Cytokines and chemokines in systemic and urinary tract infection by Escherichia coli
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Chapter 1

General introduction and outline of the thesis
Chapter 1

INTRODUCTION

The research described in this thesis focused on the role of cytokines and chemokines in urinary tract infection (UTI) caused by *Escherichia (E.) coli*. Furthermore, investigations on the regulation of systemic inflammation induced by endotoxin derived from *E. coli* are presented. In this introductory chapter, we briefly discuss the general background of the most important topics of this thesis.

1. Urinary tract infection

UTIs are responsible for as many as eight million visits to physicians a year in the United States alone [1]. The clinical spectrum of UTI ranges from asymptomatic bacteriuria to acute pyelonephritis and gram-negative sepsis [for reviews see references 2-6]. Hospital-acquired UTIs are the most common nosocomial infections, giving rise to approximately 500,000 cases a year in the United States [7, 8]. The presence of a urethral catheter is a recognized risk factor for these infections and is associated with 80 to 95 per cent of the cases. In community-acquired UTI, ascending infection usually is caused by the entry of bacteria colonizing the anterior urethra and/or the vaginal introitus into the bladder. During adolescence, UTI is predominantly a disease of women. In women of 16-35 years of age, UTIs are 50-fold more common than in men, and by age 25, over 80% of women have experienced one or more UTIs. At older ages, the relative incidence of UTIs rises in men, at least in part due to predisposing conditions such as prostate hypertrophy and catherization.

UTIs are most frequently caused by bacteria, in particular *E. coli*. *E. coli* accounts for more than 75 percent of cases of uncomplicated cystitis and pyelonephritis. In recurrent or complicated UTIs, *E. coli* remains the most common pathogen, although other organisms such as *Klebsiella, Proteus* and enterococci become more common in these conditions. A gram-positive bacterium associated with UTI is *Staphylococcus saprophyticus*, which causes infection predominantly in young women in spring and summer. In hospital-acquired UTIs, a much broader range of organisms is isolated, among which *E.coli* and yeasts are the most common.
The pathogenesis of community-acquired UTI has been studied most carefully in young women. *Host factors* that contribute to the development of UTI in this group include the short female urethra, allowing bacteria colonizing its distal end to enter the bladder, and the proximity of the urethral meatus to the rectum. Indeed, bacteria that eventually cause UTI, usually first colonize the vaginal introitus and the periurethral area. Another host factor that may predispose to UTI is the stronger binding of bacteria to uroepithelial cells, as indicated by studies demonstrating that uroepithelial cells from women prone to recurrent UTI bind more bacteria than cells from women without a history of UTI. Host factors that protect against UTI include the dynamics of urine flow and a functional vesicourethral junction, the acidity, high urea concentration, and extremes of osmolality in urine. Once urinary infection has been established, a local inflammatory response occurs which is considered to reflect an attempt of the host to limit the further development and spreading of the infection. This inflammatory response is characterized by an influx of granulocytes into the urinary tract. Conceivably, cytokines and chemokines play a role in this phenomenon.

*Bacterial factors* also play an important role in the pathogenesis of UTI. Uropathogenic *E. coli* possess specific virulence factors, such as pili (or fimbriae) that mediate adherence to vaginal and uroepithelial cells, resistance to the bactericidal activity of human serum, production of hemolysin, and increased amounts of K capsular antigen. Adhesion is mediated by bacterial ligands (usually small proteins located at the tips of bacterial fimbriae) that attach to cell-wall carbohydrate residues serving as receptors. After attachment has been accomplished, hemolysin may be important for tissue invasion and lysing leukocytes, while the presence of K-antigen protects bacteria from complement-mediated killing and from phagocytosis.

2. **Endotoxin**

Endotoxin (lipopolysaccharide, LPS) is part of outer membrane of gram-negative bacteria. LPS is a potent proinflammatory agent, which is thought to play central role in gram-negative sepsis [9]. LPS is composed of a lipid
moiety, designated lipid A, and a hydrophilic polysaccharide chain. The polysaccharide portion of LPS consists of the O-chain, that protrudes from the bacterial membrane, and a core part, connecting the O-chain with Lipid A. While the O-chain consists of a series of structurally and antigenically diverse oligosaccharides that determine the many different O-specific serotypes, the core part is identical for many different bacteria. Lipid A is the highly conserved biologically active part of LPS which is completely embedded in the bacterial membrane, shielded by the O-chain. Proinflammatory effects of gram-negative bacteria can therefore not be readily explained by the effects of cell-bound LPS. However, LPS can be shed by gram-negative bacteria via several mechanisms, including destruction of the bacterial cell wall by complement factors.

