Endotoxin-induced mortality in bile duct ligated rats after administration of reconstituted high-density lipoprotein

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ABSTRACT

Cholestatic patients have substantial morbidity due to increased susceptibility to endotoxin (lipopolysaccharide [LPS]). While Reconstituted HDL (rHDL) can bind and neutralize LPS, cholestasis is associated with a near complete absence of HDL. Effects of rHDL infusion on the outcome of LPS-induced inflammatory responses in cholestatic rats were determined. Bile duct ligated (BDL) and sham rats were treated with rHDL or saline and challenged with LPS. Distribution of cholesterol over the lipoprotein subclasses changed by ligation: levels in LDL and VLDL were increased 2-fold and 5-fold, respectively and were decreased in HDL 2-fold. RHDl treatment did not affect cholesterol distribution. LPS was mainly found in the HDL fraction and ligation affected only levels of HDL-bound LPS (50% decrease; p<0.05). Although rHDl infusion effectively normalized the lipoprotein-bound LPS distribution, it resulted in increased sensitivity (mortality: 88% in the ligation + rHDl group versus 44% in the ligation + saline group, 25% in the sham + saline group, and 0% in the sham + rHDl group, p<0.05). In accordance with these results, plasma TNF was significantly highest in the BDL + rHDl group at several hours after LPS challenge as well as the accumulation of LPS in the liver (p-values<0.05).

RHDl infusion leads to increased LPS-induced mortality in cholestatic rats. These results sharply contrast with the protective effects of rHDL supplementation in experimental endotoxemia in animals and human volunteers without biliary obstruction and suggest that there may be danger in administration of rHDL to cholestatic patients.

INTRODUCTION

Periampullary cancer, a common cause of biliary obstruction, is the fifth most common cause of cancer related death,(1) and the incidence has increased up to 10-15 in past decades.(2) A radical surgical resection is the only treatment with prospect for cure.(3) While postoperative mortality has been reduced from 20 to 5% in experienced centers, morbidity remains as high as 50%.(4-6) Most complications have a septic etiology and are thought to be related to increased translocation of endotoxin from intestinal lumen into the portal and then systemic circulation where an inflammatory cascade is triggered.(7,8) Potential causes of increased translocation of endotoxin include lack of bile salts in the intestinal lumen,(9,10) resulting in increased bacterial translocation,(10) a decreased function of Kupffer cells, with failure to intercept endotoxin,(10) and changes in plasma concentrations of lipoproteins which bind endotoxin.(11)

Initial recognition of endotoxin, the lipopolysaccharide (LPS) constituent of the outer membrane of Gram-negative bacteria, is dependent on monomerization of endotoxin complexes by the acute phase protein lipopolysaccharide binding protein.(12) LPS-binding protein presents monomeric endotoxin molecules to membrane-bound CD14,
which is necessary for binding to the Toll-4 receptor that is responsible for endotoxin signal transduction\cite{13,14} leading to production of pro-inflammatory cytokines that activate a wide range of inflammatory pathways. Alternatively, LPS-binding protein can present endotoxins to plasma lipoproteins, a pathway considered to constitute an endogenous endotoxin-scavenging system\cite{15}. In vitro studies have proven that all four classes of lipoproteins bind and neutralize LPS\cite{16} possibly by insertion of the biologically active lipid A moiety of the endotoxin molecule into the phospholipid surface layer of the lipoprotein. Anti-inflammatory effects of lipoproteins have also been shown in animal models of endotoxemia\cite{17,18}. Indeed, infusion with reconstituted high-density lipoprotein (rHDL) attenuated inflammatory responses induced by challenging healthy human volunteers with LPS\cite{19}.

Therefore, the present study was designed to investigate the effects of LPS-induced inflammation after rHDL administration in an animal model of obstructive jaundice.

**MATERIALS AND METHODS**

**Rats**

Adult male Wistar rats (350-400 g) were purchased from Harlan CPB, Zeist, The Netherlands. All animals were housed in the same animal room in individual cages, maintained under a 12-hour light/dark cycle, and provided regular rat chow (SRM-A, Hope Farms, Woerden, The Netherlands) and water *ad libitum*. The Animal Ethics Board of the Academic Medical Center, Amsterdam, The Netherlands approved the experiments.

**Materials**

LPS, *E. coli* serotype O111: B4, was purchased as a lyophilized powder from Sigma (St. Louis, MO, USA), resuspended in a 0.5 ml sterile pyrogen-free isotonic saline and injected intraperitoneally (2 mg/kg). Control rats received sterile pyrogen-free isotonic saline. HTC labeled LPS O111: B4 was also obtained from Sigma. RHDL, Lot # 4.955.006.0, was a generous gift from Dr. J. E. Doran, Blood Transfusion Service SRC, Bern, Switzerland and was supplied as a sterile, pyrogen-free, virus-inactivated, lyophilized product with 91% human apo A-I purity. It was isolated from plasma and reconstituted by cholate dialysis with soybean phosphatidylcholine to form rHDL\cite{20}. The appropriate amount of rHDL, in a solution containing 2% protein and 10% saccharose, was aspirated into dark-colored Amberlite sterile syringes (Plastipak: Becton-Dickinson, Mountain View, CA, USA) and infused via the tail vein during 1 h at a dose of 120 mg/kg. The placebo solution consisted of sterile pyrogen-free isotonic saline and was administered identically. Rats were kept under light isoflurane (Abbott Laboratories Ltd., Kent, UK) / O2 (2%/2 l) anesthesia during infusions.
**Surgical procedures**

After five days of acclimatization, surgery was performed under sterile conditions (t = 7 days). Rats were divided randomly into a sham operated control group and a bile duct ligated (BDL) group. They were anaesthetized by intraperitoneal injection of 2.7 ml/kg FFM mixture (Fentanyl (0.315 mg/ml)-Fluanisone (10 mg/ml) (Janssen, Beerse, Belgium), Midazolam (5 mg/ml) (Roche, Mijdrecht, The Netherlands) and 2 ml sterile water. After a midabdominal incision the common bile duct was ligated and dissected as described by Bemelmans et al. (21). In rats undergoing sham operation, the bile duct was prepared free from surrounding tissue and ligatures were placed (but not tied) and removed again.

At the end of each experiment, rats were sacrificed by cardiac puncture and aortic incision under isoflurane anesthesia.

**Blood sampling procedure**

Under light isoflurane anesthesia (0.2-0.5%), 500 µl blood was drawn from a tail vein into a sterile syringe, transferred to tubes containing either heparin or EDTA (K$_3$ (155/1)), and immediately placed on ice. Blood samples were replaced by 500 µl isotonic saline solution administered subcutaneously.

