



## UvA-DARE (Digital Academic Repository)

### CXC chemokine receptor 2 contributes to host defense in murine urinary tract infection

Olszyna, D.P.; Florquin, S.; Sewnath, M.E.; Branger, J.; Speelman, P.; van Deventer, S.J.H.; Strieter, R.M.; van der Poll, T.

**DOI**

[10.1086/322030](https://doi.org/10.1086/322030)

**Publication date**

2001

**Published in**

The Journal of Infectious Diseases

[Link to publication](#)

**Citation for published version (APA):**

Olszyna, D. P., Florquin, S., Sewnath, M. E., Branger, J., Speelman, P., van Deventer, S. J. H., Strieter, R. M., & van der Poll, T. (2001). CXC chemokine receptor 2 contributes to host defense in murine urinary tract infection. *The Journal of Infectious Diseases*, *184*, 301-307. <https://doi.org/10.1086/322030>

**General rights**

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

**Disclaimer/Complaints regulations**

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

*UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)*

## CXC Chemokine Receptor 2 Contributes to Host Defense in Murine Urinary Tract Infection

Dariusz P. Olszyna,<sup>1,2</sup> Sandrine Florquin,<sup>3</sup>  
Miguel Sewnath,<sup>1,4</sup> Judith Branger,<sup>1,2</sup> Peter Speelman,<sup>1</sup>  
Sander J. H. van Deventer,<sup>1</sup> Robert M. Strieter,<sup>5</sup>  
and Tom van der Poll<sup>1,2</sup>

<sup>1</sup>Department of Experimental Internal Medicine, <sup>2</sup>Department of Internal Medicine, Division of Infectious Diseases, Tropical Medicine, and AIDS, and Departments of <sup>3</sup>Pathology and <sup>4</sup>Surgery, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; <sup>5</sup>Department of Medicine, Division of Pulmonary and Critical Care Medicine, University of California, Los Angeles

**CXC chemokines have been implicated in the recruitment of neutrophils to sites of infection. To determine the role of CXC chemokines in the host response to urinary tract infection (UTI), female mice were treated with an antibody against the major CXC chemokine receptor in the mouse, CXCR2, before intravesical inoculation with *Escherichia coli*. Anti-CXCR2 prevented the influx of neutrophils in urine and kidneys. The absence of a neutrophil response only temporarily impaired the clearance of bacteria from the urinary tract, as indicated by 100- and 1000-fold more *E. coli* colony-forming units in urine and kidneys of anti-CXCR2-treated mice at 24 h, but not at 48 h, after the infection. UTI induced increases in the renal concentrations of the CXCR2 ligands macrophage inflammatory protein-2 and KC, which were not influenced by anti-CXCR2 administration. CXC chemokines play an important role in the development of a local inflammatory response to UTI.**

Urinary tract infections (UTIs) are responsible for as many as 8 million visits to doctors a year in the United States alone [1]. They comprise entities such as pyelonephritis, cystitis, and asymptomatic bacteruria. *Escherichia coli* is the most common pathogen, causing 85% of community-acquired UTIs [2] and 50% of UTIs in hospitalized patients [3]. Furthermore, the urinary tract is identified as the most common source of bacteremia [4–6].

Chemokines are a group of small chemotactic proteins. They play an important role in the host response to bacterial infections by attracting leukocytes to the infection site [7, 8]. CXC chemokines represent a subfamily of chemokines, which can be further divided into 2 subclasses on the basis of the presence or absence of a 3-amino acid motif termed “ELR” (glutamic acid–leucine–arginine). ELR-positive CXC chemokines are chemotactic for neutrophils and include interleukin (IL)–8, epithelial cell–derived neutrophil-activating protein (ENA)–78, growth-related oncogene (GRO)– $\alpha$ , – $\beta$ , and – $\gamma$  in humans, and KC and macrophage inflammatory protein (MIP)–2 in mice [9].

UTI is associated with an influx of neutrophils in the urinary tract. Several lines of evidence indicate that ELR-positive CXC chemokines are involved in this characteristic inflammatory re-

sponse. First, high concentrations of IL-8, ENA-78, and GRO- $\alpha$  were detected in the urine of patients with UTI [10–13]. Second, infected urine was chemotactic for neutrophils, and its chemotactic activity could be inhibited by neutralizing antibodies against IL-8, ENA-78, or GRO- $\alpha$  [11, 13]. Third, anti-MIP-2 antibody treatment of mice with experimental UTI resulted in a reduction of neutrophil numbers in urine [14].

In humans, 2 receptors for ELR-positive CXC chemokines have been identified on the surface of granulocytes, the CXC chemokine receptors 1 and 2 (CXCR1 and CXCR2) [15, 16]. CXCR2 is a promiscuous chemokine receptor, which binds to all ELR-positive CXC chemokines [17]. Mice lack CXCR1, and CXCR2 exclusively mediates granulocyte responses to ELR-positive CXC chemokines in this species [18]. In the present study, we sought to determine the importance of CXCR2 in neutrophil recruitment and antibacterial host defense in a murine model of ascending UTI with *E. coli*.

