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Chemotactic Activity of CXC Chemokines Interleukin-8, Growth-Related Oncogene- α , and Epithelial Cell-Derived Neutrophil-Activating Protein-78 in Urine of Patients with Urosepsis

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CXC chemokines are chemotactic cytokines that specifically act on neutrophils. To obtain insight into the extent of local production of CXC chemokines during acute pyelonephritis, interleukin (IL)-8, growth-related oncogene (GRO)- α , and epithelial cell-derived neutrophil-activating protein (ENA)-78 were measured in urine and plasma samples from patients with culture-proven urosepsis ($n = 33$), healthy human control subjects with sterile urine ($n = 31$), and human volunteers intravenously injected with endotoxin ($n = 11$). Patients had profoundly elevated urine concentrations of chemokines with no (GRO- α and ENA-78) or little (IL-8) elevation in plasma. Endotoxin-challenged subjects demonstrated transient increases in plasma chemokine concentrations, with no (GRO- α) or little (IL-8 and ENA-78) elevation in urine. Urine from patients exerted chemotactic activity toward neutrophils, which was partially inhibited by neutralizing antibodies against IL-8, GRO- α , or ENA-78. During urosepsis, CXC chemokines are predominantly produced within the urinary tract, where they are involved in the recruitment of neutrophils to the urinary compartment.

Chemokines are a family of small chemotactic proteins that play an important role in inflammatory responses as mediators of leukocyte trafficking and activation [1–4]. Depending on their structure, chemokines can be classified into several families. Interleukin (IL)-8, growth-related oncogene (GRO)- α , and epithelial cell-derived neutrophil-activating protein (ENA)-78 belong to the CXC chemokine family and act primarily on granulocytes, cells that often are found in the urine of patients with urinary tract infection (UTI).

The infected urinary tract is a frequent source of gram-negative sepsis [5, 6]. Urosepsis is usually diagnosed on the basis of a positive urine culture and of clinical findings associated with sepsis. Although increased urinary and serum IL-8 levels have been widely reported in UTI [7–9], little is known about the production of chemokines, other than IL-8, in this condition.

Accumulating evidence suggests that CXC chemokines play

an important role in neutrophil migration during UTI. In vitro studies have demonstrated that epithelial cells stimulated with pathogens can secrete chemokines and that IL-8 stimulates neutrophil migration across uroepithelial cell layers infected with *Escherichia coli* [10]. Recently, Hang et al. [11] showed that macrophage inflammatory protein (MIP)-2, a murine homologue of human IL-8, is required for neutrophil migration across the epithelium of the infected urinary tract in mice in vivo.

Patients with urosepsis, whose source of infection is located (by definition) in the urinary tract, potentially provide the opportunity for obtaining insight into the extent of chemokine production at the site of the infection, namely by measurements in urine. Therefore, in the present study, we sequentially measured plasma and urine concentrations of IL-8, GRO- α , and ENA-78 in patients with urosepsis during the first 8 h after the initiation of antibiotic treatment and compared these findings with chemokine plasma and urine concentrations in healthy humans intravenously (iv) injected with *E. coli* endotoxin (i.e., without a local inflammatory stimulus in the urinary tract). We also examined the migration of polymorphonuclear leukocytes (PMNL) induced by urine from patients with urosepsis and the extent to which each of these 3 chemokines (IL-8, GRO- α , and ENA-78) contributed to the chemotactic activity exerted by patient urine.

Materials and Methods

Patients with urosepsis and control subjects. Patients >18 years old who were suspected of having gram-negative urosepsis and for

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The study was approved by the institutional scientific and ethics committees. Written informed consent was obtained from all patients and healthy subjects.

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Table 1. Median (range) concentrations of interleukin (IL)-8, growth-related oncogene (GRO)- α and epithelial cell-derived neutrophil-activating protein (ENA)-78 in urine from healthy control subjects ($n = 16$), patients with urosepsis ($n = 33$) and healthy subjects injected with endotoxin ($n = 11$).

