Tumour necrosis factor soluble receptors I and II and interleukin-1 receptor antagonist in acute pyelonephritis in relation to bacterial virulence-associated traits and renal function

S. H. Jacobson¹, Y. Lu² and A. Brauner²

¹Division of Nephrology, Department of Medicine, and ²Department of Microbiology, Karolinska Hospital, Stockholm, Sweden

Abstract Urinary tract infections activate both mucosal and systemic inflammatory responses reflected by elevation of cytokine concentrations in serum and urine. We determined urine and serum concentrations of tumour necrosis factor soluble receptors I and II (sTNFR I and sTNFR II) and interleukin-1 receptor antagonist (IL-1ra) in 41 women with acute pyelonephritis caused by Escherichia coli, 2 weeks after the infection, during a subsequent episode of cystitis or asymptomatic bacteriuria and also later when the same patients were free from bacteriuria. Concentrations of sTNFR I, sTNFR II and IL-1ra were related to the expression of five virulence markers of E. coli, glomerular filtration rate (GFR) and to the concentration of C-reactive protein (CRP) in serum. Patients with acute pyelonephritis had elevated serum concentrations of sTNFR I and sTNFR II compared to healthy women (P<0.001 for both comparisons). The concentrations of sTNFR I and sTNFR II in urine were significantly higher in patients with acute pyelonephritis compared to controls (P<0.001 in both cases). The concentration of sTNFR II in urine was higher in patients infected by E. coli producing haemolysin (P=0.05) and in patients infected by E. coli expressing hydrophobic properties (P=0.05) compared to patients infected by strains without these virulence traits. Patients who had high concentrations of sTNFR II in serum during acute pyelonephritis had lower GFR at follow-up (r=-0.48, P=0.05). Patients who responded with a marked increase in CRP had higher sTNFR I and sTNFR II in urine (r=0.58, P<0.01) and sTNFR II in urine (r=0.48, P<0.01, respectively). The concentrations of sTNFR I and sTNFR II in serum and urine decreased during follow-up and were lower 2 weeks after the infection when all patients were free from bacteriuria. IL-1ra in serum was elevated during pyelonephritis (P<0.001) while that in urine was significantly lower compared to controls (P<0.001). It is concluded that the increased concentrations of TNF receptors may block the cytotoxic and inflammatory actions and reduce the sensibility of renal cells to TNFa-mediated effects.

Key words: cytokine receptors; interleukin-1; acute pyelonephritis; renal function; tumour necrosis factor; urinary tract infection

Introduction

Gram-negative bacteraemia or endotoxaemia activate the immune apparatus with the production of a broad spectrum of cytokines. Injection of lipopolysaccharides in human volunteers results in a rapid increase in the serum concentrations of tumour necrosis factor α(TNF), interleukin (IL)-1β, IL-1 receptor antagonist (IL-1ra) and soluble TNF receptors I and II (sTNFR I and sTNFR II) [1,2].

Urinary tract infections activate both mucosal and systemic inflammatory responses, including elevation of IL-2, IL-2 inhibitor, IL-6 and IL-8 in urine and serum [3–5]. Proinflammatory cytokines are thought to be essential for an adequate host defence. However, excessive production of TNF and IL-1 causes shock, and high serum concentrations of TNF, IL-1 and IL-6 are associated with increased mortality rates [6]. This deleterious action of the proinflammatory cytokines is balanced by factors able to inhibit the activity of cytokines in biological fluids. IL-1ra blocks the proinflammatory action of IL-1 by competitive binding to the IL-1 receptor [7]. Infusion of IL-1ra in experimental shock improves survival [8].

Two soluble TNF receptors (sTNFR) that are able to bind to free TNFα have been described. sTNFR I and sTNFR II represent the extracellular domains of two TNF receptors that can be released from the cell surface by proteolytic cleavage [9]. In contrast to the short half-life and rapid clearance of IL-1 and TNFα, the soluble TNF receptors and IL-1ra remain in circulation for a longer period of time [2,10]. This suggests
either an ongoing production or a slow clearance of these substances.

We therefore decided to study the concentrations of sTNFR I and sTNFR II and IL-1 ra in patients with acute non-obstructive pyelonephritis caused by Escherichia coli. We also examined the relationship between the concentrations of these soluble cytokine receptors and the expression of five bacterial virulence markers [expression of P-fimbriae, cell surface hydrophobic properties, haemolysin synthesis, production of cytotoxic necrotizing factor (CNF) and aerobactin], levels of C-reactive protein (CRP) and renal function after the renal infection.

