P-glycoproteins and hepatobiliary secretion
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CHAPTER 4

UPREGULATION OF THE MULTIDRUG RESISTANCE GENES Mrp1 and Mdr1b and Downregulation of the Organic Anion Transporter Mrp2 and Bile Salt Transporter Bsep/Spgp in Endotoxemic Rat Liver

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Endotoxin-induced cholestasis is mainly caused by an impaired canalicular secretion. Mrp2, the canalicular multispecific organic anion transporter, is strongly down-regulated in this situation and also canalicular bile salt secretion is reduced. We hypothesized that other adenosine triphosphate-binding cassette (ABC) transporters may compensate for the decreased transport activity to protect the cell from cytokine-induced oxidative damage. Therefore, we examined the expression of ABC-transport proteins in membrane fractions of whole liver and of isolated hepatocytes of endotoxin-treated rats and performed RT-PCR on mRNA isolated from these livers. In addition, the localization of these transporters was examined using confocal scanning laser microscopy. Already 6 hours after endotoxin-administration, we found a clear increase of Mrp1 mRNA and protein, whereas Mrp2 mRNA and protein were decreased. This was confirmed in isolated hepatocytes. In addition, Mdr1b mRNA was strongly increased, whereas Mdr1a and Mdr2 mRNA did not change significantly. Both the mRNA and protein levels of the recently cloned bile salt transporter Bsep decreased. After endotoxin-treatment, the normally sharply delineated canalicular staining of Mrp2 and Bsep had changed to a fuzzy pattern suggesting localization in a subapical compartment. We conclude that endotoxin-induced cholestasis is caused by decreased Mrp2 and Bsep levels as well as an abnormal localization of these proteins. The simultaneous upregulation of Mrp1 and Mdr1b may confer resistance to hepatocytes against cytokine-induced metabolic stress.
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INTRODUCTION

Excretion of a large variety of endogenous and exogenous compounds from hepatocytes into bile is an adenosine triphosphate (ATP)-dependent process, predominantly performed by members of the P-glycoprotein (Pgp) subfamily and the Multidrug Resistance Protein (MRP) subfamily of the ATP-binding cassette (ABC) protein superfamily.1,2. At least four members of the Pgp-subfamily are located at the canalicular membrane of rodent liver: Mdr1a, Mdr1b, Mdr2, and Bsep/Spgp. In normal rodent liver, Mdr1a and Mdr1b are present at low levels. Overexpression of mouse Mdr1a/Mdr1b confers multidrug resistance against a broad variety of natural product drugs.3,4. The main physiological function of these multidrug resistance proteins is presumably the transport of bulky amphiphilic compounds, such as cationic drugs, hydrophobic peptides, steroids, and atypical glycolipids, across the canalicular membrane1,4,5. The expression of Mdr2 in normal rodent liver is high. This transporter functions as a flippase that translocates phosphatidylcholine across the membrane6. From pig liver, Childs et al. cloned part of another member of the Pgp-subfamily: the sister gene of Pgp (Spgp)7. Most recently, Gerloff et al. cloned the full-length cDNA of Spgp from rat liver.8 They provided evidence that Spgp, which is exclusively present in the liver, most likely is the major canalicular bile salt export pump of mammalian liver. Spgp is therefore preferably named Bsep.

So far, the presence of four members of the mrp-subfamily has been demonstrated in liver9,10. MRP1/Mrp1 is present at very low levels at the lateral membrane of the hepatocyte11,12. The human MRP1 and its murine homologue Mrp1 have been shown to confer multidrug resistance to natural product drugs13,14. Moreover, MRP1 is able to transport glutathione disulfide15 and glutathione (GSH) S-conjugates including the lipid peroxidation products leukotriene C4 and 4-hydroxynonenal S-GSH.16 The recently cloned Mrp1 homologue Mrp219,20 is strongly expressed at the canalicular membrane and functions as cmoat (canalicular multispecific organic anion transporter)21,22. Kool et al. recently demonstrated the presence of two other MRP1 homologues, MRP3 and MRP5 in human liver.10 The function and subcellular localization of these proteins are yet unknown.

Cholestasis due to the action of LPS seems to be mainly due to a decreased bile salt independent bile flow, which is most probably caused by downregulation of the transport capacity for Mrp2-substrates (polyanionic organic conjugates) and GSH.23-25. Recently, downregulation of Mrp2 during endotoxemia has been reported which may explain the defective anion secretion found after endotoxin-treatment.26. Secretion of monovalent bile salts is also impaired during endotoxemia,23,27-29 suggesting down-regulation of the bile salt transporter, but this has not been studied yet.

