

## Elevated Interleukin-8 Levels in the Urine of Patients with Urinary Tract Infections

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Pyuria is one of the main features of urinary tract infections (UTI). Nevertheless, the mechanism of polymorphonuclear leukocyte (PMN) recruitment into the urine remains to be investigated. We examined whether interleukin-8 (IL-8), a potent neutrophil chemoattractant and activator, was involved in pyuria seen in UTI. Of 113 patients, 112 had elevated levels of IL-8 in their urine ( $1,078.0 \pm 181.5$  pg/ml), regardless of whether they had an upper or lower UTI; this was in contrast to undetectable levels (less than 16 pg/ml) in the urine of all of the 20 normal individuals and 74 control patients without UTI. A concomitant study revealed increases in urine IL-6, but not IL-1 $\beta$ , and tumor necrosis factor alpha levels in patients with UTI. In addition to gram-negative bacteria, a wide spectrum of microorganisms was capable of inducing IL-8 production in urine. Local production of IL-8 in the urinary tract was suggested by a urine IL-8 level that was higher than the paired serum IL-8 level. The urine IL-8 level correlated with the number of PMN in the urine, and an average of half of the chemotactic activity in urine from patients with UTI could be abrogated by anti-IL-8 antibody treatment in vitro. Furthermore, urine IL-8 purified from patients was bioactive and showed multiple forms on immunoblotting analysis. This is the first documentation of IL-8 in the urine of patients with UTI, and these results imply that IL-8 is involved in inducing PMN migration into the urinary tract.

Urinary tract infection (UTI) is one of the most common infectious diseases that affects humans throughout their lifespan (16). It is characterized by the evidence of uropathogens and pyuria and is accompanied by various clinical manifestations depending on the areas of involvement. Although the detection of pyuria in patients with suspected UTI is readily available via a laboratory test, little is known about the mechanism of neutrophil recruitment into the urine.

The elevation of urinary levels of cytokines, including interleukin-6 (IL-6) (13), IL-2 (11), and IL-2 inhibitor (9), has been observed in patients with UTI. However, none of these molecules possesses neutrophil chemotactic activity. IL-8, a potent chemoattractant and activator of neutrophils, is produced by a variety of cells, including monocytes, endothelial cells, and mesangial cells, upon stimulation by lipopolysaccharide, IL-1, and tumor necrosis factor alpha (TNF- $\alpha$ ), etc., in vitro (5, 20, 23, 24). The participation of IL-8 in infection is evidenced by an increase of the IL-8 level in the blood of a human volunteer or primate during intravenous endotoxin or *Escherichia coli* administration, respectively (22, 25). A local infection in the amniotic cavity is observed to be associated with an elevated IL-8 level in the amniotic fluid (26). Moreover, IL-8 has been detected in the urine of superficial bladder cancer patients to whom *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) cells were administered intravesically after transurethral resection of tumors (4). It is therefore reasonable to postulate that IL-8 may play a role in UTI.

In this study, we demonstrated elevated levels of IL-8 in the urine of almost all of the patients with UTI, compared with undetected levels in the controls. Furthermore, the

presence of IL-8 in urine contributed to neutrophil chemotactic activity in vitro which was specifically blocked by anti-IL-8 antibody treatment, suggesting the involvement of IL-8 in neutrophil recruitment into urine.

### MATERIALS AND METHODS

**Patients.** A total of 113 patients (48 male, 65 female; 1 to 87 years old; mean age, 54 years), including 15 with upper UTI and 98 with lower UTI (either acute or chronic), were studied. The diagnosis of UTI was based on the presence of  $\geq 10^4$  CFU of microorganisms per ml in urine culture. Patients with systemic symptoms such as fever combined with localized flank pain were diagnosed as having upper UTI, while patients with complaints of dysuria, frequency, and urgency only were considered as having lower UTI. The control group who had normal urinalyses consisted of 74 patients without bacteriuria (32 male, 42 female; 1 to 75 years old; mean age, 43 years) and 20 healthy volunteers (10 male, 10 female; 20 to 37 years old; mean age, 25 years). The control patients had a similar distribution of underlying illness as the patient group.

**Urine samples.** Either catheterized or spontaneously voided midstream unused urine specimens were obtained. Urinalysis, including determinations of specific gravity, pH, glucose, protein, blood, leukocyte esterase, and nitrite, was done by dipstick analysis (Ames-N; Miles-Sankyo, Tokyo, Japan) as described in the manufacturer's instructions. For microscopic examination, 10 ml of the specimen was spun at  $200 \times g$  for 5 min, the supernatant was aspirated, and the urine sediment was resuspended with the residual 0.2 ml of urine. The cellular elements of 15  $\mu$ l of urine were expressed as the average number of cells per high-powered field (HPF) of five separate areas (magnification,  $\times 400$ ). For microbiological studies, an aliquot was inoculated into the appropri-

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ate medium and colonies were counted 24 and 48 h later. The rest of the specimen was kept at  $-20^{\circ}\text{C}$  until needed for another assay.

