Inflammatory response in obstructive jaundice and peritonitis
Sewnath, M.E.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Download date: 11 Dec 2018
Chapter 1

Introduction

Miguel E. Sewnath\textsuperscript{1}, Tom van der Poll\textsuperscript{2,3}, and Dirk J. Gouma\textsuperscript{1}

Department of Surgery\textsuperscript{1}, Laboratory of Experimental Internal Medicine\textsuperscript{2}, and the Department of Infectious Diseases, Tropical Medicine and AIDS\textsuperscript{3}
Introduction and outline of the thesis

The research described in this thesis focused on the host response to obstructive jaundice. In particular, the role of several cytokines in responsiveness toward Escherichia coli endotoxin during jaundice was investigated. In addition, investigations seeking to establish the role of cytokines in host defense against Escherichia coli peritonitis were performed. In this introductory chapter, we will briefly discuss the most important topics of this thesis.

Cytokines

Cytokines are small proteins that orchestrate a variety of inflammatory reactions. Many different cell types are able to produce cytokines upon stimulation with bacteria and other stimuli. Cytokines interact in a highly complex network in which they influence each other's production and activity. Roughly, the cytokine network can be divided into proinflammatory cytokines and inhibitors of proinflammatory cytokines. As reflected by the term proinflammatory, these cytokines stimulate a variety of inflammatory reactions. Prototypic proinflammatory cytokines are tumor necrosis factor-α (TNF) and interleukin (IL)-1, which exert highly overlapping biological activities. IL-1 is the designation for two proteins, IL-1α and IL-1β, each encoded by a separate gene. The IL-1 gene family further consists of IL-1 receptor antagonist (IL-1ra), which inhibits the activity of both IL-1α and IL-1β by competing for their binding to the (biologically active) type I IL-1 receptor. Both IL-1s are translated as 31 kD precursor proteins, which are subsequently processed to mature proteins. Pro-IL-1α is fully active and mainly stays intracellular. Pro-IL-1α can also be transferred to the cell membrane, where it can exert cell-associated effects. In contrast, pro-IL-1β has little biological activity. mature IL-1β is released from the precursor protein through cleavage mediated by IL-1β converting enzyme (ICE). ICE also liberates mature IL-18, a cytokine that is structurally related to the IL-1 cytokine family, from pro-IL-18 (see further). IL-6 can not be considered a pure proinflammatory cytokine. Indeed, IL-6 has been considered an anti-inflammatory cytokine by some investigators since it can inhibit endotoxin-induced TNF and IL-1 production by mononuclear cells in vitro and can reduce TNF release in endotoxemic mice in vivo. IL-6 is able to influence a number of inflammatory responses that have relevance for the pathogenesis of jaundice and bacterial infection. IL-6 is considered a major regulator of the acute phase protein response. It stimulates human hepatocytes to produce several acute phase proteins, including C-reactive protein, serum amyloid A, α1-acid glycoprotein, α1-antitrypsin and fibrinogen. The capacity of IL-6 to induce an acute phase protein response has been confirmed in cancer patients infused with recombinant IL-6.
Chapter 1

Other proinflammatory cytokines studied in this thesis and implicated in the pathogenesis of hepatic injury during cholestasis and the pathophysiology of severe bacterial infection include interferon-γ (IFN-γ) and IL-18, which at least in part function as a "cytokine axis" in which IL-12 and IL-18 potently enhance the production of IFN-γ.\(^5\) The main producers of IFN-γ are activated natural killer cells, T helper 1 cells and cytotoxic T cells. Biologically active IFN-γ exists as a noncovalent homodimer. IFN-γ actions related to inflammation are induction of class II major histocompatibility complex antigen expression on different cell types and macrophage activation. Furthermore, IFN-γ likely plays an important role in the production of IgG against bacterial polysaccharides. IL-18 was first described as IFN-γ-inducing factor (IGIF). As with pro-IL-1β, the IL-18 precursor (pro-IL-18) does not contain a signal peptide required for removal of the precursor amino acids and subsequent secretion. Like pro-IL-1β, pro-IL-18 is cleaved by ICE, although recent evidence indicates that processing of pro-IL-18 can also take place via an ICE independent mechanism. Mature IL-18 shows a high degree of three-dimensional structural similarity to mature IL-1β. The importance of IL-18 in IFN-γ production has been documented in IL-18 or ICE deficient mice that produced little or no IFN-γ in spite of normal IL-12 concentrations. Although IL-18 is an inducer of IFN-γ (in particular in the presence of IL-12), IL-18 is a cytokine with proinflammatory properties not directly linked to IL-12 and/or IFN-γ. IL-12 and IFN-γ share many biological properties. IFN-γ strongly potentiates the synthesis of IL-12, and is considered to mediate many of the in vivo effects of IL-12, although at least some IL-12 effects are IFN-γ independent.

