Inflammatory response in obstructive jaundice and peritonitis

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The abnormal lipid spectrum in malignant obstructive jaundice in relation to endotoxin sensitivity and the result of preoperative biliary drainage.

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ABSTRACT

Background: Biliary obstruction changes the spectrum of lipoproteins, which are now known to bind and neutralize endotoxin. Postoperative septic complications related to an increased susceptibility to endotoxin occur frequently in patients with obstructive jaundice. The effect of preoperative biliary drainage on changes in the lipoprotein spectrum and its relation to endotoxin sensitivity was studied.

Methods: Abnormalities in the lipoprotein spectrum were assessed in 15 patients with malignant obstructive jaundice before and after three weeks endoscopic biliary drainage. Changes in endotoxin responsiveness were assessed by blocking cytokine production in whole blood cell cultures stimulated by cholestatic plasma taken before and after drainage, using reagents against endotoxin activity (anti-CD14 mAb, polymyxin B, and rBPI23).

Results: Drainage normalized VLDL, LDL, and HDL cholesterol fractions from respectively 43 to 199, 50 to 655, and 6 to 106 (p < 0.01). Ex vivo stimulation of whole blood with predrainage cholestatic plasma was 20-fold higher (p < 0.001) than with postdrainage plasma. Blocking the endotoxin response during the stimulation with predrainage cholestatic plasma with anti-CD14 mAb, polymyxin B or rBPI23 resulted in attenuation of the inflammatory response, reducing TNF-α levels at least 5-fold.

Conclusion: Preoperative biliary drainage normalizes the changed lipid profile and the endotoxin stimulating capacity of cholestatic plasma, and this may indicate a change in the sensitivity to endotoxin in these patients, thought to be related to postoperative septic complications.

INTRODUCTION

Abnormalities in the composition of the different components of the lipid spectrum and lipoproteins are common in obstructive jaundice. These abnormalities consist of a hypercholesterolemia and hypertriglyceridemia, secondary to changes in the lipoprotein spectrum. These changes include an increase in low density lipoprotein (LDL), with the appearance of abnormally formed LDL, also known as Lipid-X, and a reduction or even complete absence of high density lipoprotein (HDL), with the appearance of abnormal forms of HDL.

Recently several authors have proven that lipid particles may play a role in the protection against endotoxin (LPS). In vitro, reconstituted HDL, LDL, VLDL, and chylomicrons bind and neutralize the biological activity of endotoxin, thereby inhibiting the production and release of inflammatory cytokines by macrophages. In vivo, hypolipidemia causes an increase in LPS toxicity, suggesting that physiological levels of circulating lipids provide protection against LPS toxicity. In different animal studies, treatment with both cholesterol rich HDL and triglyceride rich VLDL or chylomicrons protected against mortality in experimental animal models of endotoxemia and gram-negative sepsis. Surgery in patients with obstructive jaundice can be complicated by septic morbidity, thought to be related to an excessive sensitivity to endotoxin. In animal models preoperative internal biliary drainage by insertion of an endoprosthesis showed a promising reduction of
endotoxemia and bacterial translocation to mesenteric lymph nodes,\textsuperscript{20} improvement of cellular immunity, and a reduction in postoperative mortality.\textsuperscript{21} The precise mechanism by which internal biliary drainage may reduce endotoxemia remains to be characterized, and in clinical studies the beneficial effects of biliary drainage are doubtful.\textsuperscript{18,22,23} In view of the established relationship between circulating lipoproteins and susceptibility to endotoxin, the present study was designed to investigate the lipid spectrum in severe obstructive jaundice and, additionally, to study the effect of preoperative internal biliary drainage.

**PATIENTS AND METHODS**

**Patients**
The study was approved by the research and ethical committees of the Academic Medical Center. After giving informed consent, fifteen patients were included with severe obstructive jaundice (bilirubin \(100\ \text{mmol/l}\)) caused by a distal biliary obstruction due to a pancreas carcinoma. Patients in whom introduction of an endoprosthesis by ERCP was not possible, and who were essentially not able to undergo a resection were not eligible for this study.

