Cytokine Profiles of Pediatric Patients Treated with Antibiotics for Pyelonephritis: Potential Therapeutic Impact

KARI KASSIR, OFELIA VARGAS-SHIRAISHI, FRANK ZALDIVAR, MONIQUE BERMAN, JASJIT SINGH, AND ANTONIO ARRIETA

Division of Pediatric Critical Care and Harbor-UCLA Department of Pediatric Critical Care, Division of Pediatric Infectious Diseases, and Division of Pediatric Immune and Inflammatory Diseases and UC Irvine Department of Medicine, Children’s Hospital of Orange County, Orange, California 92868

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Urinary tract infections are common in infants and children. Pyelonephritis may result in serious complications, such as renal scarring, hypertension, and renal failure. Identification of the timing of release of inflammatory cytokines in relation to pyelonephritis and its treatment is essential for designing interventions that would minimize tissue damage. To this end, we measured urinary cytokine concentrations of interleukin-1β (IL-1β), IL-6, and IL-8 in infants and children with pyelonephritis and in healthy children. Children that presented to our institution with presumed urinary tract infection were given the diagnosis of pyelonephritis if they had a positive urine culture, pyuria, and one or more of the following indicators of systemic involvement: fever, elevated peripheral white blood cell count, or elevated C-reactive protein. Urine samples were obtained at the time of presentation prior to the administration of antibiotics, immediately after completion of the first dose of antibiotics, and at follow up 12 to 24 h after presentation. IL-1β, IL-6, and IL-8 concentrations were measured by enzyme-linked immunosorbent assay. Creatinine concentrations were also determined, and cytokine/creatinine ratios were calculated to standardize samples. Differences between preantibiotic and follow-up cytokine/creatinine ratios were significant for IL-1β, IL-6, and IL-8 (P < 0.01). Differences between preantibiotic and control cytokine/creatinine ratios were also significant for IL-1β, IL-6, and IL-8 (P < 0.01). Our study revealed that the urinary tract cytokine response to infection is intense but dissipates shortly after the initiation of antibiotic treatment. This suggests that renal damage due to inflammation begins early in infection, underscoring the need for rapid diagnosis and intervention.

Escherichia coli is the most common organism present (80%) in UTI, although other enteric organisms such as Klebsiella sp. and enterococci, as well as staphylococci, have been identified (21). P fimbriae mediate the attachment of E. coli to intestinal and uroepithelial cells and, along with the lipid A moiety of lipopolysaccharide (endotoxin), have been shown to enhance activation of the host inflammatory response. Cytokines mediate this response (3, 33), including interleukin-1 (IL-1), IL-6, and IL-8. In general, IL-1β and IL-6 appear early in the process of inflammation and are involved in lymphocyte proliferation and differentiation, as well as neutrophil activation. IL-8 functions predominantly as a chemoattractant factor for neutrophils and is produced locally at the site of infection and resulting inflammation.

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In this study, we compare the urinary levels of IL-1β, IL-6, and IL-8 in patients with pyelonephritis and in healthy children without apparent infection to establish a cytokine profile for pyelonephritis over time and in relation to antibiotic administration. This information will be useful in determining the optimal timing for anti-inflammatory intervention.

Patient information. We obtained urine samples from 13 random patients (2 male, 11 female; age, 1 month to 8 years; mean, 29 months) admitted to Children’s Hospital of Orange County with the diagnosis of pyelonephritis during the period from February through December 1997. Urine samples were also obtained from nine random healthy children (five male, four female; age, 5 to 18 years; mean, 11 years) who presented to Children’s Hospital of Orange County outpatient clinic during the same time period. The protocol was approved by the

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Inoculation onto the Vitek UID system (bioMérieux) were obtained after inoculation of 0.001 ml of urine onto blood agar, as well as was performed by using the Iris Strip method (Iris, Chatsworth, Calif.), and antibiotics (postantibiotic), and 12 to 48 h after presentation (follow-up). Urinalysis was immediately frozen at –70°C.