Considering the potency by which proinflammatory cytokines can cause tissue damage, it is not surprising that the host can mobilize several mechanisms that can negatively influence either the production or activity of these mediators. These mechanisms include the production of anti-inflammatory cytokines, among which IL-10.\(^7\) IL-10 can be synthesized by T cells, B cells, monocytes and macrophages. Important biological effects of IL-10 are inhibition of proinflammatory cytokine production by activated mononuclear cells, inhibition of class II MHC expression by monocytes and macrophages, inhibition of killing of intracellular bacteria by macrophages and suppression of monocyte procoagulant activity. It should be realized, however, that IL-10 also has immunostimulatory properties, such as enhancement of B cell function and stimulation of development of cytotoxic T cells. IL-13, like IL-10, exerts potent anti-inflammatory effects, reducing monocyte-derived TNF, IL-1 and IL-8 production.
**Introduction**

**Obstructive jaundice**

Although obstructive jaundice can be caused by benign causes of the biliary tract, like gallstones, sclerosing cholangitis, or benign strictures, the most common cause is a malignancy of the biliary tract and pancreatic head region. In Western countries, periampullary tumors are 5th on the list of malignancies responsible for death. The incidence of periampullary tumors has increased in past decades from 10-15/100,000 in most countries. Radical surgical resection is the only curative treatment. Palliative treatment with endoscopic stenting is performed frequently, but is often accompanied with stent occlusion and cholangitis, and subsequent increased morbidity and hospital stay. Although postoperative mortality after extensive hepatopancreatobiliary surgery has decreased from 20% to 5%, morbidity remains unchanged at 40-50%. Complications primarily consist of sepsis, hemorrhage, impaired wound healing, and renal disorders. The susceptibility to infectious complications has been suggested to be related to portal and systemic endotoxemia and bacterial translocation, leading to a proinflammatory state with enhanced production of cytokines such as TNF. Endotoxemia is induced by the lack of bile in the gut and by the obstruction itself, resulting in less inhibition of endotoxin by bile salts and possibly changes in gut integrity and composition of the gut flora. The reduced cellular immunity leads to depression of the reticuloendothelial system of the liver and of the extrahepatic nonspecific and specific cell mediated immunity.

The role of neutrophils has been well defined in jaundiced patients. Neutrophil phagocytosis of Staphylococcus aureus is reduced during cholestasis, but the phagocytosis of zymosan is intact. Chemotaxis of neutrophils was shown to be impaired by some investigators, although this was not confirmed by others. In biliary obstruction increased superoxide production by neutrophils, after stimulation with FMLP, PMA or zymosan can last as long as 15 days. This has been seen both in jaundiced animals and in patients. It has been shown that jaundiced patients with a poor immediate clinical outcome have higher neutrophil superoxide production prior to death. Neutrophils from jaundiced patients, unlike controls, cannot be primed by cytokines TNF, IL-6 or IL-8. Culture of normal neutrophils with jaundiced serum fails to reproduce the increase of superoxide production observed in jaundice, indicating that other elements may be required, for example monocyte/macrophages and the cytokines they produce. Neutrophils from jaundiced patients are thought to be 'pre-primed' in vivo by bile salts and therefore produce products that are more toxic when subjected to subsequent stimulation with endotoxemia and/or a stressor such as surgical trauma. Neutrophil adhesion, an important prelude to extravasation, is also impaired during jaundice. PMA injected into bile ducts induced neutrophil
infiltration into the duct tissue and subsequent necrosis and fibrosis, suggesting a potential role for neutrophils in the pathogenesis of tissue damage during cholestasis.\textsuperscript{47} Lymphocytes also appear to play a major role during cholestasis. Circulating concentrations of soluble IL-2 receptor, an indicator of T-cell activation, were elevated in patients with obstructive jaundice.\textsuperscript{48} The inhibition of cellular immunity may be due to endotoxin.\textsuperscript{50,51} During experimental cholestasis, PHA, concanavalin A or pokeweed mitogen have been demonstrated to inhibit the lymphocyte stimulation index.\textsuperscript{51,52} This is probably due to agents in the jaundiced blood, since the impaired function is restored when jaundiced lymphocytes are cultured with control serum.

