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Chemokine response to febrile urinary tract infection

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Background. Mucosal CXC chemokines recruit inflammatory cells to the infected urinary tract. The chemokine response repertoire of the urinary tract and the relationship to disease severity have not been examined, however.

Methods. This study quantified CXC (CXCL1, CXCL3, CXCL5, CXCL8, CXCL9, and CXCL10) and CC (CCL2, CCL4, and CCL5) chemokines in sequential urine samples obtained from 50 patients with febrile urinary tract infections during 24 hours after diagnosis.

Results. All patients had elevated chemokine levels, but bacteremic infections caused higher CXCL1, CXCL3, CXCL5, CXCL8, and CCL2 responses. CCL2 and CXCL8 levels were higher in patients with acute pyelonephritis symptoms and CCL2, CXCL3, CCL4, CXCL5, and CXCL10 were significantly correlated to C-reactive protein (CRP) and temperature. Women and men showed different chemokine responses.

Conclusion. Febrile urinary tract infections are accompanied by a complex chemokine response. The response magnitude reflects disease severity, and the repertoire is influenced by gender and underlying disease.

The diverse manifestations of urinary tract infection reflect the quality, localization, and magnitude of the inflammatory response to pathogenic bacteria [1]. Acute pyelonephritis is accompanied by local symptoms from the upper urinary tract like flank pain or costovertebral angle tenderness and by fever and malaise reflecting the systemic involvement. The temperature or circulating acute-phase reactants like C-reactive protein (CRP) are used to quantify the systemic inflammatory response, but there is no tradition of measuring local host response parameters in urine, even though the local repertoire of inflammatory mediators reflects both the site and severity of infection.

Chemokines are small chemotactic proteins of 8 to 10 kD, that selectively target and activate specific cell populations and attract them to inflamed tissue sites [2]. They are divided into four subgroups (CXC, CC, C, and CX_3C), based on the arrangement of the first two of four conserved cysteine residues. The CXC family is further subdivided according to the sequence glutamic acid-leucine-arginine (ELR) near the N-terminus immediately preceding the first cysteine residue. ELR containing CXC chemokines are potent neutrophil activators and include CXCL1, CXCL3, CXCL5, and CXCL8 [previously referred to as GROα, GROγ, ENA-78, and interleukin (IL)-8]. CXC chemokines lacking the ELR motif, CXCL9 and CXCL10 (previously referred to as Mig and IP-10), have a weak if any neutrophil-activating activity, but attract and activate T cells and natural killer (NK) cells. CC chemokines like CCL2 [previously referred to as monocyte chemoattractant protein-1 (MCP-1)], CCL4 (previously referred to as MIP-1β), and CCL5 [previously referred to as regulated upon activation, normal T-cell expressed and secreted (RANTES)] attract mostly monocytes and T cells, but also neutrophils [3, 4].

Urinary tract infections elicit a mucosal chemokine response [5–8]. Epidemiologic studies of symptomatic infections showed elevated urine and serum CXCL8 levels in patients with febrile urinary tract infection [5, 7, 9–15], and both CXC and CC chemokines were detected in patients with urosepsis [12, 15, 16]. In pediatric populations, the urinary tract chemokine response was shown to be specific for febrile urinary tract infection, as elevated urine CXCL8 levels were detected in children with febrile urinary tract infection but not in those with febrile infection of unknown origin [11]. The first chemotactic signal emanates from uroepithelial cells which are efficient chemokine producers [17, 18] (for review see [19]). Uropathogenic *Escherichia coli* stimulate a chemokine response through different TLR4-dependent signaling pathways in the epithelial cells [20–22]. P fimbriae are one essential virulence factor, as shown by the rapid CXCL8 response to deliberate intravesical inoculation of the human urinary tract with P fimbriated *E. coli* [23, 24] and by the lack of chemokine production during long-term asymptomatic carriage of the nonfimbriated strain. The chemokine response is essential for the

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antibacterial defense of the urinary tract, and especially the CXC chemokines are crucial to recruit inflammatory cells to infected sites within the urinary tract [12, 18, 25–27].

The earlier studies have shown that virulent strains trigger a urinary tract chemokine response in patients with symptomatic urinary tract infection, but have not addressed how the chemokine repertoire reflects disease severity. This study investigated the chemokine response in patients with febrile urinary tract infection, the effect of bacteremia and the relationship to disease severity.