Patients were excluded if they had history of urinary tract abnormalities, vesicoureteral reflux (VUR), immunosuppression, or antibiotic use (prophylaxis or a history of previous pyelonephritis).

Control urine samples were obtained from healthy children (VUR), immunosuppression, or antibiotic use (prophylaxis or a history of previous pyelonephritis). Patients with pyelonephritis were screened. Among patients who had a positive urine culture, the diagnosis of pyelonephritis was established by the presence of pyuria (white blood cell [WBC] counts in unspun urine of ≥10 cells/high-powered field) and at least one of the following indicators of systemic involvement: temperature of ≥38.5°C, peripheral WBC count of >15,000 cells/μl, or C-reactive protein level of ≥3.0 g/dl. One patient was included who had only 5 to 9 WBCs in unspun urine/hpf because of the presence of a positive urine culture in conjunction with high fever (106°C) and an elevated C-reactive protein level (15.7 g/dl), which is highly suggestive of pyelonephritis.

Follow-up versus control P < 0.01. Preantibiotic versus control P < 0.01.

Cytokine and creatinine assays. IL-1β, IL-6, and IL-8 levels were determined by enzyme-linked immunosorbent assay kits commercially available from Immunootech (Westbrook, Maine). The lower limits of detection for IL-1β, IL-6, and IL-8 were 15, 3, and 8 pg/ml, respectively. Urinary cytokine was measured by using a commercially available kit from Sigma Diagnostics (St. Louis, Mo.), and microscopic evaluation was obtained by using the Iris system. Urine cultures were obtained after inoculation of 0.001 ml of urine onto blood agar, as well as inoculation onto the Vitek UID system (bioMérieux). Samples for cytokine analysis were immediately frozen at –70°C.

**RESULTS**

Urine samples were obtained from 13 patients diagnosed with pyelonephritis and from 9 healthy children. However, samples were not available from all patients at all three collection times. No control child was febrile at the time of sample collection. Of children diagnosed with pyelonephritis, all were febrile (38.3 to 41.2°C; mean, 39.6°C) and all had positive urine cultures (E. coli in all cases). The duration of illness prior to presentation ranged from <24 h to 22 days (mean, 4 days), with the majority of patients presenting within 5 days (six patients, ≤1 day; five patients, 1 to 5 days). Initial urinalysis revealed positive nitrite in 6 of 13 samples, a leukocyte esterase level of ≥2+ in 9 of 13 samples, and pyuria in all cases, ranging from 5 to 9 WBCs/hpf (one patient) to TNTC (too numerous to count). Peripherial blood WBC counts ranged from 7,900 to 34,700 cells/μl (mean, 16,100 cells/μl), and the C-reactive protein level was ≥3.0 g/dl in six of the seven patients tested. Nine patients were treated with broad-spectrum cephalosporins. Of the 10 voiding cystourethrogram studies performed, 3 were reportedly abnormal, demonstrating VUR.

**DISCUSSION**

The lipid A component of endotoxin and P fimbriae present in E. coli and other gram-negative bacteria promote an inflammatory reaction that has been linked to renal scarring. Prior studies have shown that IL-1β, IL-6, and IL-8 participate in this response, and all have been found in elevated quantities in the urine of patients with UTI.

IL-1 is a monokine that works synergistically with other cytokines to promote T-and B-cell proliferation. IL-1 induces cyclooxygenase and lipoxygenase gene expression, induces acute-phase response, induces production of IL-6, and acts as an endogenous pyrogen (19, 22). IL-1β has been detected in urine samples from patients with UTI (3).
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endogenous pyrogen (19, 22). It has been shown that IL-6
levels in urine increase within minutes of mucosal challenge
with E. coli expressing P fimbriae and, within hours, polymor-
phonuclear leukocytes are recruited and excreted into the
urine (23). IL-6 has been detected in significantly elevated
levels in the urine of patients with UTI (5, 6, 34).