### Materials and Methods

**Mice.** Female BALB/c mice were obtained from Harlan Sprague Dawley; 10-week-old mice were used in all experiments.

**Induction of UTI.** *E. coli* 1677, isolated from a patient with an acute febrile UTI, was donated by W. J. Hopkins (University of Wisconsin Medical School, Madison). This strain was used previously to induce pyelonephritis in mice and has virulence characteristics that include type 1 and P fimbriae, hemolysin, aerobactin, and the O6 serotype [19–21]. Bacteria were cultured for 12 h at 37°C in 5% CO<sub>2</sub> in trypticase soy broth. This suspension was diluted 1:100 in fresh medium and was grown for 4 h to mid-logarithmic phase. *E. coli* were harvested by centrifugation at 1500 g for 10 min and were washed 3 times in sterile 0.9% saline. The

Received 28 February 2001; revised 11 April 2001; electronically published 27 June 2001.

The study was approved by the animal use and welfare committee of the Academic Medical Center.

Financial support: Dutch Kidney Foundation (to D.P.O.).

Reprints or correspondence: Dr. Tom van der Poll, Academic Medical Center, Rm. G2-130, Dept. of Experimental Internal Medicine, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands (t.vanderpoll@amc.uva.nl).

The Journal of Infectious Diseases 2001;184:301–7

© 2001 by the Infectious Diseases Society of America. All rights reserved.  
0022-1899/2001/18403-0007\$02.00

bacteria were resuspended in saline at a concentration of  $1 \times 10^{10}$  cfu/mL, as determined by plating 10-fold serial dilutions of the suspensions on blood agar plates. To confirm that none of the mice had UTI before the experiments, urine was collected by applying pressure in the abdominal area and then was cultured on blood agar plates. For inoculation with *E. coli*, mice were anesthetized by administration of FFM (0.079 mg/mL fentanyl citrate, 2.5 mg/mL fluanisone, and 1.25 mg/mL midazolam in water); 7.0 mL/kg of this mixture was administered intraperitoneally. To minimize reflux of the inoculum, the bladder was emptied before infection by applying pressure to the abdominal area.

For instillation of the bacteria, a radiopaque catheter with a diameter of 0.55 mm (Abbott B.V.) was introduced into the bladder, and 100  $\mu$ L of bacterial suspension ( $1 \times 10^9$  cfu) was inoculated. This inoculum size has been used previously by other investigators examining host defense mechanisms during murine UTI [14, 20]. It should be noted that this volume likely induced vesicoureteral reflux in most of the mice [20]. Two hours before inoculation, mice received either goat anti-mouse CXCR2 antiserum [22–24] or normal goat serum (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service), both administered intraperitoneally in a volume of 500  $\mu$ L. Antiserum directed against murine CXCR2 was produced by immunizing goats with a peptide containing the ligand-binding sequence Met-Gly-Glu-Phe-Lys-Val-Asp-Lys-Phe-Asn-Ile-Glu-Asp-Phe-Phe-Ser-Gly. This protein sequence has been shown to contain the ligand-binding portion of the CXCR2 receptor [25] and has been used previously to block mouse CXCR2 in vivo [22–24].

**Quantification of leukocyturia and histologic examination.** Neutrophils were quantified in urine with a hemocytometer chamber (Emergo). Cytospin preparations stained with a modified Giemsa stain (Diff-Quick; Baxter) revealed that >99% of cells in the urine were neutrophils. Mice were killed and kidneys were removed at 6, 24, and 48 h after infection. Kidneys were fixed in 10% formalin, and were embedded in paraffin. Four-micron sections were stained with hematoxylin-eosin and were analyzed by a pathologist who was blinded for groups. The degree of renal damage (necrosis and inflammation) over the total area of each section was graded on a semiquantitative scale of 0 to 5+ [19]. For granulocyte staining, slides were deparaffinized and rehydrated. Slides were then digested by a solution of pepsin 0.25% (Sigma Chemical) in 0.01 M HCl. After being rinsed, the sections were incubated in 10% normal goat serum (Dako) and then were exposed to fluorescein isothiocyanate (FITC)-labeled anti-mouse Ly-6-G monoclonal antibody (Pharmingen). Endogenous peroxidase activity was quenched by a solution of 0.1% NaN<sub>3</sub>/0.03% H<sub>2</sub>O<sub>2</sub> (Merck). After washes, the slides were incubated with a rabbit anti-FITC antibody (Dako), then were further incubated with a biotinylated swine antirabbit antibody (Dako), were rinsed again, were incubated in a streptavidin-avidin-biotin complex solution (Dako), and were developed with 1% H<sub>2</sub>O<sub>2</sub> and 3,3'-diaminobenzidine-tetra-hydrochloride (Sigma) in Tris-HCl. The sections were mounted in glycerin gelatin without counterstaining and were analyzed.