**IL-8 assay.** The IL-8 level was determined by an enzyme-linked immunosorbent assay (ELISA), by using monoclonal antibody (MAB; clone WS 4) as a capturing antibody (Ab) and rabbit Ab as a second Ab, both of which were raised against human recombinant IL-8 (rIL-8) of 72-amino-acid form (gift from Dainippon Pharmaceutical Co., Osaka, Japan), essentially as previously described (19) except that the dilution buffer was changed to 1% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Mo.) in phosphate-buffered saline (PBS) containing 0.05% Tween 20. Urine specimens were centrifuged at  $1,000 \times g$  for 10 min, and the supernatants were assayed directly or after dilution with the dilution buffer. Serum samples were measured directly. All assays were done at least in duplicate. The detection limit of this assay was 16 pg of IL-8 per ml. The ELISA worked well for urine samples whose pH was between 5 and 10, whereas immunoreactive IL-8 was reduced to less than 60% of the original concentration for a pH either below 5 or above 10. The pH of all urine specimens was between 5 and 9. Human rIL-8 was found to be stable in urine specimens from both patients with UTI and normal subjects for at least 48 h at either room temperature or  $4^{\circ}\text{C}$ .

**Other cytokine assays.** Urine IL- $1\beta$  and TNF- $\alpha$  levels in normal subjects ( $n = 20$ ) and patients ( $n = 25$ ) were measured by using commercially available ELISA kits from Medgenix Diagnostics (Toray-Fuji-Bionics, Tokyo, Japan) and Otsuka Pharmaceuticals (Tokushima, Japan), respectively. The detection limits were 10 and 5 pg/ml for each kit, respectively. The urine IL-6 level was measured with an ELISA by using two MAbs (Fuji-Rebio, Tokyo, Japan) with a sensitivity of 4 pg/ml.

**Chemotaxis assay.** Neutrophil chemotaxis was assessed by using a multiwell chemotaxis chamber (Neuro Probe Inc., Bethesda, Md.) as described elsewhere (18). Briefly, human polymorphonuclear leukocytes (PMN) were obtained from the heparinized blood of healthy volunteers by gradient centrifugation on Ficoll-Hypaque (Nycomed, Oslo, Norway) and then subjected to sedimentation on a gelatin solution (2.5% [wt/vol] in 0.9% NaCl). The final preparation of neutrophils was suspended at  $10^6/\text{ml}$  (purity,  $>95\%$ ) in PBS containing 0.1% BSA, 0.9 mM  $\text{CaCl}_2$ , and 0.5 mM  $\text{MgCl}_2$  (dilution buffer). The viability was greater than 98%. A polycarbonate membrane with 3- $\mu\text{m}$  pores (Nuclepore Corp., Pleasanton, Calif.) without polyvinylpyrrolidone coating was used. Urine specimens were dialyzed against PBS and diluted (1:1) with the dilution buffer. Spectra/Por 3 membranes (Spectrum, Houston, Tex.) with a molecular weight cutoff of 3,500 were used for dialysis throughout the study. Twenty-five-microliter volumes of the samples were placed in the lower wells, while 50- $\mu\text{l}$  volumes of PMN were added to the upper wells in triplicate. After being incubated for 50 min at  $37^{\circ}\text{C}$ , the chamber was disassembled, and nonmigrated cells were wiped away from the upper surface. The membrane was fixed with methanol and then stained with Giemsa stain. Migrated cells per well were counted at  $\times 400$  magnification for five random fields.

For the adsorption experiment, 5 mg of either MAB anti-IL-8 (WS 4) or control MAB anti-IL- $1\alpha$  (17) was conjugated to cyanogen-bromide-activated Sepharose 4B gels (Pharmacia-LKB, Uppsala, Sweden) as described in the manufacturer's instructions. The conjugated gels were washed and resuspended in PBS containing 1% BSA. Three hundred microliters of the samples was incubated with 50  $\mu\text{l}$

of the gels at  $4^{\circ}\text{C}$  for 2 h with agitation and then centrifuged. The chemotaxis assay was performed as described above. In each assay, human rIL-8 with a final concentration of 20 ng/ml was used as a positive reference and dilution buffer was used for random migration. The results are expressed as the mean  $\pm$  standard deviation of triplicate wells in percent response of human rIL-8.

**Gel filtration.** After dialysis against PBS, urine specimens from patients were applied to a Superose 12 HR 10/30 column (Pharmacia) at a flow rate of 0.3 ml/min in PBS. For calibration, BSA and human rIL-8 were used.

**Purification of urine IL-8.** To verify the presence of IL-8 in urine, 15.5 and 6.1 liters of urine were collected separately from one patient with upper UTI and one patient with lower UTI, respectively. Specimens were precipitated with 80% ammonium sulfate, and the precipitates were dissolved in PBS and then subjected to 10% ammonium sulfate precipitation. The supernatants were consequently precipitated with 80% ammonium sulfate once again, and the final precipitates were dissolved and dialyzed against 50 mM phosphate buffer (pH 7.2). They were then applied to a heparin-Sepharose CL-6B column (2.5 cm by 10 cm; Pharmacia), washed with the same buffer, and subjected to elution with 0.5 M NaCl in phosphate buffer. ELISAs for all fractions were performed throughout all of the purification steps. The positive fractions, dialyzed against 0.04 M NaCl in 50 mM phosphate buffer, were applied to a TSK gel heparin-5PW column (TOSOH, Tokyo, Japan) equilibrated with the same buffer and eluted with a discontinuous NaCl gradient. The positive fractions eluted from the heparin-5PW column were separated into four pools. Each pool was applied to a TSK TMS-250 column (TOSOH) equilibrated with 0.05% (vol/vol) trifluoroacetic acid in high-performance liquid chromatography (HPLC)-grade water. Polypeptides eluted with a linear gradient of increasing concentrations of acetonitrile containing 0.05% trifluoroacetic acid. All fractions were lyophilized and reconstituted with 50  $\mu\text{l}$  of distilled water.

