophen challenge resulted in the classic centrilobular necrosis in both LBP KO and wild-type mice (Fig. 3). In some areas, there were large spans of hepatocytes that appeared to be necrotic or in the process of undergoing necrosis, with loss of eosinophilic staining, loss of cell architecture, and vacuolization. For the purpose of analysis, these areas were designated as “necrotic” and were quantitated. Quantitative analysis of liver necrosis showed significantly more necrosis in wild-type animals than in LBP KO animals after 6 hours; this difference persisted at the 24-hour time point (Table 2).

**Cytokine Production After Acetaminophen-Induced Hepatotoxicity.** To delineate the mechanism through which LBP mediates acetaminophen-induced liver, we examined the expression of the pro- and anti-inflammatory cytokines TNF-α, IL-10, and IL-6, all of which have previously been implicated in the pathogenesis of toxin-induced liver injury. We examined the intrahepatic expression of these cytokines using real-time PCR and found that intrahepatic messenger RNA levels of TNF-α and IL-10 were significantly increased in wild-type mice compared with LBP KO mice at both 6 and 24 hours after acetaminophen administration (Fig. 4A-B). Similarly, intrahepatic messenger RNA levels of IL-6 were increased in wild-type mice 6 hours after acetaminophen (Fig. 4C). Baseline levels of all the cytokines remained low after saline injection.

**Portal Endotoxin Levels in LBP KO Mice.** Because LBP can modulate cellular interactions with LPS, we next examined the portal endotoxin levels in LBP KO and

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**Table 2. Hepatic Necrosis (and Impending Necrosis) (%) 6 and 24 Hours After Acetaminophen Administration (350 mg/kg) in Wild-type and LBP KO Mice (n = 6 or 7 Mice/group)**

<table>
<thead>
<tr>
<th>Animals</th>
<th>6 Hours</th>
<th>24 Hours</th>
</tr>
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<tbody>
<tr>
<td>Wild-type</td>
<td>72.6 ± 2.1</td>
<td>69.2 ± 4.8</td>
</tr>
<tr>
<td>LBP KO</td>
<td>47.6 ± 8.2*</td>
<td>47.0 ± 2.8*</td>
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*P < .01 between wild-type and LBP KO animals.

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**Fig. 2.** (A) ALT levels after acetaminophen administration. Plasma ALT levels were measured at 6 and 24 hours after acetaminophen (350 mg/kg) in wild-type and LBP KO mice (n = 6 or 7 per group; *P < .05 [wild-type vs. LBP KO]). Baseline levels of ALT were measured 24 hours after saline administration. (B) AST levels after acetaminophen administration. Plasma AST levels were measured at 6 and 24 hours after acetaminophen administration (350 mg/kg) in LBP wild-type and LBP KO mice (n = 6 or 7 per group; *P < .01 [wild-type vs. LBP KO]) and 24 hours after saline administration. ALT, alanine aminotransferase; LBP KO, lipopolysaccharide-binding protein knockout; AST, aspartate aminotransferase.

**Fig. 3.** Liver histology after acetaminophen administration. Liver histology in wild-type and LBP KO mice 24 hours after saline or acetaminophen administration (350 mg/kg). (A) Wild-type, saline control; (B) LBP KO, saline control; (C) wild-type, acetaminophen; (D) LBP KO, acetaminophen (hematoxylin-eosin stain; original magnification ×10).
wild-type mice after injection with either the vehicle control saline or acetaminophen. Plasma was obtained with a 23- to 25-gauge angiocath from the main portal vein. All needles and containers were endotoxin-free. We found low levels of endotoxin after saline injection that increased slightly 2 hours after acetaminophen administration but returned to baseline by 4 hours (Table 3). Overall, the differences in endotoxin levels at different time points were minimal and of questionable significance. Furthermore, at all time points studied, there was no significant difference in portal endotoxin levels between wild-type and LBP KO mice.

