Inflammatory response in obstructive jaundice and peritonitis
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Interleukin-1 receptor type I gene-deficient bile duct ligated mice are partially protected against endotoxin

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Cholestatic liver injury is associated with an increased susceptibility toward endotoxin-induced toxicity. To determine the role of interleukin (IL-1) herein, extrahepatic cholestasis was induced by bile duct ligation (BDL) in IL-1 receptor type I-gene deficient (IL-1R-/-) mice, which are unresponsive to IL-1α and IL-1β, and normal IL-1R+ + mice. BDL elicited increases in hepatic IL-1α and IL-1β mRNA and protein. Hepatocellular injury at 2 weeks after BDL was similar in IL-1R-/- and IL-1R+ + mice as demonstrated by clinical chemistry and histopathology. Administration of endotoxin to cholestatic mice at 2 weeks after BDL was associated with enhanced cytokine release, more severe liver damage, and occurrence of death when compared to sham operated mice. Endotoxin effects in sham operated IL-1R-/- and IL-1R+ + mice were largely similar, but cholestatic IL-1R-/- mice were better protected against toxic effects of endotoxin, as reflected by lowered cytokine release, less profound liver injury, and reduced mortality. These data indicate that IL-1α and IL-1β are produced in the liver after BDL, but that these cytokines do not play a significant role in cholestatic liver damage; however, endogenous IL-1 activity is an important denominator of enhanced endotoxin sensitivity that is observed during cholestasis induced by BDL.

INTRODUCTION

Surgery in jaundiced patients with periumbilical tumors carries an increased risk of postoperative complications. Whereas in experienced centers, postoperative mortality has been reduced from 20 to 5%, morbidity remains as high as 50%. Most complications have a septic etiology and are considered to be related with translocation of endotoxin from the intestinal lumen into the portal and systemic circulation where an inflammatory cascade is triggered. Potential causes of translocation of endotoxin include lack of bile salts in the intestinal lumen, resulting in increased bacterial translocation, decreased function of the resident macrophages of the liver, the Kupffer cells, leading to inadequate interception of endotoxin, and changes in plasma concentrations of lipoproteins which bind endotoxin. In addition, cholestasis is associated with enhanced susceptibility toward endotoxin-induced toxicity.

Endotoxin exerts its biological effects by activating immunocompetent cells such as monocytes and macrophages. After exposure to endotoxin, these cells readily release various inflammatory mediators such as the cytokines tumor necrosis factor (TNF) and interleukin-1 (IL-1), which leads to an inflammatory response. The Kupffer cells are also capable of releasing these cytokines in response to endotoxin. IL-1α and IL-1β are pleiotropic proinflammatory cytokines that are considered to play a proximal role in initiation and regulation of the inflammatory cascade triggered in response to endotoxins or bacteremia. Both IL-1α and IL-1β can interact with two specific cell surface receptors, the type I and type II IL-1 receptor (IL-1R), of which only the former is involved in intracellular signaling of IL-1 effects. As a consequence, mice that lack the gene encoding the type I IL-1R (IL-1R-/- mice) can not respond to IL-1α or IL-1β.

The role of IL-1 in pathophysiological consequences of cholestasis and the ensuing increased
susceptibility to septic complications is still not understood. Therefore, we used the model of extrapheatic cholestasis induced by bile duct ligation (bdl) in IL-1R/-/- mice to study whether (a) IL-1 contributes to hepatic pathology associated with cholestasis, and (b) endogenous IL-1 is involved in the enhanced susceptibility to endotoxin that accompanies cholestasis.

MATERIALS AND METHODS

Animals
IL-1R/-/- mice backcrossed 6 times to a C57BL/6 background (kindly provided by Immunex, Seattle, WA) and their normal C57BL/6 wild type counterpart (IL-1R+/+, Harlan, Zeist, The Netherlands) were used. Each experimental group consisted of 8-12 mice (sex and age matched; 8-10 weeks old) per time point. IL-1R/-/- mice were normal in size, wt and fertility, and displayed no abnormalities in leukocyte subsets. In addition, IL-1R/-/- mice have normal serum Ig levels and generate normal levels of primary and secondary Abs. All mice were housed in the same animal room, maintained under a 12-hour light/dark cycle, and provided regular mouse chow (SRM-A; Hope Farms, Woerden, The Netherlands) and water ad libitum.