LPS-induced changes in hepatocytes are mainly due to the action of tumor necrosis factor a (TNFα) and possibly interleukin-1, interleukin-6 and other cytokines. TNFα binding to its receptor activates a complex intracellular signaling pathway that includes translocation of the transcription factor nuclear factor-kB (NF-kB) to the nucleus and the production of reactive oxygen species.30 We have hypothesized that transporters such as Mrp1 and Mrp2 play a role in the GSH-dependent cellular response to oxidative stress.1 As a result of the decreased Mrp2 activity during endotoxemia, a significant increase of liver damage by products of cytokine-induced oxidative stress would be expected. However, cholestasis and sepsis usually occur without much hepatocellular damage. Indeed, we observed only a minimal increase of aminotransferase levels during endotoxemia.31 These observations suggest that the hepatocyte may compensate for the Mrp2 down-regulation by upregulation of other transport systems.
The aim of the present study was to evaluate the expression of the presently known rat liver ABC-transporters during endotoxemia using specific antibodies against Mrp1, Mrp2 and Bsep and semiquantitative RT-PCR for Mrp1, Mrp2, Mdr1a, Mdr1b, Mdr2, and Bsep. We also investigated the subcellular localization of these transporters in normal and endotoxemic rat liver using Confocal Scanning Laser Microscopy (CSLM).

**EXPERIMENTAL PROCEDURES**

**ANIMALS**
Specified pathogen-free male Wistar rats (220-300 g) were purchased from Harlan-CPB, Zeist, the Netherlands. They were kept under routine laboratory conditions at the Central Animal Laboratory of the University of Groningen. The rats received standard laboratory chow and had free access to food and water. The study as presented was approved by the Local Committee for Care and Use of Laboratory Animals.

**EXPERIMENTAL DESIGN**
Rats were injected intraperitoneally (IP) with 5 mg/kg endotoxin (LPS, E. coli, serotype 0127:B8, Sigma, St. Louis, MO) or the same volume of PBS (control group, n=6 for each experimental group). In the experimental groups, no mortality was observed due to endotoxin administration. At indicated time points the rats were anaesthetized with pentobarbital (60 mg/kg, IP). The livers were perfused with PBS, removed, cut into small pieces, snap-frozen in liquid nitrogen and stored at -80 °C until used for isolation of crude membranes and RNA. For isolation of hepatocytes, rats were injected with 2 mg/kg LPS (IP, Sigma) or PBS and rat hepatocytes were isolated 15 hours later by two-step collagenase perfusion as described previously32.

**ANTIBODIES**
Specific polyclonal antibodies against Mrp1 (k5) were raised by immunizing rabbits with the peptide RGLFYSMAKDAGLV located at the carboxy terminal of human MRP1. This antibody does not cross-react with Mrp2, as described by Roelofsen et al. Also no cross reactivity with rat Mrp3 was found (unpublished data). Polyclonal antibodies against Mrp2 (k4) were raised by immunizing rabbits with the peptide AKEAGIENVNHTEL located at the carboxy terminal of rat Mrp2. On Western blot, the Mrp2 antibody specifically recognizes a protein of approximately 190 kilodaltons in enriched canalicular membrane fractions from normal rat liver. This protein is absent in the canalicular membrane fractions from Mrp2-deficient (TR-) rats. A polyclonal antibody against Bsep (k12) was raised by immunizing rabbits with the peptide KGAYYKLVTGAPIS from the carboxy terminal of the pig Bsep sequence. This peptide differs in one amino acid with the recently published rat Bsep; a threonine (underlined) in pig Bsep is substituted by an isoleucine in rat Bsep. The Bsep sequence exhibits no homology with Mrp1, Mrp2, Mdr1a, Mdr1b, or Mdr2 sequences. On Western blot, the Bsep antibody recognizes a protein of approximately 170 kilodaltons in canalicular membrane fractions from rat liver, but not in the basolateral enriched fraction. No specific signals were seen in membrane subfractions from cell lines overexpressing MRP1 or MDR1 (data not shown). Mouse monoclonal antibody C219 (Signet Laboratories Inc, Dedham, MA, USA) was used to detect all P-glycoproteins (Pgps). Mouse monoclonal antibody against dipeptidyl peptidase IV (DppIV/CD26) was purchased from Endogen (Woburn, MA, USA).

