Inflammatory response in obstructive jaundice and peritonitis
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Cholestatic IL-6 deficient mice challenged with endotoxin are more susceptible to endotoxin-induced liver injury, pulmonary inflammation, and death

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ABSTRACT

Circulating and hepatic interleukin (IL)-6 levels are strongly increased during clinical and experimental cholestasis. Cholestatic liver injury is associated with an increased susceptibility to endotoxin-induced toxicity. To determine the role of IL-6 herein, extrahepatic cholestasis was induced by bile duct ligation (bdl) in IL-6-gene deficient IL-6-/- and normal IL-6+/+ mice. Bdl elicited increased levels of hepatic IL-6 mRNA and protein in normal mice. Hepatocellular injury at 2 weeks after bdl was similar in IL-6-/- and IL-6+/+ 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, severe liver damage and death when compared to sham-operated mice. Effects of endotoxin were largely similar in sham-operated IL-6-/- and IL-6+/+ mice, but cholestatic IL-6-/- mice were more susceptible to the toxic effects of endotoxin, as reflected by increased cytokine release, more profound liver and lung injury, and higher mortality. In conclusion, although endogenous IL-6 is not important in the development of liver injury after experimentally induced obstructive jaundice, this cytokine plays an important role in decreasing hypersensitivity to endotoxin in cholestatic mice.

INTRODUCTION

Cholestatic liver disease is associated with a high perioperative morbidity and mortality, and linked with an increased occurrence of pulmonary inflammation and multiple organ failure. A factor that has been implicated to contribute to the increased risk of perioperative complications during cholestasis is the enhanced sensitivity to the toxic effects of endotoxin, the proinflammatory moiety of the outer membrane of Gram-negative bacteria, which accompanies obstructive jaundice. During cholestasis, endotoxemia is reported in up to 50-70% of patients, often related to the bilirubin content of the blood, and the presence of endotoxin in the blood stream is an important risk factor for patients undergoing surgery for obstructive jaundice. Endotoxin is a potent inducer of many inflammatory cascades, including the cytokine network. Previous studies have documented exaggerated release of several cytokines after endotoxin administration to cholestatic animals, including TNF-α, IL-1, and IL-6. This altered cytokine response to a given dose of endotoxin likely plays a role in the eventual effect of this bacterial component on organ damage and lethality. Indeed, we recently established that the enhanced release of IL-1 in response to endotoxin plays a major part in liver injury and mortality in cholestatic mice.

IL-6 is a pleiotropic cytokine stimulating a variety of cell types, including hematopoietic and neuronal cells, hepatocytes and non-oval biliary epithelial cells. IL-6 also modulates the cytokine levels and hepatic expression of acute-phase response genes during inflammation. Hepatic and circulating concentrations of IL-6 are profoundly elevated during experimental and clinical cholestasis. Knowledge of the role in this endogenously produced IL-6 in cholestatic liver injury and the ensuing enhanced susceptibility to endotoxin is limited. Therefore, in the present study we used mice with a targeted disruption of their IL-6 gene (IL-6-/- mice) to determine the role of IL-6 in hepatic and lung injury, and mortality during extrahepatic cholestasis induced by bile duct ligation (bdl) in the presence or absence of endotoxemia.
MATERIALS AND METHODS

Animals and surgical procedures
Male IL-6−/− mice on a BALB/c background (16-20 g body weight) were kindly provided by Dr. Manfred Kopf (Basel Institute for Immunology, Basel, Switzerland). IL-6−/− mice have a normal development of organ systems and normal expression of functional cell surface markers on immune cell types with only slightly lower numbers of thymocytes and peripheral blood T cells.17 BALB/c IL-6+/+ mice were purchased from Harlan Sprague Dawley Inc (Horst, The Netherlands). They were all housed in one animal room, maintained under a 12-hour light/dark cycle with regular mice chow (SRM-A; Hoppe Farms, Woerden, The Netherlands) and water ad libitum.

