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

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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 pre-antibiotic and follow-up cytokine/creatinine ratios were significant for IL-1β, IL-6, and IL-8 (P < 0.01). Differences between pre-antibiotic 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 (8, 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 chemotactic factor for neutrophils and is produced locally at the site of infection and resulting inflammation.

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.

MATERIALS AND METHODS

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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TABLE 1. Mean cytokine/creatinine ratios

| Group          | Mean ratio ± SEM* (range; n) | IL-1β/Cr | IL-6/Cr | IL-8/Cr |
|----------------|------------------------------|----------|---------|---------|
| Preantibiotic  | 125.17 ± 23.2^c (18.6–2.145; 9) | 133.28 ± 51.5^c (23.9–548.8; 10) | 745.46 ± 212.7^c (140.4–2195.9; 10) |
| Postantibiotic | 86.32 ± 16.3 (18.7–185.2; 13)  | 100.72 ± 36.4 (10.3–468.8; 13)  | 700.26 ± 196.2 (99.6–2625.2; 13)  |
| Follow-up      | 23.24 ± 6.4^c (0–61.6; 11)     | 20.53 ± 4.9^c (4.0–58.0; 11)     | 117.55 ± 39.4^c (0–404.1; 11)     |
| Control        | 6.08 ± 2.2 (0.9–21.4; 9)       | 6.62 ± 1.5 (2.1–13.6; 9)         | 16.15 ± 11.9 (0–106.3; 9)         |

a Cr, creatinine; n, number of samples.  
b Preantibiotic versus follow-up P < 0.01.  
c Preantibiotic versus control P < 0.01.  
d Follow-up versus control P < 0.01.  
e Follow-up versus control P < 0.05.

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 TNC (too numerous to count). Peripheral 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 gm/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). IL-6 is a lymphokine which, among other functions, pro-
motes growth and differentiation of T and B cells and acts as an 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, polymorphonuclear 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 respiratory 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 chemotactic activity, only urine from infected subjects exhibited activity (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 acquired 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 insufficiency, 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 decrease the development of renal scars and the sequelae associated with them. The potential role for immune modulators in sepsis is being intensely evaluated. Previously, meningitis studies 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 inflammatory 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 inflammatory 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-1β, 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 Hoberman 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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