Cytokine	Healthy control subjects	Patients with urosepsis				Experimental endotoxemia		
		Admission	2 Hours	4 Hours	8 Hours	0 Hours	0–3 Hours	3–6 Hours
IL-8	<0.01 (<0.01–0.14)	0.38 (<0.01–3.13) ^a	0.43 (<0.01–2.00)	0.20 (<0.01–2.06)	0.15 (0.03–1.60)	<0.01 (<0.01–0.03)	<0.01 (<0.01–0.01)	0.01 (<0.01–0.03)
GRO- α	<0.03 (<0.03–0.29)	0.10 (<0.03–0.51) ^a	0.10 (<0.03–1.14)	0.10 (<0.03–1.11)	0.03 (<0.03–0.45)	0.17 (<0.03–1.18)	<0.03 (<0.03–0.15)	0.07 (<0.03–0.62)
ENA-78	<0.03	0.27 (<0.03–1.66) ^a	0.24 (<0.03–1.93)	0.26 (<0.03–2.86)	0.25 (<0.03–3.08)	<0.03	<0.03 (<0.03–0.11)	<0.03

NOTE. Concentrations are expressed in ng/mL.

^a $P < .05$ versus healthy control subjects. Only IL-8 decreased significantly in urine during follow-up.

whom antibiotic treatment was indicated were eligible if they also met the following criteria: acute symptoms of UTI, pyuria (>10 leukocytes/high-power field [hpf] and <5 epithelial cells/hpf), urine Gram's stain with gram-negative bacteria, and metabolic or hematologic signs of infection (including 3 of the following indicators: tachycardia [>90 beats/min], leukocytosis [$>10,000/\text{mm}^3$], and fever [$>38^\circ\text{C}$]). Exclusion criteria were antibiotic use in the previous 7 days, poor clinical condition, known hypersensitivity to any β -lactam antibiotic, any major underlying disease likely to alter baseline chemokine levels, renal insufficiency (estimated creatinine clearance, <30 mL/min), pregnancy or breast feeding, use of systemic corticosteroids or other immunosuppressive agents in the past 3 months, history of seizures, use of any investigational drug within the past 30 days, or any clinically significant medical condition that would pose a risk to the patient should he or she participate.

Clinical data were collected, and the APACHE II score was assessed before the start of treatment (0 h). Patients received iv 1 dose of ceftazidime (1000 mg) or imipenem (500 mg), followed 8 h later by an antibiotic chosen by the clinician. Blood (heparinized) and urine samples were collected before the start of treatment and at 2, 4, and 8 h thereafter. Plasma (heparinized) and urine samples were also collected from 31 healthy individuals, all of whom had sterile urine. The samples were centrifuged at 1500 g for 20 min. Supernatants were collected and stored at -75°C until assays were performed.

Experimental endotoxemia. In addition to the patients with urosepsis, 11 healthy subjects (mean age \pm SE, 24 ± 1 years) were studied after iv administration of endotoxin (lipopolysaccharide, LPS). The subjects did not smoke, use any medication, or have a febrile illness in the month preceding the study. They were admitted to the clinical research unit at the Academic Medical Center (University of Amsterdam, Amsterdam) after their medical history, physical examination, hematologic and biochemical tests, chest radiograph, and electrocardiograph proved to be normal. Endotoxin (LPS standard lot G from *E. coli*; United States Pharmacopeia Convention, Rockville, MD) was given for 1 min in an antecubital vein at a dose of 4 ng/kg body weight. Blood was collected by venipunctures directly before LPS administration and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, and 24 h thereafter. EDTA plasma was obtained by centrifugation at 1500 g for 20 min. All urine produced by the subjects was collected before and 3 and 6 h after LPS administration.

Assays. Chemokine concentrations were measured by ELISA.

All measurements of 1 chemokine in patient and control samples were done on 1 day in 1 ELISA run. IL-8 was measured according to the instructions of the manufacturer (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service [CLB], Amsterdam). For determination of GRO- α levels, purified monoclonal mouse anti-human GRO- α (4 $\mu\text{g}/\text{mL}$; R&D Systems, Abingdon, UK) was used as a coating antibody, biotinylated affinity-purified goat IgG anti-human GRO- α (20 ng/mL; R&D Systems) was used as a detecting antibody, and recombinant human GRO- α (R&D Systems) was used as the standard. For determination of ENA-78, monoclonal mouse anti-human ENA-78 (1 $\mu\text{g}/\text{mL}$; R&D Systems) was used as a coating antibody, biotinylated goat anti-human ENA-78 (80 ng/mL; R&D Systems) was used as a detecting antibody, and recombinant human ENA-78 (R&D Systems) was used as the standard.