**ISOLATION OF CRUDE MEMBRANES FROM LIVER HOMOGENATES**
Crude membranes were isolated according to Meier and Boyer, with some modifications. In brief, for each sample, 2 grams of frozen rat liver were thawed in 5 mL cold 1 mmol/L NaHCO₃/Complete protease inhibitor cocktail (1 tablet/50 mL, Boehringer Mannheim GmbH, Mannheim, Germany) and homogenized with a Polytron (Kinematica GmbH, Luzern, Switzerland) and a loose Dounce homogenizer (five strokes up-and-down). The homogenate was
further diluted in ice-cold 1 mmol/L NaHCO₃ / 0.1 mmol/L PMSF and centrifuged at 1,200 g for 15 minutes at 4 °C. The crude nuclear pellet was resuspended in 2.5 volumes of 70% (w/w) sucrose. The homogenate was poured into a SW28 rotor tube (Beckman Instruments Inc., Palo Alto, CA, USA) and overlaid with 10 mL 44% and 10 mL 36.5% (w/w) sucrose. The tube was centrifuged at 90,000 g for 90 minutes at 4 °C. The crude membrane fraction from the 36.5-44% interface was centrifuged in 1 mmol/L NaHCO₃ / 0.1 mmol/L PMSF at 7,800 g, for 15 minutes at 4 °C. The pellet was resuspended in 500 µL 1 mmol/L NaHCO₃ / protease inhibitor by repeated (15 times in and out) suctioning through a 25 G needle. The sample was stored at -80 °C until further use.

**ISOLATION OF CRUDE MEMBRANES FROM ISOLATED HEPATOCYTES**

Freshly isolated hepatocytes were washed in Krebs buffer (118 mmol/L NaCl, 5 mmol/L KCl, 1.1 mmol/L MgSO₄, 2.5 mmol/L CaCl₂, 1.2 mmol/L KH₂PO₄, 25 mmol/L NaHCO₃, 10 mmol/L D(+)-glucose, 10 mmol/L Hepes, 1% bovine serum albumin, pH 7.42) and pelleted at 500 g for 8 minutes. For permeabilization cells were stirred in 35 mL 1 mmol/L NaHCO₃ / 0.1 mmol/L PMSF for one hour at 4 °C. After centrifugation of the homogenate at 90,000 g for 30 minutes at 4 °C, the pellet was resuspended in 40 mL 250 mmol/L sucrose / 0.1 mmol/L PMSF and homogenized 50 times with a tight Dounce homogenizer. Per liver, 3 SW28 tubes were loaded with 15 mL 38% sucrose and 15 mL hepatocyte homogenate, respectively. After centrifugation at 90,000 g for 90 minutes at 4 °C, the 38% fraction was washed in 1 mmol/L NaHCO₃ / 0.1 mmol/L PMSF and centrifuged at 47,000 g for 30 minutes at 4 °C. Pellets were resuspended in 250 mmol/L sucrose / protein inhibitor by 15 times in and out suctioning through a 25 G needle and stored at -80 °C until further use.

**IMMUNOBLOT ANALYSIS**

The protein concentrations in membrane fractions were determined with the DC Protein Assay from Bio-Rad (Bio-Rad Laboratories, Hercules, California) using bovine serum albumin as standard. 50 mg of membrane proteins were fractionated on a 7.5% SDS-PAGE gel and transferred to nitrocellulose (Amersham International, Buckinghamshire, UK), using a tankblotting system according to manufacturer’s instructions (BioRad Laboratories). For DppIV detection, samples were boiled in sample buffer for 5 minutes before loading on SDS-PAGE. BDH molecular weight standards (42,700-200,000 mol wt range, BDH, Dorset, England) were used as marker proteins. The blots were stained with Ponceau-S solution (0.1% Ponceau-S (w/v) in 5% acetic acid (v/v), Sigma) to confirm similar protein concentrations in every lane. The blots were incubated with the first antibody diluted in PBS containing 4% SKIM milk (Fluka BioChemica, Buchs, Switzerland) and 0.1% polyoxyethylene sorbitan monolaurate (TWEEN-20, Sigma), washed in PBS / 0.1 % TWEEN-20, subsequently incubated with horse-radish peroxidase labeled swine anti-rabbit IgG or rabbit anti-mouse IgG diluted in PBS / 4% SKIM milk / 0.1% TWEEN-20 (dilution 1:2,000, DAKO A/S, Glostrup, Denmark) and finally developed using Pierce SuperSignal Chemiluminescent Substrate Luminol/Enhancer (Pierce, Rockford, IL, USA).

**REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION**

Total RNA was isolated from frozen rat liver and isolated hepatocytes using TRIzol Reagent (Gibco Laboratories, Grand Island, NY) according to manufacturer’s instructions. Subsequently, mRNA was isolated using the Oligotex mRNA mini-kit (Qiagen GmbH, Hilden, Germany). Single stranded cDNA was synthesized from 2.5 µg mRNA using 0.5 nmol random primers (Pharmacia, Uppsala, Sweden) and 50 U AMV Reverse-Transcriptase ((RT) Promega, Madison, WI USA) in a buffer containing 50 mmol/L Tris-HCl, 50 mmol/L KCl, 10 mmol/L dithiothreitol, 10 mmol/L MgCl₂ (Promega), 50 U RNA guard (Pharmacia), and 1.25 mmol/L of each dNTP (Pharmacia) in a total volume of 100 µL. Reverse transcription was performed for 10 minutes at 25 °C and 1 hour at 50 °C and the samples were subsequently heated for 5 minutes at 95 °C to terminate the reverse transcriptase reaction. With the cDNA obtained, a PCR reaction was performed using 4 µL of the RT reaction mix. To this 2.5 U Taq polymerase (Pharmacia), 50 pmol sense and 50 pmol anti sense primer were added. The final reaction volume was 50 µL. The tubes were incubated in a Gene Amp 2400 PCR system (Perkin-Elmer, Norwalk, CT) at 95 °C for 5 minutes to denature the primers and
The cycling program was 95 °C for 30 sec, 54/56 °C for 30 sec, 72 °C for 30 sec and for 5 minutes in the last cycle, and comprised 20-33 cycles. For each primer set, an increasing number of PCR-cycles with otherwise fixed conditions was performed to determine the optimal number of cycles to be used. This was determined to be halfway the exponential phase. The number of cycles were 20 for Mrp1, 22 for Gapdh and Mrp2, 26 for Mdr2, 29 for Mdr1b, and 33 for Mdr1a and Mrp1.

For every PCR reaction, Gapdh was used as internal control. Primer sequences and resulting PCR-products are listed in Table 1. Because of the high homology between Mrp1 and Mrp3, we sequenced the Mrp1 PCR-product. Sequence analysis confirmed the specificity of the PCR-primers for Mrp1. In each experiment, water was used as a negative control. Ten µL of PCR product was loaded on a 2.5% agarose gel and stained with ethidium bromide.

### RESULTS

#### IMMUNOBLOT ANALYSIS

To study the expression of different hepatic ABC-transport proteins during endotoxemia, specific polyclonal antibodies were raised against Mrp1, Mrp2 and Bsep and their protein levels were examined by immunoblot analysis. Results are shown in Figure 1. For sake of clarity, only immunoblots of crude membrane preparations of each time point of one representative experiment are shown. Similar results were observed with membrane preparations from other rat liver samples (n=4 per time point).
During endotoxemia, a clear increase of Mrp1 protein in crude membranes of LPS-treated rat liver was seen (Figure 1). This increase was already noticeable 6 hours after LPS and was still present after 48 hours. In contrast, a strong decrease of Mrp2 protein was observed. This decrease of the Mrp2 protein levels was observed as early as 15 hours after LPS and 24-48 hours after LPS Mrp2 was undetectable. In addition to members of Mrp-subfamily, we also examined the protein levels of members of the Pgp-subfamily. This was done by using monoclonal antibody C219 which recognizes a conserved epitope in all known members of the Pgp-subfamily. The C219 staining of crude membranes decreased during endotoxemia (Figure 1). Using a specific antibody against Bsep a similar decrease was observed. The crude membrane fractions showed a similar staining of DppIV, a major canalicular membrane protein, indicating that the recovery of crude membranes during the isolation procedure was approximately the same.

**MRNA ANALYSIS BY RT-PCR**

We chose RT-PCR to investigate the regulation of the ABC-transporter genes, because Northern blotting is not sensitive enough to detect *Mrp1*, *Mdr1a* and *Mdr1b* gene transcripts in normal rat liver. Furthermore, the specificity of the PCR-reactions can be confirmed by sequencing the PCR-products. We developed RT-PCR assays using rat...
specific PCR-primers against Mrp1, Mrp2, Mdr1a, Mdr1b, Mdr2, Bsep, and Gapdh to analyze mRNA levels of the respective transporter genes. In Figure 2 RT-PCR products are shown of a typical experiment of RNA samples from control rat liver and livers 6, 15, 24, and 48 hours after LPS-treatment. The results were similar for the other mRNA samples examined (n=4 per time point).