The Institutional Animal Care and Use Committee of the Academic Medical Center, Amsterdam, the Netherlands approved all experiments. Bill and sham surgery were performed exactly as described previously.17 Briefly, after 5 days of acclimatization, surgery was performed under sterile conditions (t = 0). Mice were divided randomly into sham-operated control groups and bill groups. They were anaesthetized by intraperitoneal injection of 0.07 ml/kg FFM mixture (Fentanyl 0.315 mg/ml, Flumisol 10 mg/ml, both Janssen, Beersen, Belgium, and Midazolam 5 mg/ml (Roche, Midborder, 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. In total, 184 IL-6+/+ mice and 184 IL-6−/− mice were used. Postoperative analgesia was achieved with subcutaneous injection of 0.1 μL/kg Temgesic (Reckitt, Kingston-Upon-Hill, United Kingdom). Half of the mice in both groups were sham operated and half underwent bill.

Materials
Endotoxin, lipopoly saccharide E. coli serotype O111 B4, was purchased as a lyophilized powder from Sigma (St. Louis, MO), resuspended in 0.5 ml sterile pyrogen-free isotonic saline and injected i.p. (4 mg/kg body weight) at 2 weeks after bill or sham surgery. Control mice received sterile pyrogen-free isotonic saline.

Blood and organ sampling
Mice were sacrificed at 1 and 2 weeks after operation, and 12, 24, 72, 120, and 24 h after endotoxin challenge. Blood was collected from the vena cava inferior. samples were transferred to tubes containing heparin, and immediately placed on ice; plasma was prepared by centrifugation (3000 x g, 10 min, 4 °C), and aliquots were stored at −20 °C. Bronchoalveolar lavage fluid (BALF) was obtained by flushing the lungs twice with 500 μL sterile saline using an endotracheal cannula (Abbocath-T catheter; Abbott, Sligo, Ireland). Samples from all liver lobes and both lungs were removed rapidly after killing the animals, dissected in small portions, and either stored at −20 °C for biochemistry and histochemistry or fixed for histology.

Histology and Immunohistochemistry
Small pieces of liver tissue from left and right lateral liver lobes and lungs were fixed in 4% buffered freshly-prepared paraformaldehyde, embedded in paraffin and stained for routine histology (hematoxylin and eosin (H&E)). Histological examination was performed on coded samples by 2 independent investigators, blinded for treatment groups and biochemical and histochemical parameters. For endotoxin-induced liver injury, 2 parameters were scored: the area of necrosis and hepatic neutrophil sequestration, stained with the chloroacetate esterase technique,17 and quantitated as
described previously.11 Briefly, 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 4910; San Diego, CA), a
frame grabber (LG-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.12 Cell damage was quantitated 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 (x100). SE Quantitative analysis with the use of digital
microscopy was described as described by Jonker et al.12
For lung tissue damage, 4 histopathologic features were scored semi-quantitatively in 4 grades of severity (0-3), according
to previously described methods: 0 = normal; 1 = edema, congestion, few areas of increased alveolar macrophages, small
areas of hemorrhage; 2 = large areas of hemorrhage, multiple areas of alveolar macrophage proliferation, lipid-laden
macrophages, hemosiderin deposition, consolidation of over half of the lung; 3 = severe hemorrhage and consolidation
involving virtually the whole lung.12 The 4 features were equally weighted and summed. Granulocyte staining in lung
tissue was performed using fluorescein isothiocyanate-labeled anti-mouse Ly-6-G mAb (Pharmingen, San Diego, CA) exactly as described previously.1

Cell counts and edema assessment
Total cells present in BALF were counted in a hemocytometer, and leukocyte differentiation was determined on cytopsin
preparations stained with modified Giemsa stain (Diff-Quick products; Dade, Düdingen, Switzerland).
Liver and pulmonary edema were evaluated by measuring the wet-to-dry weight ratio. A portion of the right lateral liver
lobe and the right upper lobe of the lungs were removed immediately after death, trimmed of fat, and weighed. The water
content was determined from the initial weight (wet weight) and its weight after incubating at 110°C for 24 h (dry
weight).

