Inflammatory response in obstructive jaundice and peritonitis
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Chapter 11

Summary
Summary and conclusions

Surgical patients are at risk to develop postoperative complications that often are of infectious nature. Especially patients with extrahepatic obstructive jaundice are prone to develop sepsis. This thesis focused on the host inflammatory response to obstructive jaundice, in particular in the setting of endotoxemia. In addition, the regulation of the host response to *E. coli* peritonitis was investigated.

In Chapter 2, the benefit of preoperative biliary drainage (PBD) was studied in a cohort of 311 patients undergoing pancreaticoduodenectomy. Of this cohort, 21 patients with external and/or surgical biliary drainage were excluded and 232 patients who had received PBD were divided in 3 groups corresponding with severity of jaundice, according to preoperative plasma bilirubin levels: <40 μmol/L (n = 177), 40-100 μmol/L (n = 32) and >100 μmol/L (n = 23), respectively group 1, 2, and 3. These groups were compared with patients who underwent immediate surgery (n = 58) without PBD (mean preoperative plasma bilirubin level of 24 μmol/L). Although patients in group 1 were better drained than patients in groups 2 and 3 (median reduction of bilirubin levels respectively 82%, 57% and 37%) (*p* < 0.01), there was no difference in overall morbidity among the drained groups, respectively 50%, 50% and 52%. Finally, there was no significant difference in overall morbidity between patients with and without PBD, respectively 50% and 55%. In conclusion, this analysis shows that stented patients with ‘normal’ preoperative bilirubin values have equal postoperative morbidity compared with stented patients with a relatively ‘high’ preoperative bilirubin level. On the other hand, despite the co-morbidity of the drainage procedure itself, PBD can be performed safely in jaundiced patients. Nevertheless, biliary drainage should not be used routinely in patients presenting with a tumor in the periampullary region awaiting surgical resection, unless more time is required for other investigations or visiting referral centers because of centralization of high risk surgery.

In Chapter 3, the effectiveness of PBD in patients with obstructive jaundice due to tumors was reviewed systematically, including a meta-analysis. This meta-analysis combined data from five randomized controlled and 18 retrospective trials designed to compare the benefit of PBD. This systematic review does not provide evidence for a clinical benefit of PBD in jaundiced patients with tumors planned for surgery. It even shows that this treatment strategy increases overall morbidity. Moreover, the increase of total hospital stay due to these concurrent problems add more inconvenience for the patient, despite the considerable improvement of success rates and decreased morbidity of biliary drainage during the past decade. Decreasing drainage related complications could be a way of improving the beneficial effects of PBD that may be expected based on experimental data. Due to the lack of uniformity of the studies, it might be that a well selected high risk subgroup of patients does benefit from PBD:
however, this cannot be identified by use of this meta analysis. Therefore, new properly designed randomized controlled trials are necessary to identify patients who might or might not benefit from PBD.

In view of the established relationship between circulating lipoproteins and susceptibility to endotoxin, the study in Chapter 4 was designed to investigate the lipid spectrum in severe obstructive jaundice and, additionally, to study the effect of PBD. Abnormalities in the lipoprotein spectrum were assessed in 15 patients with malignant obstructive jaundice before and after three weeks of 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). Drainage normalized VLDL-, LDL-, and HDL cholesterol fractions from respectively 43 to 19%, 50 to 65% and 6 to 16% (p < 0.01). Ex vivo stimulation of whole blood with predrainage cholestatic plasma yielded 20-fold higher cytokine levels (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 levels at least 5-fold. In conclusion, PBD 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.

Cholestatic patients have substantial morbidity due to increased susceptibility to endotoxin. (Reconstituted) HDL (rHDL) can bind and neutralize endotoxin; however, cholestasis is associated with a near complete absence of HDL. Effects of rHDL infusion on the outcome of endotoxin-induced inflammatory responses in cholestatic rats were determined in Chapter 5. The study confirmed that bile duct ligation (bdll) 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 non-jaundiced animals as well as humans this leads to endotoxin neutralization, rhDL infusion unfortunately leads to an increased release of TNF and significantly increased mortality in bdll rats. These results suggest that there may be danger in administration of rHDL to humans with obstructive jaundice.