**Determination of bacterial outgrowth.** At 6, 24, and 48 h after infection, urine was collected as described above. The mice were anesthetized by FFM and were killed by cervical dislocation. Blood was collected in tubes containing EDTA. The left kidney and part of the right kidney from each mouse were homogenized at 4°C in 4 volumes of sterile saline with a tissue homogenizer (Biospec Prod-

ucts), which was carefully cleaned and disinfected with 70% alcohol after each homogenization. Serial 10-fold dilutions were made in sterile saline, and 15- $\mu$ L volumes of urine, blood, and homogenized kidneys were plated onto blood agar plates, which were incubated at 37°C at 5% CO<sub>2</sub>. Colony-forming units were counted after 16 h. The remaining part of the right kidney was used for histologic examination.

**Preparation of kidney tissue for chemokine measurements.** Kidney homogenates were diluted 1:2 in lysis buffer containing 300 mM NaCl, 30 mM Tris, 2 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, 1% Triton X-100, and pepstatin A, leupeptin, and aprotinin (all 20 ng/mL; pH 7.4) and were incubated at 4°C for 30 min. Homogenates were centrifuged at 1500 g at 4°C for 15 min, and supernatants were stored at -20°C until assays were performed.

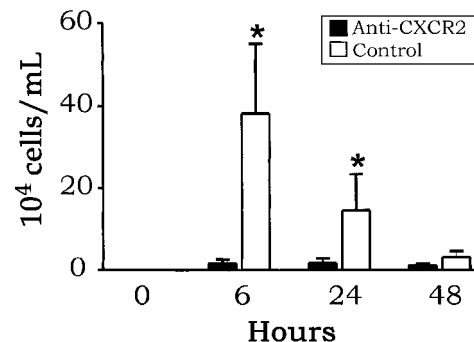
**Assays.** MIP-2 and KC levels were measured by ELISA, according to the instructions of the manufacturer. All reagents were obtained from R&D Systems. Detection limits were 20 pg/mL (MIP-2) and 25 pg/mL (KC).

**Statistical analysis.** Data are expressed as mean  $\pm$  SE, unless stated otherwise. Differences between groups were analyzed by the Mann-Whitney *U* test. The presence of leukocytes in urine was analyzed in time by 1-way analysis of variance, then by Dunnett's test. *P* < .05 was considered to be a statistically significant difference.

## Results

### Defective neutrophil recruitment in anti-CXCR2-treated mice.

None of the mice had leukocyturia before the experiment. To determine the role of CXCR2 in the recruitment of neutrophils to the urinary tract during UTI, mice pretreated with either anti-CXCR2 or preimmune serum were inoculated intravesically with *E. coli* via the urethra. In control mice, UTI with *E. coli* resulted in a transient increase in the number of granulocytes in urine, peaking at 6 h ( $38 \pm 17 \times 10^4$  cells/mL; *P* < .05 vs. baseline; figure 1). Thereafter, neutrophil numbers decreased, and very few neutrophils remained in the urine 48 h after infection. In contrast, mice treated with anti-CXCR2 dem-



**Figure 1.** Influx of neutrophils to urine inhibited by anti-CXCR2 treatment during experimental urinary tract infection (UTI). UTI was induced by intravesical administration of *Escherichia coli* at time 0; 2 h before inoculation, mice received an intraperitoneal injection of either anti-mouse CXCR2 antiserum (solid bars) or preimmune serum (open bars). Data are mean  $\pm$  SE. \* $\alpha$  vs. anti-CXCR2.

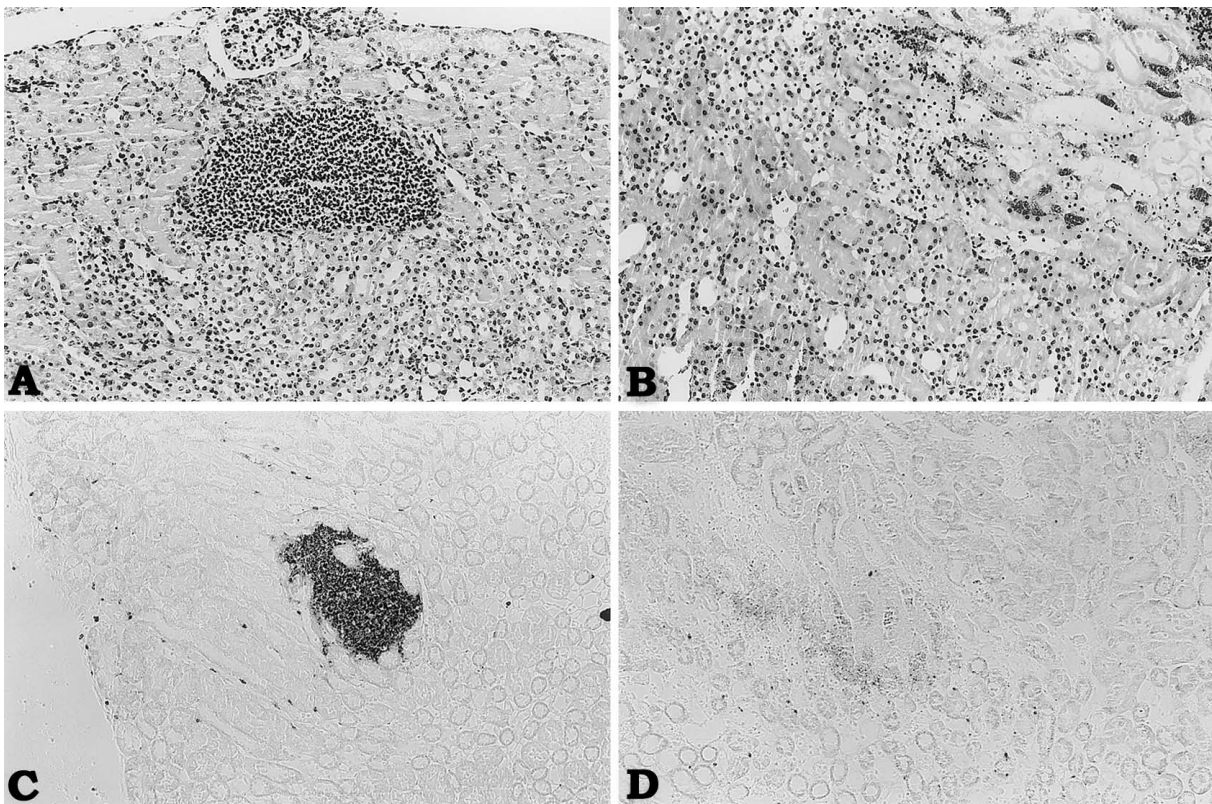
onstrated no influx of neutrophils in urine at any time after intravesical inoculation with *E. coli* ( $P < .05$ , vs. mice treated with preimmune serum at 6 and 24 h).