Surgical procedures
The Institutional Animal Care and Use Committee of the Academic Medical Center, Amsterdam, the Netherlands approved the experiments. After 5 days of acclimatization, surgery was performed under sterile conditions (t = 0). Mice were divided randomly into a sham-operated (sham control) group and a bile duct ligated (bdl) group. They were anaesthetized by intraperitoneal injection of 0.07 ml/kg FFM mixture (Fentanyl 0.315 mg/ml), Flumison (10 mg/ml) (both Janssen, Beersen, Belgium), and Midazolam (5 mg/ml) (Roche, Mijdrecht, The Netherlands). A midabdominal incision was made, and the common bile duct was ligated and divided as previously described. In mice undergoing sham operation, the bile duct was identified and ligatures were placed (but not tied) and removed again. Postoperative analgesia was achieved with subcutaneous injection of 0.1 μl/kg Temgesic (Reckitt, Kingston-Upon-Hill, United Kingdom).

Materials
Endotoxin, lipopolysaccharide E. coli serotype O111: B4, was purchased as a lyophilized powder from Sigma (St. Louis, MO), resuspended in a 0.5 ml sterile pyrogen-free isotonic saline and injected intraperitoneally (4 mg/kg). Control mice received sterile pyrogen-free isotonic saline.

Blood sampling and organ preparation
At the designated time points, mice were anaesthetized by intraperitoneal FFM (0.07 ml/kg) anesthesia and blood was drawn from the inferior caval vein into a sterile syringe. One part of the blood was transferred to tubes containing heparin, and plasma was prepared by centrifugation (3000 g, 20 min, 4°C) for measurement of levels of cytokines, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and γ-glutamyl transferase (γ-GT). All samples were aliquoted and stored immediately at -20°C until analysis.

Livers were immediately dissected and homogenized at 4°C in 4 volumes of sterile saline using a tissue homogenizer.
Endotoxemia in IL-1R/-/- cholestatic mice

Liver homogenates were diluted 1:2 in lysis buffer containing 300 mM NaCl, 30 mM Tris, 2 mM MgCl2, 1% Triton X-100, and pepstatin A, leupeptin and aprotonin at 20 ng/ml; pH 7.4; Gibco/BRL, Berlin, Germany) and incubated at 4 °C for 30 min. Homogenates were centrifuged at 190 x g at 4 °C for 10 min to remove cell debris, after which supernatants were stored at -20 °C.

RNA preparation and reverse transcriptase PCR

Tissue samples were homogenized in 1 ml of Trizol (Gibco) according to the manufacturer's instructions and total RNA was isolated using chloroform extraction and isopropanol precipitation. RNA was dissolved in diethyl pyrocarbonate-treated water and quantified by spectrophotometry. cDNA was synthesized by mixing 2 μg of RNA with 0.5 μg oligo-dT (Gibco) and by incubating the solution total volume 12 μL for 10 min at 72 °C. Subsequently, 8 μL of a solution containing 5× first strand buffer (Gibco), 1.25 mM each of dNTPs (Amersham Pharmacia, Amersham, UK), 10 mM DTT (Gibco), and the SuperScript Pre-amplification system (Gibco) was added, and the final solution was incubated for 60 min at 37 °C. For RT-PCR of IL-1α and IL-1β mRNA, equivalent amounts of cDNA (5 μL) were amplified using a solution (20 μL) containing 4× DMSO, 12.5 μg BSA (Dako, Detroit, MI), 1.25 mM of each dNTPs, 10× PCR buffer (0.67 M Tris-HCl pH 8.8), 67 mM MgCl2, 0.1 M β-mercaptoethanol, 67 mM EDTA, 0.166 M (NH4)2SO4, 0.5 U of AmpliTaq DNA polymerase (Perkin Elmer, Branchburg, NJ), and the forward (F) and the reverse (R) primers (100 μM each).

The PCR reactions were carried out in the thermocycler Gene Amap® PCR System 9700 (Perkin Elmer, Norwalk, CT). For IL-1α, IL-1β, and β-actin mRNAs, the number of cycles that resulted in linear amplification was determined (data not shown). Cycling conditions for PCR amplification of IL-1α, IL-1β, and β-actin mRNAs were: 94 °C for 5 min (1 cycle), before 30 (IL-1α), 30 (IL-1β) or 24 (β-actin) cycles of 95 °C for 60 s, 55 °C for 60 s and 72 °C for 60 s followed by a final extension phase at 72 °C for 10 min. The primers used were: IL-1α: (F): 5'-CTCAGACCCCATGCTCAGA-3' (R): 5'-TGGGGATCCAGGGGAAACACTG-3'; IL-1β: (F): 5'-TCATGGGATGATGAAACCTGCT-3' (R): 5'-CCTACTATTTAGGAAGACGGAT-3'; β-actin. (F): 5'-GTCAGAAGGCTCTCCTATG-3' (R): 5'-GCTGCGTGCCATATGTGATG-3'. The PCR products were separated on 1.5% agarose gels containing 0.5 x TBE (50 mM Tris, 45 mM boric acid, 0.5 mM EDTA, pH 8.3) and 0.5 μg/ml ethidium bromide.