**Study model**
Before ERCP and endoprosthesis placement, when the patients were severely jaundiced, blood was obtained \(t_{10}\). Following insertion of a Teflon endoprosthesis, the patients were subsequently discharged and underwent biliary drainage for approximately three weeks \((3.8 \pm 0.3\ \text{weeks})\) before returning to the hospital for surgery (pancreatoduodenectomy or bypass procedure). At this time blood for time point \(t_3\) was taken. All blood samples were obtained after an overnight fast.

**Sampling**
For determination of routine parameters blood was collected in vacuum tubes containing 30 units lithium heparin. Bilirubin and other routine liver function parameters were assessed using routine methods. For determination of cholesterol, triglycerides, lipoprotein fraction, and apoprotein A and B, blood was taken in plain vacuum tubes. After coagulation for approximately 30 minutes, serum was prepared by centrifugation at room temperature at \(2000\ \times\ \text{g}\) for 20 minutes. Serum was aliquoted and stored immediately at \(-70\ ^\circ\text{C}\) until analysis.

For endotoxin assessment and ex vivo whole blood stimulation tests, blood was collected in sterile syringes and then immediately transferred into pyrogen free plastic tubes (Falcon 2063, Becton Dickinson, Mountain View, CA) containing pyrogen free heparin (Thromboliquine, Organon, Oss, The Netherlands; final concentration of 30 IU/ml) and immersed in melting ice. Plasma was prepared within 30 minutes after the blood collection, by centrifugation at \(1500\ \times\ \text{g}\) for 15 minutes at \(4\ ^\circ\text{C}\). All plasma samples were harvested in a laminar flow cabinet to prevent contamination, and aliquots were then stored at \(-70\ ^\circ\text{C}\) until testing.

**Lipid determination**
Total cholesterol concentrations were determined enzymatically using Cholesterol Enzymatique PAP reagent 250...
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Triglycerols were measured enzymatically without glycerol blanking using Triglyceride Enzymatique PAP reagent 1000 (BioMérieux, Marcy-I'Etiole, France).

Size exclusion chromatography (SEC) was used to separate the different lipoprotein fractions. The lipoproteins were separated over a Superose column (HR 10/30, Pharmacia, Uppsala, Sweden) in an isocratic system with a mobile phase consisting of 0.1 M NaHPO₄ and 0.2 M NaCl, pH 7.4. Post-column detection was performed by either an enzymatic procedure analyzing cholesterol (Cholesterol Enzymatique PAP reagent 250, BioMérieux, Marcy-I'Etiole, France) or an enzymatic procedure analyzing triglycerides (Triglyceride Enzymatique PAP 1000, BioMérieux, Marcy-I'Etiole, France). The separate conditions were essentially as described by Marz et al. with slight modifications. The column eluate was passed through a TFE tubing with a diameter of 0.5 mm and a length of 6 m. The flow rate of the reagent was 0.1 ml/min. The passage of the column eluate through the reaction cell was completed in 3 minutes. Relative quantities for the different lipoproteins were obtained by measuring the area under the curve for each fraction, while the absolute concentration of each fraction was calculated from this relative concentration and the total concentration in serum. The approximate molecular weight of the particles separated by SEC was estimated by the measurement of particles of known molecular weight to produce a standard curve. (Molecular weight gel filtration calibration kits, Pharmacia, Uppsala, Sweden). A peak at 57 minutes, indicates a molecular weight of approximately 79,000, a peak at 54 minutes, a larger aggregate of approximately 200,000 molecular weight.

Apolipoproteins A-I and B-100 were quantified using rate-immunonephelometry.