Assays
Levels of total plasma bilirubin, alkaline phosphatase, -glutamyl transferase, alanine aminotransferase (ALT), and
aspartate aminotransferase (AST) 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 enzyme-linked immunosorbent assay (ELISA) according to the instructions of the manufacturer with detection limits
in pg mL: TNF-α (31.3), IL-1β (8), IL-6 (0.8) (all from R&D Systems, Abingdon, United Kingdom), and IL-6 (88)
(Pharmingen). Myeloperoxidase assay was performed as described previously.12 Briefly, tissue from the right lower lobe
of the lung (40 mg) was homogenized in 2 mL phosphate buffer. The suspension was then pelleted at 10,000 x g for 20
min. The pellet was resuspended in phosphate buffer containing ethylenediamine tetraacetic acid (EDTA) and a mild
detergent (H-5882; Sigma) to solubilize myeloperoxidase. The samples were heated at 60°C for 2 h to eliminate
inhibitory substances and were then incubated with 3,3',5,5'-tetramethyl benzidine (Sigma) and read at 655 nm. The
samples were quantitated by comparison with a standard curve of a human myeloperoxidase standard (Sigma).
Organ homogenate preparations
For measurements of IL-6, liver and lung homogenates were suspended in 2 volumes of lysis buffer containing 300 mM NaCl, 30 mM Tris, 2 mM MgCl₂, 1% Triton X-100, and peptatin A, leupeptin and aproptinin (all 20 μg/ml; pH 7.4; Sigma) and incubated at 4 °C for 30 min. Homogenates were centrifuged at 3,000 x g at 4°C for 10 min to remove cell debris, and supernatants were stored at -20°C until assays were performed.

RNA preparation and reverse transcriptase PCR
Tissue samples were homogenized in 1 ml of TRIzol Reagent (Gibco, Berlin, Germany) according to the manufacturer’s instructions and total RNA was isolated using chloroform extraction and isopropanol precipitation. RNA was dissolved in diethylpyrocarbonate-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 x Superscript First Strand Synthesis System for reverse transcription-PCR (Gibco), 1.25 mM of each of dNTPs (Amersham, Amersham, United Kingdom), 10 mM dithiothreitol (Gibco), and the Superscript Pre-amplification system (Gibco) were added, and the final solution was incubated for 60 min at 37 °C. For RT-PCR of IFN-γ, equivalent amounts of cDNA (5 μl) were amplified in a solution (20 μl) containing 4% DMSO (Merck, Müchen, Germany), 12.5 μg BSA (Biolabs, Carle Place, NY), 1.25 mM of each of dNTPs, 10 x PCR buffer (0.67 M Tris-HCl (pH 8.8), 67 mM MgCl₂, 0.1 M β-mercaptoethanol, 67 μM 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 mM each).

The PCR reactions were carried out in the thermocycler Gene AMP® PCR System 9700 (Perkin Elmer, Branchburg, NJ). Cycling conditions for PCR amplification were: at 94 °C for 5 min (1 cycle) followed by 33 (IL-6) or 24 (β-actin) cycles at 95 °C for 60 sec, at 58 °C for 60 sec and 72 °C for 60 sec, followed by a final extension phase at 72 °C for 10 min. The primers used were: IL-6 (F): 5'-CTGGTGACAACCCTCCCC-3', (R) 5'-ATGCTTAGCATAACGCACTAGGT-3'; and β-actin (F): 5'-GTCAGAAGGACTCCTATGTG-3', (R): 5'-GCTCGTTGCCAATAGTGATG-3'. The PCR products were separated on 1.5% agarose gel containing 0.5 x TBE (50 mM Tris, 45 mM boric acid, 0.5 mM EDTA, pH 8.3) with 0.5 μg/ml ethidium bromide. The number of cycles that resulted in linear amplification was determined (data not shown). Single band intensities were analyzed using the Eagle Eye II video system (Eagle® Software System; Stratagene, La Jolla, CA) using the negative image as described previously.¹

Statistical analysis
Statistics were performed using the SPSS’ Base 11.0 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 Bonferroni’s test for multiple comparisons where appropriate. A 2-tailed p value < 0.05 was considered to indicate significant differences.
RESULTS

Role of IL-6 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. 1A). 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. 1B, C). IL-6 mRNA and protein was never detected in plasma and livers of IL-6-/− mice. Neither sham surgery nor bdl was associated with mortality during a 1-month follow-up. 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-glutamyl transferase at 2 weeks after surgery (Fig. 2). However, significant differences between IL-6+/+ and IL-6-/− mice were not found. Sham operation did not affect normal liver parenchyma of IL-6+/+ and IL-6-/− mice up to 14 days after surgery as determined by histopathological examination. In contrast, bdl led to typical cholestatic changes in liver parenchyma which were similar in both mouse strains. (Fig. 3).