**SDS-PAGE.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for polypeptides was performed by the method described by Schaeffer et al. (29) by using a separation gel containing 16.5% T and 6% C in the presence of 6 M urea at pH 8.45 as previously described (19).

**Immunoblotting.** Immunoblotting was performed as previously described (19). A transblotted membrane was incubated with MAB WS 4 immunoglobulin G (3.6  $\mu\text{g}/\text{ml}$ ) and then reacted with peroxidase-conjugated rabbit anti-mouse Ab diluted 1/500 (Dakopatts, Glostrup, Denmark), and the membrane was developed by using the Konica immunostaining HRP kit (Konica, Tokyo, Japan).

**Statistical analysis.** All values were expressed as the mean  $\pm$  standard error of the mean unless otherwise stated. Analyses of data were performed by using Student's unpaired  $t$  test or simple regression. Specimens with values below the detection limit were excluded from comparisons involving that variable. Differences were considered significant for  $P$  of  $<0.05$ .

## RESULTS

**Determination of urinary level of cytokines.** No detectable IL-8 was found in urine specimens from either healthy volunteers or control patients (Fig. 1). On the contrary, 112 of the 113 patients with UTI showed elevated urine IL-8 levels ( $1,078.0 \pm 181.5$  pg/ml). The differences in the urine IL-8 levels of patients with upper ( $1,138.9 \pm 375.0$  pg/ml,  $n = 15$ ) and lower ( $1,068.7 \pm 201.9$  pg/ml,  $n = 97$ ) UTI, with

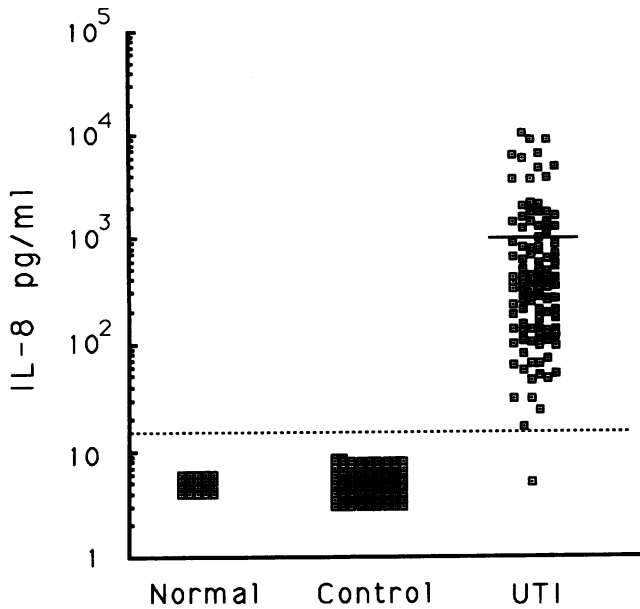


FIG. 1. Concentrations of IL-8 in urine specimens from control subjects and patients with UTI. Abbreviations: normal, normal individuals ( $n = 20$ ); control, control patients without UTI ( $n = 74$ ); UTI, patients with UTI ( $n = 113$ ). Mean values are indicated by the solid horizontal line. The dotted line represents the detection limit ( $<16$  pg/ml) of the ELISA.

( $1,138.7 \pm 268.0$  pg/ml,  $n = 60$ ) and without ( $1,008.0 \pm 241.4$  pg/ml,  $n = 52$ ) underlying illness, catheterized ( $1,586.0 \pm 448.3$  pg/ml,  $n = 29$ ) and noncatheterized ( $900.5 \pm 186.2$  pg/ml,  $n = 83$ ), and who were male ( $1,300.7 \pm 329.4$  pg/ml,  $n = 48$ ) and female ( $911.0 \pm 199.4$  pg/ml,  $n = 64$ ) were not significant.

The most common isolated microorganisms were *Escherichia coli* ( $n = 31$ ) in 50 cases of gram-negative bacterium infection, *Enterococcus faecalis* ( $n = 9$ ) in 22 cases of gram-positive bacterium infection, and *Candida* species in all 6 cases of fungus infection; polymicrobial infections were found in the remaining 34 cases. The urine IL-8 levels of each group were  $915.0 \pm 258.5$ ,  $1,071.3 \pm 420.8$ ,  $1,764.0 \pm 870.2$ , and  $1,201.1 \pm 348.8$  pg/ml, respectively, with no significant difference in the IL-8 response between different uropathogens. Polymicrobial infections were found especially in patients with an indwelling catheter associated with underlying illness. However, our preliminary study indicated that catheterization alone did not cause IL-8 elevation in five patients without UTI.

Furthermore, when we continued monitoring urine IL-8 levels in eight patients, the elevated IL-8 level detectable during infection became undetectable after recovery, which indicated the specific response of IL-8 to UTI (data not shown). As shown in Fig. 2, among the 15 paired serum and urine specimens measured, IL-8 levels in serum specimens were apparently lower than those of the corresponding urine specimens, which suggested the local production of IL-8 in the urinary tract. Although five arbitrary ranks instead of actual PMN counts were used for classifying the degree of pyuria, a correlation existed between PMN numbers and IL-8 levels in urine (Fig. 3).

