Proinflammatory Cytokine Production in Liver Regeneration Is Myd88-Dependent, but Independent of Cd14, Tlr2, and Tlr4

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*J Immunol* 2006; 176:2522-2528; doi: 10.4049/jimmunol.176.4.2522
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Proinflammatory Cytokine Production in Liver Regeneration Is Myd88-Dependent, but Independent of Cd14, Tlr2, and Tlr4

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TNF and IL-6 are considered to be important to the initiation or priming phase of liver regeneration. However, the signaling pathways that lead to the production of these cytokines after partial hepatectomy (PH) have not been identified. Enteric-derived LPS appears to be important to liver regeneration, possibly by stimulating proinflammatory cytokine production after surgery. To determine whether LPS signaling pathways are involved in the regulation of the proinflammatory cytokines TNF and IL-6 during the priming phase of liver regeneration, we performed PH on mice lacking the TLRs Tlr4 and Tlr2, the LPS coreceptor, Cd14, and Myd88, an adapter protein involved in most TLR and IL-1R pathways. In Myd88 knockout (KO) mice after PH, both liver Tnf mRNA and circulating IL-6 levels were severely depressed compared with heterozygous or wild-type mice. Activation of STAT-3 and three STAT-3 responsive genes, Socs3, Cd14, and serum amyloid A2 were also blocked. In contrast, Tlr4, Tlr2, and Cd14 KO mice showed no deficits in the production of IL-6. Surprisingly, none of these KO mice showed any delay in hepatocyte replication. These data indicate that the LPS receptor TLR4, as well as TLR2 and CD14, do not play roles in regulating cytokine production or DNA replication after PH. In contrast, Myd88-dependent pathways appear to be responsible for TNF, IL-6, and their downstream signaling pathways. The Journal of Immunology, 2006, 176: 2522–2528.

Liver regeneration following resection or injury requires a multitude of signaling and endocrine pathways that are carefully coordinated to reconstitute liver mass (reviewed in Refs. 1 and 2). The initiation of liver regeneration has been termed the priming phase, in which normally quiescent hepatocytes gain proliferative competence and become responsive to hepatic growth factors. Hepatocyte growth factor, heparin binding-epidermal growth factor-like growth factor, and TGF-α stimulate cell cycle progression, leading to DNA replication and cell proliferation (1–3). One hallmark of the priming phase is the induction of immediate early genes such as the proto-oncogenes c-myc and c-jun, and of proinflammatory cytokines (4, 5). Shortly after partial hepatectomy (PH), both serum levels of TNF and hepatic Tnf mRNA increase, leading to activation of TNFR type I and subsequent activation of the transcription factor NF-κB in nonparenchymal cells in the liver (1, 2, 6–8). Active NF-κB, in part, induces IL-6 production, which results in the activation of STAT-3. Active hepatic STAT-3 induces a number of genes involved in regulating the cell cycle, the acute phase response, and the STAT-3 pathway itself, such as the suppressor of cytokine signaling 3 (Socs3) (9–11). Socs3 is part of a negative feedback loop that is rapidly induced after PH and blocks STAT-3 signaling (12, 13).

The mechanisms by which PH results in cytokine gene induction and transcription factor activation in the liver are unclear. A number of studies implicate enteric-derived LPS as the stimulating agent for proinflammatory cytokine production at the start of liver regeneration. Rats in which LPS has been depleted by antibiotic administration or other methods display impaired hepatocyte DNA synthesis following PH (14). Liver regeneration is also delayed in athymic nuclui mice and C3H/HeJ mice, a naturally occurring strain that is hyporesponsive to LPS (15, 16). Hepatocyte DNA synthesis is enhanced if these animals receive LPS 24 h before surgery (15, 17). In addition, Webber et al. (18) demonstrated that LPS primes hepatocytes for growth factor-induced replication in nonoperated rats.