Isoflurane was chosen because it undergoes little biotransformation, is almost completely eliminated with exhaled air, and does not affect liver microsomal enzymes. So, isoflurane does not interfere with the metabolism of drugs and toxic compounds (22).

Heparinized blood was used for hematologic and chemical analyses and EDTA-treated blood was used for assessing LPS-binding to lipoproteins and TNF-α levels. Platelet-poor plasma was prepared for these determinations by centrifugation (3000 x g, 10 min, 4 °C) and aliquots were stored at -20 °C.

**Histological studies**

Samples from left and right liver lobes, and other parenchymal organs, were removed rapidly after killing the animals and fixed in neutral buffered formaldehyde (4%). Tissues were trimmed, processed routinely, embedded in paraffin, and 3-5 µm thick sections were cut and stained with haematoxylin and eosin.

Histologic examination was performed on coded samples by two independent investigators, blinded for the treatment groups or plasma determinations.

For liver tissue, five histopathologic features were scored semiquantitatively in four grades of severity (0-3): mitotic activity, portal inflammation, ductular proliferation, fibrosis and necrosis. The five features were equally weighed and summed. This scoring system is the same as described previously (23) except evaluation of necrosis, which parameter was added here. Necrosis is defined as 0, no necrosis present; 1, involvement of less than half of the pericentral areas; 2, involvement of almost the entire pericentral areas without areas of confluent necrosis; 3, extensive confluence of necrosis from adjacent pericentral areas.

**Biochemical analyses**

Total plasma bilirubin and glutamic-pyruvic transaminase were determined with commercially available kits (Sigma), using a Hitachi analyzer (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions.
Protocol 1: Hepatic toxicity of rHDL in BDL rats (Figure 1A)

One week after bile duct ligation at t = 0, eight BDL rats received 3 ml rHDL (120 mg/kg; 0.75 ml/h) intravenously, and eight BDL rats received vehicle alone during 1 h. Animals were sacrificed 24 h later. Blood samples were taken before BDL (t = -7 d), before (t = 0) and at the end (t = 1 h) of the infusion, and at 4 and 24 h after the end of the infusion. Plasma glutamic-pyruvic transaminase activity, expressed as Units/l (μmol mm1), was used to assess liver parenchymal damage. A rat was considered to have serological proof of 'significant' hepatic necrosis when successive blood samples revealed plasma glutamic-pyruvic transaminase activity that exceeded mean baseline levels by more than twice the standard deviation.

Protocol 2: Effects of BDL and rHDL on plasma lipoproteins and their binding of LPS (Figure 1B)

After sham operation or bile duct ligation, 32 rats were randomly divided into four groups. Group one: sham + saline; group two: sham + rHDL; group three: BDL + saline; group four: BDL + rHDL. Three ml rHDL (120 mg/kg; 0.75 ml/h) or saline was administered at seven days after surgery at t = 0 during 1 h. Blood was sampled before (t = 0) and at the end (t = 1 h) of the infusion period and seven days later (t = 7 d). Each plasma sample was then used to study in vitro binding of LPS.

High performance gel chromatography (HPGC) of lipoproteins with fluorescence detection for lipoprotein-bound FITC-LPS

LPS-binding capacity of plasma lipoprotein fractions was determined by HPGC with fluorescence detection according to Levels et al.(24) Briefly, plasma samples (200 μl) were incubated with FITC-LPS, 10 μg/ml, for 30 min at 37° C. The lipoproteins were then separated on a Superose 6 HR 10/30 column (Pharmacia, Uppsala, Sweden) using a system consisting of a PL-980 Ternair pump and an LG-9080-02 linear degasser (Jasco, Tokyo, Japan). A 0.15 M Tris buffered saline (TBS) solution, pH 7.4, containing 0.005% v/v Tween-20, was used as eluent. Samples of 60 μl, diluted 1:1 with an eluent buffer, were applied on the column and eluted at a flow rate of 0.3 ml/min. Lipoprotein-bound FITC-LPS in the eluate was detected in-line using a fluorescence detector (Jasco TP-920) at λex = 494 nm and λem = 530 nm. All chromatogram calculations were performed using Borwin Chromatographic software (version 1.25, IMBS Developments, Seyssinet, France). Samples incubated with FITC-LPS were also fractionated with conventional KBr density gradient ultracentrifugation as described by Groen et al.(25) Finally, the change of fluorescence was measured at 425 nm (λex = 327 nm) in a Cobas Bio centrifugal analyzer (Roche Diagnostica, Basel, Switzerland) to validate the results obtained with HPGC.

Simultaneously, postcolumn in-line cholesterol detection was established by addition of a cholesterol reagent (PAP Cholesterol Enzymatique, Bio-Merieux, Marcy l'Etoile, France) to the eluent at 0.1 ml/min by means of a P-50 pump (Pharmacia). Briefly, cholesterol esters were hydrolyzed with cholesterol esterase. Subsequently, free cholesterol was oxidized by cholesterol oxidase to yield cholesterol-4-en-3-one and H2O2. The latter was assayed by polymerization of a peroxidase substrate (4-aminophenazone + phenol) and absorbency of the polymerized product was determined at 505 nm.
**Protocol 3: LPS-induced mortality after rHDL administration (Figure 1C)**

Rats were randomly divided over four groups: sham + saline (n = 16), sham + rHDL (n = 10), BDL + saline (n = 16), and BDL + rHDL (n = 16). Three ml rHDL (120 mg/kg, 0.75 ml/h) or saline was administered at seven days after surgery at t = 0. At the end of the infusion period (t = +1 h), 2 mg/kg LPS was injected intraperitoneally. At t = 30 min, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h later, rats were monitored for rectal temperature, general well being (e.g., activity, food intake, skin care, and presence of Harder's secretions), and mortality. Blood was sampled at t = 0, +1, +2, +4, and +24 h. Additionally, six satellite animals were included in each treatment group. These satellite animals were sacrificed at t = +4 h after LPS challenge for necropy and immunohisto pathological examinations.

**Measurement of TNF-α bioactivity**

TNF-α bioactivity in plasma samples obtained in experiment three was measured in duplicate at t = 7 days, 0, 1, 2 and 24 h after LPS challenge using a rat TNF-α ELISA kit purchased from Biosource (Flers, Belgium) according to manufacturer’s instructions. The ELISA had a lower detection limit of approximately 40 pg/ml.