Concomitantly, we examined the concentrations of TNF- $\alpha$  and IL-1 $\beta$ , two well-known stimulants of IL-8, in the urine specimens (Fig. 4). No significant difference was found

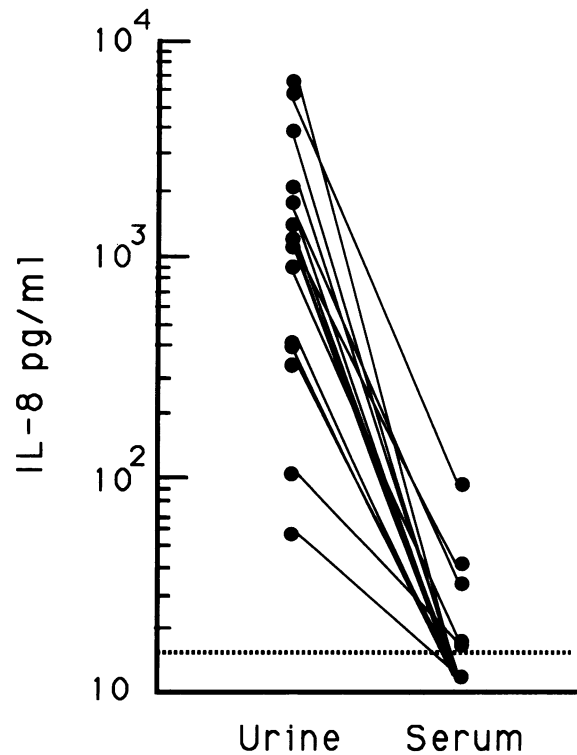


FIG. 2. Concentrations of IL-8 in paired urine and serum specimens from patients with UTI ( $n = 15$ ). Urine IL-8 levels were higher than the corresponding serum IL-8 levels in all of the patients.

between the patients with UTI and the controls for both TNF- $\alpha$  and IL-1 $\beta$ . On the other hand, another important inflammatory cytokine, IL-6, was undetected in all 20 normal subjects but elevated in 16 of 25 patients (Fig. 4). The elevated urine IL-6 level did not, however, correlate significantly with the level of TNF- $\alpha$ , IL-1 $\beta$ , or IL-8 or with the number of PMN (data not shown).

**Chemotactic activity in urine specimens from patients with UTI.** Since the urine from patients contained increased levels of IL-8, we then determined whether these urine specimens had neutrophil chemotactic activity in vitro. While no neutrophil chemotactic activity was found in urine specimens from normal subjects ( $n = 3$ ), urine specimens from all patients exhibited significant levels of neutrophil chemotactic activity (90.1 to 405.6%; average, 224.0%;  $n = 8$ ) comparable to or higher than the reference standard of 20 ng of rIL-8 per ml (Fig. 5). No apparent correlation was observed between neutrophil chemotaxis and IL-8 levels in the urine specimens. To prove that neutrophil chemotactic activity in urine specimens from patients was ascribable to IL-8, we performed adsorption experiments by using a Sepharose 4B gel coupled with either anti-IL-8 or anti-IL-1 $\alpha$  MAbs. Anti-IL-8 Ab adsorption treatment reduced the urinary IL-8 level to less than 160 pg/ml, at which level rIL-8 had no neutrophil chemotactic activity. Adsorption onto the anti-IL-8 Ab column reduced the chemotactic activity in the urine from patients by 24.9 to 93.4% (average, 54.7%), under the conditions where the same treatment reduced that of human rIL-8 by 85.8%, compared with the untreated samples (Fig. 5). As a control, anti-IL-1 $\alpha$  Ab adsorption slightly reduced the chemotactic activity of urine specimens by 1.3 to 30.3% (average, 14.0%) and that of human rIL-8 by

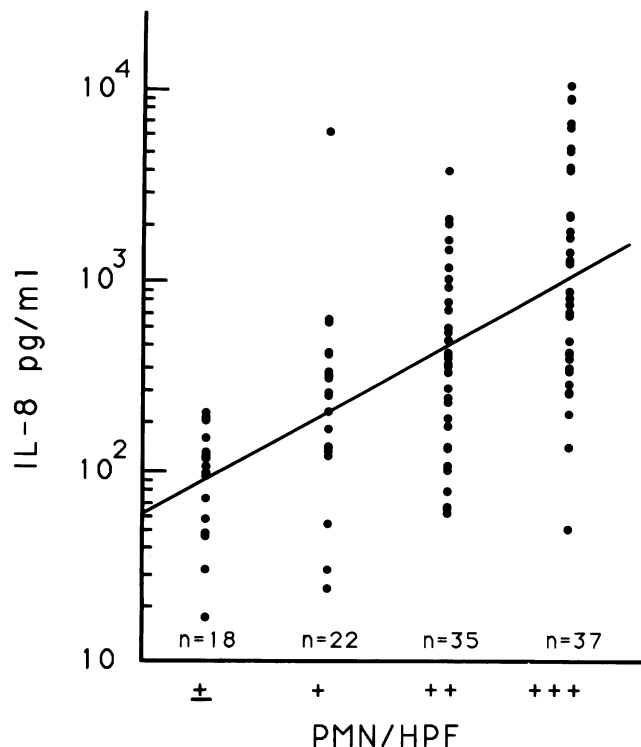


FIG. 3. Correlation between IL-8 level and PMN count in urine specimens from patients with UTI. Urine IL-8 levels correlated with the numbers of PMN ( $y = 719.0x - 1,663.1, r = 0.623, P = 0.0001$ ). The quantitation of PMN in centrifuged urine is expressed as the number of cells per HPF ( $\pm$ , 5 to 9; +, 10 to 29; ++, 30 to less than half of the count of the whole HPF; +++, more than half of the count of the whole HPF), as recommended by the UTI research group, Japan.