METHODS

Patients

Fifty patients with community-acquired febrile urinary tract infection were included in this study [28]. The patients were 18 to 85 years old, with febrile urinary tract infection requiring hospitalization and parenteral antibiotic therapy. All patients had significant bacteriuria at admission, a temperature ≥38°C, and either focal symptoms from the urinary tract, a positive nitrite test at admission, increased leukocyte counts in urine, or instrumentation of the urinary tract/acute urinary retention preceding the onset of fever. The patients were hospitalized and treated with parenteral ceftazidime [28]. Patients with bacteremic febrile urinary tract infection had at least one positive blood culture with the same bacterial species in blood and urine. Symptoms, signs, and medical history were registered by standardized questionnaire. The patients assigned to the compromised group had diabetes, chronic lymphocytic leukemia, systemic lupus erythematodes, alcoholism, Morbus Crohn, cancer, treatment with corticosteroids, or urinary tract abnormalities. Patients with urinary tract abnormalities had prostatic hyperplasia, operations of the urogenital tract, renal stones, prolapse of the uterus, urinary tract devices, indwelling urinary catheter, intermittent catheterization or an artificial urinary sphincter. Focal symptoms from the upper urinary tract included flank pain, costovertebral angle tenderness and symptoms from the lower urinary tract included dysuria, frequency, and suprapubic pain.

The study was approved by the Research Ethics Committee of the Medical Faculty, at the University of Lund.

Bacterial cultures

Freshly voided urine samples were semiquantitatively cultured. Blood samples for aerobic and anaerobic culture were taken twice at inclusion [29]. Significant bacteriuria was defined by growth of a single bacterial species at $\geq 10^4$ cfu/mL in women and $\geq 10^3$ cfu/mL in men.

Host response parameters

Blood and urine samples were obtained at inclusion, and at 6 to 8, 12 to 14, and 24 hours after the onset of antibiotic therapy. Serum and urine samples were immediately frozen at −70°C. CRP was quantified at inclusion, after 12, 24, and 48 hours, and on day 3. Total white blood cell counts, neutrophil counts, erythrocyte sedimentation rate (ESR), and orosomucoid were analyzed at inclusion and on day 3.

Urine leukocytes were microscopically counted in centrifuged urine. Pyuria was defined as ≥5 leukocytes/microscopic field, and abundant pyuria as ≥30 leukocytes/microscopic field.

Chemokine assays

Antigenic CXCL1, CXCL3, CXCL5, CXCL8, CXCL9, CXCL10, CCL2, CCL4, and CCL5 were quantified using a modification of the double ligand method previously described [30, 31]. Briefly, flat bottomed 96-well microtiter plates were coated with 50 μL/well of the appropriate polyclonal antibodies for 24 hours at 4°C. Samples were added, followed by incubation for 1 hour at 37°C. Biotinylated polyclonal rabbit antihuman CXCL1, CXCL3, CXCL5, CXCL8, CXCL9, CXCL10, CCL2, CCL4, and CCL5 antibodies were added (50 μL/well), and incubated for 45 minutes at 37°C followed by streptavidin-peroxidase conjugate for 30 minutes at 37°C. Chromogenic substrate was added, and the reaction was terminated with 3 mol/L H_2SO_4 (50 μL/well). Plates were read at 490 nm in an automated microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). The enzyme-linked immunosorbent assay (ELISA) detects cytokine concentrations >10 pg/mL. The chemokine concentrations were determined in urine samples obtained at inclusion, and 6, 12, and 24 hours after onset of antibiotic therapy. The 24 hours peak value was the highest concentration recorded during the first 24 hours after onset of antibiotic therapy.

Statistics

The Mann-Whitney *U* test, the Wilcoxon signed ranks test for paired data, the Fisher exact test and the Pearson correlation test were used. *P* values <0.05 were considered statistically significant (two-tailed).

RESULTS

Patients

Patients with febrile urinary tract infection were enrolled in the study [28]. Twenty-four patients with bacteremia and 26 nonbacteremic patients were matched according to gender, age (±10 years) and date of inclusion in the study. *E. coli* were found in 47 patients and *Citrobacter*