In Chapter 6, we sought to determine the role of endogenous IL-1 in the development of hepatic inflammation and injury following biliary obstruction, and in the increased susceptibility toward endotoxin-induced toxicity associated with obstructive jaundice. For this, extrahepatic cholestasis was induced by bdll in IL-1 receptor type I-gene deficient (IL-1R-/-) mice, that are unresponsive to IL-1α and IL-1β, and normal IL-1R+/+ mice. We demonstrate that production of IL-1α and IL-1β occurs in livers of mice with bile duct obstruction, but that endogenously synthesized IL-1 does not
Chapter II

Contribute to hepatic inflammation and injury in this condition. However, exaggerated IL-1 release is found in bdl mice exposed to endotoxin, and eliminating this IL-1 response protects animals against the proinflammatory and lethal effects of endotoxin. These results suggest that endogenous IL-1 plays an important role in the hypersensitivity towards endotoxin during biliary obstruction.

IFN-γ is a dimeric glycoprotein that has many immune regulatory functions and induces cell proliferation and apoptosis. In Chapter 7, we used IFN-γ receptor type I-gene deficient (IFN-γR,/-) mice, that are unresponsive to IFN-γ, and normal IFN-γR,+/+ mice to determine the role of IFN-γ in hepatic responses to acute biliary obstruction induced by bdl. We show that murine cholestasis induced by bdl is associated with enhanced production of IFN-γ in the liver. More importantly, this endogenous IFN-γ appeared to play an essential role in controlling early cholestatic liver injury. The increased liver injury in bdl IFN-γR,/- mice, as reflected by both clinical chemistry and histopathology, was associated with mortality within 2 weeks after induction of cholestasis, whereas bdl IFN-γR,+/+ mice survived easily up to 4 weeks of cholestasis. Furthermore, liver damage in bdl IFN-γR,+/+ mice was associated with increased apoptosis and liver regeneration, whereas liver damage in bdl IFN-γR,/- mice was characterized by necrosis, large inflammatory infiltrates, and a diminished hepatic regenerative capacity. Furthermore, as far as we know this is the first study that reports in vivo IFN-γ production by mouse liver macrophages. These data suggest that IFN-γ is an important regulator of apoptosis and regeneration in the liver in response to extrahepatic cholestasis, and that apoptosis protects the host against excessive liver injury in this condition.

Besides its association with a high perioperative morbidity and mortality, cholestatic liver disease is also linked with an increased occurrence of pulmonary inflammation and multiple organ failure. Endotoxin is a potent inducer of many inflammatory cascades, including the cytokine network. In Chapter 8, we determined the role of IL-6 herein, by bdl in IL-6-gene deficient (IL-6/-/) and normal IL-6+/+ mice. Bdl elicited increased levels of hepatic IL-6 mRNA and protein in normal mice. Hepatocellular injury at 2 weeks after bdl was similar in IL-6/-/ and IL-6+/+ mice as demonstrated by clinical chemistry and histopathology. Administration of endotoxin to cholestatic mice at 2 weeks after bdl was associated with enhanced cytokine release, severe liver damage and death when compared to sham-operated mice. Effects of endotoxin were largely similar in sham-operated IL-6/-/ and IL-6+/+ mice, but cholestatic IL-6/-/ mice were more susceptible to the toxic effects of endotoxin, as reflected by increased cytokine release, more profound liver and lung injury and higher mortality. In conclusion, although endogenous IL-6 is not important in the development of liver injury after experimentally induced obstructive jaundice, this cytokine plays an important role in decreasing hypersensitivity to endotoxin in cholestatic mice.
A number of mediators of the innate system contribute to local host defense mechanisms during peritonitis. However, knowledge of the extent of production of these mediators at the site of the infection (i.e. within the peritoneal cavity) is limited. The studies outlined in Chapters 9 and 10 sought to obtain more insight in this "compartmentalized" expression of these mediators, in particular of cytokines.