**Hepatic Cytochrome P450 Levels in LBP KO Mice.** To examine whether the differences in hepatic injury in LBP KO mice might have been due to the initial conversion of acetaminophen to its toxic metabolite, NAPQI, we examined the levels of cytochrome P450 (CYP) in these mice. Acetaminophen is metabolized by CYP to the toxic metabolite NAPQI. Through a number of different mechanisms, the presence of NAPQI leads to hepatic necrosis.21 In men and mice, CYP2E1 is the major enzyme responsible for the conversion of acetaminophen to this toxic metabolite.22 However, CYP1A2 and CYP3A2 may also play a role.21 Antibodies to rat CYP3A2 cross-react with mouse CYP3A11. In addition, there are some data that support a role of LPS signaling pathways in mediating changes in CYP1A2 and CYP3A11 levels in mice.14 For these reasons, we sought to determine whether the relative concentrations of CYP2E1, CYP1A2, and CYP3A11 are similar in wild-type and LBP KO animals. We isolated hepatic microsomes from wild-type and LBP KO animals 6 hours after either saline or acetaminophen administration. Immunoblotting for CYP2E1, CYP1A2, and CYP3A2 (mouse CYP3A11) showed no significant difference between wild-type and LBP KO animals after saline or acetaminophen administration (Supplementary Fig. 1). The lack of CYP2E1 induction with acetaminophen administration is consistent with previous reports.23

**Hepatic GSH Levels After Acetaminophen Administration.** We also examined hepatic GSH levels after acetaminophen administration in LBP KO and wild-type mice. We found that baseline GSH levels were similar in both groups. GSH levels fell to more than 90% by 2 hours after acetaminophen administration, which is consistent with previous reports.24 The early GSH depletion is a result of the formation of NAPQI. Our results show that both LBP KO and wild-type mice had the same initial drop in hepatic GSH levels, suggesting that the initial metabolism of acetaminophen is similar in both types of mice (Fig. 5).

**Table 3. Portal Endotoxin Levels in Wild-type and LBP KO Mice 2 Hours After Control (Saline) Injection or Acetaminophen Injection (n = 3 to 4 Per Group)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acetaminophen 2</th>
<th>Acetaminophen 4</th>
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<tr>
<td></td>
<td>Hours</td>
<td>Hours</td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>0.49 ± 0.18</td>
<td>0.62 ± 0.07*</td>
<td>0.35 ± 0.05</td>
</tr>
<tr>
<td>LBP KO</td>
<td>0.25 ± 0.06</td>
<td>0.89 ± 0.21*</td>
<td>0.33 ± 0.04</td>
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*P < .05 (control vs. acetaminophen via ANOVA).
Acetaminophen Protein Adduct Formation in LBP KO Mice. To assess if decreased injury in LBP KO mice was due to a decrease in acetaminophen bioactivation and protein adduct formation, immunoblots of whole liver homogenates were examined 2 hours after administration of acetaminophen (350 mg/kg) or saline. No significant difference in the amount or pattern of acetaminophen adduct formation was noted between wild-type and LBP KO mice (Fig. 6).

Reconstitution of LBP KO Animals With LBP Increased Liver Injury. To reconstitute LBP in LBP KO animals, we developed a recombinant adenovirus that expressed rat LBP (Supplementary Fig. 2). An intramuscular injection of adenovirus (recombinant adenovirus expressing the control-irrelevant protein β-galactosidase [Ad-βgal] or recombinant LBP adenovirus [Ad-LBP], $5 \times 10^9$ pfu/mouse) was given 3 days before acetaminophen administration in LBP KO mice. Analysis of the plasma revealed expression of a 60-kd protein in the Ad-LBP treated mice (Supplementary Fig. 3A). Plasma LBP levels were measured via enzyme-linked immunosorbent assay and showed that the levels of LBP achieved following intramuscular delivery of Ad-LBP were slightly above that found in normal murine plasma from C57Bl/6 mice ($4.0 \pm 0.2 \mu g/mL; n = 3$) (Supplementary Fig. 3B). No detectable plasma LBP was found in the LBP KO animals given Ad-βgal, as expected.