Histopathologic examination of kidneys revealed a profound reduction in neutrophil recruitment to the site of the infection in mice treated with anti-CXCR2 (figure 2). Six hours after *E. coli* inoculation, 30% of control mice and 40% of anti-CXCR2-treated mice showed multifocal lesions localized in the medulla but also in the cortex (grade 3–4). However the type of inflammatory reaction was strikingly different in the 2 groups. Indeed, control mice displayed abscesses that were predominantly composed of neutrophils, with few lymphocytes and little tubular necrosis. In contrast, mice treated with anti-CXCR2 presented large necrotic areas with almost no influx of neutrophils. These findings were even more evident 24 h after infection. At 24 h, 60% of mice in both groups experienced overt pyelonephritis. Two days after inoculation, no signs of pyelonephritis could be observed in most mice (70% in both groups).

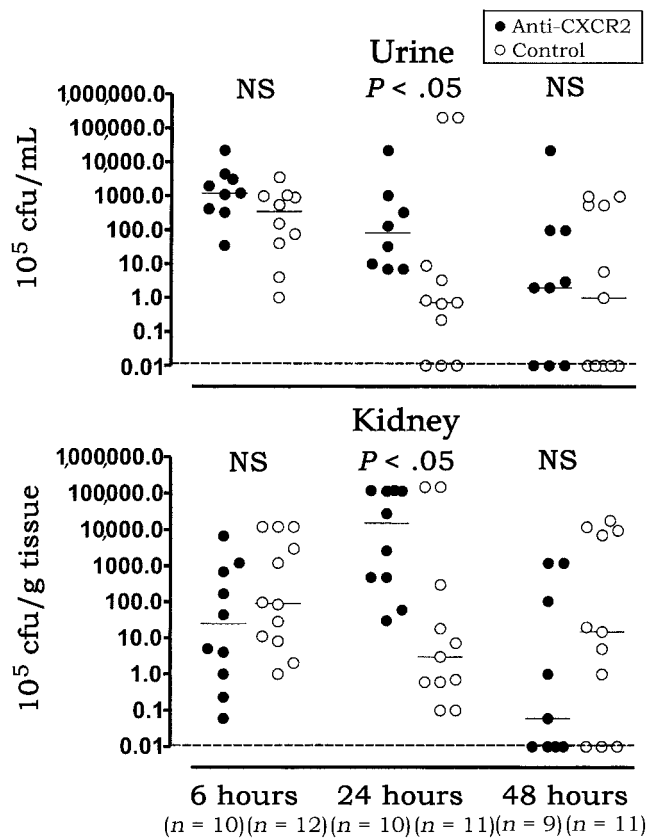
*Enhanced bacterial outgrowth in anti-CXCR2-treated mice.* Before induction of UTI, all mice had negative urine cultures. To determine the role of CXCR2 in antibacterial host defense during UTI, the bacterial outgrowth in urine, kidneys, and blood

of mice with blocked and functioning CXCR-2 was compared at 6, 24, and 48 h after intravesical inoculation with *E. coli* (figure 3). At 6 h after infection, mice treated with anti-CXCR2 or preimmune serum had similar bacterial counts in urine and kidneys. However, at 24 h, mice pretreated with anti-CXCR2 had significantly more *E. coli* colony-forming units in urine and kidneys than did mice injected with control antiserum (both  $P < .05$ ). The relatively impaired clearance of *E. coli* from the urinary tract of anti-CXCR2-treated mice was temporary: at 48 h, the recovery of *E. coli* from urine and kidneys of mice from both treatment groups was similar again. Blood cultures were positive for *E. coli* in 20% of mice treated with anti-CXCR2 and 25% of control mice at 6 h postinfection. At 24 h, these numbers were 10% and 8%, respectively, whereas at 48 h after inoculation, none of the mice in either group had bacteremia.