Detection limits in plasma and urine diluted 1:2 with ELISA high-performance buffer (CLB) were 1.2 pg/mL (IL-8) and 28.6 pg/mL (GRO- α and ENA-78). Urine concentrations of chemokines are expressed both in nanograms per milliliter urine (table 1) and, to correct for dilution of urine, per micromole creatinine (figures 1–4).

Chemotaxis assay. The migration of PMNL was measured by use of a multiwell chemotaxis chamber (Neuro Probe, Cabin John, MD) and a 5.0 μm -pore polycarbonate membrane (Nuclepore; Costar, Cambridge, MA), as described elsewhere [12]. In brief, PMNL were obtained from heparinized blood samples of healthy volunteers, using Polymorphprep (Nycomed Pharma, Oslo), according to the manufacturer's instructions, and were suspended in endotoxin-free RPMI 1640 (BioWittaker, Verviers, Belgium) containing 5% normal human serum (BioWittaker).

Urine samples from 8 patients (those with the highest leukocyte counts in urine) were pooled and diluted 1:2 in RPMI 1640, with or without the following reagents: monoclonal mouse-derived anti-human IL-8 neutralizing antibody (R&D Systems; final concentration 10 $\mu\text{g}/\text{mL}$), monoclonal mouse-derived anti-human GRO- α neutralizing antibody (R&D Systems; final concentration, 10 $\mu\text{g}/\text{mL}$), monoclonal mouse-derived anti-human ENA-78 neutralizing antibody (R&D Systems; final concentration, 50 $\mu\text{g}/\text{mL}$), or mouse IgG1 (R&D Systems; final concentration, 50 $\mu\text{g}/\text{mL}$). During in vitro cell stimulation, these concentrations of anti-chemokine antibodies completely neutralize activity of recombinant IL-8, GRO- α , and ENA-78 when added at 2–3-log higher concentrations than those detected in urine from patients with urosepsis (information

on the neutralizing capacities of the antibodies provided by the manufacturer). The respective antibodies used do not have cross-reactivity with other chemokines (information provided by the manufacturer).

Urine from 16 healthy control subjects was also diluted 1:2 in RPMI 1640, to serve as a negative control, and, in combination with recombinant human IL-8 (rhIL-8; R&D Systems; final concentration 5 ng/mL), it served as a positive control. Urine specimens (25- μ L volumes) were placed in the lower chamber in triplicate. Volumes of 50 μ L containing 4.0×10^4 PMNL were added to the upper well. The chamber was incubated for 55 min in an humidified environment at 37°C. The membrane was removed, nonmigrated cells were washed off, and the membrane was fixed in methanol and stained with DiffQuik (Dade Behring, Duding, Switzerland). Migrated cells in each well were counted at 1000 \times magnification for 5 random fields.

Statistical analysis. Log-transformed concentrations of chemokines were analyzed in time by 1-way analysis of variance (ANOVA) and followed (for uroseptic patients and healthy subjects given endotoxin) by Dunnett's test. The difference between the 2 antibiotic regimens in chemokine concentrations was assessed by use of the repeated-measures ANOVA. The Mann-Whitney *U* test was used to compare chemokine concentrations in urosepsis patients (at 0 h) with those in healthy control subjects and concentrations in patients with positive blood culture results with those in patients with negative results. Correlations between chemokine concentrations and duration of symptoms were assessed by calculating Spearman's correlation coefficient. Comparisons of numbers of migrated cells in the chemotaxis assay between patients with urosepsis and healthy control subjects were done by using the Mann-Whitney *U* test. The effect of neutralizing antibodies on the migration of cells in urine from patients with urosepsis was assessed by use of the Wilcoxon signed rank test. $P < .05$ was considered significant.

Results

Healthy subjects. IL-8 was detectable in urine (median, <2.0 ng/mmol; range, <2.0–8.5 ng/mmol) but not in plasma from healthy individuals. Median GRO- α levels in plasma were 0.20 ng/mL (range, <0.03–0.45 ng/mL) and in urine they were 2.7 ng/mmol (range, <2.0–48.3 ng/mmol). ENA-78 concentrations were detectable in plasma (median, 0.03 ng/mL; range, <0.03–0.41 ng/mL) but not in urine.