**Immunohistochemical studies**

Immunohistochemistry was performed as described previously (26). All incubations were carried out at room temperature in a moist chamber. Between each step, sections were rinsed three times in 0.01 M PBS, pH 7.4, unless specified otherwise. Cryostat sections of uniform thickness (4 μm) were thawed and air-dried overnight, fixed in acetone, and preincubated with 0.3% H₂O₂ to block endogenous peroxidase activity, before incubation for 60 min with a panel of monoclonal antibodies (Table 1). Sections were rinsed thoroughly and then incubated for 45 min with the secondary rabbit anti-mouse IgG conjugated with horseradish peroxidase (Dako, Glostrup, Denmark). Incubation media included 0.25% (v/v) bovine serum albumin (BSA) and 1% (v/v) normal rat serum to block nonspecific background staining.

Peroxidase activity was visualized with either 3-amino-9-ethyl-carbazole (AEC, Sigma) or 3,3′-diaminobenzidine-tetrachloride (DAB, Sigma). Counterstaining was performed with haematoxylin before mounting of the sections in glycerin-gelatin.

Immunoenzyme double-staining methods were used to localize different cellular populations as shown in Table 1 by performing sequentially two entire immunoenzymatic methods (27).

**Cell counting**

Periportal and pericentral regions of liver lobules were compared. The latter regions were defined as parenchymal layers five hepatocytes thick surrounding terminal branches of the hepatic vein. For each liver, 12 sections were used and three periportal and pericentral zones were randomly selected in each section. Number of ED1-, ED2-, ED9-, and ON3-positive cells per unit area were determined in a standardized microscopic field using a light microscope (Zeiss, Oberkochen, Germany), a 10 × 10 raster viewer and a Neofluar objective 25 x.
Legend to figure 1 on next page
**rHDL administration in endotoxin-challenged cholestatic rats**

Fig. 1. Experimental protocols: rats were randomized to undergo BDL or sham operation at t = -7 days. At t = 0, rats in each group were first matched for weight and then randomized in order to receive an one hour lasting infusion of either rHDL or 0.9% saline. A. In protocol one, only BDL rats were used. Blood was sampled at t = -7 days, 0, +1, +4 and +24 h, and then animals were sacrificed. B. In protocol two, at t = 0, BDL and sham rats were randomized to receive an infusion during 1 h of either rHDL or 0.9% saline. Blood was sampled at t = -7 days, 0, +1 h, and +7 days. At t = +7 days, animals were sacrificed. C. In protocol three, four groups of rats were used (sham + saline, sham + rHDL, BDL + saline, and BDL + rHDL). All rats were challenged with LPS (5 mg/kg) intraperitoneally, directly following the infusion period (t = +1 h). Blood was sampled at t = -7 days, 0, +1, +2, +4, and +24 h, and then animals were sacrificed. Parallel, satellite animals were sacrificed at t = +4 h for immunohistopathology. D. In protocol four, 4 groups of rats were used (sham + saline, sham + rHDL, BDL + saline, and BDL + rHDL). All rats were challenged with LPS (5 mg/kg) intraperitoneally, directly after the infusion period (t = +1 h). Blood was sampled at t = -7 days, 0, +1, +3, +5, +7, and +24 h, and then animals were sacrificed. Parallel, satellite animals, challenged with FITC-LPS, were sacrificed at t = +5 h for determination of the liver distribution of endotoxin.

### Table 1. Monoclonal antibodies to demonstrate Kupffer cells and newly recruited MΦ's in relation to their activation state in rat livers of LPS-challenged sham and BDL rats treated with rHDL or saline (protocol 3)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Kupffer cells</th>
<th>Newly recruited MΦ</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activated</td>
<td>Not activated</td>
<td>Activated</td>
</tr>
<tr>
<td>ED1&lt;sup&gt;+&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ED2&lt;sup&gt;+&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>OX3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ED9&lt;sup&gt;+&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Monoclonal antibodies obtained from Serotech, Hilversum, The Netherlands.
* Monoclonal antibody obtained as a generous gift by Prof. Dr. C.D. Dijkstra, Department of Cell Biology and Immunology, Medical Faculty, Free University, Amsterdam, The Netherlands
* Positivity (+) or negativity (-) for staining with the antibody

### Protocol 4: Liver clearance of FITC-LPS and rHDL (Figure 1D)

In order to obtain insight in the fate and distribution of LPS in BDL rats treated with rHDL, 24 rats were randomly and equally divided over four groups: sham + saline, sham + rHDL, BDL + saline, and BDL + rHDL. Three ml rHDL (120 mg/kg, 0.75 ml/hr) or saline was administered at seven days after surgery at t = 0. At the end of the infusion period (t = +1 hr), 2 mg/kg LPS was injected intraperitoneally. Blood was sampled before operation (t = -7 days), before (t = 0) and at t = +1, +2, +3, +5, +7, and +24 h after the infusion period. Blood samples were drawn from the tail vein into a sterile syringe, and immediately transferred to pyrogen-free tubes (Falcon 2063, Becton Dickinson, Mountain View, CA) containing either pyrogen-free heparin (Thromboliquine®; Organon, Oss, The Netherlands; final concentration 30 U/ml), or EDTA (K<sub>3</sub>) (150i), and immediately placed on ice.

82
Platelet-rich plasma (PRP) of the heparin samples was prepared by centrifugation at 190 x g for 10 min at 4 C and was used for the determination of endotoxin in the LAL assay. After sterile removal, PRP was subsequently aliquoted and stored at -20 C.

EDTA plasma samples were used to measure human apolipoprotein A-I. Platelet-poor plasma was prepared for these determinations by centrifugation (3000 x g, 10 min, 4 C) and aliquots were stored at -20 C. Plasma levels of apolipoprotein A-I were determined by immunonephelometry with a commercially available kit (Sigma) using a Hitachi analyzer (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions.

Additionally, in order to obtain insight in the liver distribution of endotoxin after rHDL treatment, satellite animals were included in each treatment group and injected with FITC-LPS (Sigma). These satellite animals were sacrificed at t = 3, 5, and 7 h (n = 6 per group) by extrasanguination. These time points were chosen since it had become evident from the survival studies that mortality occurred as early as 8 h after endotoxin challenge. The liver was then excised, snap-frozen, and stored at -80 C for immunofluorescence detection of FITC-LPS. Liver samples were kept in the dark as much as possible to avoid light exposure in order to prevent fading of FITC fluorescence.

**Determination of endotoxin levels**

Endotoxin was batch-wise determined by the Limulus assay, performed with slight modifications as described previously. This assay has a detection limit in blood of endotoxin levels <35 EU/PRP (approximately 3.5 pg/ml). A control of each plasma sample was measured chromogenically without the addition of LAL reagents to check for the color of jaundiced plasma. Each sample was measured in duplicate and the results were expressed as the mean of the two tests.