IL-10 is a potent anti-inflammatory cytokine. In Chapter 9, we determined the role of endogenous IL-10 in local antibacterial host defense and in the development of a systemic inflammatory response syndrome during abdominal sepsis. For this, IL-10 gene deficient (IL-10/-) and wild type (IL-10+/+) mice received an intraperitoneal injection with *Escherichia coli*. We demonstrate the seemingly paradoxical role of endogenous IL-10 during septic peritonitis. The absence of IL-10 reduced the survival of mice, accompanied by profound multiple organ damage, in spite of a more effective bacterial clearance and a reduced dissemination of bacteria to distant organs. These data exemplify the complex role of IL-10 in bacterial infection, and indicate that the net effect of endogenous IL-10 on the outcome of a bacterial infection is determined by the balance between its local effects (facilitating the outgrowth of microorganisms) and its systemic effects (attenuating inflammation).

The role of endogenous IL-18, a potent pro-inflammatory cytokine, during peritonitis, was determined in Chapter 10. IL-18 gene-deficient (IL-18/-) mice and wild-type (IL-18+/+) mice were intraperitoneally infected with *E. coli*. Infection with *E. coli* increased IL-18 release in the peritoneal cavity and plasma. After infection, IL-18/-mice had significantly more bacteria in the peritoneal lavage fluid and were more susceptible for progressing to systemic infection during the first 20 h post-inoculation. These data suggest that endogenous IL-18 plays an important role in the early antibacterial host response during *E. coli* induced peritonitis.

**Conclusions**

Based upon the results of a retrospective series of jaundiced patients with periampullary tumors and operated on at the Academic Medical Center from 1992 - 1999, **preoperative biliary drainage** did not influence the incidence of postoperative complications, and although it can be performed safely in jaundiced patients, it should not be used routinely. This conclusion is confirmed in a **meta-analysis**. The potential benefit of PBD on postoperative mortality and morbidity does not outweigh the disadvantage of the drainage procedure. Only if PBD related complications could be reduced dramatically and consequently reduce hospital stay, than PBD might be beneficial. Further randomized controlled trials with improved PBD techniques are necessary and such one will start soon at the AMC.
In spite of this, PBD 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. While reconstituted HDL (rHDL) can bind and neutralize endotoxin, cholestasis is associated with a near complete absence of HDL. Nevertheless, rHDL infusion leads to increased endotoxin-induced mortality in cholestatic rats. These results sharply contrast with the protective effects of rHDL suppletion 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.

From the studies with cytokine gene deficient mice subjected to bdl, the following conclusions can be drawn. Extrahepatic cholestasis is associated with the local production of several cytokines in the liver, including IL-1α, IL-1β, IFN-γ and IL-6. The investigations presented in Chapters 6 to 8 indicate that IFN-γ, but not IL-1α, IL-1β or IL-6, plays an important role in the protection of the liver against hepatocellular damage during a 2-week period of obstructive jaundice. In addition, IL-1α and/or IL-1β contribute significantly to the enhanced sensitivity of the jaundiced host to endotoxin, whereas IL-6 diminishes the increased endotoxin responsiveness in this condition. Hence, distinct cytokines are intimately involved in the orchestration of the host response to obstructive jaundice and the ensuing altered endotoxin responsiveness. Possible interventions during obstructive jaundice are perioperative suppletion of anti-IL-1 antibodies or suppletion of IFN-γ or IL-6, and further research into these therapeutic measures are warranted.

The dual role of the cytokine network in the host response to bacterial peritonitis is also illustrated by the studies reported in Chapters 9 and 10. The anti-inflammatory arm of the cytokine network (represented by IL-10 in Chapter 9) impairs the clearance of bacteria from the site of infection, yet attenuates the systemic inflammatory response syndrome that accompanies the peritonitis. On the other hand, the proinflammatory arm of the cytokine network (represented by IL-18 in Chapter 10), facilitates bacterial defense. Further research needs to be performed to establish which interventions directed at the cytokine network will help to decrease mortality and morbidity during peritonitis.