Patients with urosepsis. Thirty-three of 35 patients (mean age \pm SE, 42 ± 3 years) who had been randomized to treatment with imipenem or ceftazidime completed the study. The duration of symptoms before the first urine and plasma samples were taken was 2.9 ± 0.3 (mean \pm SE) days. All patients had systemic symptoms, and there was no correlation between the duration of symptoms before inclusion in the study and chemokine levels at admission. *E. coli* was cultured from the urine of all but 1 patient. Ten patients had bacterium-positive blood culture results (1 for *Proteus mirabilis* and 9 for *E. coli*). At admission, the median APACHE II score was 6 (range, 0–17).

All patients fully recovered after treatment. The type of antibiotic regimen did not significantly influence chemokine levels in urine or plasma (data not shown); therefore, these 2 patient groups were combined in further analyses.

Compared with control subjects, patients with urosepsis had higher levels of IL-8 in plasma (median, 0.03 ng/mL; range, 0.01–0.65 ng/mL) and urine (median, 60.0 ng/mmol; range, <2.0–1237.0 ng/mmol; $P < .001$ for both) at admission (figure 1). In addition, compared with healthy control subjects, patients with positive and negative blood cultures had higher plasma concentrations ($P < .001$ for both) and urine concentrations

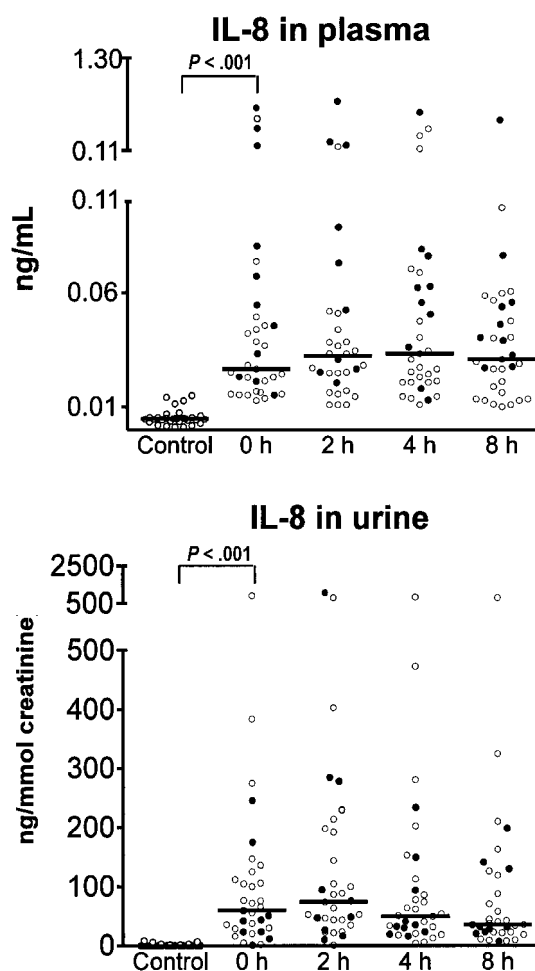


Figure 1. Plasma and urine levels of interleukin (IL)-8 in healthy subjects ($n = 31$) and patients with urosepsis ($n = 33$) at admission and 2, 4, and 8 h after initiation of antibiotic therapy. Horizontal lines represent medians. ●, Patients with positive blood culture results (determined on admission). ○, Patients with negative blood culture results and healthy control subjects. Patients at admission had higher plasma and urine levels than did control subjects (both $P < .001$). In patients, urine, but not plasma, IL-8 concentrations decreased after admission ($t = 0$) and during follow-up ($P < .05$ vs. $t = 0$). Because of the low detection limit of the assay, a line indicating the limit is not visible.

