Studies on coagulation-induced inflammation in mice
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Summary
The interaction between blood coagulation and inflammation as part of the innate host defense mechanism is becoming more and more apparent. In particular in the field of infectious disease there has been increasing interest in this subject, since major complications of sepsis (i.e. disseminated intravascular coagulation (DIC) and multiple organ failure) are strongly linked with excessive disturbances in the balance between coagulation factors and their inhibitors. Thrombi formed during these complications are often accompanied by massive inflammation. The interaction between coagulation and inflammation is a two directional process of which inflammation-induced coagulation is best established. Coagulation-induced inflammation has only recently gained extensive attention and inhibition of coagulation during gram-negative sepsis might be an important target for therapeutic interventions. In chapter 1, an overview of the current knowledge concerning coagulation-induced inflammation is given. Recently it has become evident that beyond the role of coagulation factors in haemostasis, their role in intracellular signaling cascades might be of major importance for coagulation-induced inflammation. Unfortunately, in an experimental setting it is impossible to discriminate between coagulation dependent and coagulation independent functions of the individual proteins. Ideally, to study coagulation-induced inflammation inhibitors that specifically inhibit single coagulation factors are needed, and more importantly, the precise inhibitory effect on both coagulant and inflammatory properties should be known. The current available inhibitors are well characterized regarding their anti-coagulant properties but whether they inhibit (for instance) intracellular signaling of the targeted proteins remains unclear for most of the inhibitors. As an alternative approach numerous transgenic and knockout mice are now available. Especially knockout mice may yield important insight into the role of individual coagulation factors in inflammation. The advantage of knockout mice over inhibitors lies in the fact that these mice completely lack a single protein and therefore there will be no uncertainty about which functions of the protein are inhibited. The major objective of the studies described in this thesis was to study the interaction between coagulation and inflammation in more detail using (knockout) mice. To this end, experiments were performed using infectious disease models like endotoxemia and peritonitis as model systems for the interaction between coagulation and inflammation. Hypoxia has been studied for its potential role as a model system for the cross-talk between coagulation and inflammation in the absence of infectious agents. In chapter 2, a short review of the dual characteristics of disseminated intravascular coagulation (DIC), as both a contributor to multiple organ failure as well as a symptom of severe underlying disease associated with systemic vascular changes, is provided, based on both published literature and unpublished data of our research group. In this chapter it is also hypothesized that hypoxia (lowered levels of oxygen) might be involved in DIC, thereby explaining why exposure of mice to hypoxia might be a suitable model system to study coagulation-induced inflammation in the absence of infectious agents. In chapter 3-5 the effects of alterations in coagulant properties during either endotoxemia or septic peritonitis in mice have been studied. The role of the initiator of coagulation, tissue factor (TF), in infectious disease has been
investigated using several tools like knockout mice and inhibitors. In chapter 3 the role of blood-borne TF in endotoxemia is described. Mice that lack TF on their blood cells have been generated by bone marrow transplantation using TF knockout embryonic liver cells as donor material. These hematopoietic cell-specific TF knockouts react less to endotoxemia as visualized by improved clinical symptoms, less cytokine production and less coagulation activation upon endotoxin administration when compared to wildtype littermates. The effect of TF haploinsufficiency during endotoxemia has been studied both in vitro and in vivo and is described in chapter 4. In vitro stimulation of heterozygous TF blood cells resulted in lower IL-6 and KC levels than stimulation of wildtype blood cells, thereby suggesting a role for TF in endotoxin-induced IL-6 and KC production. However, injection of heterozygous TF deficient mice with endotoxin did not result in differences between heterozygous TF deficient mice and wildtype mice. More in-depth analysis of coagulation activation in these heterozygous mice showed endotoxin-induced differential upregulation of TF levels to levels found in wildtype mice. In chapter 5 we investigated whether TF’s procoagulant function or its signaling properties are involved in the outcome of septic peritonitis. To this end NAPc2, an inhibitor of TF/FVIIa induced coagulation that does not inhibit TF’s signaling properties, has been used. rNAPc2 strongly attenuated the procoagulant response caused by peritonitis, but did not influence the inflammatory response. Moreover, rNAPc2 did not alter bacterial outgrowth, and survival was not different in rNAPc2 treated and control mice, evidently implicating that TF induced coagulation is not involved in host-defense against E.coli, suggesting an important role of TF’s intracellular capacity.

In chapter 6 the role of the coagulation system itself during inflammation was investigated by determining whether hemophilia or thrombophilia determine host defense during septic peritonitis. Hemophilic FVIII deficient mice showed slightly reduced coagulation activation, bacterial outgrowth and disseminated inflammation. Upon induction of peritonitis, prothrombotic FVLeiden mice showed increased coagulation activation and an impaired host-defense. However, like FVIII deficiency, FVLeiden did not influence sepsis-induced mortality. These data demonstrate that inherited tendencies to bleeding or thrombosis modify host-defense during septic peritonitis, but are of no importance for the final outcome of sepsis.

To investigate whether hypoxia can be used as a model system to study the cross-talk between coagulation and inflammation without using infectious agents, we studied the time course of coagulation activation and cytokine production during and after cessation of oxygen deprivation (chapter 7). As expected, exposure of mice to 8% oxygen led to coagulation activation and altered cytokine profiles. However, in these experiments coagulation activation took place in the circulation, whereas cytokine production was observed locally in several tissues. As described in chapter 3-6, coagulation activation and inflammation during endotoxemia or peritonitis take both place in the circulation as well as in tissues. The locally altered cytokine profile remained for at least 10 days after cessation of hypoxia. The results obtained in chapter 7 made us doubt the bioactivity of the cytokines produced during hypoxia. Tissue cytokines play an important role in the
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Pathogenesis of *Pseudomonas aeruginosa* pneumonia and therefore we investigated as described in chapter 8 whether a hypoxic period influences host defense during *Pseudomonas aeruginosa* pneumonia. Inoculation with *P. aeruginosa* resulted in lower bacterial outgrowth from lung tissues in hypoxic mice than in mice that were held in normal oxygen tensions before instillation of bacteria, indicating that a preceding hypoxic episode boosts host defense during *P. aeruginosa* pneumonia, most likely via local induction of bioactive cytokine levels in the lung.

Finally, in chapter 9 we tried to answer an important question regarding factor VIII synthesis. FVIII is mainly produced in the liver, however, FVIII mRNA has also been found in blood cells. We investigated whether blood cells are also capable of producing factor VIII. Therefore, bone marrow transplantations in FVIII-deficient mice using hematopoietic stem cells from wildtype mice were performed. After bone marrow transplantation, FVIII deficient mice showed low, but detectable plasma FVIII levels. The source of plasma FVIII remains difficult to define, however, as after transplantation the presence of FVIII mRNA was not limited to hematopoietic cells, most likely results due to stem cell plasticity.