Different intervention strategies have been investigated in order to reduce the inflammatory state and thus postoperative infectious complications. While external biliary drainage was disappointing,\textsuperscript{53,54} internal biliary drainage showed many promising results in experimental studies by reducing endotoxemia,\textsuperscript{55} bacterial translocation, the depression of the cellular immune system,\textsuperscript{57,58} and most importantly by reducing the postoperative mortality.\textsuperscript{59} However, in the clinical setting, the biliary drainage procedure itself has certain complications, such as cholangitis, and until now has not been more beneficial to patients with respect to lowering postoperative infectious complications.\textsuperscript{54,57,58}

Interventions like lactulose syrup also have promising results,\textsuperscript{60,61} especially in the experimental situation, but is however less effective than anti-TNF treatment in reducing TNF levels in a model of cholestatic mice undergoing an operative trauma.\textsuperscript{62} Bile-salts administered to cholestatic animals were demonstrated to inhibit bacterial overgrowth and to reduce the rate of positive bacterial cultures from mesenterial lymph nodes.\textsuperscript{63} Another seemingly promising intervention, bowel preparation and irrigation, appears disappointing since it has not been shown to prevent endotoxemia in jaundiced patients.\textsuperscript{18}

Early studies using anti-endotoxin agents polymyxin B, neomycin and lactulose to reduce endotoxin in the blood failed to prevent endotoxin-induced damage to the liver and biliary system.\textsuperscript{64,65} Recently Houdijk et al. reported that cholestyramine may be used in preventing endotoxemia related renal damage in jaundiced rats by significant reversal of the reduced renal blood flow.\textsuperscript{66} This may also prevent the further reduction of renal blood flow in jaundiced rats after surgery.\textsuperscript{67}

Several anti-TNF strategies have been evaluated for their efficacy to improve the outcome of surgical trauma in experimental biliary obstruction in mice.\textsuperscript{62,68} Both a monoclonal anti-TNF antibody and pentoxifylline significantly reduced the levels of TNF in these cholestatic mice, neither however had a significant effect on mortality. Another intervention, PAF receptor antagonist, reduced free radical levels and modified the liver damage in jaundiced rats.\textsuperscript{69}

Due to increased insights in the inflammatory response in local infection, it is important to investigate the role of cytokines and the possibility to use them as
immunomodulating therapy in addition to the treatment for obstructive jaundice. Identifying the exact phase of the inflammatory response during obstructive jaundice and endotoxemia to will help to improve perioperative measures to reduce postoperative morbidity in cholestatic patients.

Sepsis and Peritonitis

Sepsis is a clinical syndrome defined as the systemic response of the host to an infection. In recent clinical trials involving patients with severe sepsis, pneumonia was the most common source of infection followed by peritonitis. Especially abdominal sepsis bears a grim prognosis. Cytokines play an eminent role in the pathogenesis of bacterial infection and sepsis. In 1985 the pivotal role of early TNF production in the systemic toxicity elicited by high dose endotoxin was recognized for the first time. Pretreatment with antiserum to TNF was found to protect mice against the lethal effect of intravenous endotoxin. Two years later, a monoclonal anti-TNF antibody was reported to protect baboons against lethal Gram-negative bacteremia. Since then anti-TNF strategies have been found protective in a number of sepsis models in which bacteria or bacterial products were administered systemically as a bolus or a brief infusion. Neutralization of IL-1 activity, by administration of recombinant IL-1ra, also reduced lethality induced by endotoxin or living bacteria in various species. Importantly, administration of recombinant TNF or IL-1 to laboratory animals can reproduce many characteristics of the sepsis syndrome and the simultaneous injection of TNF and IL-1 resulted in synergistic toxicity in rabbits. These hallmark studies formed the basis of the design and performance of several clinical trials with TNF and IL-1 neutralizing agents. The anti-inflammatory cytokine IL-10 represents an important autoregulatory mechanism controlling the production of proinflammatory cytokines and endotoxin toxicity in vivo. Indeed, neutralization of endogenously produced IL-10 in endotoxemic mice was associated with an increased production of several proinflammatory cytokines, including TNF, and an enhanced mortality. and IL-10 gene deficient mice showed an increased mortality after endotoxin administration together with elevated levels of TNF, IL-1, and other inflammatory mediators.

