Cytokines and chemokines in systemic and urinary tract infection by Escherichia coli
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Citation for published version (APA):
Chapter 13

Summary and General Discussion
Chapter 13

Summary

*Escherichia coli* is a gram-negative bacterium that is a common pathogen in a number of infectious diseases, including urinary tract infections (UTIs). Infection with *E. coli* is associated with induction of inflammatory responses which are considered to play an important role in the pathogenesis of infectious disease syndromes caused by this bacterium. Endotoxin (lipopolysaccharide, LPS) is a constituent of the outer membrane of *E. coli* (and other gram-negative bacteria) that is a major factor in *E. coli*-induced inflammation. The studies described in this thesis focused on the induction and the role of cytokines and chemokines in the inflammatory response to either live *E. coli* or LPS derived from *E. coli*. The first part of this thesis (Chapters 2 to 6) involves studies on clinical and experimental UTI caused by *E. coli*. The second part of this thesis (Chapters 7 to 11) deals with regulation of systemic inflammation induced by intravenous injection of *E. coli* LPS in healthy human subjects. Finally, Chapter 12 describes an investigation in a mouse model of abdominal sepsis caused by live *E. coli*.

In **Chapter 2**, we sought to increase the insight into the kinetics of interleukin (IL)-6 and IL-8 release into urine shortly before and directly after the microbiological documentation of UTI. We therefore prospectively followed a group of 156 patients undergoing major abdominal surgery, who were at risk for developing UTI because of the fact that they all received a urinary catheter. Ten patients developed UTI, in all cases caused by *E. coli*. In these patients, urine IL-6 and IL-8 concentrations were highest on the day the urine culture became positive. IL-6 concentrations already increased in the two to four days preceding UTI; however, a similar increase in urine IL-6 levels was found in control patients from the same population without UTI who were matched for the duration of catheterization. In accordance, we found that these patients with uncomplicated abdominal surgery demonstrated a gradual rise in urine IL-6 from postoperative day four and onward. In contrast, urine IL-8 concentrations were elevated only on the day UTI was documented, and did not increase in controls without UTI. These data suggest that in postoperative patients who develop UTI, IL-8 but not IL-6 marks the early phase of the local host response to infection.
Summary and General Discussion

In Chapter 3, we evaluated local and systemic concentrations of a number of inhibitors of proinflammatory cytokines, in particular of tumor necrosis factor (TNF) and IL-1, in 30 patients with culture proven urosepsis. In these patients, soluble TNF receptors types I and II, IL-1 receptor antagonist (IL-1ra), soluble IL-1 receptor type II and IL-10, were measured in urine and serum obtained on three consecutive days after admission to the hospital, and compared with values measured in urine and serum from 20 healthy controls with sterile urine. By calculating the urine/serum ratios, we sought to obtain insight into the extent of local (within the urinary tract) versus systemic production of these anti-inflammatory mediators. Urosepsis patients had elevated serum and urine concentrations of soluble TNF receptors types I and II, and higher urine/serum ratio’s than healthy controls. In contrast, IL-1ra and IL-10 concentrations were only elevated in serum of patients, whereas soluble IL-1 receptor type II levels were not elevated at all. These data suggest that during acute UTI the anti-inflammatory cytokine response is generated largely at the systemic level, and that TNF and IL-1 inhibitors are not produced in significant quantities within the urinary tract with the possible exception of soluble TNF receptors.

In Chapter 4 we used the same patient population to examine the local and systemic release of monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β and interferon-γ inducible protein (IP)-10, all chemokines that mainly act on mononuclear cells, during acute UTI. In addition, we included urine and plasma samples from 11 healthy subjects intravenously injected with *E. coli* LPS in our analyses, representing humans exposed to *E. coli* LPS at the systemic level (contrasting with patients with urosepsis, who were exposed to *E. coli* within their urinary tract). We argued that comparison of urine and plasma levels of chemokines in patients with urosepsis and humans intravenously injected with LPS could provide insight in the extent of local production of these mediators (i.e. within the urinary tract) versus renal excretion of systemically produced mediators. Urine and serum concentrations of MCP-1, MIP-1β and IP-10, but not of MIP-1α, were elevated in urosepsis patients. Intravenous injection of LPS into healthy subjects was associated with transient rises in the plasma and urine levels of all four chemokines. Together these results suggest that MCP-1, MIP-1β
and IP-10 are produced both at the systemic level and in the urinary tract during acute UTI.

In Chapter 5 we studied plasma and urine concentrations of the ELR positive CXC chemokines IL-8, growth related oncogene (GRO)α and epithelial-cell derived neutrophil-activating protein (ENA)-78 in 33 patients with culture proven urosepsis during the first eight hours after initiation of antibiotic therapy, in 31 healthy controls and in 11 subjects intravenously injected with LPS. Patients had markedly elevated urine concentrations of CXC chemokines, with no (GROα, ENA-78) or little (IL-8) elevation in plasma. LPS injection induced transient rises in the plasma levels of CXC chemokines, with no (GROα) or little (IL-8, ENA-78) elevation in urine. In additional in vitro experiments, we found that urine from patients exerted chemotactic activity toward neutrophils, which was inhibited in part by antibodies directed against either IL-8, GROα or ENA-78. These results suggest that during acute UTI, IL-8, GROα and ENA-78 are produced predominantly within the urinary tract, where they contribute to the recruitment of neutrophils to the urinary compartment.