Assays

Total plasma bilirubin, AST, ALT, alkaline phosphatase, and γ-GT were determined with commercially available kits (Sigma), using a Hitachi analyzer (Boehringer, Mannheim, Germany) according to the manufacturer’s instructions. Cytokines were measured in duplicate by ELISA according to the instructions of the manufacturer (with detection limits in pg/ml): IL-1α (82), IL-1β (156), TNF-α (31.3) (all from R&D Systems, Abingdon, UK) and IL-6 (9.8) (Pharmingen, San Diego, CA).

Histology and histochemistry

Samples from each liver lobe, and other parenchymal organs, were removed rapidly after killing the animals, fixed in 4% formaldehyde, and embedded in paraffin for routine histology (3.5 μm thick sections, stained with haematoxylin-eosin (H&E). Histologic examination was performed on coded samples by 2 independent investigators, blinded for treatment groups and cholestatic parameters. Five histopathologic features were scored semi-quantitatively in 4 grades of
severity: portal inflammation, ductular proliferation, fibrosis, mitosis and necrosis. The 5 features were equally weighted and summed. This scoring has been described previously. Periportal and pericentral regions of the liver lobe were compared, with the latter defined as concentric layers of 5 hepatocytes surrounding the terminal branches of the hepatic vein. For each liver lobe, 12 sections were used and 3 periportal and pericentral zones were randomly selected in each section.

For endotoxin-induced liver injury, 2 parameters were scored: the area of necrosis and hepatic neutrophil sequestration. Neutrophils were stained with the chloroacetate esterase technique. Neutrophils were identified on the basis of positive staining and morphology and were counted in 50 high-power fields (400x). Images of the sections were captured using a Vanox-T microscope (Olympus, Tokyo, Japan) attached to a charge-coupled-device camera with an 8-bit resolution (Cohu, San Diego, CA), a frame grabber (L.G.3, Scion, Frederick, MD), and a Power Macintosh 8100/110 computer (Apple, Cupertino, CA) using the public domain NIH Imaging-software program (version 1.57; written by Wayne Rasband at the National Institutes of Health and available via internet from http://rsb.info.nih.gov). Settings of the camera and frame grabber were adjusted according to Jonker et al. Cell damage was quantified in parallel sections stained with H&E by measuring pixel intensities by blinded investigators. The percentage of necrosis was calculated by evaluating the number of microscopic fields with necrosis compared with the entire histological section x (100) SE. Quantitative analysis with the use of digital microscopy was performed as described by Jonker et al.

Statistical analysis

Statistics were performed using the SPSS® Base 9.0 for Windows® Statistical Software Package (SPSS, Chicago, IL). All results are given as mean ± SE. Survival curves were compared with the log-rank test. Differences in biochemical, immunological, immunohistochemical and histological data were analyzed using the Mann-Whitney U test. Changes in time were analyzed by one-way analysis of variance. Differences at indicated time points were assessed by Dunnett’s t-test for multiple comparisons where appropriate. A 2-tailed p value < 0.05 was considered significant.

RESULTS

Role of IL-1 in cholestatic liver injury

RT-PCR was performed on liver homogenates to determine whether IL-6 is produced after bdl. IL-6 mRNA was detectable in livers of IL-6+/+ mice after induction of extrahepatic cholestasis and in sham-operated animals (Fig. 1). However, mRNA levels were distinctly higher in the bdl group. Similarly, IL-6 protein levels were higher in liver and plasma of bdl IL-6+/+ mice (Fig. 2).