*Release of the CXCR2 ligands MIP-2 and KC.* To further assess the role of CXCR2 in the pathogenesis of UTIs, we determined local and systemic levels of the CXCR2 ligands MIP-2 and KC. UTIs with *E. coli* elicited increases in MIP-2 and KC concentrations in kidney and blood, peaking 6–24 h after the infection (all  $P < .05$  vs. 0 h, except for plasma MIP-2, which was nonsignificant; figure 4). Anti-CXCR2 treatment



**Figure 2.** Absence of neutrophilic infiltrates in infected kidneys as result of anti-CXCR2 treatment. *A* and *C*, At 24 h after *Escherichia coli* inoculation, control mice present small abscesses predominantly composed of granulocytes in renal parenchyma (*A*, hematoxylin-eosin [HE]; *C*, antigranulocyte immunostaining; original magnification,  $\times 40$ ). *B* and *D*, Anti-CXCR2-injected mice develop large necrotic areas in the renal cortex with few inflammatory infiltrates, in the absence of granulocytes (*B*, HE; *D*, antigranulocyte immunostaining; original magnification,  $\times 40$ ).



**Figure 3.** Transient increase in bacteria loads in urine and kidneys associated with anti-CXCR2 treatment. Urinary tract infection was induced by intravesical administration of *Escherichia coli* at time 0; 2 h before inoculation, mice received an intraperitoneally administered injection of either anti-mouse CXCR2 antiserum (●) or preimmune serum (○). Horizontal lines represent medians. NS, nonsignificant.

did not influence renal MIP-2 and KC concentrations (figure 4). In plasma, anti-CXCR2 treatment was associated with MIP-2 and KC levels higher than those measured after treatment with the control antibody (both  $P < .05$  at 6 and 24 h for MIP-2 and at 24 h for KC; figure 4).

## Discussion

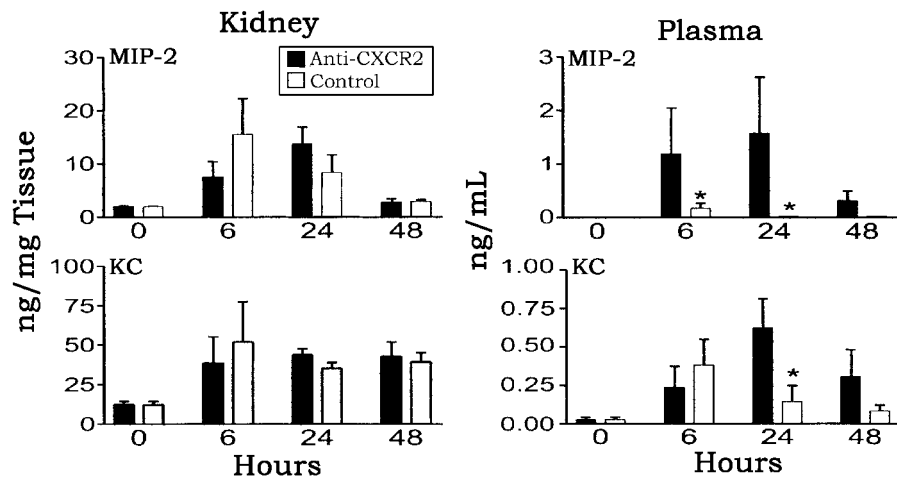
Neutrophils constitute an important component of early host defense against bacterial infection. UTI results in a rapid appearance of neutrophils in urine, a response that, at least in part, is mediated by effects of locally produced ELR-positive CXC chemokines on specific neutrophil receptors [13, 14, 26]. The present study examined the role of CXCR2, the sole receptor that is responsive to ELR-positive CXC chemokines in the mouse, in ascending UTI. Administration of a blocking CXCR2 antibody was found to virtually completely prevent the recruitment of neutrophils to the urinary tract, as reflected by the absence of leukocyturia and the lack of neutrophilic

infiltrates in kidneys. The clearance of *E. coli* from the urinary tract was impaired only transiently, with 100- and 1000-fold higher bacteria loads in urine and kidneys, respectively, at 24 h, but not at 48 h, after the bacterial inoculation. Furthermore, anti-CXCR2 treatment was not associated with an enhanced dissemination of the infection, considering that blood cultures were positive in a similarly small subset of anti-CXCR2-treated and control mice, and only at early times. These data suggest that, although CXCR2 is essential for the attraction of neutrophils to the urinary tract, this chemokine receptor does not have a major impact on host defense during UTIs.