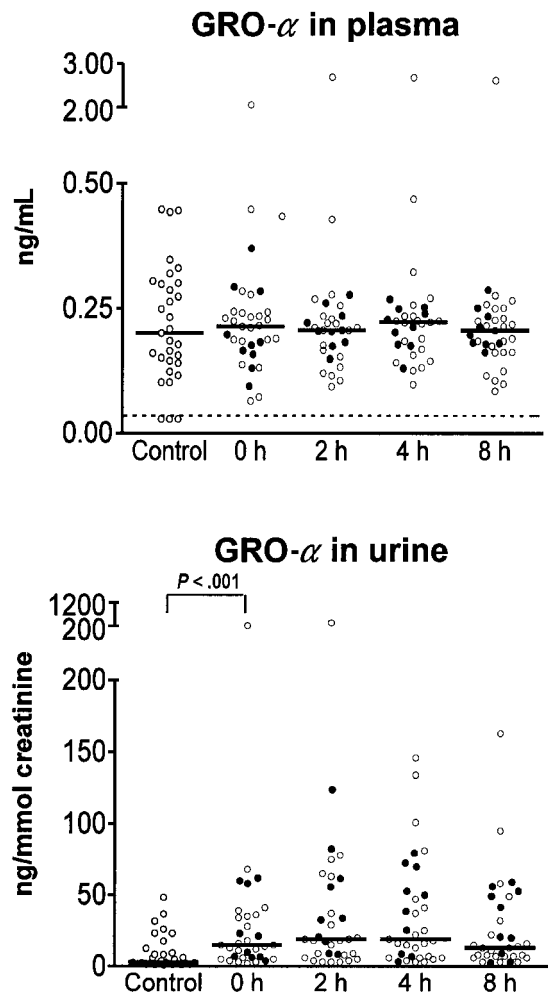


Figure 2. Plasma and urine levels of growth-related oncogene (GRO)- α in healthy subjects ($n = 31$) and in patients with urosepsis ($n = 33$) at admission and 2, 4, and 8 h after initiation of antibiotic therapy. Horizontal lines represent medians. ●, Patients with positive blood cultures (determined at admission). ○, Patients with negative blood culture results and healthy control subjects. Urine, but not plasma, levels were higher in patients at admission than in control subjects ($P < .001$). In patients, urine and plasma concentrations did not change significantly during follow-up. Dotted line depicts the detection limit of the assay.

($P < .001$ for those with positive blood cultures and $P = .005$ for those with negative blood cultures) of IL-8. Furthermore, plasma but not urine IL-8 levels were higher in patients with positive blood cultures than in patients with negative blood cultures ($P < .05$; table 2). GRO- α was elevated only in urine (median, 15.0 ng/mmol; range, <2.0–217.0 ng/mmol; $P < .001$ vs. healthy control subjects; figure 2). This increase was significant in patients with positive and negative blood culture results (both $P < .001$).

Only patients with positive blood culture results had elevated plasma levels of ENA-78 (median, 0.32 ng/mmol; range,

<0.03–6.57 ng/mmol; $P < .005$ vs. healthy control subjects; figure 3). Urine ENA-78 levels were significantly elevated in patients with positive and those with negative blood culture results ($P < .001$ for both) and were higher in patients with positive blood culture results than in patients whose blood cultures were negative ($P < .005$; table 2). Of all 3 chemokines measured in plasma and urine, only urine levels of IL-8 decreased significantly during follow-up ($P < .05$; figure 1). Urine concentrations of chemokines expressed per milliliter of urine are given in table 1.