Mrp1 mRNA was increased 6 hours after LPS-administration and remained increased for at least 48 hours, whereas Mrp2 mRNA completely disappeared 6 hours after LPS and returned after 48 hours (Figure 2). In the Pgp-subfamily, the mRNA levels of Mdr1a and Mdr2 did not change significantly during endotoxemia. Compared to Mrp2, Bsep showed a moderately decreased mRNA expression. A clear increase of Mdr1b mRNA was observed from 6 until at least 48 hours after LPS-treatment (Figure 2). Levels of Gapdh were not influenced by LPS-treatment.

### MRNA AND PROTEIN LEVELS OF MRP1 AND MRP2 IN ISOLATED HEPATOCYTES

In spite of the detection of Mrp1 in crude membrane fractions of total rat liver (Figure 1), we were not able to detect the protein in frozen liver sections from LPS-treated rats (CSLM). This is most likely a result of the relatively low expression of Mrp1 in rat liver hepatocytes. To prove that the Mrp1 upregulation takes place in hepatocytes, we isolated hepatocytes from normal and LPS-treated rats (2 mg/kg, 15 hours) and analyzed the mRNA and protein levels of Mrp1 and Mrp2. Also in isolated hepatocytes, Mrp1 protein (Figure 3) as well as Mrp1 mRNA (Figure 4) were increased, whereas Mrp2 protein (Figure 3) and Mrp2 mRNA (Figure 4) were decreased. Moreover, mRNA levels of Mdr1a and Mdr2
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did not change, whereas Mdr1b mRNA increased and Bsep mRNA decreased (Figure 4). On Western blot, Bsep and C219 signal decreased, whereas DppIV signal did not change (Figure 3). These results are comparable to the results obtained from total rat liver (Figure 1 and 2).

LOCALIZATION STUDIES USING CONFOCAL SCANNING LASER MICROSCOPY

CSLM was used to assess the localization of hepatic ABC-transport proteins during endotoxemia. The staining of Bsep, C219 (detecting all Pgps), and Mrp2 was examined in frozen liver sections of control rats and rats 6 hours after LPS-administration. Comparison of the Bsep and C219 labeling showed a clear colocalization at the canalicular membrane of normal rat liver (Figure 5A and C). The same pattern was observed when Mrp2 staining was compared with C219 (not shown). However, already 6 hours after LPS, the staining of Bsep and C219 became irregular and “fuzzy” as shown by double labeling (Figure 5B and D). Likewise, Mrp2 staining was normal in control (Figure 5E) and irregular in LPS-treated rat liver (Figure 5F). When detectable, this “fuzzy” staining was also present at later time points after LPS (not shown).

Figure 3. MRP1, MRP2, C219, BSEP, AND DPPIV PROTEIN LEVELS IN ISOLATED HEPATOCYTES DURING ENDOTOXEMIA. Western blot of crude membranes (50 µg) from isolated hepatocytes of control rats or rats 15 hours after LPS-administration.
In the present study, we have demonstrated that Mrp1 and Mdr1b expression are increased in the early phase of endotoxemia (6 hours after LPS), whereas Mrp2 expression is decreased. In addition, 15 hours after LPS-treatment also the expression of the recently cloned bile salt transporter, Bsep, is decreased. These results are the first indications of increased expression of the two multidrug resistance genes Mrp1 and Mdr1b in hepatocytes of LPS-treated animals. Furthermore, the decreased levels of Bsep may explain the reported decreased transport of bile salts after endotoxin-treatment.

It is known that LPS-induced changes in hepatocytes are mainly mediated by TNFα. The transcription factor NF-κB has been recognized as a major modulator of gene regulation induced by TNFα and other cytokines. A κB-element (mdr-κB) has recently been identified in the promoter of the rat Mdr1b gene. Thus, on basis of our results and data from the literature, we speculate that TNFα-binding to hepatocytes results in the rapid translocation of NF-κB to the nucleus where it is in part responsible for upregulation of transporter genes such as Mdr1b and Mrp1. Interestingly, as demonstrated recently, TNFα induces Mrp1 in human colon carcinoma cells and TNFα induces Mdr1 in a rat hepatoma cell line. The finding that Mrp1 induction in cultured hepatocytes takes place at the mid G1-phase of the cell cycle further supports our finding, because cytokines such as TNFα and interleukin-6 are known to initiate a proliferation trigger in hepatocytes in vivo.