In Chapter 6, we utilized a mouse model of ascending UTI to determine the role of CXC chemokines in host defense against UTI. In this model UTI was induced by intravesical inoculation of female mice with live E. coli via the urethra. Mice were pretreated with either an antibody against the sole receptor in the mouse reactive to ELR positive CXC chemokines (CXCR2) or with a control antibody. Anti-CXCR2 treatment virtually completely prevented the influx of neutrophils to the urinary tract, as reflected by the absence of leukocyturia and the lack of neutrophilic infiltrates in kidneys. Anti-CXCR2 only transiently impaired the clearance of bacteria from the urinary tract, as indicated by significantly more E. coli colony forming units in urine and kidneys at 24 hours, but not at 48 hours, after the infection. Further, the absence of a neutrophilic response was not accompanied by dissemination of the infection. These data suggest that ELR positive CXC chemokines play a major role in the development of a local inflammatory response to ascending UTI.

A number of chemokines can interact with the Duffy antigen receptor for chemokines (DARC) on erythrocytes. In Chapter 7 we determined the extent to which chemokines circulate in cell-associated form during
human endotoxemia. In particular we compared the concentrations of chemokines known to interact with DARC (IL-8, GROα and MCP-1) with those of MIP-1β (which does not show affinity for DARC) in plasma and in lysates of red blood cells (RBCs), polymorphonuclear cells (PMNs) and mononuclear cells (PBMCs) isolated from peripheral blood before and after intravenous administration of *E. coli* LPS to eight healthy humans. LPS injection induced transient increases in the plasma levels of all four chemokines. IL-8 was detected at high concentrations in the RBC, PMN and PBMC fractions after LPS administration, whereas GROα and MCP-1 were only recovered in significant quantities from RBCs. MIP-1β was detectable in plasma only. These results suggest that DARC binding chemokines circulate in association with blood cells during endotoxemia, in particular with RBCs. The measurement of cell-associated chemokines in conditions of inflammation and infection may provide more accurate information on the extent of chemokine production that the mere measurement of plasma levels.

In Chapter 8 we evaluated the role of CD14, a pattern recognition receptor for a number of bacterial antigens including LPS, in the release of chemokines during human endotoxemia. In this investigation 16 healthy humans were intravenously injected with *E. coli* LPS preceded by either an intravenous infusion of IC14, an anti-human CD14 monoclonal antibody, or placebo. IC14 profoundly attenuated the rises in the plasma concentrations of IL-8, MCP-1 and MIP-1β, but not of MIP-1α. IC14 also diminished the increases in the IL-8 levels measured in RBC, PMN and PBMC fractions, and in RBC-associated MCP-1. MIP-1α and MIP-1β, which do not bind DARC, were not detected in cell fractions. These results suggest that the release of chemokines during human endotoxemia is mediated in part by an interaction between LPS and CD14.

Severe infection frequently results in an anergic state which has been referred to as “LPS tolerance” or “immunoparalysis”. This refractory state is characterized by a reduced capacity of monocytes to produce cytokines upon stimulation with LPS. In Chapter 9 we examined whether PMNs are also rendered hypo-responsive during an ongoing inflammatory response, using the model of human endotoxemia. For this PMNs were isolated from peripheral blood from six healthy humans before and one, two or 24 hours after intravenous injection of *E. coli* LPS, and stimulated “ex vivo” with
Chapter 13

either LPS or heat-killed bacteria. As measures of PMN responsiveness, GROα and ENA-78 were measured in culture supernatants, and IL-8 in culture supernatants and in PMN lysates. At one and two hours after LPS injection, the capacity of PMNs to produce CXC chemokines was strongly reduced. Serum obtained two hours after LPS administration did not influence chemokine release by PMNs. These results indicate that intravenous exposure to LPS induces a refractory state of PMNs that is not caused by soluble mediators secreted in response to in vivo exposure to LPS.