13.5%. These data suggest the variable but significant participation of IL-8 in neutrophil recruitment into urine.

**Characterization of urine IL-8.** The results described above indicated the presence of immunoreactive and bioactive IL-8 in urine specimens from patients with UTI. To characterize urine IL-8, we performed the gel chromatography which demonstrated that immunoreactive IL-8 eluted at a position similar to that of human rIL-8 (Fig. 6). Consequently, we purified urine IL-8 through sequential ammonium sulfate precipitation, heparin-Sepharose CL-6B column chromatography, heparin-5PW column chromatography, and reversed-phase HPLC (RP-HPLC). The final yields were 18.5% (137.3 ng) and 31.7% (1,814.2 ng) for the patients with lower and upper UTI, respectively. As shown in Fig. 7a, four IL-8 peaks were observed in the heparin-5PW chromatography. These four peaks were separately pooled and applied to the RP-HPLC. The retention time of the IL-8 peak eluted for rIL-8 (data not shown) as well those of all four pools were the same (Fig. 7b). These four IL-8 peaks on RP-HPLC were tested for their chemotactic activity; all were neutrophil chemoattractive and specifically inhibited by the treatment with anti-IL-8 Ab (data not shown). Upon immunoblotting analysis with anti-IL-8 MAb (WS 4), these peaks showed molecular masses of about 6 to 5.5 kDa and were estimated to represent IL-8 of between 69 and 72 amino acids (Fig. 7c). The purified urine IL-8 from patients with upper and lower UTIs showed similar results

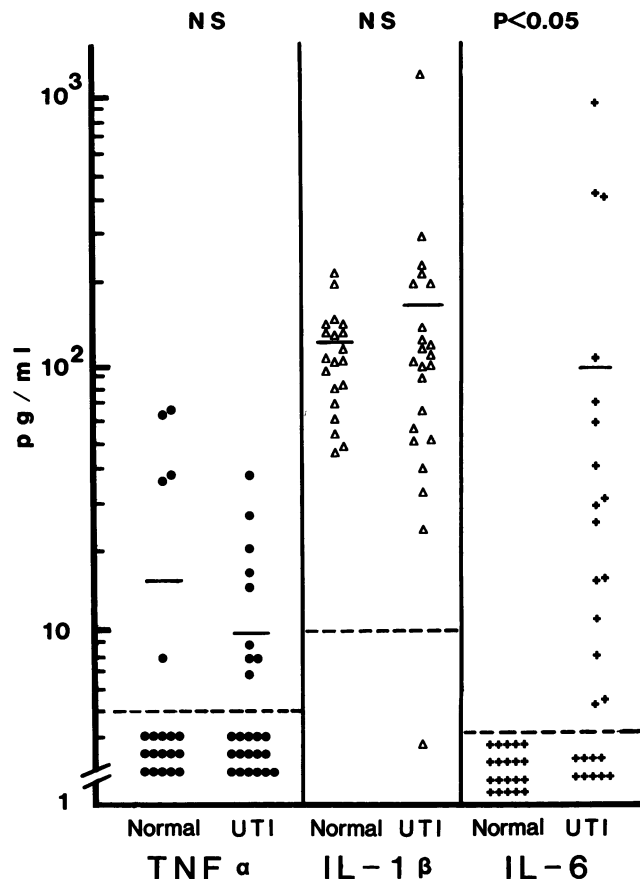


FIG. 4. TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 levels in urine specimens from normal individuals ( $n = 20$ ) and patients with UTI ( $n = 25$ ). The dotted lines represent the detection limits of each assay. Mean values are indicated by the solid horizontal lines. The means  $\pm$  standard errors of the mean of urine TNF- $\alpha$  levels for normal subjects and patients were  $14.9 \pm 4.7$  and  $9.3 \pm 1.7$  pg/ml, respectively. Similarly, urine IL-1 $\beta$  levels were  $115.1 \pm 10.7$  and  $161.9 \pm 49.8$  pg/ml, respectively. The urine IL-6 level was  $92.5 \pm 43.3$  pg/ml for patients ( $n = 16$ ), while it was undetectable in normal subjects. NS, not significant.

except that the proportion of each peak varied slightly (data not shown).

DISCUSSION

The present study describes evidence of the presence of IL-8 in urine specimens from patients with UTI and the partial characterization of urine IL-8. A correlation was observed between the semiquantitative counts of PMN and the IL-8 levels in urine. Furthermore, even though individual variance existed, an average of half of the chemotactic activity in the urine was abrogated by anti-IL-8 Ab in vitro. These data suggested the involvement of IL-8 in neutrophil recruitment into urine.