Neither sham surgery nor bdl were associated with mortality during 1-month follow-up. Bdl mice became slightly ill (ruffled fur, anorexia) from 3 weeks onwards, but mortality did not occur up to 4 weeks after bdl, and differences between both mouse strains were not observed. Bdl mice showed biochemical evidence of hepatocellular injury as reflected by elevated plasma levels of ALT and AST, and significant cholestasis as reflected by elevated plasma levels of bilirubin, alkaline phosphatase, and γ-GT levels (Fig. 3). However, significant differences between IL-6+/+ and IL-6−/− mice were not found. Sham operation did not affect normal liver parenchyma

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of IL-6+/+ and IL-6-/− mice up to 14 days after operation as determined by histopathological examination (Fig. 4). In contrast, bdl lead to typical cholestatic changes in liver parenchyma which were similar in both mice strains (Fig. 4).

In conclusion, our data demonstrate that IL-6 was produced in the liver after bdl, but did not play an important role in cholestatic liver injury.

Bdl IL-6-/− mice are protected against endotoxin induced lethality

To determine the role of endogenously produced IL-6 in the immune response to endotoxin, IL-16+/+ and IL-6-/− mice were challenged with endotoxin at 2 weeks after bdl or sham operation and monitored during 24 h. All mice that survived the endotoxin challenge during the first 24 h proved to be permanent survivors. As shown in Fig. 5, during the first 6 h after endotoxin challenge, lethality was not observed in any group. Moreover, endotoxin challenge in sham operated mice was not associated with mortality at all at the endotoxin dose given. However, after bdl, both mouse strains demonstrated significant mortality after administration of endotoxin (both p < 0.05 vs. sham). Importantly, a significant difference was found in mortality between both mouse strains. A 75% mortality was observed in IL-6-/− mice and only 44% mortality in IL-6+/+ mice (p = 0.014).

Increased production of proinflammatory cytokines in bdl IL-6-/− mice after endotoxin injection

IL-6+/+ and IL-6-/− mice were challenged intraperitoneally with endotoxin at 2 weeks after sham surgery or bdl, and IL-1α, IL-1β, IL-6, and TNF levels were measured in plasma at different time intervals during the first 6 h, i.e. before any animal died. In response to endotoxin, proinflammatory cytokine levels were significantly higher in bdl than in sham mice (all p values < 0.05; Fig. 6). Compared with endotoxemic sham mice, IL-1α levels were 20-fold higher, IL-1β levels were up to 40-fold higher, IL-6 levels were 4-fold higher, and TNF levels were 10-fold
higher in endotoxemic cholestatic mice.
Overall, IL-6-/− mice demonstrated a relatively higher release of cytokines upon exposure to endotoxin as compared with IL-6+/+ mice. The difference in cytokine release between the 2 mouse strains was especially apparent in bdl mice (Fig. 6).

Endotoxin induced liver damage is increased in bdl IL-6-/− mice
Endotoxin challenge resulted in influx of neutrophils in the liver, development of hepatocellular necrosis, and an increase in plasma ALT levels (Table 1; Figs. 7, 8), which were more pronounced in bdl mice as compared with sham mice \( (p < 0.001 \) for all responses). After bdl and subsequent challenge to endotoxin, neutrophil influx, hepatocellular necrosis, and the rise in plasma ALT levels were all significantly more pronounced in IL-6-/− than in IL-6+/+ mice \( (p < 0.05 \) for all responses), whereas in sham animals these responses were not significantly different in IL-6+/+ and in IL-6-/− mice.

![Figure 2](image-url)

**Figure 2.** Mean ± SE levels of IL-1α (A) and IL-1β (B) in livers of IL-1R−/− and IL-1R+/+ mice after bile duct ligation (bdl) or sham operation \( ( n = 8 ) \). *P* values indicate differences between bdl and sham operated mice (either IL-1R−/− or IL-1R+/+). Differences between IL-1R−/− and IL-1R+/+ mice within bdl and sham operated groups were not significant.
DISCUSSION

Surgical procedures in patients with obstructive jaundice are more frequently associated with complications than similar operations in patients without biliary obstruction, including the occurrence of sepsis syndrome. Endotoxin has been implicated as an etiologic factor in such complications in jaundiced patients. In the present study, we sought to determine the role of endogenous IL-1 in the development of hepatic inflammation and injury following biliary obstruction, and in the increased susceptibility toward endotoxin-induced toxicity associated with
obstructive jaundice. The main findings of our study were that although IL-1 does not play an important role in hepatic pathology resulting from biliary obstruction, this cytokine contributes to a significant extent to the pathogenesis of endotoxin-induced liver injury and death in jaundiced mice.