Measurement of endotoxin levels in cholestatic plasma

Endotoxin was assayed by the chromogenic Limulus amebocyte lysate test (LAL) (Chromogenix, Amsterdam, The Netherlands), performed with minor modifications as described previously. Inhibitors and activated clotting factors were removed by dilution and heating at 75 °C for five minutes. Standard curves were made with Escherichia coli O55: B5 endotoxin (Mallinckrodt Inc., St. Louis, Missouri, USA). The assay had a detection limit in blood of less than 35 EU/LPS/plasma (approximately 3.5 pg/ml). A plasma control of each sample was used to prevent false positive results owing to the intrinsic color of jaundiced plasma. Each sample was assayed either in duplicate or quadruplicate and the results were expressed as the mean of the two or four tests.

Endotoxin-induced stimulation of human whole blood cultured with ex vivo 'cholestatic' plasma and cytokine assays

To assess the presence of biologically active endotoxins in the plasma of each patient, plasma samples from each patient, taken at t₀ and t₃, were tested for their potency to induce the release of proinflammatory cytokines by human mononuclear cells as described previously. Human whole blood of healthy volunteers was collected aseptically using a sterile collecting system consisting of a butterfly needle connected to syringe (Becton Dickinson & Co., Rutherford, NJ). For anticoagulation pyrogen free heparin (Thrombolytique, Organon) (final concentration of 10 U/ml blood) was used. Whole blood, diluted 1:2 was stimulated for 24 hours at 37 °C and at a final dilution of 1:1 in sterile 200 µl flat-bottom wells (Corning Inc., Corning, NY) in sterile RPMI-1640 (GibcoBRL, Life Technologies Inc., Grand Island, NY) containing 100 U/ml penicillin, 100 µg/ml streptomycin, in the presence of serial dilutions of 'cholestatic' plasma samples (t₀ and t₃) to be tested. Each diluted sample before drainage (t₀) was tested alone as well as in the presence of either five µg/ml anti-CD14 mAb (a generous gift from Dr. J. Pugin, and...
Division of Medical Intensive Care, University Hospital of Geneva, Geneva, Switzerland, two ng/ml polymyxin B (Sigma Chemical Co., St. Louis, MO) or two µg/ml recombinant bacterial permeability increasing protein (rBPI: XOMA Corp., Berkeley, CA). After incubation culture supernatants were harvested and stored at −70°C until tested for the presence of cytokines. A 24-hour incubation period was chosen since TNF levels have reached a plateau by then.7 As a control, normal human plasma containing LPS (from Escherichia coli serotype 0111:B4; Sigma) at concentrations of 0.1 to 1000 pg/ml, was repeatedly tested under similar conditions and in the same assay as the cholestatic samples to validate the results.

The following cytokines were measured in the supernatant by a sandwich-type enzyme-linked immunosorbent assay (ELISA) according to the instructions of the manufacturer (with detection limits): TNF-α (6.8 pg/ml), IL-6 (2.2 pg/ml), and IL-1β (1.2 pg/ml) all purchased from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, C.B., Amsterdam, The Netherlands.

All samples were assayed in duplicate in the same culture experiment using blood from one healthy donor and this was repeated using blood from another donor, to assess donor dependent variability. All ELISA's used had intra-assay variation coefficients of less than 10%, as was estimated from the variation of dose-response curves obtained on at least three different days over a three-month period. All supernatants were tested within one assay procedure to minimize the inter assay variation of each cytokine.

Statistical analysis

All results are given as mean ± SEM. A 2-tailed p-value < 0.05 was considered significant. Statistical differences between the time points were analyzed using the Wilcoxon-Rank Sum test for two related samples. All statistical calculations were conducted with standard biomedical statistical programs (SPSS 9.0; SPSS, Chicago, IL).

RESULTS

Routine measurements

Adequate biliary drainage was achieved in all patients. The mean bilirubin concentration decreased from 244 ± 33.8 at t0 to 34.6 ± 14.9 µmol/l at t3 (p=0.003), and alkaline phosphatase was reduced from 404.1 ± 46.3 (t0) to 171.1 ± 23.3 U/l at t3 (p=0.003). Albumin levels were 42.4 ± 1.4 at t0 and 38.8 ± 1.9 g/l after 3 weeks (NS).