The explanations for the wide range in the reduction of chemotactic activity when anti-IL-8 columns were used and the lack of correlation between neutrophil chemotactic and IL-8 levels may be identical or different. The existence of an additional neutrophil chemotactic factor(s) in urine specimens from patients with UTI is one possible explanation. Within the growing list of IL-8-related molecules, GRO

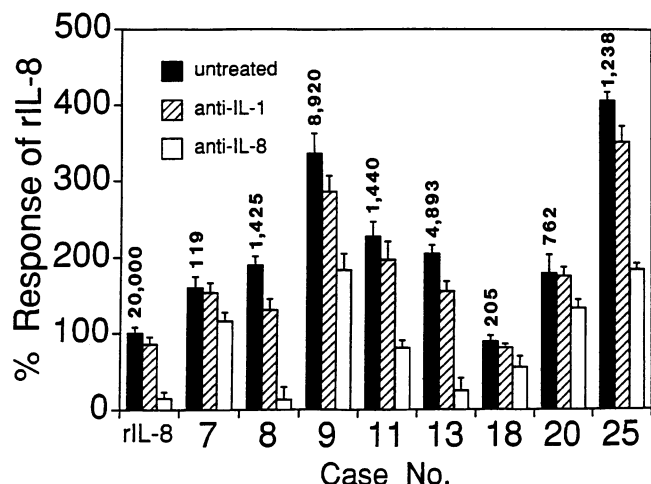


FIG. 5. Chemotaxis assays of urine IL-8. PBS-dialyzed urine before and after adsorption with either anti-IL-8 or anti-IL-1 $\alpha$  Ab-conjugated gels were assayed. Pooled normal urine specimens ( $n = 3$ ) showed no chemotactic activity (data not shown). All urine from patients with UTI ( $n = 8$ ) showed significant neutrophil chemotaxis expressed as the percent response (mean  $\pm$  standard deviation) of 20 ng of rIL-8 per ml. Random migration, which was less than 10%, was subtracted from each value. Numerals above the black bars indicate IL-8 levels (in picograms per milliliter) in each untreated sample. Data from one of two representative experiments are shown.

protein (28), which bears heparin binding and neutrophil chemoattractive properties similar to those of IL-8, may be involved since several IL-8-negative fractions of the RP-HPLC were found to have neutrophil chemotactic activities (our unpublished observations). The contribution of products of microorganisms such as fMet-Leu-Phe should also be considered. The single patient with cystitis who had pyuria (5 to 9 PMN per HPF) but an undetectable urine IL-8 level may be explained by the above speculations. Our data suggest the notion that IL-8 is not the sole chemoattractant in urine and it works with other molecules in neutrophil emigration during infection.

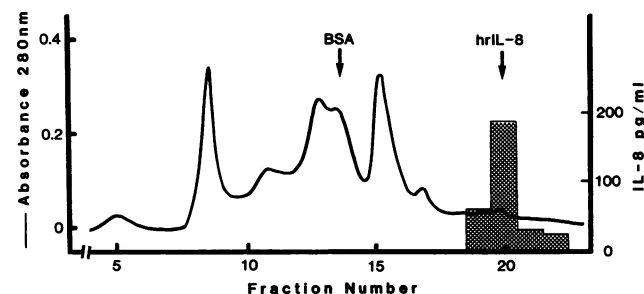


FIG. 6. Gel filtration chromatogram (Superose 12 HR 10/30) of urine of patients with UTI. Two hundred microliters of PBS-dialyzed urine was applied, and fractions of 0.9 ml were collected at a flow rate of 0.3 ml/min in PBS. As molecular weight markers, human rIL-8 (hrIL-8, 8 kDa) and BSA (67 kDa) were applied to the same column in a separate experiment. All fractions were measured for IL-8 by using an ELISA, and positive fractions are indicated by the hatched areas. The elution peak of urine IL-8 coincided with that of the human rIL-8 control. Similar results were obtained from three other patients, and a representative one is shown.

In addition to chemotactic activity, IL-8 is known to promote the in vitro neutrophil adhesion to endothelium cells and transepithelial migration in a concentration gradient manner (7, 15). In patients, the higher IL-8 level in urine than in serum may provide a concentration gradient which aids the transmigration of neutrophils from the bloodstream to the urinary tract lumen.

We postulate that IL-8 is produced locally in the urinary tract since urine IL-8 levels were higher than the levels in sera in all of the patients examined. Several transitional-cell carcinoma-derived cell lines produce IL-8 in vitro either upon stimulation (1) or constitutively (our unpublished observations). Hence, uroepithelial cells such as transitional cells may be responsible for IL-8 production in cystitis. During purification of urine IL-8, the urine from a patient with upper UTI was obtained directly through a catheterized left-kidney nephrostomy because of the complete obstruction of the ureter. In this patient, the constantly undetectable serum IL-8 level (more than four occasions) precluded the possibility of systemic production, suggesting the local production of IL-8 within the infected kidney. These data are substantiated by several recent reports on the capability of mesangial cells (5, 20), renal cortical epithelial cells (30), and renal-cell carcinoma (1) to secrete IL-8 upon stimulation in vitro. The emigrated neutrophils probably produce IL-8 after exposure to microorganisms (3, 10) in the urinary tract lumen. Nevertheless, we believe that the cell sources of urine IL-8 are multiple and include the endothelial cells (31) and uroepithelial cells mentioned above as well. The definitive identification of producing cells, however, requires the biopsy of the urinary tract accompanied by immunohistological and/or in situ hybridization methods.

It is worthy of note that a wide spectrum of microorganisms was identified as the causative pathogen of UTI in this study. That all of these urine specimens showed elevated levels of IL-8 implies that almost all microorganisms can stimulate the production of IL-8, irrespective of whether they contain lipopolysaccharide or not. This is further supported by the observation of IL-8 production upon stimulation by a streptococcal preparation immunomodulator (33), yeast (3), virus (34), BCG (4), or fastidious organisms such as *Ureaplasma* spp. (26).