Three cloned molecules expressed on the surface of mononuclear cells have been documented to bind the lipid A part of LPS, i.e. CD14, the β2 leukocyte integrins (CD11a/CD18, CD11b/CD18, and CD11c/CD18), and the macrophage scavenger receptor [10]. Binding of LPS to CD14 or CD11/CD18 will eventually result in a cellular effect, while scavenger receptors do not seem to function as signaling receptors. Spontaneous binding of LPS to CD14 occurs at very slow rates. LPS-CD14 binding is greatly accelerated in the presence of LPS binding protein (LBP), an acute phase reactant mainly derived from the liver and present in blood at concentrations in the μg/mL range [10, 11]. LBP can also bind intact gram-negative bacteria via LPS in the outer membrane, and can facilitate attachment of bacteria to CD14. CD14 is present in serum in a soluble form. Cell types that do not express membrane bound CD14 can be rendered LPS responsive by a mechanism that involves soluble CD14 and LBP. It was recognized many years ago that CD14, which is a glycosphatidylinositol (GPI)-anchored membrane protein and does not have an intracellular domain, is not the LPS receptor signaling element. Recent research has identified Toll-like receptor 4 as a signaling receptor for LPS [12].

3. Cytokines

Cytokines are small proteins important for the orchestration of inflammatory processes [13]. They are produced by a number of cells of the
immune system in response to various infectious stimuli. Cytokines can be divided into proinflammatory cytokines, anti-inflammatory cytokines and soluble inhibitors of cytokines. Whereas the former group of cytokines stimulates inflammatory processes, the two latter groups act to inhibit either the production or the activity of proinflammatory cytokines. Important proinflammatory cytokines are tumor necrosis factor-\(\alpha\) (TNF) and interleukin (IL)-1 [14, 15], while IL-10 is a major anti-inflammatory cytokine [16]. Examples of soluble inhibitors of TNF and IL-1 include soluble TNF receptors types I and II, and IL-1 receptor antagonist (IL-1ra) and the type II soluble IL-1 receptor respectively. IL-6 is a cytokine with both pro- and anti-inflammatory properties [13].

A delicate balance between the three branches of the cytokine network is decisive for the outcome of a bacterial infection [13]. In a localized bacterial infection, proinflammatory cytokines are required for an adequate host defense leading to clearance of the pathogen, whereas anti-inflammatory cytokines can impair antibacterial effector mechanisms. On the other hand, overwhelming sepsis may result in a systemic inflammatory response, during which excessive systemic activity of proinflammatory cytokines may harm the host and anti-inflammatory cytokines may protect against organ damage caused by abundant inflammation. As such, the cytokine network seems to act as a double-edged sword, i.e. local activity of proinflammatory cytokines is important for local antibacterial defense, whereas systemic activity of these mediators may lead to tissue toxicity.

4. **Chemokines**

Chemokines are a group of small chemotactic proteins that play an important role in the host response to bacterial infections by attracting leukocytes to the site of infection [17-19]. Chemokines may further contribute to the inflammatory response by activation of leukocytes through induction of oxygen burst and degranulation [20]. Depending on their structure, chemokines can be divided into distinct families. Two of these subfamilies are the subject of studies presented in this thesis, i.e. CXC- and CC chemokines. In the former family, one amino acid separates the first two cysteine residues adjacent to each other (cysteine- X amino acid-cysteine, or CXC), whereas in the latter family, the first two cysteine
residues are adjacent to each other (cysteine-cysteine, or CC). CXC chemokines can be further subdivided into ELR positive chemokines, which possess a three amino acid motif termed ELR (glutamic acid - leucine - arginine) near the N-terminal end, and ELR negative CXC chemokines. ELR positive CXC chemokines are chemotactic for neutrophils and include IL-8, growth-related oncogene (GRO)-α, -β, -γ and epithelial cell-derived neutrophil-activating protein (ENA)-78 in humans, and keratinocyte (KC) and macrophage inflammatory protein (MIP)-2 in mice. Examples of ELR negative CXC chemokines, which act primarily on lymphocytes, are monokine induced by interferon-γ (Mig) and interferon-inducible protein (IP)-10. CC chemokines mainly act on mononuclear cells. Members of this subfamily are monocyte chemoattractant protein (MCP)-1, MIP-1α and MIP-1β.

Each of the chemokine subfamilies is recognized by its own group of receptors. In humans, two receptors for ELR positive CXC chemokines have been identified on the surface of granulocytes, the CXC chemokine receptor – 1 and - 2 (CXCR1 and CXCR2) [21, 22]. While CXCR1 binds exclusively IL-8, CXCR2 is a promiscuous chemokine receptor, which binds all ELR positive CXC chemokines [23]. Mice do not express CXCR1, and in this species CXCR2 exclusively mediates granulocyte responses to ELR positive CXC chemokines [24]. Interestingly, both CXC- and CC-chemokine subfamilies share a promiscuous receptor, the Duffy antigen receptor for chemokines (DARC) [25, 26]. DARC is present on erythrocytes and endothelial cells and was initially characterized as a receptor for Plasmodium vivax [27]. IL-8 has been reported in association with erythrocytes and other blood cells during sepsis [28, 29], suggesting that DARC may trap certain chemokines in the circulation [30].