IL-8 is a chemokine that acts as a chemotactic factor for
neutrophils, T-lymphocyte subsets, and basophils and activates
neutrophils to release lysosomal enzymes, undergo a respira-
tory burst, and degranulate (19, 22). Production of IL-8 by
mesangial cells has been demonstrated in response to IL-1β
and tumor necrosis factor alpha but not to lipopolysaccharide
(7). Elevated levels of IL-8 have been found in the majority of
urine specimens tested from patients with UTI (6, 18, 34). In
addition, when specimens were tested for neutrophil chemo-
tactic activity, only urine from infected subjects exhibited ac-
tivity (18).

Previous studies with animal models have demonstrated that
renal scarring is a result of the acute infection rather than the
continued presence of microorganisms in the urinary tract (10,
11, 26, 28). Further, early institution of antibiotic therapy has
been shown in animal experiments to mitigate the extent of
renal scarring (11, 26).

Rushton and colleagues have demonstrated in children with
DMSA scintigraphy that the renal parenchyma is capable of
recovery from the acute inflammatory changes associated with
pyelonephritis (29). These researchers also concluded that ac-
quired renal scarring occurs only at sites corresponding to
previous areas of acute pyelonephritis. It follows that early and
accurate identification and treatment of patients with acute
pyelonephritis would provide the best opportunity for reversal
of the inflammatory changes and thus the prevention of renal
scarring. In one study of febrile infants less than 1 year of age,
7.5% with no apparent source of infection were found to have
UTI (14). This underscores the need to include evaluation for
UTI in febrile children, so that appropriate therapy can be
initiated in a timely fashion.

Renal scarring has been reported in up to 65% of patients
with pyelonephritis. The development of scars in early life,
particularly in patients with VUR, has been correlated with the
development of hypertension, pre-eclampsia, renal insuf-
ciency, and end-stage renal disease. Tullus et al. showed that
the initial IL-6 level in urine of children with pyelonephritis
correlated with DMSA uptake defects in the acute phase as
well as 1 year later (35). These data suggest that modulating
the inflammatory response in patients with UTI should de-
crease the development of renal scars and the sequelae associ-
ated with them. The potential role for immune modulators in
sepsis is being intensely evaluated. Previously, meningitis stud-
ies showed that the use of steroids blunted the inflammatory
response and decreased the frequency of hearing loss (20). The
timing of the anti-inflammatory intervention appeared to be
crucial in these studies. Arditi et al. demonstrated in an animal
model that the use of antibiotics in meningitis resulted in the
release of endotoxin, which mediated an increase in inflam-
matory cytokines (2). These data provide insight into the best
time for steroid use in patients with meningitis.

If any intervention is to be instituted to modulate the in-
flammatory response and decrease the renal scarring that oc-
curs during pyelonephritis, it is critical that we understand the dynamics of cytokine release during this infectious process. To our knowledge, we are the first ones to attempt to establish the point at which peak cytokine release occurs in infection, if antibiotic treatment has any impact on it, and if this process is protracted or short-lived. We had anticipated that we would see an increase in the level of urinary cytokines after the addition of antibiotics, similar to what Arditi et al. observed in meningitis. We had hoped that this would provide a window of opportunity for the potential use of anti-inflammatory intervention. Our results, however, showed that a significant inflammatory process, as evidenced by the levels of IL-6, IL-8, and IL-1α, was already present at the time of diagnosis. Our results also showed that, when appropriate antibiotic treatment is initiated, the inflammatory process ceases rapidly, as noted by cytokine levels returning to control values. Our data strongly suggest, as did those of Hoferman et al. (15), that early recognition and treatment of UTI decrease the occurrence of renal scarring. It is imperative that awareness is raised regarding the frequency of UTI in febrile children, even when infection is not apparent. It also may be inferred from our data that children at risk for recurrent UTI and its sequelae may be candidates to evaluate the safety and efficacy of long-term anti-inflammatory intervention that would blunt cytokine release and minimize the damaging effects of the inflammation that occurs during each recurrence of infection.

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REFERENCES