Endotoxemia in healthy humans. The iv injection of *E. coli*

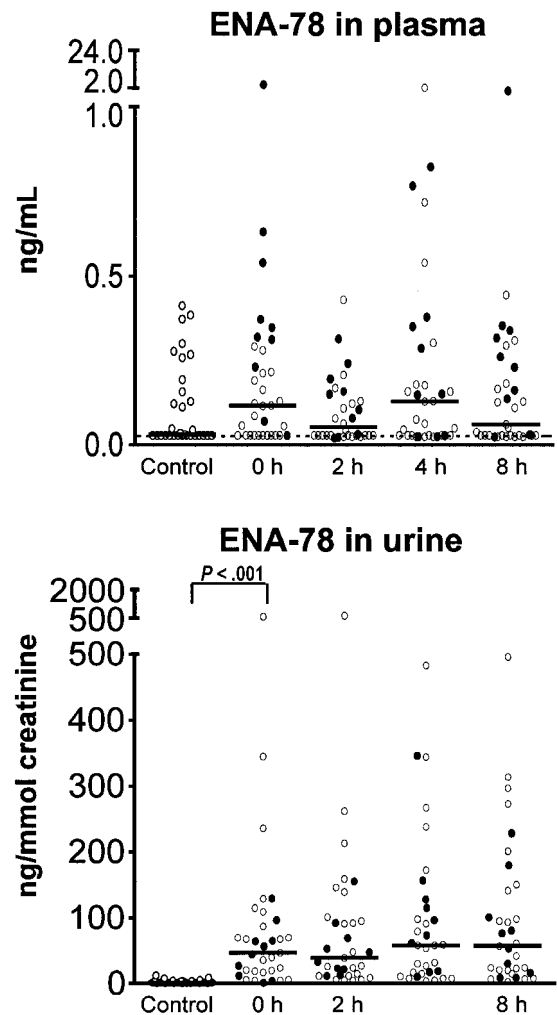


Figure 3. Plasma and urine levels of epithelial cell-derived neutrophil-activating protein (ENA)-78 in healthy subjects ($n = 31$) and patients with urosepsis ($n = 33$) at admission and 2, 4, and 8 h after initiation of antibiotic therapy. Horizontal lines represent medians. ●, Patients with positive blood culture results (determined on admission). ○, Patients with negative blood culture results and healthy control subjects. Urine, but not plasma, levels were higher in patients at admission than in control subjects ($P < .001$). In patients, urine and plasma concentrations of ENA-78 did not change significantly during follow-up. Dotted line (*upper panel*) depicts the detection limit of the assay.

Table 2. Median concentrations (range) of interleukin (IL)-8, growth-related oncogene (GRO)- α and epithelial cell-derived neutrophil-activating protein (ENA)-78 in plasma and urine of patients with urosepsis with both positive and negative blood cultures.

Cytokine	Positive blood culture (n = 10)	Negative blood culture (n = 23)	P
IL-8			
Plasma, ng/mL	0.06 (0.02-0.51)	0.02 (0.01-0.65)	<.05
Urine, ng/mmol	37.0 (<2.0-246.0)	76.0 (<2.0-1237.0)	NS
GRO- α			
Plasma, ng/mL	0.19 (0.09-0.37)	0.23 (0.07-2.05)	NS
Urine, ng/mmol	14.0 (<2.0-68.0)	15.0 (<2.0-217.0)	NS
ENA-78			
Plasma, ng/mL	0.32 (<0.03-6.57)	0.07 (<0.03-0.32)	<.005
Urine, ng/mmol	54.0 (<2.0-129.0)	47.0 (<2.0-1029.0)	NS

NOTE. Urine concentrations are expressed per mmol creatinine. NS, not significant

LPS was associated with transient increases in the plasma concentrations of all 3 chemokines measured. In addition, urine IL-8 and ENA-78 concentrations modestly increased after iv administration of LPS (figure 4). LPS induced an increase in plasma IL-8 levels, starting at 1.5 h and peaking at 3 h after administration (mean \pm SE, 1.79 \pm 0.16 ng/mL; P < .001). Urine IL-8 increased between 3 and 6 h after LPS administration (mean \pm SE, 0.7 \pm 0.2 ng/mmol; P < .005). Plasma levels of GRO- α increased significantly within 3 h after LPS (mean \pm SE, 0.47 \pm 0.05 pg/mL; P < .005). Urine GRO- α did not change significantly after LPS. Plasma levels of ENA-78 increased from 3 h onwards, reaching peak concentrations at 4 h (mean \pm SE, 0.45 \pm 0.05 ng/mL; P < .001). Urine concentrations of ENA-78 increased to 8.3 \pm 1.6 ng/mmol (P < .001) within 3 h after LPS administration. No leukocytes were found in urine from any of the subjects injected with LPS at any time

point. Urine chemokine concentrations expressed in ng/mL urine are given in table 1.

Chemotactic activity of urine from patients with urosepsis. Urine from healthy control subjects attracted few PMNL (mean \pm SE, 0.2 \pm 0.1 cells/hpf). Significantly more PMNL migrated toward pooled urine from patients with urosepsis (24.8 \pm 3.0 cells/hpf; P < .005 vs. normal urine). Addition of either anti-IL-8, anti-GRO- α , or anti-ENA-78 to urine from patients with urosepsis significantly reduced chemotactic activity of this urine on neutrophils (all P < .05 vs. urine from patients with or without control antibody; figure 5). None of the anti-chemokine antibodies completely neutralized the chemotactic activity of urine from patients. In fact, anti-IL-8, anti-GRO- α , and anti-ENA-78 antibody treatment inhibited neutrophil chemotaxis toward patient urine to a similar extent (to ~60% of migration in the absence of anti-chemokine antibodies). Addition of all 3 anti-chemokine antibodies together did not further reduce urine chemotactic activity, compared with either antibody alone (data not shown).

