Interaction between inflammation, coagulation and fibrinolysis during infection

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Chapter 13

Summary of the study results
Summary and General Discussion

The research described in this thesis is focused on three primary subjects. The first part of this thesis discusses the role of cytokines, the second part discusses the role of the phospholipid platelet activating factor (PAF) in the host response to (myco)bacterial pathogens and their products; the third part of this thesis examines possible connections between the inflammatory response to infection and the coagulation-anti-coagulation system.

The thesis starts with a general introduction, followed by the chapters 2, 3, 4 and 5 that discuss the role of the pro-inflammatory cytokines IFN-γ, IL-12 and IL-18 in infection and inflammation. Cytokines are a family of small proteins that play an essential role in the regulation of the immune response. Several studies in experimental models of infectious diseases have demonstrated the importance of locally produced proinflammatory cytokines for an adequate host response against bacteria. IFN-γ, IL-12 and IL-18 at least in part function as a “cytokine axis” in which IL-12 and IL-18 potently enhance the production of IFN-γ. In chapter 2 and 3, we used a murine model of septic peritonitis to investigate the role of IL-12 and IL-18 in abdominal sepsis in mice. In chapter 2, the role of endogenous IL-12 was analyzed in the host response to septic peritonitis induced by E. coli by making use of p35 gene deficient mice, animals in which IL-12 is the only cytokine that cannot be produced. It was found that endogenous IL-12 contributes to an adequate host response by limiting the bacterial outgrowth and thereby reducing secondary tissue injury. In chapter 3 we showed that IL-18 gene deficient have a phenotype in this model of abdominal sepsis that resembles the phenotype of p35 gene deficient mice, i.e. IL-18 facilitated the host response by reducing the outgrowth of E. coli, thereby diminishing secondary tissue injury. Thus, IL-12 and IL-18 production are part of a protective early immune response to abdominal sepsis caused by E. coli. In chapter 4 and 5, two clinical studies are presented aiming to elucidate the role of IFN-γ in two different inflammation settings. In chapter 4, we investigated the role of IFN-γ in LPS induced immunoparalysis. It has been proposed that immunoparalysis may contribute to the enhanced susceptibility to nosocomial infections and late mortality of patients after surgery and patients who survive the initial acute phase of sepsis syndrome. Administration of recombinant IFN-γ has been advocated as a treatment of patients with immunoparalysis. In this chapter we demonstrated that the production capacity of IFN-γ is greatly diminished after LPS administration to healthy humans, and that the experimentally induced immunoparalysis in this model can not or only partially be restored by addition of recombinant IFN-γ. In chapter 5, the responsiveness to IFN-γ in patients recovered from L. pneumophila pneumonia was investigated. In this study, whole blood of these patients was incubated with non-specific stimuli or specific stimuli to evaluate IFN-γ production, and with IFN-γ to evaluate IFN-γ responsiveness. We showed that the blood of patients released less IFN-γ than the
blood of controls in response to the different stimulations, suggesting that impaired IFN-γ production may contribute to susceptibility to *L. pneumophila* infection.

In the second part of this thesis we discuss the role of the PAF in two different murine models of lung inflammation. PAF is a potent phospholipid mediator that plays an important role in inflammatory and immune responses. In chapter 6 we investigated the role of PAF in a murine model of lung tuberculosis. We found, contrary to our expectations, that both mouse strains were indistinguishable with respect to histopathology, the recruitment and activation of lymphocytes and cytokine concentrations in the lung. These data suggest that PAF is not involved in the protective immune response to tuberculosis. In chapter 7 we investigated the role of PAF in a murine model of bacterial pneumonia. For this purpose, we compared host responses in PAF receptor (PAFR) gene deficient and normal wild type mice after intranasal infection with live *S. pneumoniae*. It has previously been shown that *S. pneumoniae* needs the PAFR to enter epithelial cells. In line, we here demonstrated that PAFR gene deficient mice are less susceptible for developing invasive disease and have an improved host defense during the pneumococcal pneumonia.