Subjects and methods

Subjects

Blood and urine samples were collected from 41 women who were admitted to the Karolinska Hospital because of clinical and laboratory signs of acute, non-obstructive pyelonephritis. Mean age of the patients was 32 years (range 16–70 years). The diagnostic criteria for acute pyelonephritis were bacteriuria (>10^5 bacteria/ml urine) increased CRP>20 mg/l, fever >38°C and clinical signs of acute pyelonephritis including flank pain without concurrent symptoms or signs of other infections. Two patients had both acute pyelonephritis and E. coli bacteremia. All patients underwent intravenous urography during the follow-up period and six patients had signs of pyelonephritic renal scarring, i.e. calyceal clubbing in combination with a corresponding reduction of the renal parenchyma. All these patients had a history of previous acute pyelonephritis. All episodes of acute pyelonephritis were caused by E. coli. Patients with acute pyelonephritis were treated with antibiotics intravenously for 3–5 days followed by 2 weeks of oral medication. If infection recurred, treatment was reinitiated with the appropriate agent for 7–10 days and the patient was then usually established on a long-term low-dose prophylactic regime.

Urine and blood (n=19) samples were also collected approximately 2 weeks after the episode of acute pyelonephritis (mean 16 days, range 10–39 days). The patients were then encouraged to attend the renal ward at any time without appointment if they had symptoms or signs of urinary tract infection (UTI). A mid-stream urine sample was cultured at the outpatient clinic, usually 6 months after the infection. Blood and urine samples were also collected approximately 2 weeks after the episode of acute pyelonephritis (mean 16 days, range 10–39 days). The patients were then encouraged to attend the renal ward at any time without appointment if they had symptoms or signs of urinary tract infection (UTI). A mid-stream urine sample was cultured at the outpatient clinic, usually 6 months after the infection.

Viral characteristics of E. coli

Numbers of bacteria in the urine were estimated by the standard loop technique. Isolates were identified biochemically by means of the API 20 E-system (API, La Balme-les Grottes, France). The following five virulence characteristics of E. coli were sought in the present study: expression of P-fimbriae, expression of cell surface hydrophobic properties, haemolysin synthesis, CNF production and production of the iron-binding siderophore aerobactin. These were analysed as previously described [11–13].

Cytokine determination

Cytokines were determined by enzyme immunoassay (EIA). TNFz and IL-1/3 kits were obtained from Medgenix Diagnostics (Fleurus, Belgium), sTNFR-I, sTNFR II and IL-1ra kits from R&D systems (Abingdon, Oxon, UK). The limit of detection in urine and serum was defined as 15 pg/ml for TNFz, 10 pg/ml for IL-1/3 and 7.8 pg/ml for sTNFR I and sTNFR II. IL-1ra was detected in urine at 31.3 pg/ml and in serum at 46.9 pg/ml. Both free receptors and receptors bound to TNFz were measured with the employed assays. There is no cross-reaction observed between the two receptors.

Renal function

Glomerular filtration rate (GFR) was determined by the plasma clearance of ^{51}Cr-EDTA, 2–3 month after the renal infection.

Statistical analyses

Results are given as means±SEM, medians and range. Simple regression analyses, the Mann–Whitney U-test, Wilcoxon signed-rank test and χ2 analysis with continuity correction were used for paired and unpaired observations.

Results

The patients were admitted to the hospital a mean of 2 days (range 1–6 days) after the onset of clinical signs and subjective symptoms of infection. Mean CRP on admission was 75±7 mg/l, mean leukocyte count was 11.7±0.7x10^9/l and mean erythrocyte sedimentation rate was 33±3 mm/h.

sTNFR I and sTNFR II in urine and serum during and after acute pyelonephritis

The concentrations of sTNFR I and sTNFR II in urine during acute pyelonephritis were significantly higher compared to those in healthy subjects (P<0.001 in both cases, Table 1). The concentration of these soluble receptors in urine decreased during follow-up and was significantly lower 2 weeks after the infection (P<0.05 in both cases, Table 1). The urine concentrations of sTNFR I and sTNFR II during subsequent episodes of cystitis or ABU are presented in Table 1. The serum concentrations of sTNFR I and sTNFR II decreased during follow-up (P<0.001 in both cases, Table 2). The serum concentrations of sTNFR I and sTNFR II decreased during follow-up (P<0.001 in both cases, Table 2) but were also at that time point significantly higher than in healthy subjects (P<0.05 and P<0.001, respectively).
Soluble tumour necrosis factor receptors in acute pyelonephritis