Although in these preclinical sepsis studies anti-inflammatory therapies exerted strongly protective effects, in clinical trials with patients with sepsis anti-TNF and anti-IL-1 strategies were not successful. In this context, it should be realized that these intravenous challenge models are associated with a relatively acute syndrome, unlike many cases of sepsis in the clinic. Furthermore, these models are devoid of a localized infectious source, i.e. an infected organ or cavity, from which the infection disseminates. Cytokine production occurs primarily at the site of the infection.
Infection models, that make use of an initially localized source of infection, such as pneumonia and peritonitis, have indicated that proinflammatory cytokines play a crucial role in host defense against bacterial infection. Neutralization of endogenous TNF during murine pneumonia caused by either Gram-positive or Gram-negative bacteria, resulted in an accelerated course of the infection associated with an enhanced outgrowth of bacteria in the lungs and a decreased survival. Conversely, the elimination of IL-10, which converts a sublethal model of endotoxemia into a lethal shock model, improved survival during murine pneumonia, reducing the bacterial load within the pulmonary compartment. Endogenous TNF also plays a protective role in the pathogenesis of Gram-negative peritonitis. Hence, the role of cytokines in acute models (bolus administration of endotoxin or bacteria) and more realistic infection models (pneumonia, peritonitis) can be completely different. The severity of the bacterial challenge appears to be an pivotal denominator of the eventual effect of certain cytokines on survival. In general, the action of proinflammatory cytokines seems important for an adequate antibacterial host defense at the site of an infection, whereas their systemic action can harm the host and cause tissue damage. Anti-inflammatory cytokines, such as IL-10, impair local antibacterial effector mechanisms, yet may diminish systemic toxicity produced by bacteria.

Like TNF and IL-1, IL-18 is a proinflammatory cytokine. Systemic inflammation such as induced by endotoxin is in part mediated by endogenous IL-18. Indeed, treatment with an anti-IL-18 antiserum protected mice against the lethal effects of both E. coli and Salmonella endotoxin, which in the case of E. coli endotoxin was associated with a reduced accumulation of neutrophils in lungs and liver. In accordance, treatment of mice with a fusion construct consisting of recombinant human IL-18BPα and human IgG1 Fc also conferred a strong protective effect against lethality after administration of endotoxin. On the other hand, local production of IL-18 is required for an optimal antimicrobial defense during experimentally induced bacterial infection in mice, such as induced by Salmonella typhimurium, Yersinia enterocolitica, Listeria monocytogenes and Streptococcus pneumoniae. The role of IL-18 in bacterial peritonitis has not been established.

Outline of the thesis

The general aim of the studies described in this thesis is to gain more insight in the pathophysiology of postoperative septic complications in relation to obstructive jaundice. In addition, in some of the investigations presented we sought to determine the role of cytokines in the host response to bacterial peritonitis. The specific objectives of the different studies are given in the respective chapters of this thesis.
Chapter 2 presents an analysis of the outcome of preoperative biliary drainage in a consecutive series of 311 patients undergoing surgery for a tumor of the pancreatic head region. The incidence of postoperative complications in jaundiced patients who underwent preoperative biliary drainage is compared with the incidence of complications after surgery in jaundiced patients in whom preoperative biliary drainage was not applied. In addition, the association between severity of jaundice according to preoperative bilirubin plasma levels and the incidence of postoperative infectious is studied.

A meta-analysis on the efficacy of preoperative biliary drainage for tumors causing obstructive jaundice is presented in Chapter 3. Randomized controlled trials and comparative cohort studies conducted worldwide are classified on methodological strength and subdivided into level 1 (randomized controlled trials) and level 2 (comparative cohort studies). Preoperative biliary drainage is compared with no preoperative biliary drainage in jaundiced patients undergoing resection of a tumor, using in-hospital death rate, overall complications resulting from the treatment modality (drainage and operation related complications) and hospital stay as outcome parameters.