Lipid parameters

Table. Results of lipid parameters

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<th>Parameter</th>
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<th>T3</th>
<th>p-value</th>
<th>Reference</th>
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<td>Total cholesterol (mmol/l)</td>
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<td>LDL-cholesterol (mmol/l)</td>
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<tr>
<td>HDL-cholesterol (mmol/l)</td>
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<td>0.8</td>
<td>0.1</td>
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<th>Lipoprotein Fraction</th>
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<th>LDL-triglyceride (mmol/l)</th>
<th>HDL-triglyceride (mmol/l)</th>
<th>Apo A-I (g/l)</th>
<th>Apo B-100 (g/l)</th>
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<td>&lt; 0.58</td>
<td>&gt; 0.22</td>
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<td>0.5-1.1</td>
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</table>

*Reference values in healthy controls.

Total cholesterol and triglyceride levels

Total cholesterol (reference values: < 6.0 mmol/l) was increased in jaundiced patients at 8.9 ± 1.0 mmol/l. Total triglyceride levels (reference values: < 2.0 mmol/l) were also increased to 3.6 ± 0.5 mmol/l. After biliary drainage total cholesterol decreased to 5.4 ± 0.5 mmol/l (p=0.001). Total triglyceride was also significantly reduced to 1.8 ± 0.3 mmol/l (p=0.0008). (Table 1)

Qualitative and quantitative lipoprotein fractions

Cholesterol fractions

Size exclusion chromatography analysis of lipoprotein cholesterol fractions showed significant differences in relative percentages, which were abnormal during obstructive jaundice, when compared to reference values, and returned toward reference values after drainage. At t0 42.9 ± 2.4 % of total cholesterol consisted of VLDL-cholesterol (reference: 13%). This was significantly reduced to 18.9 ± 2.4 % after biliary drainage (p=0.0008). 49.8 ± 1.9 % was LDL cholesterol (reference: 70%) and increased toward normal to 65.1 ± 2.5 % at t3 (p=0.0015). In the jaundiced patients the percentage HDL cholesterol (reference 17%) was very low (6.3 ± 1.5 %) and increased to 15.7 ± 2.2 at t3 (p=0.0008).

As total cholesterol and the relative percentage VLDL-cholesterol decreased after drainage, the reduction in the absolute amount of this cholesterol fraction present in serum was highly significant, 3.6 ± 0.3 mmol/l at t0 versus 1.1 ± 0.2 mmol/l at t3 (p=0.0007). Total cholesterol decreased after drainage, in there the LDL-cholesterol fraction relatively increased resulting in levels of 4.6 ± 0.6 at t0 and 3.5 ± 0.3 mmol/l at t3, which was not significant (p=0.14). The result is similar for HDL-cholesterol levels, namely 0.6 ± 0.1 and 0.8 ± 0.1 mmol/l at t0 and t3 respectively, and did not reach significance (p=0.18).

HDL cholesterol particles were smaller at t0 than 3 weeks after drainage. In 12 of the 15 patients a HDL cholesterol peak with a molecular weight of approximately 79 000 was found, in the remaining 3 patients a larger aggregate was encountered of approximately 200 000 molecular weight. After biliary drainage the HDL cholesterol peak contained the larger aggregate in 13 of the patients. In 2 patients the particles remained smaller. Healthy controls produce a peak approximately around that of the large HDL particle.

Triglyceride fractions

Relative lipoprotein triglyceride fractions showed 36.8 ± 3.9 % of triglyceride at t0 to be
VLDL-triglyceride (reference 55%). After drainage there was no significant change with 39.7 ± 2.9 % (p=0.7). LDL-triglyceride made up 38.4 ± 2.5 % of total triglyceride at t0 (reference 29%) and 33.3 ± 2.7 % at t3 (p=0.18). HDL-triglyceride was 15 ± 1.7 % during jaundice (reference 11%), and 11.0 ± 1.7 after drainage (p=0.29).