LPS and other bacterial or viral by-products bind to a family of specific receptors, the TLRs, which regulate both innate and adaptive immunity (19–21). Studies using Tlr knockout (KO) mice and primary cell cultures from such mice have delineated the signaling pathways used by LPS (reviewed in Refs. 20–22). Circulating LPS forms complexes with LPS-binding protein and CD14, forming a ternary complex that binds to TLR4 on the surfaces of many different cell types, resulting in receptor dimerization and activation (23). Activation of TLR4 initiates both Myd88-dependent and -independent signaling cascades (20, 24–26). Broadly, the TLR4 signaling pathway and transcription factor activation in the liver are unclear. A number of studies implicate enteric-derived LPS as the stimulating agent for proinflammatory cytokine production at the start of liver regeneration. Rats in which LPS has been depleted by antibiotic administration or other methods display impaired hepatocyte DNA synthesis following PH (14). Liver regeneration is also delayed in athymic nuclui mice and C3H/HeJ mice, a naturally occurring strain that is hyporesponsive to LPS (15, 16). Hepatocyte DNA synthesis is enhanced if these animals receive LPS 24 h before surgery (15, 17). In addition, Webber et al. (18) demonstrated that LPS primes hepatocytes for growth factor-induced replication in nonoperated rats.

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Although TLRs and their signaling pathways are critical for the first line of defense against bacterial and viral infection, and as
reviewed above, there is suggestive evidence that LPS may trigger cytokine production to initiate liver regeneration, there is no direct evidence linking LPS and the TLRs to the initiation of liver regeneration. Using KO mice for Cd14, Tlr2, and Tlr4, we show that the lack of these receptors does not influence liver regeneration. By contrast, there is blockage of cytokine pathways after PH in Myd88 KO mice.

Materials and Methods

KO mice

Tlr2, Tlr4, and Myd88 KO mice were obtained from Dr. T. Hawn (Department of Medicine, University of Washington, Seattle, WA) and originally generated by Dr. S. Akira (Osaka University, Osaka, Japan) (24, 27, 28). Cd14 heterozygous mice (29) were purchased from The Jackson Laboratory. All mouse strains (C57BL/6) were initially housed in a specific pathogen-free facility with 12-h light/dark cycles with free access to standard food and water. After initial experiments were done, we found that all strains harbored Helicobacter species, which can induce low-level activation of the TLR pathway (30). We then generated Helicobacter-free mice by neonatal rederivation (31) using foster dams housed in a Helicobacter-free specific pathogen-free-room. DNA extracted from fecal samples was analyzed for Helicobacter hepaticus, Helicobacter bilis, and Helicobacter muridarum as described (32) and confirmed by the Research Animal Diagnostic Laboratory (RADIL) at the University of Missouri (Columbia, MO). All animal studies were conducted under approved Institutional Animal Care and Use Committee protocols at the University of Washington.

Surgery

PH resulting in removal of two-thirds of the liver was performed on 8- to 11-wk-old male KO, heterozygous (Het), and wild-type (WT) littermates. Surgeries were performed under inhalational isoflurane anesthesia in the morning (33) after a night of fasting, and consisted of midline laparotomy with separate ligation and removal of the left and anterior (median) lobes (7, 13, 34). Laparotomy with gentle liver manipulation was performed as a sham operation. At various times after surgery, mice were sacrificed by CO2 inhalation, and livers were harvested for analysis. BrdU (50 mg/kg) was given by i.p. injection 2 h before killing. In some cases, 3-day continuous osmotic pumps (Alzet; 1 μl/hour) containing BrdU (10 mg/ml) were implanted s.c. at the time of surgery and mice were killed 48 h later.

Histology and BrDU labeling

At the indicated time points, liver tissue was fixed in 10% neutral-buffered formalin or methacarn (60% methanol, 30% chloroform and 10% acetic acid; vol/vol/vol) (Fisher Scientific) overnight. BrdU immunohistochemistry was performed as described (37). Data are presented as the number of nuclear BrdU-labeled hepatocytes present in 30–40× fields (1.3 mm2; ~3000 hepatocytes). Mitotic figures were also counted in 30–40× fields.

Immunoblotting

Whole liver homogenates were prepared using 1% Triton X-100 lysis buffer and quantified using Bradford reagent with BSA as a standard. Fifty micrograms of total protein was subjected to SDS-PAGE and transferred to polyvinylidene difluoride membranes as described (13). After blocking with 5% milk, membranes were probed overnight with Abs that detect STAT-3 phosphorylated on tyrosine 705 (Cell Signaling; no. 9131). Protein-Ab complexes were detected using anti-rabbit HRP-conjugated secondary Ab (Amersham) and visualized with an ECL reagent (Pierce). To detect total STAT-3, membranes were first stripped with glycine stripping buffer (0.2 M glycine, 0.1% SDS, 1% Tween 20; pH 2.2), reblocked with milk and then probed with STAT-3 (Cell Signaling; no. 9132) and anti-rabbit HRP-conjugated secondary Ab. Autoradiographic film representing each phospho- and total STAT-3 blot was scanned and NIH Image analysis (version X; National Institutes of Health) was used to quantify the density of the appropriate bands. Immunoblot data are presented as the ratio of density of tyrosine 705 STAT-3 divided by the density of total STAT-3.