Liver injury was assessed by analyzing plasma aminotransferase levels and histology at 6 and 24 hours after acetaminophen administration. In the LBP KO mice given Ad-βgal or Ad-LBP, plasma ALT and AST levels increased after acetaminophen at 6 hours and peaked at 24 hours. The LBP KO mice given Ad-LBP had a significantly greater amount of liver injury as assessed by both AST and ALT 24 hours after acetaminophen administration (Fig. 7A,B). Consistent with the higher levels of plasma aminotransferases, quantitative hepatic necrosis was also significantly greater in the mice given Ad-LBP compared with those given Ad-βgal (Table 4).

Discussion

LBP is an acute-phase protein produced predominantly by hepatocytes, although low levels of production have been noted at extrahepatic sites. In vitro, LBP binds with high affinity to the lipid A portion of LPS and facilitates its transfer to multiple target sites such as membrane-bound CD14, soluble CD14, and high-density lipoprotein. In vivo, the role of LBP is less understood. LBP KO mice do not exhibit in vivo alterations in response to exogenous LPS, but ex vivo, serum from LBP KO mice is deficient in facilitating activation of monocytes by LPS. An important role for LBP in modulating innate immune response to

<table>
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<th>Treatment</th>
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<th>24 Hours</th>
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<tr>
<td>Ad-βgal</td>
<td>46.3 ± 8.0</td>
<td>41 ± 4.3</td>
</tr>
<tr>
<td>Ad-LBP</td>
<td>57.8 ± 1.9</td>
<td>57.2 ± 0.9*</td>
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*P < .01 (Ad-βgal vs. Ad-LBP).
bacteria is demonstrated by finding that LBP KO animals are more susceptible to gram-negative infections such as *Klebsiella pneumoniae* or intraperitoneal injection of *Salmonella* typhimurium. In our studies, we have found that LBP—which mediates an innate immune response to gram-negative bacteria—also has a role in modulating liver injury from acetaminophen. We found that LBP KO mice experience less liver injury than wild-type mice given the same dose of acetaminophen (350 mg/kg). At 350 mg/kg of acetaminophen, this difference in hepatic injury resulted in a significant difference in mortality. The modulating role of LBP is shown with repletion studies in the LBP KO mice that are given adenovirus containing recombinant LBP.

The pathogenesis of acetaminophen-induced hepatotoxicity has been well studied. At therapeutic doses, acetaminophen is rapidly metabolized in the liver, mainly by glucuronidation and sulfation. A small amount of acetaminophen is oxidized by the CYP system to the highly reactive and cytotoxic-intermediate NAPQI. Under normal conditions, NAPQI is detoxified by the presence of GSH; however, following large doses of acetaminophen, the glucuronidation and sulfation pathways are overwhelmed and hepatic GSH is depleted. NAPQI is then present in excess, leading to liver injury. Because the metabolism of acetaminophen is very important in determining the extent of liver injury, we sought to examine whether quantitative differences in the initial metabolism of acetaminophen were present between wild-type and LBP KO mice. Immunoblotting revealed that relative concentrations of CYP2E1, CYP3A2, and CYP1A2 were similar in both groups. Furthermore, the time course of GSH depletion appeared to be similar between these two groups of mice, suggesting that the initial metabolism of acetaminophen could not account for the subsequent differences in liver injury. Although the results of these studies indirectly suggest that LBP KO animals do not have altered metabolism of acetaminophen, they are not direct measurements of acetaminophen metabolism and thus have limitations.

The mechanism through which NAPQI causes hepatocyte necrosis is not entirely understood. A number of pathways have been proposed that may not be exclusive of each other. NAPQI can have direct hepatocellular effects by binding to a number of intracellular proteins and DNA leading to cell death via mitochondrial dysfunction and oxidant stress. Furthermore, NAPQI is a highly reactive intermediate that by itself can contribute to oxidative damage. The increase in oxidative stress and the presence of necrotic hepatocytes can lead to activation of Kupffer cells, which in turn further exacerbates liver injury.