These data demonstrate that IL-6 is produced in the liver after bdl, but does not play an important role in cholestatic liver injury.
Figure 2. Mean ± SE levels of bilirubin (A), alkaline phosphatase (B), γ-glutamyl transferase (C), ALT (D), and AST (E) in plasma of IL-6+/+ and IL-6-/− mice at 2 weeks after bdl or sham operation (n = 8 per group). P values indicate differences between bdl and sham-operated mice (either IL-6+/+ or IL-6-/−). Differences between IL-6+/+ and IL-6-/− mice within bdl and sham-operated groups were not significant.

Figure 3. Comparable histology in cholestatic IL-6+/+ and IL-6-/− mice
Representative liver sections from sham and bdl animals (n = 8) at 14 days after operation (H&E). Note the normal liver architecture in sham mice (A, B) compared to bdl mice (C, D). The latter exhibit infrequent focal areas of necrosis (arrowheads) and scarce cellular infiltrates with marked bile duct proliferation (arrows), comparable in both IL-6+/+ (A, B) and IL-6-/− mice (C, D). CV = central vein, PV = portal vein. (Bar = 100 μm).
Endotoxemia in cholestatic IL-6-/- mice

Increased endotoxin-induced lethality in bdl IL-6-/- mice
At 2 weeks after bdl or sham, mice were challenged with endotoxin 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. 4, during the first 6 h after endotoxin challenge, death 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 $p < 0.01$ vs. sham). Importantly, a significant difference was found in mortality between both cholestatic mouse strains, i.e., mortality was 81% (13/16) in IL-6-/- mice and only 44% (7/16) in IL-6+/+ mice ($p < 0.05$).

![Figure 4](image_url)

**Figure 4.** Endotoxin-induced lethality in bdl and sham IL-6+/+ and IL-6-/- mice ($n = 16$ per group). At 2 weeks after operation, mice were injected with endotoxin (4 μg/kg body weight, i.p.), and survival was assessed every hour during 24 h.

Increased cytokine release in bdl IL-6-/- mice after endotoxin challenge
After endotoxin challenge, IL-1α, IL-1β, IL-6, and TNF-α levels were measured in plasma at different time intervals during the first 6 h, i.e., before any animal died. Proinflammatory cytokine levels were significantly higher in bdl than in sham mice (all $p$ values $< 0.05$; Fig. 5). As compared with endotoxemic sham mice, IL-1α levels were 10-fold higher, IL-1β levels were up to 30-fold higher, IL-6 levels were 10-fold higher, and TNF-α levels were 6-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.
Figure 5. Mean (± SE) plasma cytokine levels after injection of endotoxin in IL-6+/+ and IL-6-/- mice (n = 8 per group) at 2 weeks after sham or bdl; plasma samples were obtained at the time points indicated. IL-1α (A, B), IL-1β (C, D), IL-6 (E, F), and TNF-α (G, H) concentrations were determined by ELISA. IL-6 was not detectable in IL-6-/- mice (E, F). Note the differences in scale of the y-axes for sham and bdl mice.* P < 0.05 vs. bdl IL-6+/+ mice.
Increased endotoxin-induced liver damage in bdl IL-6-/- mice

Endotoxin challenge resulted in influx of neutrophils in the liver, development of hepatocellular necrosis, increased plasma ALT levels, and increased liver edema (Fig. 6, 7), which were more pronounced in bdl mice as compared with sham mice (p < 0.05 for all responses). After bdl and subsequent challenge with endotoxin, neutrophil influx, hepatocellular necrosis, rises in plasma ALT levels, and increased liver edema 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 between IL-6+/+ and IL-6-/- mice, except for the higher liver wet/dry ratio in IL-6-/- mice (p < 0.05).

![Figure 6](image.png)

Figure 6. Mean (± SE) neutrophil influx detected by chloroacetate esterase activity (A) and area of necrosis (B) in liver, plasma level of ALT (C), and liver edema (D) in IL-6+/+ and IL-6-/- mice (n = 8 per group) at 2 weeks after sham or bdl and 6 h after endotoxin challenge.