IL-1 and TNF- $\alpha$ , two proinflammatory cytokines, have been shown to be capable of inducing IL-8 production in various types of cells (24). The effects of these molecules on IL-8 production are presumed to be crucial in the inflammatory cascade. However, no significant elevation of the IL-1 $\beta$  or TNF- $\alpha$  level was observed in urine specimens from patients with UTI. This is consistent with the previous results of murine studies (6, 27). We assume that IL-8 production is induced mainly by microorganisms, receiving a minor paracrine effect from these two cytokines. This supposition is not unrealistic when the report of TNF-independent IL-6 production during murine listeriosis by Havell and Sehgal (12) is considered. A UTI model of animal study will provide a clue to the answer. Induction of IL-6 production in gram-negative bacterial UTI has been described by several investigators (6, 13, 27). Hedges et al. showed that urinary IL-6 was not continuously secreted in patients (13), which may explain the undetected IL-6 in 9 of 25 patients in our study. Consistent with our results, they show no correlation between the count of PMN in urine and the IL-6 level in the individual patient.

IL-8 also activates neutrophils in terms of respiratory burst, enzyme release, enhanced PMN-mediated anti-*Candida* activity, and phagocytosis (2, 7, 8), which are all

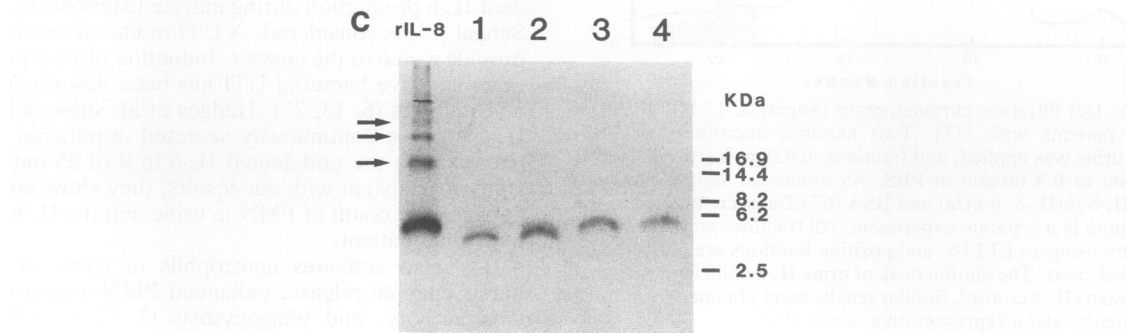
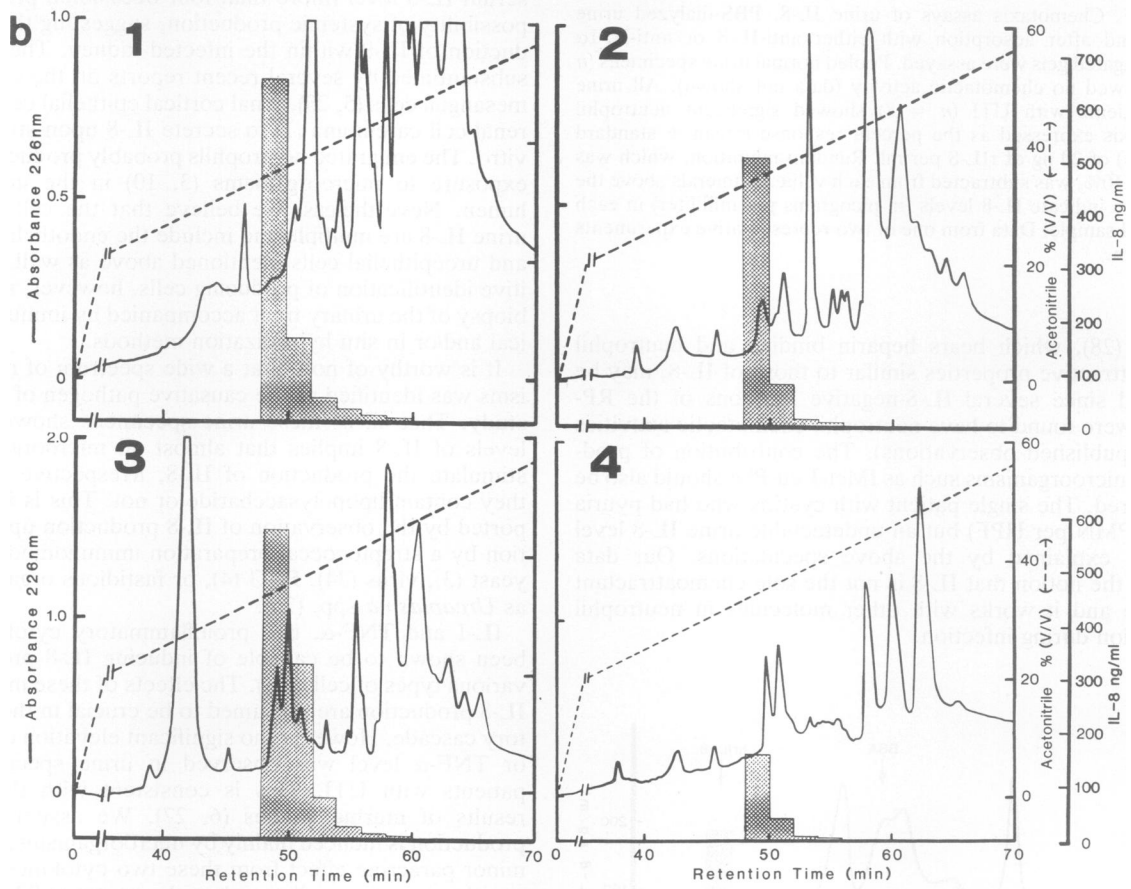
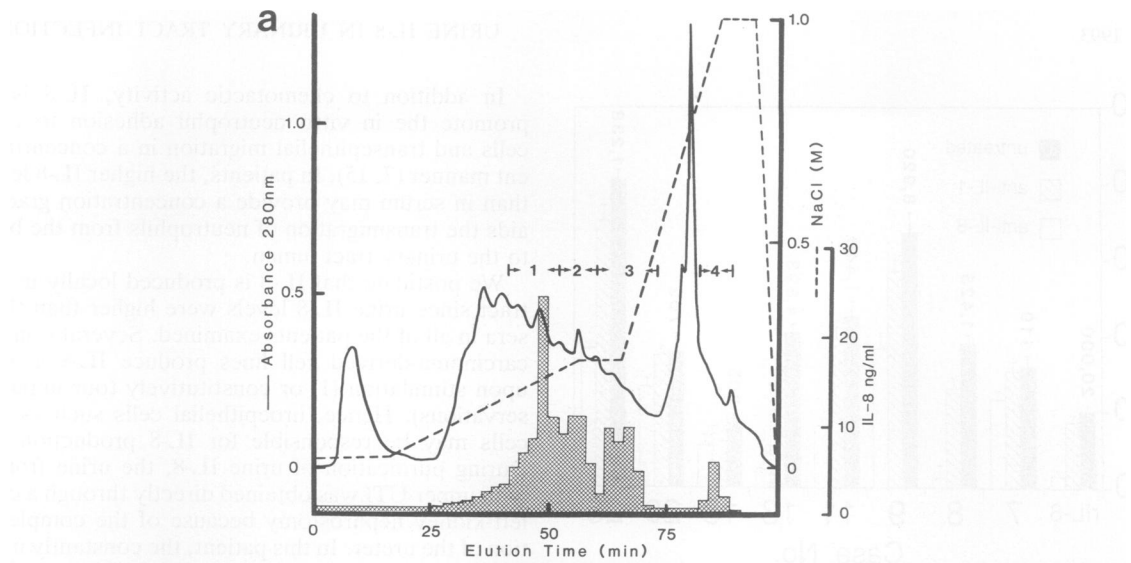