**Confocal laser scanning microscopy.**

Liver parenchymal distribution patterns of FITC-LPS were visualized with a confocal laser scanning microscope (Leica Lasertechnik, Heidelberg, Germany). All experiments were performed immediately after sectioning of the livers. Sections (8 μm) were kept in the dark whenever possible to prevent fading of FITC fluorescence.

Air-dried sections were counterstained with the DNA-specific dye propidium iodide (SIGMA; diluted 1:20: 20 min, 20 C) to clearly mark the nuclei. Subsequently, sections were washed with PBS and mounted in Vectashield (Vector Laboratories, Burlingame, CA), and evaluated using the dual excitation and detection mode of the confocal microscope equipped with an argon-krypton laser. The images were optimized for voltage, offset, and merged using multicolor analysis software (Leica Lasertechnik). Serial optical sections were obtained in 0.5-μm steps along the z-axis over the total thickness of the section. The stored images were overlaid to create a single integrated image referred to as a "volume projection" using the manufacturer’s proprietary software (Leica Lasertechnik). The gain level was adjusted to obtain a range of 0 to 255 gray levels. The images were photographed with Ektachrome 320 JT color slide film (Kodak, Rochester, NY). All fluorescence patterns were read independently by two investigators.
Quantitation of lobular distribution patterns of FITC-LPS

Differences in lobular fluorescence of FITC-LPS at 3, 5, and 7 h after challenge were quantified as follows: parenchyma within 100 μm distance from the portal tract or central vein was defined as periportal or pericentral parenchyma, respectively. Confocal images of sections from each liver were obtained from periportal and pericentral areas (22 images each) from randomly selected sites in all liver lobes according to a rigid scheme as described previously (29) from 12 cryostat sections. Images were captured from an area diagonally between a portal tract and a central vein. All images were coded to avoid bias during quantitative analysis. In periportal and pericentral parenchyma after sham + saline/rHDL, or BDL + saline/rHDL treatment, differences in fluorescence of FITC-LPS were quantitated by measuring pixel intensities by blinded investigator using a Power Macintosh 7600/200 computer (Apple, Cupertino, CA) using the public domain NIH Image program developed at the National Institutes of Health and available from the Internet by anonymous FTP from zippy.nimh.nih.gov, or on floppy disk from the National Technical Information Service, Springfield, VA, part no. PB95-500195(GE).

The number of FITC-LPS positive cells was expressed per unit volume of liver tissue ± SE. The percentage area of FITC-LPS positive cells was expressed as: ratio of the area of FITC-LPS bearing cells and the total area of liver tissue including FITC-LPS bearing cells (×100) ± SE.

Statistical analysis

All statistical calculations were conducted with standard biomedical statistical programs (SPSS 8.01; SPSS, Chicago, Ill.). All are given as mean ± SEM, but for histological, and histochemical data mean and range are used. A 2-tailed p value < 0.05 was considered significant. Mortality data, determined at 24 h after LPS-challenge, was evaluated by Kaplan-Meier analysis. For biochemical, immunohistochemical and histologic data, mean values were compared between groups by Student's t test for independent samples. Significance of differences in plasma TNF-α levels was determined using analysis of variance (ANOVA).

RESULTS

Hepatic toxicity of rHDL in BDL rats

Sham and BDL rats infused with rHDL suffered neither clinical morbidity nor mortality during the 24 h observation period. rHDL administration in sham and BDL rats did not result in elevations of plasma glutamic-pyruvic transaminase levels at t = 0, 1 and 4 h, suggesting that there was no hepatotoxicity due to rHDL administration. Livers of both saline treated and rHDL treated BDL rats showed comparable degrees of increased mitotic activity near periportal areas, portal inflammation, bile duct proliferation, fibrosis, and necrosis because of eight days of cholestasis. These results show that rHDL treatment did not have any significant effects on histological parameters (data not shown). Injury to other parenchymal organs was not evident in either group (data not shown).
Chapter 5

Effects of BDL and rHDL on plasma lipoproteins and their binding of endotoxin

Confirmation of cholestasis

Plasma bilirubin levels were similar (0-1 µmol/l) in all four experimental groups before surgery (t = -7 days), with no significant changes afterwards in both sham subgroups. In the BDL subgroups, plasma bilirubin levels increased to 120 ± 18 µmol/l at t = 0 and this was not influenced by rHDL treatment (124 ± 24 µmol/l, 1-4 hours after infusion). Bilirubin plasma levels increased to 170 ± 29 µmol/l at t = +7 days.

The mean preoperative body weight of sham and BDL rats was 370 ± 2 g and 372 ± 2 g, respectively. Rats in both sham subgroups progressively gained weight in a similar way from the third postoperative day onwards, and showed an increase of approximately 15% during two weeks after surgery (t = +7 days). Rats in both BDL subgroups, lost approximately 5% of their weight during the first week after surgery (t = 0), but their weight stabilized during the second week. Total body weight of rats in both sham subgroups was significantly higher than the weight of rats in both BDL subgroups on t = -3 days and onwards (p < 0.0001 at t = +7 days).

The lipoprotein spectrum in sham and BDL rats and the effects of rHDL

Cholesterol levels in plasma lipoprotein fractions VLDL, LDL and HDL after separation by HPGC are shown in Table 2. Preoperative cholesterol levels in lipoproteins in the sham subgroups and the BDL subgroups at t = -7 days were similar. At t = 0, sham operated rats showed no change in cholesterol levels in lipoproteins, whereas in BDL rats cholesterol shifted from HDL to LDL and VLDL (p < 0.0001 versus sham), because the levels in HDL decreased 2-fold and in LDL and VLDL increased 2 to 3-fold. RHDl infusion affected only the cholesterol level in HDL. It was restored to preoperative levels, whereas LDL and VLDL levels were unaffected by rHDL treatment. The effect of rHDL infusion was gone seven days after infusion (t = +7 days) (data not shown).