Electromobility shift assays

Liver tissue was snap-frozen at various time points after PH, and nuclear extracts were prepared using two different methods. For analysis of STAT-3, we used methods previously described (7, 13). Because NF-κB activation occurs predominately in nonparenchymal cells after PH (7, 8), we used a nuclear extract preparation method described by Zeini et al. (35) that lacks a sucrose gradient step and thus allows nuclear extracts to be made from all liver cell types. Protein concentration was determined by the Bradford method using BSA as a standard. Five micrograms of nuclear protein was incubated with the 32P-labeled oligonucleotide probe for NF-κB or STAT-3 for 30 min at room temperature, and samples were then subjected to electrophoresis through 5% polyacrylamide Tris-glucine-EDTA gel. Gels were then dried under a vacuum and exposed to Kodak X-AR film at ~80°C with intensifying screens. For supershift experiments, 1 μg of specific Abs to p50 NF-κB (sc-114X), p65 NF-κB (sc-372X), STAT-1 (C-Cruz Biotechnology), STAT-3 (C-20X), and STAT-3A (C-1X) (all from Santa Cruz Biotechnology) were used as described (7, 13). A nuclear extract prepared from a WT mouse 3 h after PH was run on every STAT-3 gel as a control. We used rabbit reticulocyte lysate (1 μg; Promega) as a control for NF-κB. The dried gels were used for Phosphorimager analysis (Storm; Molecular Dynamic) and the data are presented as the density of the NF-κB p50/p65 heterodimer and STAT-3 homodimer DNA-protein complexes divided by the density of the same bands in the control sample.

Determination of IL-6 in serum, and Tnf, Socs3, Cd14, and Saa2 mRNA in liver

Serum was obtained by cardiac puncture and IL-6 levels determined using an ELISA kit (BD Pharmingen) as described (7, 13). Liver RNA was prepared using TRIzol (Ambion) according to the manufacturer’s instructions and quantified by A260 measurement.Murine Tnf and Socs3 mRNA levels were determined by RT-PCR with specific primers: Tnf sense: 5′-AGT AGC ACA GAA AGC ATG ATC CGC GAC-3′, antisense: 5′-GAG ATA GCA AAT CGG CTG ACCG-3′; Socs3: sense: 5′-ACC TTC AGC TCG AAA GAG GAG TA-3′, antisense: 5′-GCA GCT GGG TCA TTG TCT CAT AG-3′. Two micrograms of RNA was reverse-transcribed, and the cDNA was then used as a template for a radioactive PCR. In all cases, PCR cycle analysis was first performed to determine the linear range of each amplification for a given primer pair. Tnf and Socs3 expression levels were normalized to that of β-actin (primer to competitor ratio of 2:8) as described in the Quantum RNA kit (Ambion). Cd14 and Saa2 mRNA were detected using murine cDNA probes (provided by Drs. M. Chaisson, Amgen, Seattle, WA, and O’Brien, University of Washington, Seattle, WA, respectively) using established Northern blotting procedures and normalized to cyclophilin (Ambion) (13). All radioactive amplification and detected bands in Northern blots were quantified using Phosphorimager analysis.

Statistical analysis

Statistical analysis was done by nonparametric analysis (Mann-Whitney or an unpaired t test with Welch’s correction). Data are presented as average ± SEM with the following symbol indicating the level of significance: p < 0.05. Statistical analysis was performed using GraphPad Prism software.