FIG. 7. (a) Chromatogram of urine from a patient with a lower UTI on TSK gel heparin-5PW. Positive fractions from a heparin-Sepharose column were dialyzed against 0.04 M NaCl in 50 mM phosphate buffer and applied. Elution with a discontinuous gradient of NaCl is indicated by a dotted line. Flow rate, 0.5 ml/min; 1 ml per fraction. Positive fractions were collected separately as four pools (labeled 1 to 4) and are indicated by the hatched areas. (b) RP-HPLC. Each pool (1 to 4) described above was applied separately onto a TSK TMS-250 column in 0.05% trifluoroacetic acid and eluted with increased gradients of acetonitrile in 0.05% trifluoroacetic acid (---). Flow rate, 0.5 ml/min; 1 ml per fraction. (c) Immunoblot of urine IL-8 by using MAb WS 4. Each IL-8 peak of the RP-HPLC described above was separated by SDS-PAGE and then analyzed by immunoblotting. Samples applied per lane (1 to 4) contained 2.0, 1.9, 2.2, and 0.9 ng of IL-8 (as determined by ELISA), respectively, while 10 ng of rIL-8 was used as a control (which gave 6 kDa in Tricine-SDS-PAGE). Polymerized forms of rIL-8 (arrows) were observed in the positive control. Similar results were obtained with specimens from the patient with upper UTI (data not shown).

essential in antimicrobial functions. On the other hand, in experimental pyelonephritis, infiltration with PMN has been presumed to be responsible for tubular destruction and renal scarring, and, therefore, colchicine and cyclophosphamide ameliorated irreversible renal damage by diminishing PMN infiltration (14). Thus, the role of IL-8 in the pathogenesis of UTI remains to be defined.

Recent studies propose that a threshold of  $10^2$  (32) or  $10^3$  (21) CFU/ml may be a more sensitive indicator of UTI. Under such circumstances, a urine IL-8 measurement may help to differentiate the bacteriuria of true infection from that due to contamination. In addition, such a measurement may be beneficial in certain atypical cases such as those of UTI in granulocytopenic patients without pyuria or in cases of negative culture in symptomatic patients either undergoing treatment or infected by a fastidious organism. Of the patients with lower UTI, five asymptomatic bacteriuric patients with catheters were included. Their urinary IL-8 levels measured  $1,869.0 \pm 1,211.8$  pg/ml ( $n = 5$ ), showing no significant difference from those of symptomatic patients ( $1,025.2 \pm 203.6$  pg/ml,  $n = 92$ ). However, it will be of interest to evaluate whether asymptomatic bacteriuric patients without pyuria have lower urinary IL-8 levels.

We conclude that the determination of urine IL-8 is more sensitive and specific than that of other inflammatory cytokines and is useful in the diagnosis of UTI. The confirmation of the presence of bioactive IL-8 in urine suggests the participation of IL-8 in UTI, providing additional evidence of the role of IL-8 in inflammation.

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