Lipoprotein-associated FITC-LPS in lipoprotein fractions

The distribution of FITC-LPS, after addition to normal and jaundiced plasma in vitro, over the lipoprotein and non lipoprotein fractions is depicted in Table 2. It was first determined that the signal and the applied amount of FITC-LPS showed a linear relationship, and that the distribution of FITC-LPS over the lipoprotein fraction was not influenced by the applied amount (data not shown). The mean preoperative percentages of FITC-LPS bound to VLDL, LDL and HDL fractions and the non lipoprotein fraction in sham and BDL subgroups, and in the preinfusion sham subgroups were similar (Table 2). Before infusion of saline or rHDL (t=0), biliary obstruction caused a significant 3.2-fold increase in cholesterol levels in the VLDL and LDL regions: ex vivo incubation with FITC-LPS resulted in a similar 3.5-fold
increased FITC-LPS association with the VLDL and LDL fractions. At t = 0, biliary obstruction had also caused a 2.4-fold decrease in HDL cholesterol levels; FITC-LPS association with the HDL fraction decreases also 2-fold. Of note was the 6.7-fold increased FITC-LPS association with the non-lipoprotein fraction due to bile duct ligation prior to rHDL or saline infusion. RHDl infusion of BDL rats almost completely restored the percentage FITC-LPS associated to the HDL fraction, yielding distributions of FITC-LPS similar to those in sham rats. The 2.5-fold decrease of FITC-LPS association with the non-lipoprotein fraction in the rHDL-infused BDL rats was striking. Saline infusion was without any effect. Seven days after infusion, distribution of lipoprotein-associated FITC-LPS returned to preinfusion values in all experimental groups (data not shown).

Similar results were obtained when samples were fractionated by density gradient ultracentrifugation (data not shown).

LPS challenge in BDL rats after rHDL administration

Clinical manifestations and 24 h mortality rate

After LPS challenge, all animals were weak, lethargic, and anorexic, with ruffled fur, huddling behavior and diarrhea. At 24 h after LPS challenge, all these signs had disappeared in the survivors.

Mortality differed significantly among the 4 groups and was 0% (0/10) in the sham + rHDL group, 25% (4/16) in the sham + saline group, 44% (7/16) in the BDL + saline group and 88% (14/16) in the BDL + rHDL group (p < 0.05). In the latter 3 groups, animals died during the first 8 h, with a peak between 5 and 6 h after LPS challenge. Death was preceded by progressive respiratory distress between 4 and 6 h after LPS challenge. Necropsy showed that all diseased animals had suffered from sepsis, and the lungs appeared congested, red, wet, and firmer than normal. Histologically, pulmonary vessels were congested and they contained many neutrophils and vast perivascular edema.

Circulating TNF-α levels

Circulating TNF-α levels as measured by ELISA were below the detection limit in plasma of rats in sham and BDL subgroups before operation and immediately before infusion (Table 3). LPS challenge resulted in a sharp increase in circulating TNF-α levels after 2 h in all groups, being the highest in the rHDL treated BDL rats and the lowest in rHDL treated sham rats (p < 0.001). TNF-α levels in plasma of surviving rats at 24 h after LPS challenge had decreased significantly in all groups, but were still higher in both BDL subgroups as compared with TNF-α levels in the sham group (p < 0.05).
<table>
<thead>
<tr>
<th>Group</th>
<th>(Lipo) protein fraction</th>
<th>t = -7 days</th>
<th>t = 0</th>
<th>t = +1 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol (μmol/l)</td>
<td>FITC-LPS (%)</td>
<td>Cholesterol (μmol/l)</td>
<td>FITC-LPS (%)</td>
</tr>
<tr>
<td>Sham saline</td>
<td>VLDL</td>
<td>450 ± 63</td>
<td>8 ± 2</td>
<td>449 ± 59</td>
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<tr>
<td></td>
<td>LDL</td>
<td>394 ± 91</td>
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<td>390 ± 95</td>
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<td></td>
<td>HDL</td>
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<td>756 ± 137</td>
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<td>Non lipoproteins</td>
<td></td>
<td>3 ± 1</td>
<td></td>
<td>2 ± 1</td>
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<tr>
<td>Sham rHDL</td>
<td>VLDL</td>
<td>442 ± 60</td>
<td>7 ± 2</td>
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<tr>
<td></td>
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<td>Non lipoproteins</td>
<td></td>
<td>3 ± 2</td>
<td></td>
<td>3 ± 1</td>
</tr>
<tr>
<td>BDL saline</td>
<td>VLDL</td>
<td>451 ± 66*</td>
<td>8 ± 1*</td>
<td>1036 ± 164†</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>387 ± 95*</td>
<td>3 ± 3*</td>
<td>1638 ± 364†</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>765 ± 135*</td>
<td>86 ± 5*</td>
<td>318 ± 12†</td>
</tr>
<tr>
<td>Non lipoproteins</td>
<td></td>
<td>3 ± 1*</td>
<td></td>
<td>20 ± 3†</td>
</tr>
<tr>
<td>BDL rHDL</td>
<td>VLDL</td>
<td>448 ± 67*</td>
<td>8 ± 2</td>
<td>1329 ± 250‡</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>392 ± 87*</td>
<td>5 ± 2</td>
<td>1865 ± 196‡</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>751 ± 131</td>
<td>85 ± 3</td>
<td>349 ± 203</td>
</tr>
<tr>
<td>Non lipoproteins</td>
<td></td>
<td>2 ± 1</td>
<td></td>
<td>20 ± 3‡</td>
</tr>
</tbody>
</table>

NOTE: Mean cholesterol level (μmol/l) in lipoproteins and mean % of fluorescence levels of FITC-LPS in the lipoprotein and non lipoprotein fractions at 7 days after operation at t = 0, before rHDL administration, and at the end of the rHDL infusion period, t = +1 h. The fraction with "non lipoproteins" in fact consists of LPS associated or co-eluted (HPGC) with plasma-proteins (ultracentrifugation: density > 1.21 g/ml). *: Binding capacity of FITC-LPS to (non) lipoprotein fractions was quantified by calculating the peak area of the different protein fractions for eight rats in each treatment group.