Several antibacterial mechanisms may contribute to the clearance of bacteria from the urinary tract in the absence of neutrophils [27]. The epithelial surfaces of the urinary tract are covered by a thin layer of fluid containing a number of antimicrobial proteins and peptides, including lysozyme, peroxidases, and defensins. Although the role of these proteins in host defense against UTI has not been studied systematically, it is conceivable that they contribute to bacterial clearance. In this context, it is of interest that human  $\beta$ -defensin (HBD)-1 is produced in the kidney (in distal tubules, loops of Henle, and collecting ducts) [28], and that the release of HBD-1 is increased during pyelonephritis [29]. Although direct evidence is not available, it is tempting to speculate that HBD-1 works together with other constitutive components of the mucosal lining of the urinary tract to form a barrier to tissue penetration by bacteria.

Histopathologic studies revealed that mice treated with anti-CXCR2 had large necrotic areas in their kidneys, whereas little or no necrosis was observed in mice treated with control antibody, suggesting that the presence of neutrophils protects against the development of necrosis during pyelonephritis. Conceivably, *E. coli* can exert direct cytopathic effects on renal tissue when they are not controlled by the antibacterial activity of neutrophils. Along this line, bacteria were concentrated around necrotic tubuli. It should be noted that, although the number of *E. coli* colony-forming units in kidneys was similar in anti-CXCR2-treated and control mice at 48 h after infection, the presence of renal necrosis in the former group of mice may result in reduced renal function at later times. Further studies are warranted to investigate this possibility.

The role of neutrophils and chemokines in experimental UTI has been studied in previous investigations. Systemic depletion of neutrophils by administration of a monoclonal antibody specifically targeting neutrophils and eosinophils was associated with a complete absence of neutrophil influx in urine during murine UTI and with an increased outgrowth of bacteria in kidneys and bladders at 24 h after the intravesical inoculation with *E. coli*; the numbers of bacteria recovered from the urinary tract at other times during the infection were not reported in that study [30]. Treatment of mice with an antibody directed against MIP-2, an important ELR-positive CXC chemokine and CXCR2 ligand in the mouse, resulted in a reduced neutrophil influx in urine [14]. Interestingly, in anti-MIP-2-treated



**Figure 4.** Macrophage inflammatory protein (MIP)-2 and KC concentrations in kidneys and plasma during experimental urinary tract infection (UTI). UTI was induced by intravesical administration of *Escherichia coli* at time 0; 2 h before inoculation, mice received an intraperitoneal injection of either anti-mouse CXCR2 antiserum (solid bars) or preimmune serum (open bars). Data are mean  $\pm$  SE. \* $P < .05$ , vs. anti-CXCR2.

mice, neutrophil recruitment to kidneys remained intact, with neutrophils accumulating on the tissue side of the pelvic urothelium, which suggests that MIP-2 may have a role in neutrophil migration across the urothelium into urine [14]. This finding contrasts with our observations in anti-CXCR2-treated mice, which demonstrated a lack of neutrophil accumulation in infected renal tissue, indicating that CXCR2 ligands different from MIP-2 may be involved in the influx of neutrophils into renal tissue during UTIs.

Notably, a similar difference between effects of anti-MIP-2 and anti-CXCR2 antibodies was reported in a mouse model of pulmonary *Nocardia asteroides* infection, in which blocking of CXCR2 strongly inhibited neutrophil influx in lungs, concurrently facilitating bacterial outgrowth, whereas anti-MIP-2 was without effect [22]. Moreover, and in accordance with these findings, anti-CXCR2 treatment had a much larger negative influence on host defense against pulmonary infections with *Aspergillus fumigatus* or *Pseudomonas aeruginosa* than did treatment with anti-MIP-2 [23, 24]. We showed earlier that different ELR-positive CXC chemokines—that is, IL-8, GRO- $\alpha$ , and ENA-78—all contribute to the chemotactic activity on neutrophils in urine derived from patients with urosepsis, which further supports the notion that multiple CXCR2 and potentially CXCR1 ligands may play a role in host defense against UTIs [13]. In murine UTIs, anti-MIP-2 treatment did not hamper the clearance of *E. coli* from kidneys [14]. Together, these data suggest that neutrophils that accumulate under the urothelium because of a reduced capacity to cross the epithelial barrier remain able to protect the host against outgrowth of bacteria in the urinary tract [14], whereas an overall reduced recruitment of neutrophils to the kidneys results in a reduced ability to eliminate bacteria [30] (present study).

While our investigation was in progress, C. Svanborg's group

of investigators reported a study in which UTI was induced in CXCR2 knockout mice [31, 32]. These mice were found to have a strongly reduced influx of neutrophils in urine and demonstrated accumulation of neutrophils in the submucosa in kidneys [32]. In addition, CXCR2 knockout mice were unable to control local bacterial outgrowth, resulting in bacteremia and symptoms of severe disease. Our results with an anti-CXCR2 antibody differ from those obtained with CXCR2 knockout mice with respect to the absence of neutrophil recruitment into renal tissue in anti-CXCR2-treated mice and the impact on the clearance of bacteria from the urinary tract; that is, anti-CXCR2 treatment was associated with transiently increased bacterial loads in urine and kidneys and did not result in dissemination of the infection. It is unlikely that an incomplete blockade of CXCR2 by the antibody explains the different effect on antibacterial defense, considering that anti-CXCR2 virtually completely prevented neutrophil influx in kidneys and urine, and considering that the same antibody used in the same amount also profoundly diminished neutrophil recruitment to the pulmonary compartment during lung infections with different pathogens [22–24].