IL-1α and IL-1β are encoded by separate genes. Both IL-1 forms can interact with 2 types of receptors. Type I IL-1R is expressed on virtually all cell types and is responsible for transducing cellular effects of IL-1α and IL-1β. In contrast, biological effects generated by IL-1α or IL-1β are thus far not known to be mediated by type II IL-1R, which has led to the concept that this receptor type is inactive and serves as a negative regulator of IL-1 activity. Therefore, mice that lack type I IL-1R are functionally deficient for both IL-1α and IL-1β responses. As far as we know, the present study is the first to examine the role of IL-1 during inflammation during biliary obstruction.

We first documented that both IL-1α and IL-1β are produced in the liver after biliary obstruction. Hepatic IL-1 concentrations were already markedly elevated at 1 wk after bdl and did not
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Figure 5. Endotoxin-induced lethality in bdl and sham IL-1R+/+ and IL-1R−/− mice. Sham IL-1R+/+ (■, n = 12), sham IL-1R−/− (○, n = 12), bdl IL-1R+/+ (●, n = 16), and bdl IL-1R−/− (○, n = 16) mice were injected with endotoxin (4 μg/kg, i.p.), and survival was assessed every hour during 24 h.

increase further during the second wk of obstruction of the bile duct. We then evaluated the role of this endogenously produced IL-1 in hepatic inflammation and injury during bile duct obstruction by comparing several parameters in IL-1R+/+ and IL-1R−/− mice. Each parameter analysed, i.e. mortality, levels of transaminases, alkaline phosphatase, and γ-GT in plasma and histology of the liver proved to be similar in both mouse strains. Hence, neither IL-1α nor IL-1β appears to influence pathological consequences of biliary obstruction in the liver. It should be noted, however, that at least some extrahepatic effects of IL-1 may be exaggerated in jaundiced animals. Indeed, an increased responsiveness of the central nervous system to IL-1β has been implicated in “sickness behavior” that is commonly observed in patients with cholestatic liver disease, characterized by lethargy, fatigue and malaise. This notion is derived from experiments in cholestatic rats, in which infusion of IL-1β into the central nervous system caused a marked reduction in their activity, whereas such decline was not observed in control rats. However, in another investigation, it was found that the febrile response to IL-1β was unaltered in cholestatic rats. Recently, IL-6 was reported to be produced in livers of mice subjected to bdl, which is in line with earlier reports of elevated plasma levels of IL-6 in mice with experimentally induced obstructive jaundice. Similar to the absence of differences between IL-1R+/+ and IL-1R−/− mice as described here, IL-6 gene-deficient mice did not demonstrate clear phenotypic differences after bdl when compared to wild type mice. Experimentally induced obstruction of the bile duct was associated with a profoundly increased susceptibility towards endotoxin, as indicated by the occurrence of mortality after administration of endotoxin in a dose that did not cause mortality, enhanced cytokine release or more severe liver damage in sham-operated mice.
Figure 6. Plasma cytokine concentrations after injection of endotoxin in sham IL-1R+/+ (■) and IL-1R-/- (○) mice and in bile duct ligated (bdl) IL-1R+/+ (●) and IL-1R-/- (○) mice. IL-1R+/+ and IL-1R-/- mice were treated with endotoxin (4 μg/kg, i.p.), and plasma samples were obtained at the time points indicated. IL-1α (A, B), IL-1β (C, D), IL-6 (E, F), and TNF (G, H) concentrations were determined by ELISA. Each value represents the mean ± SE of 8 mice. Absence of error bars for a given data point in this and subsequent figures indicates that SE is smaller than size of symbol. Note the differences in scale of the y-axes for sham and bdl mice. * P < 0.05 vs. IL-1R+/+ mice.
These data confirm and extend previous studies in bdl rodents. In earlier studies in rodents, we showed that endotoxin sensitivity is increased after bdl, and rises further when endotoxin transport to the cholestatic liver is increased by binding with (reconstituted) HDL. Humans with biliary obstruction also demonstrate evidence for enhanced susceptibility to endotoxin. Possible causes for this phenomenon include alterations in circulating levels of endotoxin-binding proteins, a general activation state of neutrophils, and the fact that biliary obstruction influences the reticuloendothelial system in the liver as well as extrahepatic phagocytic cells.