Table 1. Concentration (median value and range) of sTNFR I (pg/ml), sTNFR II (pg/ml) and IL-1ra (pg/ml) in urine during and after an episode of acute pyelonephritis

<table>
<thead>
<tr>
<th>Condition</th>
<th>sTNFR I</th>
<th>sTNFR II</th>
<th>IL-1ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute pyelonephritis (n=41)</td>
<td>2500±5±</td>
<td>6300±6±</td>
<td>40±3±</td>
</tr>
<tr>
<td>Two weeks after acute pyelonephritis (n=19)</td>
<td>1125±3±</td>
<td>2260±6±</td>
<td>nd±3±</td>
</tr>
<tr>
<td>Patients without bacteriuria (n=20)</td>
<td>1360±4±</td>
<td>2720±5±</td>
<td>nd±3±</td>
</tr>
<tr>
<td>Cystitis (n=6)</td>
<td>460±2±</td>
<td>1470±5±</td>
<td>42±5±</td>
</tr>
<tr>
<td>ABU (n=6)</td>
<td>460±2±</td>
<td>980±3±</td>
<td>nd±3±</td>
</tr>
<tr>
<td>Healthy subjects (n=26)</td>
<td>1125±2±</td>
<td>1980±3±</td>
<td>8400±4±</td>
</tr>
</tbody>
</table>

*P<0.001 vs healthy subjects (Mann–Whitney U-test). †P<0.05 vs 2 weeks after acute pyelonephritis (Wilcoxon signed-rank test). ‡P<0.002 vs patients without bacteriuria (Wilcoxon signed-rank test). **P<0.002 vs healthy subjects (χ² analysis).

There was a significant correlation between the serum and urine concentrations of sTNFR I (r=0.46, P<0.05) but no correlation was observed between the serum and urine concentrations of sTNFR II.

The concentration of TNFα was determined in serum and urine in the present study but was undetectable in all patients with acute pyelonephritis at the time of sampling.

IL-1ra in urine and serum during and after acute pyelonephritis

The urine concentration of IL-1ra was significantly lower during and 2 weeks after acute pyelonephritis compared to healthy subjects (P<0.001 and P<0.002, respectively, Table 1). The median concentration of IL-1ra in urine was lower 2 weeks after pyelonephritis compared to during infection but the differences in concentrations were not significantly different. Table 1 shows the concentration of IL-1ra in urine during subsequent episodes of cystitis or ABU.

On admission because of acute pyelonephritis, IL-1ra in serum was significantly higher than in healthy controls (P<0.001, Table 2). The IL-1ra concentration in serum decreased during follow-up and was significantly lower 2 weeks after the infection when antibiotic treatment was completed (P<0.001). The concentration was also elevated at this time point compared to healthy subjects (P<0.001).

We also determined the IL-1β concentrations in serum and urine in the present investigation. However, most patients had low or undetectable concentrations.

Correlation between bacterial virulence-associated traits of the infecting strain and sTNFR I, sTNFR II and IL-1ra in serum and urine

Virulence markers of the infecting E. coli are presented in Table 3. The concentration of sTNFR II in urine was higher in patients infected by E. coli producing haemolysin (15753±3652 pg/ml) compared to patients infected by haemolysin-negative strains (9774±2954 pg/ml, P<0.05). Patients infected by E. coli expressing hydrophobic properties had higher concentrations of sTNFR II in urine compared to those infected by less hydrophobic strains (P=0.05). No significant differences were observed for P-fimbriation, or CNF or aerobactin production with regard to cytokine receptor production. There were no differences in the urine concentrations of sTNFR I or IL-1ra with respect to the expression or production of the other virulence markers examined. Likewise, no differences were found in serum for sTNFR I, II or IL-1ra.

Renal function in relation to sTNFR I, sTNFR II and IL-1ra

The mean GFR was 99±4 ml/min/1.73 m². The serum concentration of sTNFR II during acute pyelonephritis correlated negatively to GFR after the infection (r = -0.48, P=0.05, Fig. 1). Thus, patients with a high concentration of sTNFR II during pyelonephritis had lower renal function 2–3 months after the renal infection. No correlations were observed between urinary concentrations of soluble TNF receptors, serum and urine levels of IL-1ra and renal function.