Discussion

IL-8, GRO- α , and ENA-78 are members of the CXC chemokine family, in which the first 2 cysteine residues are separated by a single amino acid. They primarily target neutrophils, cells that are found in urine from patients with UTI. The aim of this study was to obtain insight into the extent of local production of these chemokines in patients with urosepsis and into the contribution of these mediators to the chemotactic activity toward neutrophils of urine from patients with urosepsis.

Urine IL-8, GRO- α , and ENA-78 concentrations were

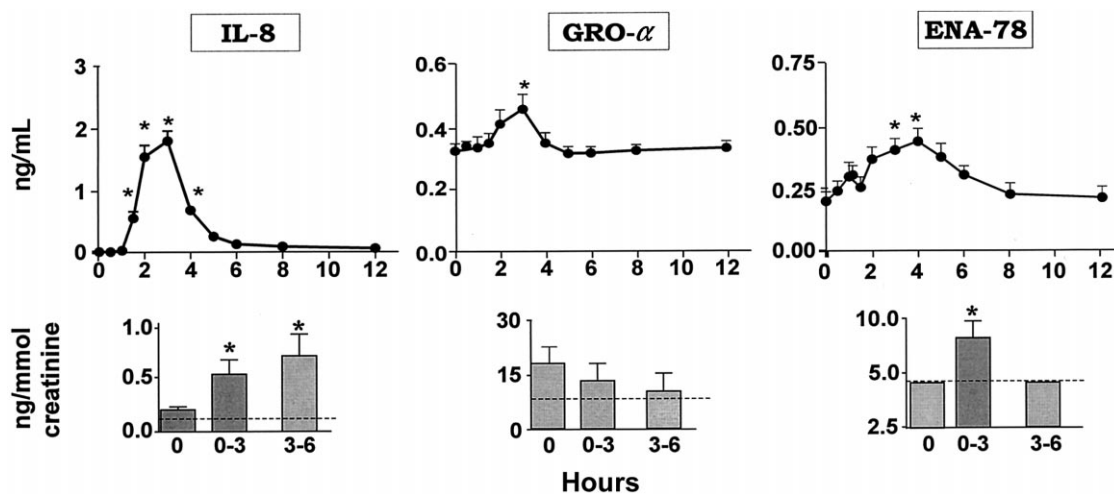


Figure 4. Mean \pm SE plasma and urine concentrations of interleukin (IL)-8, growth-related oncogene (GRO)- α , and epithelial cell-derived neutrophil-activating protein (ENA)-78 in healthy subjects (n = 11) after intravenous administration of endotoxin. Plasma concentrations were measured before endotoxin injection (t = 0) and during a 12-h follow-up. Urine concentrations were measured before endotoxin injection and in all urine excreted within 3 h after infusion of endotoxin (t = 0-3) and between 3 and 6 h after endotoxin administration (t = 3-6). *Significant difference from the levels at t = 0. Dotted lines depict the detection limit of the assay.

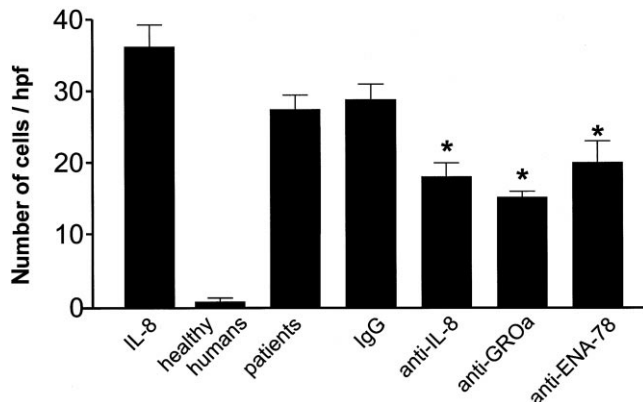


Figure 5. Mean \pm SE number of polymorphonuclear lymphocytes (PMNL) per high-power field (hpf) that migrated toward urine (from healthy control subjects) containing 5 ng/mL recombinant human interleukin (IL)-8 ($n = 16$), urine from healthy control subjects alone ($n = 16$), and urine from patients with urosepsis with pyuria ($n = 8$). Neutralization of IL-8, growth-related oncogene (GRO)- α or epithelial cell-derived neutrophil-activating protein (ENA)-78 in urine from patients with urosepsis resulted in attenuation of this migration. *Significant difference in migration of PMNL toward patient urine either with or without control antibody ($P < .05$).

strongly elevated in patients but not (or less elevated) in healthy subjects after iv injection of *E. coli* LPS. In vitro, IL-8, GRO- α , and ENA-78 contributed to the chemotactic activity of infected urine toward neutrophils. These data suggest that CXC chemokines are produced within the urinary tract during urosepsis and that they are involved in the recruitment of neutrophils to the urinary compartment.