Biliary obstruction changes the spectrum of lipoproteins, which are known to bind and neutralize endotoxin. In particular High Density Lipoprotein (HDL) levels are decreased during cholestasis. In Chapter 4, the effect of preoperative biliary drainage on changes in the lipoprotein spectrum and its relation to endotoxin responsiveness are studied. Abnormalities in the lipoprotein spectrum are assessed in patients with malignant obstructive jaundice before and 3 weeks after endoscopic biliary drainage. Changes in endotoxin responsiveness are assessed using various endotoxin-neutralizing reagents in whole blood cell cultures stimulated in the presence or absence of cholestatic plasma taken before and after biliary drainage.

Next, treatment with an anti-endotoxin therapy, i.e. reconstituted HDL (rHDL), an endotoxin binding and neutralizing lipoprotein, is studied in Chapter 5. 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 are determined.

In the following in vivo animal studies, the role of several cytokines during cholestasis and subsequent endotoxemia is investigated. In Chapter 6, the role of IL-1 during cholestasis is determined. Cholestatic liver injury is associated with an increased susceptibility toward endotoxin-induced toxicity. Extrahepatic cholestasis is induced by bdl in IL-1 receptor type 1 gene-deficient (IL-1R−/−) mice, which are unresponsive to IL-1α and IL-1β, and normal IL-1R+/+ mice. After two weeks of cholestasis, sham operated and cholestatic mice are challenged with endotoxin intraperitoneally, thereby evaluating whether (a) IL-1 contributes to hepatic pathology associated with
cholestasis, and (b) endogenous IL-1 is involved in the enhanced susceptibility to endotoxin that accompanies cholestasis.

Cholestasis is a main feature of many human liver diseases, including primary biliary cirrhosis, primary sclerosing cholangitis, and chronic allograft rejection following liver transplantation. In many of these cholestatic diseases, the initial insult is directed towards cells composing the wall of bile ducts and often mediated by T lymphocytes and cytokines. IFN-γ is a dimeric glycoprotein that has many immune regulatory functions and induces cell proliferation and apoptosis. Generation of mice with a homozygous disruption of the IFN-γR1 chain gene (IFN-γR1/-/- mice) enables the evaluation of the tissue protective role of IFN-γ in immune response and host defense during cholestasis. In Chapter 7, IFN-γR1/-/- and wild type mice are used to determine the role of IFN-γ in hepatic responses to acute biliary obstruction induced by bdl.

Preexisting liver dysfunction, especially cholestatic liver disease, has been linked to enhanced pulmonary inflammation and multiple organ failure. Interactions between liver and lungs early after endotoxemia are held responsible, particularly those altering the metabolism of circulating endotoxin. With the availability of mice harboring a targeted disruption of the gene encoding for IL-6, we were able to evaluate the protective role of IL-6 in immune responses and host defense during cholestasis in Chapter 8. IL-6/-/- and IL-6+/+ mice are used to determine the role of IL-6 in hepatic and lung injury during acute biliary obstruction induced by bdl, and to determine the role of IL-6 in the enhanced susceptibility to endotoxin during cholestasis.

IL-10 is a potent anti-inflammatory cytokine. The role of endogenous IL-10 in host defense against bacterial infection is unclear; i.e. neutralization of IL-10 improved survival during murine pneumonia, whereas it increased lethality during peritonitis induced by cecal ligation and puncture. In Chapter 9, the role of endogenous IL-10 in local antibacterial host defense and in the development of a systemic inflammatory response syndrome during abdominal sepsis is studied by challenging IL-10 gene deficient (IL-10/-/-) and wild type (IL-10+/+) mice intraperitoneally with *Escherichia coli*.

IL-18 is a proinflammatory cytokine of which the production is increased during infection. In Chapter 10, the role of this endogenously produced IL-18 in the host response to *Escherichia coli* peritonitis is determined using IL-18/-/- and IL-18+/+ mice.

Chapter 11 concludes this thesis with a summary and a general discussion.

REFERENCES

Introduction


Chapter 1