Knockout mice may differ from wild-type mice not only with respect to the product of the deleted gene, but also with respect to compensatory changes, resulting from hereditary deficiency of a protein, that have little to do with the original defect. Indeed, CXCR2 knockout mice demonstrate lymphadenopathy resulting from an increase in B cells and splenomegaly resulting from an increase in metamyelocytes and band and mature neutrophils [18]. The number of neutrophils in the peripheral blood of CXCR2 knockout mice is 12-fold higher than that in normal wild-type littermates [18], whereas anti-CXCR2 treatment does not influence peripheral neutrophil counts [22–24]. One cannot exclude the possibility that these secondary alterations in

CXCR2-deficient mice influence their behavior during experimental UTIs.

In addition, differences in the *E. coli* strains used by Svanborg's group [31, 32] and in the present investigation may have played a role in the different effects on bacterial outgrowth. The strain used in the previous investigation, *E. coli* 1177 of serotype O1:K1:H7, expresses P and type 1 fimbriae but is hemolysin negative. Our strain was of the O6 serotype, which also expresses P and type 1 fimbriae but is hemolysin positive [19–21]. It should be noted, however, that in normal control mice, both strains induced a self-limiting disease with transient leukocyturia and spontaneous clearance of the infection [19–21, 30–32].

UTI was associated with an elevation of the CXCR2 ligands MIP-2 and KC within the urinary tract, confirming an earlier report [14]. These increases coincided with peak granulocyte influx in urine. Anti-CXCR2 treatment did not influence renal concentrations of MIP-2 and KC. In plasma, MIP-2 and KC concentrations were higher in anti-CXCR2-treated mice. The explanation for this finding is not certain, although other investigators have described elevated plasma levels of a ligand in the absence of its receptor [33, 34], which suggests that at least some receptors may exert a negative feedback effect on the production of ligand.

The pathogenesis of UTI is multifactorial. Host factors, such as the short female urethra and the proximity of the urethral meatus to the rectum, and bacterial factors, such as the presence of fimbriae and the production of hemolysin, are considered to contribute significantly to the susceptibility to UTI [35]. We here demonstrate that, in the mouse, CXCR2 plays an essential role in the recruitment of neutrophils to the urinary tract during experimental UTI, and that the absence of neutrophils at the site of the infection only temporarily reduces host resistance. Further studies are warranted to examine the individual contribution of different CXCR2 ligands in the inflammatory response to UTI.