We hypothesize that ABC-transporters like MDR1 and MRP1 and their rodent homologues function as an aspecific protection systems to maintain cellular membrane integrity by extruding cytotoxic compounds such as lipid peroxidation products. Furthermore, MRP1/Mrp1 may contribute to the regulation of the intracellular redox state by transporting glutathione disulfide. In addition, MRP1/Mrp1 has been
demonstrated to transport the GSH S-conjugate of the major lipid peroxidation product 4-hydroxynonenal.\textsuperscript{18} In contrast to increased \textit{Mrp1} and \textit{Mdr1b} levels, we observed an almost complete disappearance of \textit{Mrp2} and a moderate decrease of Bsep on mRNA as well as protein levels. A decreased canalicular expression of \textit{Mrp2} is in agreement with the observed reduction in transport of \textit{Mrp2} substrates into bile after LPS-treatment\textsuperscript{23,25,29,47}. Trauner

\textbf{Figure 5.} LOCALIZATION OF BSEP, C219 AND MRP2 IN CONTROL RAT LIVER, AND RAT LIVER 6 HOURS AFTER LPS-TREATMENT USING CSLM. A canalicular colocalization of Bsep (A) and C219 (C) is seen in frozen rat liver sections of control rats. Six hours after LPS treatment the localization of Bsep (B) and C219 (D) appears irregular and fuzzy. Also the localization of Mrp2 is normal in control rat liver (E) and changed to a fuzzy pattern in rat livers 6 hours after LPS (F). Typical staining patterns of \textit{n}=3 per group. Bar is 10 µm.
et al.\textsuperscript{26} recently described downregulation of Mrp2 mRNA and protein 18 hours after LPS. We can add to this, that Mrp2 mRNA was undetectable as early as 6 hours after LPS and started to return to normal levels 48 hours later.

GSH is an important cytosolic factor for protection of cells against reactive oxygen species and toxic electrophiles resulting from peroxidation reactions. It has been suggested that MRP2 is involved in canalicular secretion of GSH\textsuperscript{1,48}, the molecular driving force of bile-salt independent bile flow. If this is the case, the rapid downregulation of Mrp2 during sepsis may prevent secretion of GSH into bile. Indeed, after LPS-administration decreased biliary GSH-output and decreased GSH concentrations in bile have been reported\textsuperscript{47}. Interestingly, MRP1 may also transport GSH\textsuperscript{49}. Thus, upregulation of lateral Mrp1 may result in an increased GSH-secretion into the blood. Extracellular GSH may serve as scavenger for reactive oxygen species released by activated neutrophils.

In our hands, Bsep and Pgps levels showed a comparable decrease during endotoxemia, whereas Trauner et al.\textsuperscript{26} described an unchanged signal when the C219 antibody was used. In normal rodent liver, Bsep and Mdr2 are present at high levels, whereas the expression of Mdr1a and Mdr1b are very low. Therefore, the signal detected by C219 in normal liver will predominantly represent Bsep and Mdr2. The decrease seen with the C219 antibody after LPS-treatment will therefore result from the combination of a downregulated Bsep, an increased Mdr1b and unchanged Mdr1a and Mdr2. Our finding that Bsep is decreased after LPS-treatment may explain the reported reduced bile salt transport after LPS-administration\textsuperscript{23,28,29,50}.

Not only a variation of the amount of protein can lead to an altered canalicular transport, it is obvious that also the subcellular localization of the protein is important. Trauner et al.\textsuperscript{26} reported on a punctuate staining of Mrp2 in LPS-treated rat livers 18 hours after LPS. In their study, this punctuate pattern was not observed with C219 or an antibody against ecto-ATPase/cell CAM105. Already 6 hours after LPS-treatment, we found a fuzzy staining pattern in frozen liver sections of endotoxemic rats for Mrp2, Bsep, and Pgps (C219). The origin of the fuzzy staining could be due to localization of transporters in a subapical vesicular compartment, as speculated by Trauner for Mrp2\textsuperscript{26}.

In summary, we conclude that LPS-induced cholestasis is the result of a downregulation of Mrp2- and Bsep-expression as well as an altered localization of the transporters. The expression of the multidrug resistance genes Mrp1 and Mdr1b is strongly increased. By conferring resistance to hepatocytes these transporters likely play a role in the cellular response against cytokine-induced stress.

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