* p < 0.05, bdl vs. sham mice; ‡ p < 0.05, IL-6-/- vs. IL-6+/+, bdl mice; † p < 0.05, IL-6-/- vs. IL-6+/+, sham mice

Increased endotoxin-induced lung inflammation in bdl IL-6 deficient mice

At 6 h after endotoxin challenge, IL-6 levels in the lungs of cholestatic IL-6+/+ mice were significantly elevated as compared with sham-operated IL-6+/+ mice (p < 0.05; Fig. 8A). IL-6 was not detectable in IL-6-/- mice. Endotoxin challenge resulted in influx of neutrophils in the lungs, an increase in MPO and TNF-α levels in homogenized lung tissue, and increased lung edema (Figs. 8B-E), which were more pronounced in bdl mice than in sham-operated mice (p < 0.01 for all responses). After challenge with endotoxin, neutrophil influx, MPO levels in lung
tissue, and lung edema were all significantly more pronounced in IL-6-/− than in IL-6+/+ mice ($p < 0.05$ for all responses), whereas TNF-α levels were only significantly higher in bdl IL-6-/− mice ($p < 0.05$). Lung pathology was exaggerated in cholestatic mice as compared with sham-operated mice ($p < 0.05$). However, no difference was found between cholestatic IL-6+/+ and IL-6-/− mice (Fig.’s. 8F, 9).

**Figure 7.** Representative liver sections from sham (A, B) and bdl (C, D) animals ($n = 8$ per group) at 6 h after administration of endotoxin (H&E). Necrosis was quantitated by measuring pixel intensities. Note the relatively normal liver architecture in sham-operated mice as compared with bdl mice, with infrequent focal areas of necrosis (arrowheads) and sparse cellular infiltrates (arrows); most necrosis and cellular infiltrates are found in IL-6-/− mice after bdl (C) and less necrosis and infiltrates are found in IL-6+/+ mice after bdl (D). CV = central vein, PV = portal vein. Bar = 100 μm.

**DISCUSSION**

Obstructive jaundice is associated with enhanced susceptibility to toxic effects of endotoxin (the present study, and references 11,19,23-26). Increased endotoxin responsiveness has been implicated in the pathogenesis of postoperative complications that frequently occur in patients with extrahepatic biliary obstruction, including sepsis and the systemic inflammatory response syndrome.5,6,27 In the present study, we determined the role of endogenous IL-6 in the development of hepatic inflammation and injury following biliary obstruction, and the increased
susceptibility to endotoxin-induced toxicity associated with obstructive jaundice in a mouse model. The main findings were that IL-6 does not play an important role in hepatic pathology resulting from biliary obstruction during a 2-week follow up, but that this cytokine contributes to a significant extent to the occurrence of hepatocellular damage, lung injury and death after endotoxin challenge in jaundiced mice. These data suggest that endogenous IL-6 plays an important role in the increased endotoxin responsiveness during cholestasis.

Figure 8. Mean (± SE) plasma level of IL-6 in IL-6+/+ mice (A; IL-6 was not detectable in IL-6-/- mice), mean (± SE) neutrophil count in BALF (B), MPO levels in lungs (C), lung edema (D), lung TNF-α levels (E), and lung histology score (F) in IL-6+/+ and IL-6-/- mice (n = 8 per group) at 2 weeks after sham or bdl and at 6 h after endotoxin challenge. * P < 0.01, bdl vs. sham mice; † P < 0.05, IL-6-/- vs. IL-6+/+, bdl mice; ‡ P < 0.05, IL-6-/- vs. IL-6+/+, sham mice

IL-6 is a cytokine with many different functional properties that can be produced by a variety of cell types. Patients with obstructive jaundice have elevated IL-6 levels in their circulation, which decrease after cholangio-drainage. In accordance, IL-6 levels were increased in plasma and liver homogenates during bdl induced jaundice in the present study, confirming previous investigations. This endogenously produced IL-6 did not contribute to cholestatic liver
damage ensuing extrahepatic obstruction. Indeed, each parameter measured at 2 weeks after bdl, i.e., transaminases, alkaline phosphatase, γ-glutamyl transpherase in plasma and histopathology of the liver, proved to be similar in IL-6-/− and IL-6+/+ mice. Our findings are in line with a recent study in which IL-6-/− mice did not demonstrate clear phenotypic differences at 1 week after bdl when compared to IL-6+/+ mice. However, IL-6 may play a role in more chronic cholestatic liver injury, as was indicated by observations that IL-6-/− mice developed a more advanced stage of biliary fibrosis at 12 weeks after bdl, which was associated with higher serum bilirubin levels and mortality.

![Representative sections of lungs with anti-granulocyte immunostaining of IL-6+/+ and IL-6-/− mice](image)

**Figure 9.** Representative sections of lungs with anti-granulocyte immunostaining of IL-6+/+ and IL-6-/− mice (n = 8) at 2 weeks after bdl or sham surgery and 6 h after endotoxin challenge. Bdl mice displayed more intense and diffuse inflammatory infiltrates, with more intense staining for PMNs (arrows) and thrombotic areas (arrowheads); the absence of IL-6 however, did not affect lung pathology in sham nor in bdl mice.