Results

Deficits in circulating levels of IL-6 and activation of STAT-3 signaling pathways in Myd88 KO mice after PH

Tlr2, Tlr4, Cd14 and Myd88 KO mice and macrophages obtained from these animals fail to produce TNF and IL-6 after LPS treatment (24, 27–29). We thus used Tlr2, Tlr4, Cd14, and Myd88 KO mice to determine whether the rapid increases in IL-6 and STAT-3 that occur after PH would be blocked in animals deficient in LPS signaling. Before performing experiments with KO mice, we ascertained that Het animals lacking a single copy of each of these genes showed no alterations in cytokine induction after PH when compared with WT littermates. For these reasons, both WT and Het mice were used as controls in subsequent experiments. As determined by ELISA, circulating levels of IL-6 determined at 4 h after PH were not affected in Tlr2, Tlr4, or Cd14 mice when compared with WT/Het mice. In marked contrast, only a small amount of IL-6 was detectable in Myd88 KO mice (Fig. 1A). An analysis of the time course of IL-6 production after PH revealed that IL-6 amounts transiently increased by 50-fold or more in WT/Het mice, whereas Myd88 KO mice lacked this induction (Fig. 1B).

IL-6 production after PH causes the activation STAT-3. In turn, this transcription factor induces a large number of genes, including those involved in hepatocyte growth and survival, and the acute
phase response (10, 11, 36). It also activates a feedback mechanism through SOCS-3 activity, which blocks further STAT-3 activation during liver regeneration (13). We used phosphoimmunoblotting of STAT-3 and EMSA to analyze whether the deficit in circulating IL-6 seen in Myd88 KO mice would result in a lack of activation of STAT-3 after PH (Fig. 2). Tyrosine 705 phosphorylated STAT-3 was 2- to 4-fold lower in the Myd88 KO mice compared with WT/Het mice at 2, 4, and 6 h after PH (Fig. 2, A and B). EMSA analysis confirmed deficient STAT-3 DNA binding in Myd88 KO mice after PH as well (Fig. 2, C and D).

We next determined whether levels of IL-6-STAT-3 target genes were affected by the decreased levels of circulating IL-6 and blunted STAT-3 activation in Myd88 KO mice. We performed RT-PCR on mRNA extracted from whole liver to evaluate Socs3 expression after PH in Myd88 KO mice. WT/Het mice showed a 7-fold induction of Socs3 mRNA at 4 h after PH, while Myd88 KO animals showed significantly less induction of Socs3 (Fig. 3A). Interestingly, at 2 h after PH, when there is only a 3-fold induction of Socs3 mRNA in WT/Het mice, there were no differences between WT/Het, Myd88 KO, and sham-operated mice, suggesting that there may be multiple pathways capable of inducing Socs3 in the liver. We next determined whether levels of Cd14 and Saa2 mRNA, two acute phase response genes, were also blunted in Myd88 KO mice, because the expression of Cd14 and Saa2 has previously been shown to be dependent on IL-6 (10, 37, 38). The levels of Cd14 and Saa2 mRNA were elevated 2- to 5-fold in WT/Het mice at 6 and 8 h after PH, but there was little to no induction of these genes in Myd88 KO mice (Fig. 3, B and C). Taken together, these data indicate that there is a block in IL-6 production in Myd88 KO mice after PH with a subsequent deficit in IL-6-signaling pathways. Importantly, there were no deficits in circulating levels of IL-6 in Cd14, Tlr2, and Tlr4 KO mice compared with WT/Het mice after PH.

FIGURE 1. Serum IL-6 levels are decreased after PH in Myd88 KO mice, but not in Tlr2, Tlr4, or Cd14 KO mice. PH or sham operations were performed on Myd88, Tlr2, Tlr4, and Cd14 KO mice with WT/Het littermates used as controls. Serum was collected at the indicated time points. A, IL-6 serum levels were measured at 4 h after PH in all strains using a specific ELISA. Data are represented as picograms per milliliter ± SEM. B, Time course of IL-6 secretion in Myd88 KO mice. *, p = 0.05 for KO vs WT/Het mice.