Table 3. Virulence characteristics of the pyelonephriogenic E. coli strains

<table>
<thead>
<tr>
<th>Virulence marker</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression of P-fimbriae</td>
<td>72</td>
</tr>
<tr>
<td>Production of Haemolysin</td>
<td>44</td>
</tr>
<tr>
<td>Cytotoxic necrotizing factor</td>
<td>26</td>
</tr>
<tr>
<td>Aerobactin</td>
<td>68</td>
</tr>
<tr>
<td>Cell surface hydrophobic properties</td>
<td>83</td>
</tr>
</tbody>
</table>
Correlation between CRP and sTNFR I, sTNFR II and IL-1ra in serum and urine

Patients with high levels of CRP in serum had higher concentrations of sTNFR I in urine ($r = 0.58$, $P < 0.01$) and sTNFR II in urine ($r = 0.48$, $P < 0.01$) during the episode of acute pyelonephritis. There were however no significant correlations between the concentration of CRP and serum concentrations of soluble TNF receptors or the concentration of IL-1ra in serum and urine.

Discussion

We have investigated the local and systemic concentrations of the naturally occurring soluble receptors of TNF and the IL-1 receptor antagonist in patients with acute pyelonephritis and during subsequent episodes of cystitis and asymptomatic bacteriuria. The concentrations of sTNFR I, sTNFR II and IL-1ra in serum and urine were also related to the expression or production of five different bacterial virulence characteristics of $E. coli$ causing the renal infection, to renal function and to the degree of systemic inflammation measured as the concentration of CRP in serum.

Patients with acute pyelonephritis had higher concentrations of sTNFR I and sTNFR II in both urine and serum during an episode with acute pyelonephritis than healthy individuals. Both the urine and serum concentrations decreased during antibiotic treatment. The urine levels were similar to those in healthy subjects 2 weeks after the infection, while a prolonged elevation in the serum concentrations was observed for sTNFR I and sTNFR II. The urine concentrations of sTNFR I and sTNFR II were not elevated in those patients who had a subsequent episode of cystitis or asymptomatic bacteriuria. We are not aware of any other study focusing on the concentrations of soluble TNF receptors in patients with acute pyelonephritis. However, circulating levels of these receptors are elevated in several disease states including sepsis, meningitis, autoimmunity, malignancy, allograft rejection, hairy cell leukaemia, type B chronic lymphocytic leukaemia, HIV infection and in patients with end-stage renal disease, as well as in patients on continuous ambulatory peritoneal dialysis or haemodialysis [2,14,15].

sTNFR I and sTNFR II are the extracellular domains of the TNF receptor types I and II and are apparently shed from the cell surface in response to many of the same inflammatory stimuli that are known to induce TNFz production [2,10]. The shedding of such receptors and resultant acute decrease in the number of TNF receptors on the cell surface may serve to transiently desensitize cells thereby providing a mechanism for inhibition of TNFz activity. Released soluble receptors may inhibit TNFz bioactivity by binding to the molecule and preventing ligand binding to the cellular TNF receptors. Hence, the appearance of such extracellular soluble receptors may provide a regulatory mechanism for modulation of excessive TNFz activity arising in response to severe infection. Indeed, a high ratio between soluble TNF receptor concentration and TNFz in serum has been shown to be associated with a better clinical prognosis [2]. It remains uncertain whether soluble TNF receptors and IL-1ra are produced locally during acute pyelonephritis.

Local production of cytokines, including TNFz, in the urinary tract has previously been examined in humans [4,5] and in a mouse model of $E. coli$ pyelonephritis [16]. The presence of mRNA for specific cytokines including TNFz was demonstrated in the mouse kidney after bacterial challenge. TNFz was however not detectable in the serum or urine during the course of the infection [16]. The soluble TNF receptors appear in human urine and have also been detected in dialysates of stable patients with peritoneal dialysis and in even higher concentrations during pyelonephritis [9,17].

There are contradictory results with regard to the correlation between TNFz concentrations in serum and mortality rate [18]. There are several possible explanations for the inconsistency in TNF data with regard to disease activity and mortality. Apart from differences in assays to detect free TNF and TNF–soluble TNF receptor complexes, TNF is present in serum only in short intervals during the sepsis syndrome [10,19]. TNFz was determined in serum and urine in the present study but was undetectable in all patients at the time of sampling. The mean time that elapsed between the start of symptoms and presentation at the renal ward was 2 days, which may explain the failure to detect elevated concentrations in this study.