* P < 0.01, BDL subgroups: t = -7 days versus t = 0 and t = +1 h; † P < 0.01, t = 0; BDL subgroups versus sham subgroups; ‡ P < 0.05, rHDL treated BDL rats: t = 0 versus t = -7 days and t = +1 h; § P < 0.05, t = +1 h: rHDL treated BDL rats versus saline treated BDL rats.
Table 3. TNF-α levels of sham and BDL rats before and after LPS challenge (protocol 3)

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α levels ± SEM (pg/ml)</th>
<th>t = -7 days</th>
<th>t = 0</th>
<th>t = +2 h</th>
<th>t = +24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham saline</td>
<td>&lt; 40</td>
<td>&lt; 40</td>
<td>913±</td>
<td>123</td>
<td>&lt; 40</td>
</tr>
<tr>
<td>Sham rHDL</td>
<td>&lt; 40</td>
<td>&lt; 40</td>
<td>352±</td>
<td>85*</td>
<td>&lt; 40</td>
</tr>
<tr>
<td>BDL saline</td>
<td>&lt; 40</td>
<td>&lt; 40</td>
<td>3673±</td>
<td>185</td>
<td>123± 25 §</td>
</tr>
<tr>
<td>BDL rHDL</td>
<td>&lt; 40</td>
<td>&lt; 40</td>
<td>7225±</td>
<td>535± §</td>
<td>115± 13 §</td>
</tr>
</tbody>
</table>

TNF-α levels (detection limit = 40 pg/ml) in sham and BDL rats are given at different time points in relation to rHDL administration (t = 0) and LPS challenge (t = +1 h). Each value is the mean ± SEM of duplicate wells. At t = +2 h rHDL treated BDL rats (n = 16) produced more TNF-α than saline treated BDL rats (n = 16) and sham rats (n = 26). At t = +24 h, TNF-α levels had decreased more in sham rats (n = 14) than in BDL rats (n = 9).

* P < 0.01 rHDL treated sham rats (n = 10) versus other groups (n = 16 in each group).
† P < 0.05 rHDL treated BDL rats versus controls (sham rHDL, n = 10), sham saline (n = 16), and BDL saline (n = 16).
‡ P = 0.013, rHDL treated BDL rats (n = 16) versus saline treated BDL rats (n = 16).
§ P < 0.05, BDL subgroups (n = 9) versus sham subgroups (n = 14).

Liver histopathology

Livers of rats in both BDL subgroups showed high mitotic activity, extensive ductular proliferation, and fibrosis, and significantly more portal inflammation and necrosis as compared with sham rats (Table 4). Differences between saline treated and rHDL treated BDL rats were not detected.

Table 4. Histological scores of rat livers from satellite animals (sham, saline treated BDL, and rHDL treated BDL rats) at 4 h following LPS challenge (protocol 3)

<table>
<thead>
<tr>
<th></th>
<th>Mitotic activity</th>
<th>Portal inflammation</th>
<th>Ductular proliferation</th>
<th>Fibrosis</th>
<th>Necrosis</th>
<th>Mean total score (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.0</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2.0</td>
<td>3.7 (3.0-6.0)</td>
</tr>
<tr>
<td>Sham rHDL</td>
<td>0.0</td>
<td>2.5</td>
<td>0.0</td>
<td>0.0</td>
<td>1.5</td>
<td>4.0 (3.0-6.0)</td>
</tr>
<tr>
<td>BDL placebo</td>
<td>2.5‡</td>
<td>2.8‡</td>
<td>3.0‡</td>
<td>3.0‡</td>
<td>2.8‡</td>
<td>13.0 (12.0-15.0)‡</td>
</tr>
<tr>
<td>BDL rHDL</td>
<td>2.8‡</td>
<td>2.5‡</td>
<td>3.0‡</td>
<td>3.0‡</td>
<td>2.9‡</td>
<td>14.0 (12.0-16.0)‡</td>
</tr>
</tbody>
</table>

Mean scores in sham + saline, sham + rHDL, BDL + saline and BDL + rHDL rats for each of the five histological parameters and the total mean scores (plus range) after LPS challenge.

* Scores on a semiquantitative scale for each parameter: † P < 0.001, BDL subgroups versus sham subgroups.
Liver immunohistopathology

Immunohistochemical scores of liver parenchyma are summarized in Table 5. To assess the effects of cholestasis, and treatment with rHDL and LPS on the composition of the hepatic mononuclear phagocytic cell pools, sections were stained for ED1-positive Kupffer cells and Mφ's and for ED2-positive Kupffer cells. After LPS challenge, the number of ED1-positive cells was higher than the number of ED2-positive cells, indicating the presence of newly recruited Mφ's and monocytes (see also table 1). Biliary obstruction was associated with an increase of the number of both ED1-positive and ED2-positive cells. The number of Kupffer cells was higher in cholestatic livers than in sham livers (p < 0.001).

In the liver, mainly Kupffer cells and endothelial cells act as antigen-presenting cells and express major histocompatibility complex (MHC) class II antigen (Ia antigen). OX3-positive activated Mφ's in all three groups were found in periportal and pericentral zones only and in low numbers in connective tissue of portal tracts. These activated Mφ's were mainly Kupffer cells and were found in increasing numbers in liver parenchyma of sham rats, saline treated BDL rats and rHDL treated BDL rats, respectively. These data were confirmed by double staining showing Kupffer cells that were both ED2-positive and OX3-positive (Table 5).

Signal regulatory protein expression on monocytes and Mφ's was detected with mAb ED9. Signal regulatory proteins constitute a subfamily within the immunoglobulin superfamily, closely related to the antigen receptors: immunoglobulin, TCR, and MHC. It's role in cellular signaling, emphasizes the role of the activated Kupffer cells during cholestasis and more importantly during endotoxin challenge. Both bile duct ligation and LPS challenge caused an increase of the number of ED9-positive cells (Table 5). The number of periportal and pericentral ED9-positive Kupffer cells in the liver parenchyma of rHDL treated BDL rats was 2-fold higher than in saline treated BDL rats and even 8-fold higher than in sham rats.

Fate and distribution of FITC-LPS and rHDL in BDL rats

Endotoxin activity in plasma

At t = -7 days, endotoxin levels of 3.4 ± 0.8 pg/ml were not significantly different (all rats). One week after operation, at t = 0, there was no change in endotoxin levels: 3.7 ± 0.6 pg/ml in the sham rats and 3.6 ± 0.5 pg/ml in all BDL rats (p = 0.2) (Fig. 2). Within two hours after induction of endotoxemia (t = +3), endotoxin levels reached a peak in all groups, being significantly highest in rats treated with BDL + saline, followed by sham + saline, BDL + rHDL, and sham + rHDL (p < 0.01). Respective endotoxin levels were 751.4 ± 150.2 pg/ml, 421.0 ± 121.3 pg/ml, 71.3 ± 12.3 pg/ml, and 35.4 ± 12.1 pg/ml. At t = +5 h, endotoxin levels had diminished significantly two-fold in both groups treated with rHDL, albeit levels were still significantly higher in BDL rats as compared with sham rats (p = 0.03). Endotoxin levels in the saline-
treated groups had decreased with only 4% in the BDL group and 11% in the sham group. At \( t = +7 \) h, endotoxin levels were still significantly lowest in the group treated with sham + rHDL, followed by BDL + rHDL, sham + saline, and BDL + saline (\( p < 0.05 \)). At \( t = +24 \) h, endotoxin levels had returned to prechallenge levels in all surviving rats (sham + rHDL, \( n = 6 \); sham + saline, \( n = 4 \); BDL + saline, \( n = 3 \); BDL + rHDL, \( n = 1 \)).