Extrahepatic cholestasis resulted in a markedly increased susceptibility to endotoxin, as indicated by mortality after administration of an endotoxin dose that did not cause mortality in sham-operated mice, enhanced cytokine release, and more severe liver and lung damage. These data confirm and extend data of previous studies on bdl rodents. Although the hepatobiliary alterations in bdl IL-6-/− mice were indistinguishable from those in bdl IL-6+/+ mice, the susceptibility of the former mice to systemic endotoxin exposure was more enhanced as compared with bdl wild type mice. The role of IL-6 in endotoxin-induced inflammation has been investigated in a number of studies using non-jaundiced IL-6-/− mice. Similar or slightly
increased mortality rates and enhanced release of TNF after systemic endotoxin administration have been reported, when compared with IL-6+/+ mice. In line with these data, we found in the present study that overt differences between IL-6-/− and IL-6+/+ mice did not occur after endotoxin challenge and sham surgery, although IL-6 deficiency was associated with marginally elevated circulating levels of TNF and IL-1. The modest anti-inflammatory role of IL-6 during endotoxemia is explained at least in part by the fact that this cytokine is capable of inhibiting endotoxin-induced TNF and IL-1 production by mononuclear cells in vitro, and reducing TNF release in endotoxemic mice in vivo. Remarkably, the role of IL-6 in endotoxin-induced tissue damage and mortality was much more important in cholestatic mice, i.e. IL-6-/− mice injected with endotoxin displayed more extensive liver and lung injury, and higher mortality rates than bdl IL-6+/+ mice at 2 weeks after bdl. Thus, obstructive jaundice results in an endotoxin-hypersensitivity state in which endogenous IL-6 activity has become an important protector of endotoxin-induced damage. Interestingly, we recently did a similar observation on the role of endogenous IL-1, albeit in the opposite direction. Indeed, IL-1-receptor deficient mice, which lack the capacity to transmit signals of both IL-1α and IL-1β, were equally sensitive to endotoxin as wild type mice in the absence of jaundice, but were partially protected against the toxic effects of endotoxin after bdl; as reflected by attenuated cytokine release, less severe liver injury, and reduced mortality. Taken together, these data indicate that IL-1α, IL-1β and IL-6, produced in the liver after bdl, are part of a delicate balance between proinflammatory and anti-inflammatory mediators, the former of which (including IL-1α and IL-1β) mediate toxicity provoked by endotoxin, whereas the latter (including IL-6) play a protective role. These two sides of the cytokine network also influence each others activity during cholestasis and endotoxemia, as indicated by our findings that cholestatic IL-1-receptor deficient mice displayed lower IL-6 levels after endotoxin challenge, whereas cholestatic IL-6-/− mice demonstrated higher IL-1α and IL-1β levels during endotoxemia. Cholestatic IL-6-/− mice showed more extensive lung inflammation than IL-6+/+ mice did after intraperitoneal administration of endotoxin. In a previous study, IL-6-/− mice without jaundice demonstrated an enhanced influx of neutrophilic granulocytes into BALF and lung tissue after administration of endotoxin per aerosol than IL-6+/+ mice. We recently found a similar anti-inflammatory role of endogenous IL-6 in the pulmonary compartment after intranasal administration of lipoteichoic acid, a major component of the gram-positive bacterial cell wall, as reflected by enhanced inflammation in lungs of IL-6-/− mice. Notably, the role of IL-6 in lung inflammation may vary depending on the inflammatory stimulus used, e.g. inflammatory responses induced by peptidoglycan were diminished in lungs of IL-6-/− mice. In conclusion, we demonstrated here that IL-6, produced in the liver during obstructive jaundice induced by bdl, does not contribute to hepatic inflammation and injury during a 2-week follow up. However, enhanced IL-6 release is found in jaundiced mice exposed to endotoxin, and eliminating this IL-6 response renders mice hypersensitive to the proinflammatory and lethal effects of endotoxin. These results suggest that endogenous IL-6 plays an important role in protecting the cholestatic host against hypersensitivity to endotoxin.
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Endotoxemia in cholestatic IL-6-/- mice