Although IL-1R/- mice were indistinguishable from IL-1R+/+ mice with respect to their response to bdl, they were partially protected against the effects of systemic endotoxin exposure. The role of endogenous IL-1 in the pathogenesis of endotoxin shock has been studied before in normal non-jaundiced animals. Repeated treatment with recombinant IL-1 receptor antagonist, the naturally occurring inhibitor of IL-1α and IL-1β, conferred some protection in mice injected with a lethal dose of endotoxin. However, IL-1 receptor antagonist did not influence hemodynamic, metabolic or inflammatory responses to a sublethal dose of endotoxin in baboons or healthy humans, and IL-1R/- mice have been found to generate responses to low and high doses of endotoxin that were similar to those registered in IL-1R+/+ mice with respect to fever, anorexia, wt loss, and hepatic acute phase protein production. Likewise, IL-1β/- mice demonstrated unaltered endotoxin responses when compared to IL-1β+/+ mice. Furthermore, neither IL-1R/- nor IL-1β/- mice were protected against lethality caused by intraperitoneal injection of either high dose endotoxin or low dose endotoxin combined with D-galactosamine. Hence, the majority of studies argue against a significant role of IL-1 in endotoxin-induced inflammation and lethality, although one study with a limited number of animals showed that D-galactosamine sensitized IL-1R/- mice were protected against a lethal intravenous endotoxin challenge. Our data obtained in sham-operated mice showed that IL-1R/- mice had a modestly reduced endotoxin responsiveness, as indicated by diminished secretion of IL-1α, IL-1β, and IL-6, but not of TNF, whereas liver pathology was similar in sham-operated IL-1R/- and IL-1R+/+ mice after administration of endotoxin. More importantly, the present study demonstrates that endogenous IL-1 plays an important role in endotoxin-induced toxicity in mice after biliary obstruction. Indeed, IL-1R/- mice were less responsive to all endotoxin-induced effects analysed, including cytokine release, liver injury and lethality. Thus, bdl results in an endotoxin hypersensitive state in which endogenous IL-1 activity is an important mediator of endotoxin-induced damage. These data suggest that attempts to block IL-1 activity as an adjunctive treatment of septic complications in patients with obstructive jaundice may be more successful than anticipated from the negative data derived from clinical trials with recombinant IL-1 receptor antagonist after general sepsis.

To date, understanding of the role of inflammatory mediators in the increased endotoxin sensitivity during cholestasis is limited. TNF is known to be involved in the changes in body temperature in response to endotoxin in cholestatic rats. However, treatments aimed at eliminating endogenous TNF activity were not able to reduce mortality in cholestatic mice.
indicating that circulating TNF is not the ultimate cause of mortality observed in these mice.\textsuperscript{15,46} IL-1 is a major proinflammatory cytokine that has been implicated in the pathogenesis of several inflammatory diseases.\textsuperscript{15} We demonstrate here that production of IL-1\(\alpha\) and IL-1\(\beta\) occurs in livers of mice with bile duct obstruction, but that endogenously synthesized IL-1 does not contribute to hepatic inflammation and injury in this condition. However, exaggerated IL-1 release is found in bdl mice exposed to endotoxin, and eliminating this IL-1 response protects animals against the proinflammatory and lethal effects of endotoxin. These results suggest that endogenous IL-1 plays an important role in the hypersensitivity towards endotoxin during biliary obstruction.

Table 1. Endotoxin induced liver changes

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<th>Time (min)</th>
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Data represent the mean ± SE from 8 animals per group, at 90-360 min after endotoxin challenge. Abbreviations: bdl, bile duct ligated; ALT, alanine aspartate transaminase.

\(\star\) \(P < 0.001\) vs. sham; \(\star\) \(P < 0.05\) vs. IL-1R−/−, bdl
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**Figure 7.** Representative liver sections from sham (A, B) and bdl (C, D) animals ($n = 8$) at 6 h after endotoxin challenge (chloroacetate-esterase activity staining for neutrophils). Note increased numbers of neutrophils (arrows) mainly in periportal areas, infrequent aggregates of neutrophils in areas of focal necrosis (arrow heads) in IL-1R+/+ (A, C) mice, and hardly any aggregates of neutrophils or necrosis in the livers of IL-1R/- mice (B, D). CV = central vein, PV = portal vein. (Bar = 100 µm).

**Figure 8.** Representative liver sections from sham (A, B) and bdl (C, D) animals ($n = 8$) at 6 h after administration of endotoxin (H&E). Note the relatively normal liver architecture in sham mice as compared with bdl mice, with infrequent focal areas of necrosis (arrowheads) and scarce cellular infiltrates (arrows); whereas most necrosis and cellular infiltrates are found in IL-1R+/+ mice after bdl (C) and diminished necrosis and infiltrates are found in IL-1R/- mice after bdl (D). CV = central vein, PV = portal vein. (Bar = 100 µm).
REFERENCES