5. Aim and outline of the thesis

The general objectives of the studies presented in this thesis were to obtain insight in (1) the production and the function of cytokines and chemokines during UTI caused by E. coli, and (2) the regulation of systemic inflammation elicited by E. coli LPS.
In Chapter 2, we describe the release of IL-6 and IL-8 in surgical patients with a urinary catheter who developed a UTI postoperatively. Patients who did not develop a UTI, matched for the duration of catheterization, served as controls. The aim of this study was to assess the kinetics of IL-6 and IL-8 release in urine before and after the clinical and bacteriological diagnosis of UTI, using a patient population known to be at risk for UTI because of the presence of a urinary catheter. In Chapter 3, we report the urine and serum concentrations of inhibitors of TNF and IL-1, i.e. soluble TNF receptors types I and II, IL-1ra, soluble IL-1 receptor type II and IL-10, in 30 patients with culture-proven urosepsis who were followed for three consecutive days after admission to the hospital. By calculating the urine/serum ratio's in patients and healthy controls, we sought to evaluate the extent of local (within the urinary tract) versus systemic production of these anti-inflammatory mediators.

Chapters 4, 5 and 6 examine the production and function of chemokines in UTI. In Chapter 4, urine and serum concentrations of the CC chemokines MCP-1, MIP-1α and MIP-1β and the ELR negative CXC chemokine IP-10 were measured in the same patient population presented in Chapter 3. In addition, chemokine concentrations were measured in urine and plasma samples from 11 healthy subjects who were intravenously injected with low dose E. coli LPS. The latter study group represented humans exposed to E. coli LPS at the systemic level (contrasting with patients with urosepsis, who had a localized infectious source within their urinary tract). We argued that comparison of urine/plasma ratio's of chemokines in patients with urosepsis and humans intravenously injected with LPS could provide insight in the extent of localized production of these mediators (i.e. within the urinary tract) versus renal excretion of systemically produced mediators. In Chapter 5, a similar approach was taken to obtain insight in the local versus systemic production of the ELR positive CXC chemokines IL-8, ENA-78 and GRO-α. The patients with urosepsis (n= 33) differed from the patients studied in Chapters 3 and 4; their urine and plasma samples were obtained with two-hour intervals during the first eight hours after the diagnosis of urosepsis. Furthermore, the relative contribution of these CXC chemokines to the chemotactic activity of patient urine toward granulocytes was studied using neutralizing monoclonal antibodies directed against either IL-8, ENA-78 or GRO-α. In Chapter 6, we sought to determine the role of CXC chemokines in host defense against UTI. For this
we used a murine model of ascending UTI, in which *E. coli* was administered to female mice intravesically via the urethra. By injecting mice with either a blocking anti-CXCR2 antibody (eliminating the activity of all ELR positive CXC chemokines) or an irrelevant control antibody we evaluated the significance of ELR positive CXC chemokines during UTI.

In Chapters 7, 8 and 9, we studied the regulation of chemokine release after intravenous injection of *E. coli* LPS into healthy humans. In **Chapter 7**, we measured the concentrations of the DARC binding chemokines IL-8, MCP-1 and GRO-α in isolated fractions of peripheral blood cells (red blood cells, granulocytes and mononuclear cells), and compared these with the cell-associated concentrations of the non-DARC binding chemokine MIP-1β. In **Chapter 8**, we determined the role of CD14 in the appearance of cell-associated chemokines during human endotoxemia by treating healthy humans with IC14, an anti-CD14 monoclonal antibody, prior to LPS in a placebo controlled investigation. In **Chapter 9**, we investigated the capacity of neutrophils, isolated from peripheral blood of healthy humans before and one to 24 hours after in vivo LPS exposure, to produce IL-8, ENA-78 and GRO-α upon stimulation with LPS or heat-killed bacteria.

Chapters 10, 11 and 12 involve studies in which the effects IL-10 on chemokine production induced by *E. coli* LPS, and on host defense against peritonitis induced by live *E. coli* were addressed. **Chapter 10** describes the effects of recombinant human IL-10, given either 2 minutes before or one hour after an intravenous injection of LPS, on the release of the CC chemokines MCP-1, MIP-1α and MIP-1β during human endotoxemia. In addition, underlying mechanisms of IL-10 effects were studied in vitro using human whole blood, and neutrophils and mononuclear cells isolated from peripheral blood. In **Chapter 11**, a similar approach was taken to determine the effects of IL-10 on LPS-induced ENA-78 and GRO-α release. 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 and normal wild type mice after intraperitoneal injection of live *E. coli*.

**Chapter 13** concludes this thesis with a summary and a general conclusion.
Introduction

References

Chapter 1