Several studies have documented elevated IL-8 concentrations in urine of patients with UTI [7–9]. In patients with acute pyelonephritis, IL-8 levels are often higher in urine than in plasma [8]. In addition, deliberate colonization of the human urinary tract with *E. coli* resulted in a rapid increase in urine IL-8 levels without a detectable rise in IL-8 in serum [13]. Together, these data suggest that IL-8 is produced locally at the site of the infection during UTI. In accordance, we found high IL-8 concentrations in the urine of patients with urosepsis caused by *E. coli* but not in volunteers injected iv with *E. coli* LPS. In this latter experiment, IL-8 concentrations were especially elevated in plasma, with little elevation (conceivably through renal clearance) in urine. Cell types that can produce IL-8 within the urinary tract include epithelial, mesangial, and endothelial cells, and renal fibroblasts [14]. To our knowledge, this study is the first to document that, besides IL-8, 2 other CXC chemokines (i.e., GRO- α and ENA-78) are released in urine during acute pyelonephritis. Of interest, GRO- α and ENA-78 concentrations were elevated in urine but not in plasma of patients with urosepsis; however, plasma levels but not urine levels of GRO- α and ENA-78 increased to a signif-

icant extent after iv injection of LPS. Hence, it is likely that GRO- α and ENA-78 also are produced at the site of the infection during acute pyelonephritis.

In a previous investigation, Ko et al. [8] reported neutrophil chemotactic activity of urine specimens from patients with UTI, whereas no such activity was found in urine from normal subjects. Adsorption of infected urine onto an anti-IL-8 column reduced the chemotactic activity by ~55% [8]. Using a slightly different approach (i.e., direct addition of anti-chemokine antibodies to urine samples), we found that anti-IL-8 inhibited the neutrophil chemotactic activity of urine from patients with urosepsis to a similar extent. Furthermore, we extend these findings by showing that anti-GRO- α and anti-ENA-78 treatments also inhibit the chemotactic activity of infected urine toward neutrophils. Concurrent addition of anti-IL-8, anti-GRO- α , and anti-ENA-78 did not reduce the chemotactic activity of urine any more than did the addition of either antibody alone, indicating that other factors present in urine, such as LPS, contribute significantly to the chemotactic potential of infected urine. In this respect, it should be noted that GRO- α and ENA-78 preferentially interact with CXCR2 (also known as IL-8 receptor type B) on neutrophils, whereas IL-8 can bind both CXCR1 (IL-8 receptor type A) and CXCR2 with high affinity [1]. Both CXCR1 and CXCR2 can mediate chemotaxis of neutrophils [15–18]. IL-8 induces neutrophil chemotaxis predominantly via CXCR1, but other CXC chemokines, including GRO- α and ENA-78, can stimulate this inflammatory response via CXCR2 [15, 19].

Our data differ from in vitro studies reported by Godaly et al. [10], who found that an anti-CXCR1 antibody, but not an anti-CXCR2 antibody, reduced *E. coli*-induced transuroepithelial neutrophil migration in vitro [10]. Apparently, in their in vitro system, CXC chemokines that preferentially interact with CXCR2 (such as GRO- α and ENA-78) are not involved in neutrophil migration. Whether CXCR2 ligands were produced in their system was not determined. Further studies are warranted to establish the respective roles of CXCR1 and CXCR2 in neutrophil recruitment to urine.

Relatively little is known about the kinetics of CXC chemokine release during infection. Of interest, injection of LPS was associated with a brisk and transient increase in the plasma concentrations of IL-8 and, to a lesser extent, of GRO- α , whereas ENA-78 increased and dissipated more gradually. Differences in the rates of production and clearance of different chemokines may obviously influence the timing of their respective effects during the innate immune response to an infection. Further research is warranted to evaluate this issue.

Chemokines play a central role in host defense against infectious diseases. Here, we report elevated levels of the CXC chemokines IL-8, GRO- α , and ENA-78 in the urine of patients with urosepsis. CXC chemokines likely are produced primarily

in the urinary tract during urosepsis, where they are involved in the attraction of neutrophils to the urinary compartment.

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