## References

1. The National Kidney and Urologic Diseases Advisory Board 1990 long-range plan: window on the 21st century. Bethesda, MD: US Department of Health and Human Services, Public Health Service, National Institutes of Health, 1990.
2. Mobley HLT, Warren JW. Urinary tract infections. Washington, DC: ASM Press, 1996.
3. Sobal JD, Kaye D, Reihart H. Host defense mechanisms in urinary tract infections. In: Schrier RV, Gottschalt CW, eds. Diseases of the kidney. Boston: Little, Brown, 1993:885–907.
4. Muder RR, Brennen C, Wagener MM, Goetz AM. Bacteremia in a long-term-care facility: a five-year prospective study of 163 consecutive episodes. *Clin Infect Dis* 1992;14:647–54.
5. Kreger BE, Craven DE, McCabe WR. Gram-negative bacteremia. IV. Reevaluation of clinical features and treatment in 612 patients. *Am J Med* 1980;68:344–55.
6. Roberts FJ, Geere IW, Coldman A. A three-year study of positive blood cultures, with emphasis on prognosis. *Rev Infect Dis* 1991;13:34–46.
7. Luster AD. Chemokines: chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436–45.
8. Adams DH, Lloyd AR. Chemokines: leucocyte recruitment and activation cytokines. *Lancet* 1997;349:490–5.
9. Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines: CXC and CC chemokines. *Adv Immunol* 1994;55:97–179.
10. Prins JM, van Agtmael MA, Kuijper EJ, van Deventer SJ, Speelman P. Antibiotic-induced endotoxin release in patients with gram-negative urosepsis: a double-blind study comparing imipenem and ceftazidime. *J Infect Dis* 1995;172:886–91.
11. Ko YC, Mukaida N, Ishiyama S, et al. Elevated interleukin-8 levels in the urine of patients with urinary tract infections. *Infect Immun* 1993;61:1307–14.
12. Benson M, Jodal U, Agace W, et al. Interleukin (IL)-6 and IL-8 in children with febrile urinary tract infection and asymptomatic bacteriuria. *J Infect Dis* 1996;174:1080–4.
13. Olszyna DP, Opal SM, Prins JM, et al. Chemotactic activity of CXC chemokines interleukin (IL)-8, growth-related oncogene (GRO) $\alpha$  and epithelial cell-derived neutrophil-activating protein (ENA)-78 in urine of patients with urosepsis. *J Infect Dis* 2000;182:1731–7.
14. Hang L, Haraoka M, Agace WW, et al. Macrophage inflammatory protein-2 is required for neutrophil passage across the epithelial barrier of the infected urinary tract. *J Immunol* 1999;162:3037–44.
15. Ahuja SK, Murphy PM. The CXC chemokines growth-regulated oncogene (GRO)  $\alpha$ , GRO $\beta$ , GRO $\gamma$ , neutrophil-activating peptide-2, and epithelial cell-derived neutrophil-activating peptide-78 are potent agonists for the type B, but not the type A, human interleukin-8 receptor. *J Biol Chem* 1996;271:20545–50.
16. Lee J, Horuk R, Rice GC, Bennett GL, Camerato T, Wood WI. Characterization of two high affinity human interleukin-8 receptors. *J Biol Chem* 1992;267:16283–7.
17. Murdoch C, Finn A. Chemokine receptors and their role in inflammation and infectious diseases. *Blood* 2000;95:3032–43.
18. Cacalano G, Lee J, Kikly K, et al. Neutrophil and B cell expansion in mice that lack the murine IL-8 receptor homolog. *Science* 1994;265:682–4.
19. Hopkins WJ, Gendron-Fitzpatrick A, Balish E, Uehling DT. Time course and host responses to *Escherichia coli* urinary tract infection in genetically distinct mouse strains. *Infect Immun* 1998;66:2798–802.
20. Hopkins WJ, Hall JA, Conway BP, Uehling DT. Induction of urinary tract infection by intraurethral inoculation with *Escherichia coli*: refining the murine model. *J Infect Dis* 1995;171:462–5.
21. Morin MD, Hopkins WJ. Treatment of mice with staphylococcal enterotoxin B enhances resolution of an induced *Escherichia coli* urinary tract infection and stimulates production of proinflammatory cytokines. *Infect Immun* 1998;66:2466–70.
22. Moore TA, Newstead MW, Strieter RM, Mehrad B, Beaman BL, Standiford TJ. Bacterial clearance and survival are dependent on CXC chemokine receptor-2 ligands in a murine model of pulmonary *Nocardia asteroides* infection. *J Immunol* 2000;164:908–15.
23. Mehrad B, Strieter RM, Moore TA, Tsai WC, Lira SA, Standiford TJ. CXC chemokine receptor-2 ligands are necessary components of neutrophil-mediated host defense in invasive pulmonary aspergillosis. *J Immunol* 1999;163:6086–94.
24. Tsai WC, Strieter RM, Mehrad B, Newstead MW, Zeng X, Standiford TJ. CXC chemokine receptor CXCR2 is essential for protective innate host response in murine *Pseudomonas aeruginosa* pneumonia. *Infect Immun* 2000;68:4289–96.
25. Hebert CA, Chuntharapai A, Smith M, Colby T, Kim J, Horuk R. Partial functional mapping of the human interleukin-8 type A receptor: identification of a major ligand binding domain. *J Biol Chem* 1993;268:18549–53.
26. Agace WW, Hedges SR, Ceska M, Svanborg C. Interleukin-8 and the neutrophil response to mucosal gram-negative infection. *J Clin Invest* 1993;92:780–5.
27. Ganz T. Defensins in the urinary tract and other tissues. *J Infect Dis* 2001;183(Suppl 1):S41–2.
28. Valore EV, Park CH, Quayle AJ, Wiles KR, McCray PB, Ganz T. Human

- beta-defensin-1: an antimicrobial peptide of urogenital tissues. *J Clin Invest* **1998**;101:1633–42.
29. Hiratsuka T, Nakazato M, Ihi T, et al. Structural analysis of human beta-defensin-1 and its significance in urinary tract infection. *Nephron* **2000**;85:34–40.
  30. Haraoka M, Hang L, Frendeus B, et al. Neutrophil recruitment and resistance to urinary tract infection. *J Infect Dis* **1999**;180:1220–9.
  31. Frendeus B, Godaly G, Hang L, Karpman D, Lundstedt AC, Svanborg C. Interleukin 8 receptor deficiency confers susceptibility to acute experimental pyelonephritis and may have a human counterpart. *J Exp Med* **2000**;192:881–90.
  32. Hang L, Frendeus B, Godaly G, Svanborg C. Interleukin-8 receptor knock-out mice have subepithelial neutrophil entrapment and renal scarring following acute pyelonephritis. *J Infect Dis* **2000**;182:1738–48.
  33. Matthys P, Froyen G, Verdot L, et al. IFN-gamma receptor-deficient mice are hypersensitive to the anti-CD3-induced cytokine release syndrome and thymocyte apoptosis: protective role of endogenous nitric oxide. *J Immunol* **1995**;155:3823–9.
  34. Juffermans NP, Florquin S, Camoglio L, et al. Interleukin-1 signaling is essential for host defense during murine pulmonary tuberculosis. *J Infect Dis* **2000**;182:902–8.
  35. Hooton TM, Stamm WE. Diagnosis and treatment of uncomplicated urinary tract infection. *Infect Dis Clin North Am* **1997**;11:551–81.