We observed a significant negative correlation between the concentration of sTNFR II in serum and GFR 2–3 months after pyelonephritis. Increased soluble TNF receptor concentrations have not only been demonstrated in patients with inflammatory diseases; increased concentrations of soluble TNF receptors have been detected in patients with chronic renal failure as well as in patients on haemodialysis and on CAPD.
Soluble tumour necrosis factor receptors in acute pyelonephritis

[15,17]. Furthermore, a positive correlation between both soluble TNF receptors and serum creatinine concentrations was demonstrated in patients with sepsis syndrome [18]. Moreover, experimental data from a mice model have shown that the kidneys play a central role in the clearance of TNF receptors and TNF-TNF receptor complexes [20]. The increased concentrations of soluble TNF receptors could be explained by increased production or reduced clearance due to renal insufficiency. It has not yet been elucidated which of these mechanisms is the most important, or if both are equally important.

We have also examined the correlation between serum and urine concentrations of soluble TNF receptors and the expression of five different virulence markers of E. coli causing acute pyelonephritis. Patients infected with E. coli with the ability to synthesize haemolysin had significantly higher concentrations of sTNFR II in urine compared to patients infected by strains without this capacity. sTNFR II was also higher in patients infected by E. coli strains with increased hydrophobic properties. This has not been reported before, but is consistent with our previous observation that patients infected with haemolysin-producing E. coli strains have higher concentrations of IL-6 in serum [4]. There were however no correlations between adherence properties of E. coli and soluble receptor concentrations in this study.

A significant correlation between CRP levels in serum and concentrations of sTNFR I and sTNFR II in urine was observed in the present study. There are conflicting results with regard to the correlation between CRP levels and cytokine concentrations in the literature [4]. The correlation in the present study may be due to the fact that CRP is synthesized in the liver after induction with a prolonged increase in serum concentration resulting in a temporal correlation with the extended period in which soluble TNF receptors appear in circulation [2]. Patients with acute pyelonephritis usually have increased concentrations of CRP while patients with bladder infections have normal concentrations. This accords with the fact that sTNFR I and sTNFR II were not elevated during a subsequent episode of asymptomatic bacteriuria or cystitis in our group of patients.

Patients with acute pyelonephritis in the present study had significantly higher IL-1ra concentrations in serum compared to healthy controls. The serum IL-1ra concentration was significantly lower after 2 weeks of antibiotic treatment compared to during pyelonephritis, but still significantly elevated also at that point compared to the concentration in healthy subjects. Similar to what has been stated about soluble TNF receptors, IL-1ra levels also decrease slowly during disease, which may reflect either slow renal clearance or ongoing production of the antagonist [15]. By contrast, only a few patients with acute pyelonephritis had elevated IL-1ra concentrations in urine. The concentration was significantly lower (smaller proportion of individuals with detectable IL-1ra concentrations in urine) compared to that in healthy controls. This accords with our observations in a recent study of children with acute pyelonephritis [21]. The highest urinary IL-1ra levels in that study were found in healthy controls. The mechanisms underlying the decrease in IL-1ra in urine observed in patients with acute pyelonephritis remain elusive. This could be due to consumption in the urine of naturally occurring IL-1ra. The finding of even lower concentrations of IL-1ra in the urine 2 weeks after the infection may support this notion. In children, the lowest concentrations of IL-1ra were observed in convalescence and in children with recurrent infections which also may support this theory [21].

High serum levels of IL-1β have been associated with high mortality rates in patients with septicemia [1]. Several studies have however failed to detect increased serum IL-1β concentrations during sepsis, and even negative correlations between the severity of disease and the concentration of IL-1β has been observed [22]. We determined the IL-1β concentrations in serum and urine in the present investigation, but most patients had low or undetectable concentrations, which probably is due to the transient early appearance of IL-1β even during severe infections [1].

In conclusion, patients with acute non-obstructive E. coli pyelonephritis have increased concentrations of sTNFR I and sTNFR II in both serum and in urine and also increased IL-1ra concentrations in serum. The highest serum concentrations of sTNFR II were observed in patients with decreased renal function at follow-up. Patients infected by E. coli with the capacity to synthesize haemolysin had higher concentrations of sTNFR II in urine. The increased concentrations of TNF receptors may block the cytotoxic actions and reduce the sensibility of renal cells to TNFα-mediated effects. The appearance of soluble cytokine receptors may thus provide a regulatory mechanism for modulation of excessive inflammatory activity arising in response to renal infections.

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References

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