**Table 5.** Immunohistochemical scores of rat livers from satellite animals (sham, saline treated BDL, and rHDL treated BDL rats) at 4 h following LPS challenge (protocol 3)

<table>
<thead>
<tr>
<th>Mean scores (range), ( n = 6 ) per group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shannon</td>
</tr>
<tr>
<td>ED1</td>
</tr>
<tr>
<td>ED2</td>
</tr>
<tr>
<td>OX3</td>
</tr>
<tr>
<td>ED2/OX3</td>
</tr>
<tr>
<td>ED9</td>
</tr>
</tbody>
</table>

*Kupffer cells as demonstrated using ED2, total population of MIP-2's (Kupffer cells, newly recruited MIP-2's and monocytes) as detected with ED1, total population of activated MIP-2's as detected with OX3 and Kupffer cells expressing signal-regulatory proteins as detected with ED9 in periportal and pericentral zones of liver parenchyma of sham, saline treated BDL, and rHDL treated BDL rats after LPS challenge.*

\( ^{1} p < 0.001 \), ED1 versus ED2 in sham livers; \( ^{2} p < 0.001 \), sham saline versus BDL subgroups; \( ^{3} p = 0.01 \), rHDL treated rats versus saline treated rats; \( ^{4} p < 0.001 \), rHDL treated BDL rats versus controls (sham saline, sham rHDL, and BDL saline rats); \( ^{5} \) Percentage of activated Kupffer cells was calculated as the ratio of the number of cells positive for both ED2 and OX3 and the number of ED2-positive cells.

**Human apolipoprotein A-I levels after LPS challenge**

Plasma levels of human apo A-I were undetectable in all rats before infusion of rHDL (t = 0), demonstrating that the reagents used for determining human apo A-I did not cross react with rat apo A-I (Fig. 3). As a result of rHDL infusion, plasma levels of human apo A-I increased comparably in sham and BDL rats at \( t = +1 \) h up to 2.3 - 0.34 mg/ml and 2.5 - 0.65 mg/ml, respectively. Although human apo A-I levels remained higher in the BDL group throughout the experiment, this difference did not reach statistical significance. At \( t = +3 \) h, human apo A-I levels decreased rapidly in the sham group by 30% and the BDL group by 24%. Thereafter, human apo A-I levels decreased more slowly in both groups. At \( t = +24 \) h, approximately half of the human apo A-I was still in the circulation in both groups treated with rHDL.
Fig. 2 Mean (± SEM) endotoxin levels before and during endotoxemia in plasma of rats treated as sham + saline (open circles, straight line), sham + rHDL (closed circles, dashed line), BDL + saline (open squares, straight line), and BDL + rHDL (closed squares, dotted line). rHDL (120 mg/kg body weight) or saline was given during 1 h infusion (t = 0), starting at 1 h prior to endotoxin challenge (t = +1). P-values indicate differences between the treatment groups. Data at each time point represent the mean of 6 animals except for t = +24 h: sham + saline, n = 4; sham + rHDL, n = 6; BDL + saline n = 3; BDL + rHDL n = 1. *P < 0.01, rHDL treated rats versus saline treated rats; † P < 0.01, sham + rHDL versus BDL + rHDL.

Quantitation of lobular differences in FITC-LPS levels
Detection of FITC-LPS was performed in liver sections of saline-treated and rHDL-treated sham and BDL rats at t = 3, 5 and 7 h. Peak fluorescence was observed at t = 7 h. In some liver cells, a diffuse partially granular staining of FITC-LPS (green fluorescence) in the cytoplasm was observed whereas nuclei (red fluorescence) were negative. The overlay of green and red fluorescence leads to yellow fluorescent staining of the respective structures. All FITC-LPS was found to be present in cellular compartments outside the nuclei because yellow fluorescence was hardly observed (Fig. 4). The staining pattern of cells containing FITC-LPS indicated that these cells were Kupffer cells. In BDL rats, the cellular FITC-LPS content was increased (cf. Figs. 4 C,D and A,B).

Peak fluorescence levels of FITC-LPS at 7 h. after infusion are listed in Table 6. FITC-LPS containing cells were twice as much present due to rHDL treatment of sham rats. However, this difference was not statistically significant. Bile duct ligation, however, lead to significantly higher numbers of FITC-LPS containing cells. The
increase was 4-fold due to bile duct ligation (p<0.05). RHDL-treatment of BDL rats lead to the highest number of FITC-LPS containing cells. Comparable results were obtained when determining the percentage area of FITC-LPS containing cells (Table 6).

**Table 6.** Number of FITC-LPS containing cell and percentage area of FITC-LPS containing cells at 7 h after infusion of rHDL or saline in sham and BDL rats, as detected with confocal scanning laser microscopy

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of FITC-LPS containing cells*</th>
<th>Percentage area‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>sham + saline</td>
<td>4 ± 3</td>
<td>1.42 ± 1.17</td>
</tr>
<tr>
<td>sham + rHDL</td>
<td>7 ± 3</td>
<td>2.51 ± 1.43</td>
</tr>
<tr>
<td>BDL + saline</td>
<td>15 ± 4 §</td>
<td>6.92 ± 0.87 §</td>
</tr>
<tr>
<td>BDL + rHDL</td>
<td>25 ± 4 §</td>
<td>11.92 ± 3.18 §</td>
</tr>
</tbody>
</table>

* Data are expressed as the number of FITC-LPS containing cells per unit volume of liver tissue ± SE (n = 6 animals per group). ‡ Percentage area is expressed as the ratio of the area of FITC-LPS positive cells per total area of liver tissue including FITC-LPS positive cells ± SE (n = 6 animals per group). § P < 0.01 for BDL + saline versus sham groups. ¶ P < 0.05 for BDL + rHDL versus other groups.