FIGURE 2. Phosphorylation and activation of STAT-3 is blocked in Myd88 KO mice after PH. Liver tissue was collected from Myd88 KO mice and WT/Het littermates at the indicated time points after PH. A, Phosphorylation of STAT-3 on tyrosine 705 (top blot) and total STAT-3 levels (bottom blot). B, Quantification of phosphorylated STAT-3 normalized to total STAT-3. C, STAT-3 EMSA. arrow indicates STAT-3 homodimer. D, Phosphoimager quantification of STAT-3 homodimer normalized to control sample. Data are presented as the average ± SEM for four to six mice per time point per strain; *, p = 0.05 for KO vs WT/Het mice.
MyD88 KO mice have a delay in NF-κB activation and a failure to induce TNF after PH

Shortly after PH, there is an increase in circulating TNF levels as well as increases in hepatic mRNA levels, which coincide with NF-κB activation (1, 2). To determine whether TNF expression is altered in Myd88 KO mice after PH, we measured Tnf mRNA levels by RT-PCR. TNF expression increased at 30 min after PH in WT/Het mice, while there was no induction in Myd88 KO mice (Fig. 4A). Tnf mRNA levels did not increase in Myd88 KO mice even as late as 8 h after PH, suggesting that a compensatory rebound from this deficit does not occur (Fig. 4A). In contrast to Myd88 KO mice, induction of liver Tnf mRNA was seen in Tlr4 KO mice to a similar level observed in WT/Het littermates (data not shown). We were unable to detect TNF protein in serum or liver lysates after PH in either control or Myd88 KO mice. We next determined whether the deficit in Tnf mRNA induction in Myd88 KO mice would have an effect on NF-κB activation. In agreement with previous work (6), NF-κB was activated at 30 min after PH in WT/Het mice, but not in Myd88 KO mice (Fig. 4, B and C). However, at 1 h after PH, Myd88 KO mice showed a recovery in NF-κB activation. Thus, there is a delay rather than an overall loss of NF-κB activation after PH in Myd88 KO mice, despite the block in Tnf induction.

Normal hepatocyte proliferation in Cd14, Tlr2, Tlr4, and Myd88 KO mice after PH

To determine whether hepatocyte DNA replication after PH might be altered in Myd88 KO mice, we injected BrdU 2 h before harvesting the livers at 24–72 h after surgery. Surprisingly, there was no statistical difference in BrdU incorporation in Myd88 KO mice compared with WT/Het littermates at any of the time points examined (Fig. 5). These results suggest that the deficits in cytokine signaling present in Myd88 KO mice are not sufficient to alter hepatocyte DNA replication.

Recently, Mandell et al. (30) demonstrated that infection with Helicobacter species results in baseline activation of TLR pathways. We wondered whether the lack of effect of LPS signaling on hepatocyte DNA replication might be due to baseline activation of the innate immune response by this rodent pathogen. We performed Helicobacter genus PCR on fecal samples from mice in all four of our KO colonies. Each KO strain was infected with H. hepaticus and H. bilis (data not shown). To remove the potential confounding effect of Helicobacter infection on the role of TLR
FIGURE 5. Normal hepatocyte DNA replication in Myd88 KO mice. PH was performed on Myd88 KO and WT/Het mice, and liver tissue was collected and fixed as described in Materials and Methods. Nuclear BrdU labeling of hepatocytes was counted, and the data are presented as the average ± SEM for four to six mice per time point per strain; *, p ≤ 0.05 for KO vs WT/Het mice.

Discussion

IL-6 and Tnf expression increases within the first 1–2 h after PH, leading to the activation of pathways that involve NF-κB and STAT-3. At 6–12 h after the operation, IL-6 production and STAT-3 activation are terminated by the induction of Socs3. This transient mechanism of cytokine expression allows the activation of multiple target genes to initiate liver regeneration, while preventing cytokine toxicity (1, 2). Although these pathways have been described in detail, there is great uncertainty about the mechanisms that lead to their activation. It has been hypothesized for many years that LPS is the main agent responsible for the activation of cytokine pathways at the start of liver regeneration. This hypothesis is based on several observations, which include the delay in liver regeneration associated with procedures that presumably decrease LPS release from the gut, and the delay that occurs in C3H/HeJ mice, a LPS hyporesponsive strain that carries a point mutation in Tlr4 (14–17, 39). Moreover, the demonstration that liver regeneration is defective in mice lacking C3 and C5 is compatible with the notion that LPS release may trigger the activation of cytokine cascades by enhancing the production of complement proteins (40). Despite this compelling evidence, the potential role of LPS in the initiation of liver regeneration has not been analyzed by direct experiments in which LPS signaling is specifically blocked. In the present work, we used mice that are deficient in Tlr2, Tlr4, Cdl4, and Myd88 to determine the relevance of LPS signaling in the activation of cytokine pathways at the start of liver regeneration.