IL-10 is a potent anti-inflammatory cytokine that is released in high amounts during infectious diseases (e.g. Chapter 3). In Chapter 10 we determined the effect of recombinant IL-10 on the release of CC chemokines during human endotoxemia. In this study, 16 healthy humans were evaluated in a cross-over design in which all subjects were studied on two occasions. On one occasion, each subject was injected with LPS in combination with placebo. On the other occasion, each subject was injected with LPS in combination with recombinant IL-10. Placebo or IL-10 were administered either two minutes before LPS (pretreatment) or one hour after LPS (posttreatment). In whole blood in vitro, IL-10 caused a dose-dependent inhibition of LPS-induced MIP-1α and MIP-1β release, whereas MCP-1 secretion was not affected. IL-10 also diminished MIP-1α and MIP-1β release stimulated by agonists derived from gram-positive bacteria. Pretreatment with IL-10 in vivo resulted in significant reductions in the plasma levels of MIP-1α, MIP-1β and MCP-1, whereas IL-10 posttreatment only reduced MIP-1β release. In final in vitro experiments we established that (1) the capacity of IL-10 to inhibit LPS-induced TNF production did not significantly contribute to the IL-10 effect on chemokine release, and (2) IL-10 also inhibited MIP-1α and MIP-1β production by both isolated PMNs and PBMCs. In Chapter 11 we determined the effect of IL-10 on the production of the ELR positive CXC chemokines GROα and ENA-78. IL-10 pretreatment modestly but significantly reduced GROα release, but was without effect on ENA-78 secretion. IL-10 posttreatment did neither influence GROα nor ENA-78 release. In whole blood and cultures of isolated PMNs and PBMCs, IL-10 was capable of inhibiting both GROα and ENA-78 release induced by LPS. The results of Chapters 10 and 11 suggest that the anti-inflammatory potential of IL-10 in vivo at least in part is related to its inhibitory effect on chemokine production.
In **Chapter 12**, we used a murine model to evaluate the production and function of endogenous IL-10 during peritonitis induced by *E. coli*. For this we compared host responses in IL-10 gene deficient (IL-10-/−) and normal wild type (IL-10+/+) mice after intraperitoneal injection of live *E. coli*. Administration of increasing doses of *E. coli* resulted in a dose-dependent increase in IL-10 concentrations in peritoneal fluid and plasma. This endogenous IL-10 hampered antibacterial effector mechanisms as reflected by an increased bacterial clearance from the peritoneal cavity and a reduced dissemination of the infection to distant organs in IL-10-/− mice. Nonetheless, IL-10-/− mice demonstrated higher TNF levels in peritoneal fluid and plasma than IL-10+/+ mice during peritonitis, and displayed more severe multiple organ damage as indicated by clinical chemistry and histopathology. The elevated TNF concentrations in IL-10-/− mice contributed to the development of multiple organ damage, since it was diminished in anti-TNF treated IL-10-/− mice. In addition, anti-TNF prevented the enhanced lethality of IL-10-/− mice during peritonitis. Hence, endogenous IL-10 protects against organ damage and lethality during abdominal sepsis induced by *E. coli* by a mechanism that involves inhibition of TNF release, in spite of the fact that it impairs antibacterial effector mechanisms. These results exemplify the paradoxical role of inflammation in the pathogenesis of bacterial infection, i.e. whereas local inflammation is required to effectively combat invading microorganisms, excessive systemic inflammation can harm the host and can contribute to tissue injury and death.

**General Discussion**

Cytokines and chemokines play a critical role in the regulation of the innate immune response to bacterial infection. Bacteria or their products, such as LPS, strongly stimulate the production of these mediators by various cell types. Subsequently, they orchestrate the inflammation that invariably accompanies infection. In doing so, cytokines and chemokines closely interact in a tightly controlled network, in which the synthesis and activity of proinflammatory mediators is regulated by a number of anti-inflammatory mechanisms, including anti-inflammatory cytokines and a phenomenon designated "immunoparalysis". In this thesis several aspects
Chapter 13

of the chemokine and cytokine response to infection by *E. coli* were studied, primarily focused on UTI and systemic LPS effects. Evidence was obtained that the anti-inflammatory cytokine response during urosepsis likely is generated predominantly at the systemic level (Chapter 3), and that hyporesponsiveness of PMNs is part of the anti-inflammatory reaction of the host to the proinflammatory stimulus delivered by *E. coli* LPS (Chapter 9). By contrast, proinflammatory responses (i.e. CXC chemokine production) for a large part originate from the urinary tract during UTI (Chapter 5). In addition, we demonstrated the importance of locally produced ELR positive CXC chemokines in the recruitment of neutrophils to the urinary tract during UTI (Chapters 5 and 6). We further established that a number of chemokines circulate in cell-associated form, which may help to improve our insight in the extent of chemokine production during inflammation and infection (Chapters 7 and 8). The interaction between the prototypic anti-inflammatory cytokine IL-10 and chemokines was established in systemic inflammation induced by *E. coli* LPS (Chapters 10 and 11), and the interplay between IL-10 and the prototypic proinflammatory cytokine TNF was revealed in the pathogenesis of abdominal sepsis caused by live *E. coli* (Chapter 12).

Early in the course of an infection a delicate balance between proinflammatory mediators and anti-inflammatory mechanisms exists, both at the site of the infection and at the systemic level, which is an important denominator of the eventual disease outcome. The studies presented in this thesis may contribute to our understanding of the highly complex role of cytokines and chemokines during infection, in particular during systemic infection and UTI by *E. coli*. 