The total reduction in triglyceride and the relative percentage changes resulted in significant triglyceride fraction concentration changes following the biliary drainage procedure. VLDL triglyceride concentrations were 1.4 ± 0.2 mmol/l (t0) and 0.9 ± 0.2 mmol/l (t3) (p=0.019).

The HDL triglyceride fraction was reduced from 0.5 ± 0.07 to 0.3 ± 0.03 mmol/l (p=0.0012).

Here too, the presence of two different sized particles was noted, which in contrast to HDL in some patients coexisted simultaneously. At t0 all patients had a small HDL triglyceride particle (~79 000 MW). At t3 all patients, again, had the same small particle (which was not detectable at t3 in HDL-cholesterol), and 6 additionally had the second peak of the large particle (~200 000 MW).

Apolipoproteins
Apolipoprotein A-I (normal values: 1-2 g/l) was 0.65 ± 0.09 at t0 and had increased toward normal after drainage to 0.98 ± 0.10 (p=0.046). Apoprotein B-100 (reference values: 0.5-1.1 g/l) was 2.41 ± 0.26 at t0 and decreased to 1.38 ± 0.15 after 3 weeks biliary drainage (p=0.0097).

Endotoxin in plasma
Endotoxin concentrations at t0, as measured by the LAL assay, was 4.3 ± 1.7 pg/ml, which is marginally above the cut off for normal concentrations. After biliary drainage at t3, no reduction in endotoxin concentrations was found (4.5 ± 2.1 pg/ml, P = 0.07, t0 versus t3).

Stimulation of human whole blood by cholestatic plasma
To assess whether the observed changes after endoscopic biliary drainage was due to circulating endotoxins, we examined the potency of cholestatic plasma, taken before and three weeks after drainage, to induce the release of cytokines in human whole blood. Using the experimental conditions described in the Methods section, 1 and 100 pg/ml LPS added to human whole blood resulted in a TNF-α concentration in the supernatant of 120 ± 45 and 595 ± 87, respectively. Addition of anti-CD14 mAb (five µg/ml), rBPI22 (two µg/ml), and to a lesser extent polymyxin B (two ng/ml) resulted in an almost complete inhibition of TNF-α, IL-6 and IL-1β in the supernatant induced by LPS concentrations up to 100 pg/ml (data not shown).

Incubation of human whole blood with diluted cholestatic plasma taken at t0, yielded significant higher levels of TNF-α in the supernatant as compared with cholestatic plasma taken at t3 (Figure 1 A: P < 0.05).
Incubation of human whole blood with a 10-fold dilution of cholestatic plasma taken at t0 and t3 caused TNF-α levels in the supernatant ranging from respectively 3654-4832 pg/ml and 125-230 pg/ml. When anti-CD14 mAb, rBPI2, or polymyxin B was added to cultures with cholestatic plasma taken at t0, levels of TNF-α were significantly reduced, consistent with the specific anti-LPS activities of the former three reagents (Figure 1 B). The supernatant TNF-α concentrations of human whole blood incubated with cholestatic plasma taken at t0 were reduced by addition of the anti-LPS reagents to levels almost equal (0.05 < P < 0.07 for polymyxin B and anti-CD14) and levels near equal (P < 0.05 for rBPI2) compared with levels induced by cholestatic plasma at t0 alone to those induced by cholestatic plasma taken at t3.

Similar results were obtained when IL-6 or IL-1β was measured in the culture supernatants. Again, cholestatic plasma taken at t0 yielded significant higher levels of these cytokines in the supernatant than those samples taken at t3. Adding rBPI2 significantly diminished these elevated levels, maximum reductions being respectively 82% for IL-6 levels and 75% for IL-1β levels. The elevated levels of IL-6 and IL-1β could only be diminished to a lesser extent by adding anti-CD14 mAb, and polymyxin B to the culture (0.05 < P < 0.08) (data not shown).