In Myd88 KO mice, a deficit in IL-6 production was associated with inhibition of STAT-3 activation and the loss of induction of three target genes, Socs3, Cdl4, and Saa2. Myd88 KO mice also had delayed activation of NF-κB and no induction of Tnf mRNA shortly after PH. Surprisingly, IL-6 secretion after PH was not altered by the lack of Tlr4, Tlr2, and Cdl4. It is puzzling that loss of Myd88 created multiple defects in these cytokine pathways while the lack of Tlr4 and Tlr2 had no effect on cytokine activation in the regenerating liver. Myd88 functions as an adapter protein for several TLRs, and also for the IL-1 and IL-18 receptors. It is possible that Myd88 signaling in the regenerating liver depends on IL-1 and IL-18 rather than LPS. However, a number of experiments in hepatocyte cultures suggest that IL-1β inhibits hepatocyte replication (41, 42), while IL-18 expression has been associated with liver injury rather than cell proliferation (43, 44). IL-18 is also known as IFN-γ-inducing factor, and recently we and others have demonstrated that IFN-γ inhibits hepatocyte proliferation and liver regeneration (45, 46). Thus, it is unlikely that either IL-1 or IL-18 is responsible for the Myd88 signaling that results in cytokine activation at the start of liver regeneration, but experiments with IL-1 and IL-18 KO mice are needed to solidify this conclusion. Our data also suggest that complement activation after PH may be independent of LPS receptors, although no experiments were conducted to specifically examine this issue. An intriguing possibility is that signaling by some complement proteins might involve Myd88.

Our data from the TLR4 KO mice are particularly surprising in light of published data demonstrating a delay in liver regeneration in C3H/HeJ mice, an inbred strain of mice with a point mutation in Tlr4 (i.e., Tlr4P712H) (15, 16). Our findings, which are consistent with recent studies by Seki et al. (58), suggest that a point mutation in Tlr4 has more profound effects on liver regeneration than the loss of the entire receptor. A number of possible mechanisms may exist for these seemingly contradictory results including the following: 1) other, non-TLR4, LPS receptors may exist (47, 48); 2) TLR4P712H appears to act as a dominant-negative receptor (49, 50); and 3) the TLR4 allele appears to be subject to tissue specific monoallelic expression (51). Alternatively, a possible key to this puzzle might lie in the hyporesponsive nature of C3H/HeJ mice after LPS injection. Sultzter (52) demonstrated that in C3H/HeJ mice, there was a bias toward the migration of mononuclear cells (e.g., lymphocytes and macrophages) after LPS injection, rather than neutrophils. This observation suggests that a different cytokine and chemokine milieu is present in C3H/HeJ mice after LPS injection through an osmotic pump implanted at the time of operation in CD14, Tlr4, Tlr2, and Myd88 KO mice that are deficient in these cytokine pathways at the start of liver regeneration. This transient mechanism of cytokine expression allows the activation of multiple target genes to initiate liver regeneration, while preventing cytokine toxicity. It is possible that Myd88 signaling in the regenerating liver depends on IL-1 and IL-18 rather than LPS. However, a number of experiments in hepatocyte cultures suggest that IL-1β inhibits hepatocyte replication, while IL-18 expression has been associated with liver injury rather than cell proliferation. IL-18 is also known as IFN-γ-inducing factor, and recently we and others have demonstrated that IFN-γ inhibits hepatocyte proliferation and liver regeneration. Thus, it is unlikely that either IL-1 or IL-18 is responsible for the Myd88 signaling that results in cytokine activation at the start of liver regeneration, but experiments with IL-1 and IL-18 KO mice are needed to solidify this conclusion. Our data also suggest that complement activation after PH may be independent of LPS receptors, although no experiments were conducted to specifically examine this issue. An intriguing possibility is that signaling by some complement proteins might involve Myd88.