Our data support a role for LBP in the pathogenesis of acetaminophen-induced liver injury. One logical hypothesis would be that LBP mediates acetaminophen-induced hepatotoxicity through its ability to facilitate LPS activation of Kupffer cells. In other models of toxin-induced liver injury, such as CCl4 and ethyl alcohol, evidence supports a role for endogenous LPS as a cofactor in liver injury through the activation of Kupffer cells. In acetaminophen-induced hepatotoxicity, however, the role for LPS has been poorly defined, although ample evidence supports a role for activated Kupffer cells. Within 24 hours of acetaminophen injection in the rat, activated mononuclear cells infiltrate the centrlobular region of the liver. Kupffer cells isolated from acetaminophen-treated animals show characteristics of activation such as increased adherence, phagocytosis, and superoxide production. A functional role for Kupffer cell activation in the pathogenesis of acetaminophen-induced hepatotoxicity is supported by studies in which the use of gadolinium chloride or dextran sulfate decreased acetaminophen hepatotoxicity and formation of nitrotyrosine protein adducts in mice. Our data support a role for Kupffer cell activation, because intrahepatic expression of both pro- and anti-inflammatory cytokines was decreased in LBP KO mice. Activated Kupffer cells are major sources of these cytokines in the liver. Our data therefore support differential activation of Kupffer cell activation in LBP wild-type and KO mice, both at baseline and after acetaminophen administration. Whether these differences in cytokine expression are merely indicators of injury or are actual mediators of hepatotoxicity is not clear and remains the subject of continued investigation in our laboratory. Furthermore, recent studies suggest a more complicated role for Kupffer cell activation in the pathogenesis of acetaminophen-induced liver injury. A study of reduced NADPH oxidase KO mice by James et al. found equal injury in these mice after acetaminophen, casting doubt on the hypothesis that Kupffer cell–derived superoxides contribute to hepatotoxicity in this model. Moreover, a study by Ju et al. showed that while pretreatment gadolinium chloride protected mice from acetaminophen, a different method of Kupffer cell depletion using liposome/clodronate increased susceptibility to acetaminophen-induced liver injury. The authors hypothesized that the difference in the two methods led to varying levels of Kupffer cell depletion, which could account for the widely different phenotypes. Thus, Kupffer cell activation may have a dual effect on liver injury and regeneration and that the overall outcome of Kupffer cell manipulation is dependent on the relative changes in cytokine production.
an effect on endogenous LPS is not clear. A small increase in portal endotoxemia was observed after acetaminophen administration, but this was not different between wild-type and LBP KO mice. This result is not surprising, because LBP has not been reported to play a role in modulating intestinal absorption of LPS. This would suggest that the changes we see in intrahepatic cytokine expression are due to alterations in Kupffer cell reactivity in the absence of changes in LPS levels. Alternatively, it may be that although LBP-mediated effects on LPS activation of Kupffer cells contribute to acetaminophen-induced liver injury, other pathways of LBP-mediated effects also exist.

The role for endogenous LPS in acetaminophen-induced hepatotoxicity is poorly defined. Many studies have shown that pretreatment of rodents with LPS decreases hepatotoxicity resulting from subsequent acetaminophen administration. However, these studies used very high concentrations of exogenous LPS that may not reflect the physiology of endogenous LPS. Exogenous administration of LPS can decrease CYP levels, including those of CYP2E1. Less CYP2E1 activity results in decreased metabolism of acetaminophen to NAPQI, and, consequently, decreased hepatotoxicity. We measured the levels of the predominant CYP responsible for murine metabolism of acetaminophen to NAPQI, CYP2E1, and found no difference in CYP2E1 levels between LBP KO and wild-type mice. Thus, the protection in LBP KO mice does not appear to be due to LPS-mediated changes in CYP2E1 expression.

In conclusion, our studies show that LBP plays a role in modulating acetaminophen-induced liver injury that is associated with a decrease in intrahepatic cytokine production.

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References