DISCUSSION

In the present study the lipid abnormalities in malignant obstructive jaundice were comparable to those reported previously in persistent primary biliary cirrhosis. Because of the lipid profile encountered, and the fact that it changed after drainage, these changes were likely due to the obstruction and not to the malignant disease. Biliary obstruction was associated with hypercholesterolemia and hypertriglyceridemia and the relative cholesterol fractions showed an increase in the VLDL fraction and a decrease in both LDL and HDL-cholesterol. Absolute VLDL-cholesterol concentrations were also greatly increased. The serum concentration of apoprotein A, which is found in HDL particles, was diminished and apoprotein B, found in LDL, was increased. Biliary drainage returned hypercholesterolemia and triglyceridemia to more normal levels and relative levels of the cholesterol fractions were changed in the direction of reference values. This also applies to absolute VLDL-cholesterol levels. Apoproteins A-I and B-100 concentrations were also significantly changed toward normal levels.

The abnormal lipid composition in obstructive jaundice could be due to a combination of mechanisms, including a reduced clearance of excess cholesterol to the gut by bile salts, and an impairment of hepatocyte function. Both VLDL synthesis and de novo HDL synthesis can be impaired in extensive liver damage. Less severe liver damage, such as occurs in short lasting biliary obstruction, significantly reduces the activity of the enzyme lecithin cholesterol acyltransferase, synthesized in the liver, which is up to 90% less active in obstructive jaundice.
jaundice. This results in the formation of immature HDL, reduced cholesterol esterification and increased free cholesterol, which cannot be transferred to VLDL or LDL for transportation to the liver. As LCAT activity may severely decrease in only two weeks, while hepatocyte function is still preserved, it is likely that LCAT synthesis or release is sensitive to intraductal pressure. The key role of this enzyme for induction of the abnormal lipid spectrum in obstructive jaundice is underscored by the observation that patients with familial LCAT deficiency have abnormalities in their lipid spectrum virtually identical to obstructive jaundice patients.

LDL uptake by hepatocytes is diminished in obstructive jaundice, due to impairment of hepatocyte function and perhaps because of alterations in the lipoproteins themselves. Lipoprotein uptake by hepatocytes results from receptors for apolipoproteins. Changes in apoproteins, as in obstructive jaundice, impair the interaction between LDL and the hepatocyte. In the present study the concentration of apoprotein A-I was decreased during jaundice and increased toward normal after drainage. Apoprotein B, on the other hand, was increased and was reduced after drainage.

The small HDL-cholesterol particle encountered in jaundiced patients doubled in weight after drainage. These small particles most likely represent nascent HDL particles that lack esterified cholesterol and have been described previously. In the present study the HDL moiety size was shown for the first time to be changed after relief of jaundice.

The consequences of the lipid abnormalities for endotoxin sensitivity in obstructive jaundiced patients may be important. In vitro studies with radiolabeled LPS indicate that most LPS added to serum is recovered from the lipid fraction, and that this fraction is responsible for inactivation of endotoxin by serum. Lipids bind to endotoxin, bound by lipopolysaccharide binding protein, by insertion of the biologically active lipid A moiety of lipopolysaccharide into the phospholipid surface layer of the lipoprotein particle. Apoproteins can also bind and neutralize endotoxin. VLDL’s and chylomicrons may also in part protect against the lethal effects of endotoxemia by increasing endotoxin-uptake by hepatocytes instead of Kupffer cells, causing more rapid clearance of lipoprotein-LPS complexes into bile. Clearly, this mechanism will be impaired in obstructive jaundice, and may represent another mechanism for an increased sensitivity to endotoxin.

In recent studies of low dose endotoxemia in human volunteers it was shown that while reconstituted HDL protected against endotoxemia and blocked the induction of clinical symptoms and cytokine induction, triglyceride rich lipoprotein treatment (Intralipid) showed no protective effect. In agreement with this finding, in studies with radioactive LPS, it was the complex of LPS with HDL that was frequently found and that most effectively blocked binding to macrophages. Similarly, the plasma LPS inactivating rate of serum from patients with liver cirrhosis was shown to be positively correlated to serum HDL-cholesterol levels.