Fig. 3. Mean (± SEM) human apo A-I levels before and during endotoxemia in plasma of rats treated as sham + rHDL (closed circles, dashed line), or BDL + rHDL (closed squares, dotted line). RHDL (120 mg/kg body weight) or saline was given during 1 h infusion (t = 0), starting at 1 h prior to endotoxin challenge (t = +1). Data at each time point represent the mean of 6 animals except for t = +24 h: sham + saline, n = 4; sham + rHDL, n = 6; BDL + saline n = 3; BDL + rHDL n = 1.
**DISCUSSION**

Various therapies have been tested to prevent septic complications in biliary obstructed patients and animals. Although internal biliary drainage reduces endotoxemia and bacterial translocation from the gut into the circulation of cholestatic rats,(32,33) the clinical benefit of drainage in humans is still unclear, due to procedure-related complications, like cholangitis or local inflammatory responses to the endoprosthesis.(34) Several types of anti-inflammatory therapies have been tested but with little or no benefit, including oral lactulose,(35) bile salts,(36) antibiotics (Polimyxin B),(8) bowel preparation and irrigation,(37) or antibodies directed against cytokines.(38) Kimmings et al. studied changes in lipid spectra of jaundiced patients before and at three weeks after biliary drainage in relation to endotoxin sensitivity and reported an increase in cholesterol levels, mostly in the VLDL fraction at the expense of HDL and LDL fractions.(39) The present study in rats confirmed that cholestasis leads to decreased levels of plasma HDL,(39) which of all lipoproteins has the highest binding affinity for endotoxin. Using a novel HPGC system, we were able to demonstrate a decrease in endotoxin-binding capacity of lipoproteins, in particular HDL, in cholestatic rats. Because obstructive jaundice is associated with such marked changes in concentrations of plasma lipoproteins, which are intimately involved in
scavenging of endotoxin, we have investigated whether infusion with rHDL protects against LPS-induced mortality in BDL rats. Although rHDL administration proved to restore the LPS-binding capacity of lipoproteins in jaundiced plasma, it significantly worsened the outcome of LPS-induced mortality in cholestatic rats.

These results are surprising, because in previous studies in not obstructed animals, infusion of rHDL led to decreased mortality after LPS challenge in vitro as well as in vivo.(16,17) Albeit that in a previous study with not obstructed dogs the beneficial effect of rHDL was overshadowed by rHDL-induced hepatotoxicity.(40,41) Nevertheless, it should be noted that rHDL-induced hepatotoxicity is dose dependent and the dose used in that study was relatively high (>500 mg/kg).(41) In the present study, we found that rHDL concentrations were not hepatotoxic. In a pilot study with 8 rats in a control (saline) group and 8 rats in a test group (3 ml of 120 mg/kg rHDL, 0.75 ml/hr), animals were observed for 14 days and then sacrificed. This dose (120 mg/kg rHDL) is twice the amount suggested for patients. Infusion of rHDL did not cause illness or death, changes in body weight or food intake, and there were no biochemical or histological indications for (hepato) toxicity. So rHDL-related hepatotoxicity as a possible explanation for the unexpected high mortality rate, has been ruled out in this study. More importantly, rHDL infusion followed by LPS administration in sham rats had no detrimental effects, instead, survival significantly improved. This experiment proves that rHDL is able to attenuate inflammatory responses after LPS challenge which is in agreement with the literature.

What could then be the cause of increased mortality associated with rHDL infusion in BDL rats? Our data show that rHDL infusion normalizes lipoprotein profiles as assessed by cholesterol distribution patterns and, as expected, in endotoxemic BDL rats, endotoxin was mainly associated with infused rHDL. The high VLDL and LDL cholesterol levels sustained at t = 1 h in BDL rats treated with rHDL did not result in higher FITC-LPS association but nearly all FITC-LPS was associated with the HDL fraction reflecting high binding capacity of HDL for LPS as compared with VLDL and LDL. Furthermore, it may be feasible that the high lipid free LPS fraction observed in the BDL groups at t = 0 is also present in the circulation rendering this LPS fraction free to react with inflammatory cells and resulting in an elevated inflammatory process. In previous studies in animals without jaundice, this phenomenon resulted in functional detoxification, prevented the release of pro-inflammatory cytokines and reduced mortality.(17,42) In contrast, rHDL infusion in BDL rats caused an increase in endotoxin-induced circulating TNF-α concentrations, and a dramatic increase in mortality. HDL is known to transport endotoxin to the liver, where it is detoxified and excreted via bile by hepatocytes; it constitutes a major pathway of endotoxin removal.(43) However, the blockade of biliary lipid secretion also leads to impaired rHDL clearance. Indeed, table 2 clearly shows that rHDL clearance is impaired in BDL rats; while in rHDL treated sham rats excess cholesterol is already removed at 1
h after infusion, in rHDL treated BDL rats still significantly increased cholesterol levels are observed at this time point as compared with saline treated BDL rats. This impaired clearance might lead to prolonged exposure of Kupffer cells to LPS, thereby causing enhanced TNF-α production. In addition, it is possible that biliary obstruction causes a preferential shift of endotoxin delivery from hepatocytes to liver Mφ’s and Kupffer cells, shown to be present in increased numbers in livers of BDL rats, as has been previously reported.(44,45) Indeed, in endotoxemia, Kupffer cells are a major source of TNF-α production.(46) Of further relevance in this respect is the finding that in endotoxemia in healthy volunteers, rHDL infusion increased the Limulus reactivity in blood (reflecting endotoxin bioactivity), while TNF-α induction was markedly suppressed.(19)

Indeed, efforts to delineate the mechanism(s) by which lipoproteins protect against endotoxin-induced death of the animals suggest that lipoproteins alter the in vivo metabolism of endotoxin. Specifically, the metabolic fate of the lipoprotein endotoxin complex appears to be directed by lipoprotein particles, which are abundantly present in the circulation up to 24 h post-infusion.(40) When administered with chylomicrons, clearance of endotoxin from plasma is elevated, and the amount of endotoxin taken up by the liver is doubled.(47,48) The same mechanism is very likely to occur when infusing rHDL.

These data are compatible with the concept that rHDL scavenges LPS from the circulation, which is followed by rapid excretion of bioactive endotoxin through the biliary tract. Paradoxically, in the presence of bile duct obstruction, the same mechanism may cause increased delivery of endotoxin to (activated) Kupffer cells, which are present in large numbers in the obstructed liver. These findings would suggest that rHDL infusion, as a therapeutic intervention in endotoxemia should be restricted to situations in which biliary excretion is not compromised.

In conclusion, the present study confirms that bile duct ligation in rats causes redistribution of lipoprotein profiles that are very similar to those during human biliary obstruction. RHDl infusion leads to normalization of the lipoprotein profile, and endotoxin rapidly associates with the infused rHDL. Whereas in not jaundiced animals as well as humans this leads to endotoxin neutralization, rHDL infusion leads to an increased release of TNF-α and significantly increased mortality in BDL rats. These results suggest that there may be danger in administration of rHDL to humans with obstructive jaundice.

REFERENCES


Chapter 5


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