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Table I. Hepatocyte proliferation after PH in CD14, Tlr4, Tlr2, and Myd88 KO mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>BrdU Incorporationa</th>
<th>Mitotic Figuresb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>het/wt</td>
<td>KO</td>
</tr>
<tr>
<td>CD14</td>
<td>761 ± 73.74</td>
<td>682.5 ± 121.6</td>
</tr>
<tr>
<td>TLR4</td>
<td>761 ± 73.74</td>
<td>750 ± 159.2</td>
</tr>
<tr>
<td>TLR2</td>
<td>697 ± 121.4</td>
<td>916 ± 205.3</td>
</tr>
<tr>
<td>MyD88</td>
<td>650 ± 100.2</td>
<td>625 ± 80.4</td>
</tr>
</tbody>
</table>

a Per 3000 cells, at 38 h for TLR4 and Myd88 KOs; 48 h for CD14 and TLR2 KOs.
b Per 3000 cells, at 48 h for all strains.
treatment compared with LPS-sensitive mouse strains. Although the importance of leukocytes in liver regeneration (53) is accepted, the impact of a proposed difference in composition of liver leukocytes in C3H/HeJ mice on liver regeneration is unknown. Clearly, any of these hypothetical possibilities would need in vivo experimentation to understand the potency of the mutant TLR4 allele in liver regeneration (15, 16). In any case, the data presented here strongly suggest that TLR2 and TLR4 are not involved in the initiation of liver regeneration. Our results do not exclude the possibility that other members of TLR family (e.g., TLR3, TLR5, TLR9, etc.) may be involved in liver regeneration.

Another surprising finding in our studies was that Myd88 KO mice did not have any defects in hepatocyte replication despite the lack of IL-6 production, absence of Tnf mRNA induction, and delayed NF-κB activation during the first 12 h after PH. Previous studies conducted with IL-6 KO mice indicated that IL-6 is essential for liver regeneration (9), although recent data suggests that IL-6 may be involved in hepatocyte survival rather than proliferation (54–57). We, however, did not see a difference in survival rates between Myd88 KO and WT mice at 7 days after PH (data not shown). The proliferative effects of IL-6 have undergone further scrutiny, as some groups have reported that IL-6 KO mice and mice deficient in gp130 (a necessary component of the receptor complex for the IL-6 ligand family) did not have dramatic defects in DNA replication after PH (36, 54–57). Our results with Myd88 KO mice suggest that a defect in IL-6 production does not affect liver regeneration in these animals.

Although we observed a lack of Tnf mRNA induction in Myd88 KO mice, we were unable to see any change in TNF protein in liver tissue or in the serum immediately after PH in either KO mice, we were unable to see any change in TNF protein in liver regeneration irrespective of the specific TNF superfamily ligand that binds and activates this receptor.

In contrast to our findings, Seki et al. (58) found that in addition to deficits in IL-6, Tnf and proto-oncogenes, DNA replication was also blocked in Myd88 KO mice after PH. It should be pointed out that in the work of Seki et al. (58), despite the observed deficit in hepatocyte DNA replication, Myd88 KO mice did eventually regenerate their livers as indicated by liver weight to body ratios. We currently do not know how to reconcile their findings with our own studies, in which we used identical strain of KO mice. It is possible that different surgical techniques, anesthetics used, or status of enteric pathogens contributed to a different outcome in the Seki study. Here, we tested two of these possibilities. Neither the re-derivation of Myd88 KO mice to remove Helicobacter nor the use of two different surgeons, who performed the PH using slightly different surgical techniques on Myd88 KO mice, altered our results with DNA replication (data not shown).

A consistent finding, however, was that none of the single or double Tlr KO mice tested (i.e., Tlr2, Tlr4, Tlr9, or Tlr2/Tlr4) or Cd14 KO mice used in either study had any defects in either cytokine signaling or liver regeneration. Other types of liver injury appear to require TLRs. For example, TLR4 and IRF-3 but not MyD88 are important in hepatic damage and inflammation after ischemia-reperfusion injury (73). Thus, to more fully understand the role of the receptors that control innate immunity, it would be of interest to determine which TLR is responsible for MyD88-dependent activation of cytokine pathways and whether there is TLR redundancy in liver regeneration.

Acknowledgments
We thank Drs. Shizuo Akira and Thomas Hawn for providing TLR-2, TLR-4, and MyD88 KO mice; Melissa Odell, Mary Nivison, and Vicki Hoagland for technical support; Andy Hieb for assistance with graphics; Dr. Tony Parks for helpful discussions; and Dr. Lillian Price and Shelby Henderson for participation in rederivation.

Disclosures
The authors have no financial conflict of interest.

References