In part three of this thesis we discuss the cross talking between the inflammation system and the coagulation-anti-coagulation system. It has been known for several years that severe infections can be associated with activation of the coagulation system, which can result in diffuse intravascular coagulation (DIC). A pivotal mechanism in the pathogenesis of DIC is the activation of the tissue factor (TF)/factor (F)VIIa dependent pathway of coagulation. Ample evidence exists however that the coagulation and fibrinolytic systems also influence the inflammation system. In chapter 8 we discuss the hypothesis that elimination of the FVIIa-TF pathway protects against death not merely by an effect on the coagulation system, but that TF may also have effects on inflammatory responses different from the procoagulant response. To determine the role of the TF/FVIIa complex in the host response to peritonitis, mice received an intraperitoneal injection of live *E. coli* with or without concurrent treatment with recombinant Nematode Anticoagulant Protein c2 (rNAPc2), a selective inhibitor of the TF/FVIIa pathway. Peritonitis was associated with an increase in TF expression at tissue level, activation of coagulation, as reflected by elevated levels of thrombin-antithrombin complexes (TATc), and by increased fibrinogen deposition in liver and lungs. rNAPc2 strongly attenuated this procoagulant response, but did not influence the inflammatory response (histopathology, leukocyte recruitment to the peritoneal cavity, cytokine and chemokine levels). Moreover, rNAPc2 did not alter bacterial outgrowth locally or dissemination of the infection, and survival was not different in rNAPc2 treated and control mice. These data suggest that TF-FVIIa activity contributes to the coagulation activation during *E. coli* peritonitis, but does not play a role in the inflammatory response or antibacterial host defense. In chapter 9 we discuss the role of TF-FVIIa in host defense against pneumococcal pneumonia. We found that human patients with unilateral community-acquired pneumonia demonstrate elevated concentrations of
FVIIa, soluble TF and TATc in broncho-alveolar lavage fluid (BALF) obtained from the infected site compared to the uninfected site. In mice infected with *S. pneumoniae* pneumonia we found an increased TF expression and fibrin deposits in lungs together with elevated TATc levels in BALF. However, inhibition of TF-FVIIa by rNAPc2 attenuated the procoagulant response in the lung, but did not impact on host defense, as reflected by an unaltered outgrowth of pneumococci and an unchanged survival. The central aim in chapter 10 was to examine the influence of thrombomodulin (TM), in particular the domain of TM that is crucial for the generation of activated protein C (APC), in the regulation of the pulmonary response to tuberculosis. APC is a crucial anticoagulant that also can exert several anti-inflammatory effects. TM is a receptor that binds thrombin, after which thrombin loses its procoagulant properties and instead becomes the driving force behind the generation of APC. In theory, a reduced capacity to produce APC (such as in the genetically modified mice, used in this research, with a single TM<sup>pro/pro</sup> mutation) could influence the local response to chronic infection with *M. tuberculosis* via two major mechanisms: in light of the anticoagulant properties of APC, the TM<sup>pro/pro</sup> mutation could result in an increased tendency to form pulmonary thrombosis, whereas in light of the anti-inflammatory properties of APC, this TM mutation could lead to an enhanced proinflammatory reaction. We here demonstrated that TM<sup>pro/pro</sup> mice do not display a diminished capacity to maintain a normal hemostatic balance during tuberculosis, but clearly have a reduced ability to control the inflammatory response to *M. tuberculosis* infection. In mice that lack the normal TM receptor, and therefore are not able to generate APC, the coordinated inflammatory response characterized by the recruitment of lymphocytes and macrophages to the site of the infection and the formation of well-shaped granulomas was disturbed. These data suggest that the presence of functional TM in the lung is of importance for the regulation of the immune response to *M. tuberculosis*. In chapter 11 we demonstrated that bacterial pneumonia was associated with coagulation activation and that these pulmonary procoagulant responses were unaltered in TM<sup>pro/pro</sup> mice. In contrast to our data in the murine tuberculosis model, TM<sup>pro/pro</sup> mice displayed unchanged antibacterial defense, neutrophil recruitment and cytokine/chemokine levels during bacterial pneumonia. These data suggest that the capacity of TM to generate APC does not play a role of importance in the (sub) acute pulmonary responses, but does play a role in chronic pulmonary diseases like tuberculosis. Finally, in chapter 12 the effect of tranexamic acid (a synthetic anti-fibrinolytic substance) on the activation of the coagulation pathway, granulocytes, endothelial cells and the cytokine network in healthy humans injected with a single dose of LPS was examined. Here, we demonstrated that although active plasmin is generated early after intravenous injection of LPS into normal subjects, it does not contribute to a significant extent to activation of the coagulation system, granulocytes, the vascular endothelium or the cytokine network.

This thesis investigated several aspects of the innate immune response to infection in different experimental models and patients. Clearly, the specific role of a mediator or a group of mediators depends on the type and origin of the infection. Peritonitis is an
acute disease that frequently results in the clinical syndrome of sepsis (hence the designation abdominal sepsis). Indeed, peritonitis is the second most frequent cause of sepsis, after pneumonia. We established that the inflammatory and antibacterial response to abdominal sepsis at least in part is regulated by proinflammatory cytokines, in particular IL-12 and IL-18. Although previous studies not presented in this thesis have indicated that the inflammatory system contributes to the activation of the coagulation system, the research in this thesis established that strong inhibition of the coagulant response (by interfering with TF activity) in this model does not influence the inflammatory and antibacterial response. Together these studies shed more light on the interplay between various host mediator systems during ongoing severe infection originating from the peritoneal cavity, where TF and coagulation may contribute to organ injury but apparently do not play a role of significance in a protective immune response against the invading pathogen. The studies using TM$^{pro/pro}$ mice clearly showed that the role of a mediator (or receptor) differs tremendously depending on the experimental setting. Whereas in (sub) acute pneumonia caused by bacterial respiratory pathogens TM did not contribute to either defense or pathology, the TM$^{pro/pro}$ mutation resulted in an exaggerated inflammatory response in the lungs of mice with tuberculosis. Finally, the studies that made use of PAFR deficient mice illustrated that certain receptors play a role in defense against specific pathogens because of the phenomenon called "molecular mimicry": the PAFR facilitates the invasion of *S. pneumoniae* because it recognizes the phosphocholine component of the cell wall of this bacterium as PAF. All together this thesis investigated several parts of host mediator systems that respond to invading pathogens. Clearly this host response is extremely complex and the result of millions of years of evolution. Unravelling the complex interactions between different mediator systems during severe infections is a major task for research to be performed in the years to come. Knowledge derived from this research may eventually pave the way for innovative and better therapies for patients with live threatening infections.