Although the jaundiced patients had hypercholesterolemia and hypertriglyceridemia, HDL and LDL levels were low, which could indicate increased sensitivity to endotoxin, as in
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simple hypolipidemia. Furthermore, the inhibiting effect of reconstituted HDL on endotoxin was recently observed partly to be the result of a reduction of CD14 expression on monocytes. If this also applies to natural HDL, a reduction in this particle, as seen in the patients studied here, may mean an increase in CD14 expression and, therefore, in the potency of the biological effects of endotoxin on monocytes.

Biliary drainage restores the enterohepatic bile salt circulation, resulting in cholesterol clearance to the gut. In addition, drainage relieves the biliary obstruction, improving liver function and ameliorating synthesis functions and hepatocyte uptake, therefore resulting in normalization of lipid levels. After drainage HDL and LDL-cholesterol levels and apolipoprotein A-I levels significantly increased, indicating a change toward a state with more protective HDL. VLDL-cholesterol levels decreased after drainage.

Circulating endotoxin levels as measured using the Limulus assay did not significantly decrease after endoscopic biliary drainage. It should be recognized that the endotoxin test used is a chromogenic assay that is potentially influenced by the yellow color of cholestatic plasma. We have noted this problem before and have used each sample measured chromogenically for endotoxin as its own control. It is unclear whether such controls have always consistently been performed in previous studies, and this could account for the high endotoxin concentrations reported in some. In the present study, we used a different approach to identify plasma endotoxin activity, i.e., cytokine production by human whole blood cultures stimulated by cholestatic plasma.

This approach was successfully used in previous studies to demonstrate endotoxin in plasma during cholestasis and liver surgery. To investigate the involvement of endotoxin and changes therein in cholestatic plasma at t0 and t3, among others the LPS receptor on monocytes (CD14) was specifically blocked by using an anti-CD14 mAb. We found that plasma samples obtained before drainage much more potently stimulated the production of cytokines than samples obtained after successful drainage. Anti-CD14 mAb, polymyxin B, and rBPL; were able to block cytokine release in human whole blood stimulated with cholestatic plasma taken at t0, in agreement endotoxins being present in cholestatic serum. From these results we concluded that both pre- and postdrainage cholestatic plasma samples stimulated human blood cells to produce TNF-α, IL-6 and IL-1β because of the presence of circulating plasma endotoxin. In addition, because the response was higher with predrainage samples compared with postdrainage samples, circulating endotoxin levels were apparently higher before endoscopic biliary drainage.

Unfortunately in clinical studies preoperative biliary drainage did not result in reduction of infectious complications, probably due to inflammatory changes related to the drainage procedure and the endoprosthesis itself. If adequate preoperative biliary drainage without inflammatory changes is not feasible, then changing the lipid abnormalities preoperatively instead of biliary drainage might affect on postoperative complications. This potential treatment should be evaluated in the future.

In conclusion, in patients with obstructive jaundice significant abnormalities of the spectrum
of circulating lipoproteins were observed. Postoperative complications in these patients are thought to be related to an increased susceptibility to endotoxin. Lipoproteins have been shown to play an important role in the physiologic protection against endotoxin and biliary drainage returns nearly all the lipid abnormalities encountered in this study toward normal. The changed lipid profile may indicate a change in the sensitivity to endotoxin in these patients, thought to be related to postoperative septic complications. The subsequent normalization following drainage may therefore indicate a change in this susceptibility to endotoxin related complications.

REFERENCES

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A-C: Endotoxin-induced stimulation of human whole blood cultures with ex vivo cholestatic plasma samples (n = 15). Induction of TNF-α (A), IL-6 (B), and IL-1β (C) in cultures of human whole blood by cholestatic plasma samples collected before drainage at t0 (squares) and after drainage at t3 (triangles). D-F: Inhibition of endotoxin-induced stimulation of human whole blood cultured with ex vivo cholestatic plasma samples taken before drainage at t0 (n = 15). Induction of TNF-α (D), IL-6 (E), and IL-1β (F) in cultures of human whole blood is inhibited by anti-CD14, polymyxin B, or rBPI23.