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ORIGINAL ARTICLE

Strong inflammatory cytokine response in male and strong anti-inflammatory response in female kidney transplant recipients with urinary tract infection

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Summary

Urinary tract infection (UTI) is the most common post-transplant infection in renal transplant recipients. The relationship of plasma and urine cytokines with UTI after kidney transplantation has not yet been delineated and literature reports on cytokine and UTI are rare. In a retrospective study, we compared post-transplant plasma and urine cytokine levels of 132 outpatient renal transplant recipients with or without UTI. Soluble interleukin-1 receptor antagonist (sIL-1RA), IL-2, sIL-2R, IL-3, IL-4, IL-6, sIL-6R, IL-8, IL-10, transforming growth factor-β2 (TGF-β2), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) levels were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits. We found gender-related urine cytokine patterns. Anti-inflammatory sIL-1RA was significantly higher in females than in males and this gender-related difference was more pronounced in bacteriuric ($P < 0.0001$) than in nonbacteriuric ($P = 0.001$) patients. Urine proinflammatory cytokines IL-6 ($P = 0.001$) and IL-8 ($P = 0.007$) were significantly higher in male patients with bacteriuria than in males without bacteriuria and sIL-2R ($P = 0.001$) and sIL-6R ($P = 0.03$) were significantly higher in males with leukocyturia than in males without leukocyturia. Bacteriuria in males was associated with higher doses of immunosuppressive drugs ($P = 0.02$). Male renal transplant recipients with UTI have a strong inflammatory cytokine response with activation of IL-6, IL-8, sIL-2R and sIL-6R producing cells, whereas female patients with UTI block the inflammatory response to UTI by production of sIL-1RA.

Introduction

Urinary tract infection (UTI) is the most common post-transplant infection in renal transplant recipients [1–3]. Risk factors include pretransplant UTI, a prolonged period of hemodialysis before transplantation, polycystic kidney disease, diabetes mellitus, postoperative bladder catheterization, allograft trauma, female sex, schistosomiasis, and technical complications associated with ureteral anastomosis [4,5]. The influence of UTI on graft survival and on triggering rejection needs further elucidation [3,6,7]. Because UTI, especially during the early post-transplant period, is commonly associated with pyelonephritis and bacteremia, careful surveillance is necessary to identify and eliminate these infections [8,9]. Patients with a renal transplant require lifelong immunosuppression and remain susceptible to infection. However, these medications also can mask the symptoms of infection, making

the diagnosis of infection based on the patient's symptoms problematic [10]. Renal transplant recipients with UTI, as well as other immunocompromised patients, are at high risk of bacteremia and should receive antibiotic therapy when they exhibit asymptomatic bacteriuria [11]. Prolonged antibiotic prophylaxis significantly reduces the incidence of UTI and bacteremia [1]. The most commonly used agents are trimethoprim-sulfamethoxazole (TMP-SMZ), and ciprofloxacin. The relationship of plasma and urine cytokines with UTI after kidney transplantation has not been clarified and literature reports on this subject is rare [12–14].

In this retrospective study, we analyzed plasma and urine levels of soluble interleukin-1 receptor antagonist (sIL-1RA), IL-2, sIL-2R, IL-3, IL-4, IL-6, sIL-6R, IL-8, IL-10, transforming growth factor (TGF)-β2, interferon (IFN)-γ, and tumor necrosis factor (TNF)α in outpatient renal transplant recipients. We hypothesized that UTIs would cause changes in plasma and urine cytokine levels during the inflammatory response. Because of the well-known preferential occurrence of UTI in females, we paid attention to the relationship of gender and cytokine patterns. Moreover, we analyzed the daily dose of immunosuppressive drugs in relation to the symptoms of UTI in males and females.

Patients and methods

Urine and plasma cytokines were determined in 388 consecutively transplanted patients between September 1995 and April 2002. 134 of the 388 patients experienced bacteriuria ($\geq 10^5$ colony-forming units of an organism per milliliter in urine cultures). 81 of 141 females (57%) and 53 of 247 males (21%) showed significant bacteriuria (relative risk = 2.7; $P < 0.0001$), and 24 of 81 bacteriuric females (30%) and eight of 53 bacteriuric males (15%) were leukocyturic (relative risk = 2.0; $P = 0.05$). For statistical analysis, patients were separated into four groups: bacteriuric men, bacteriuric women, nonbacteriuric men, and nonbacteriuric women. The diagnosis of UTI was based on the presence of more than five white blood cells (WBC) per high power field (hpf) in the urine and on the clinical decision to initiate antibiotic treatment. All patients were free of clinical symptoms of UTI such as fever and pain. Only test results obtained during ongoing UTI were evaluated in this study. If a patient was investigated several times during UTI, the first investigation was selected for statistical analysis. If a patient experienced UTI several times, only the first manifestation of UTI was analyzed. All were outpatients without evidence of acute or chronic rejection, extrarenal infection, cytomegalovirus (CMV) infection, or urologic complications. In each gender group, 33 bacteriuric patients and 33 patients without bacteriuria were matched for age and post-transplant date of investigation. The age of patients ranged between 16 and 68 years. Bacteriuric males (mean ± 1 SD: 45 ± 15 years), nonbacteriuric males (44 ± 13 years), bacteriuric females (47 ± 12 years), and nonbacteriuric females (48 ± 17 years) had similar ages. Post-transplant date of investigation was similar in bacteriuric and nonbacteriuric males (14 ± 3 months vs. 12 ± 9 months: $P = 0.103$) and females (16 ± 2 months vs. 21 ± 3 months: $P = 0.170$). All patients received prednisolone; 30 of the bacteriuric males, 27 of the nonbacteriuric males, 29 of the bacteriuric females and 26 of the nonbacteriuric females were treated with cyclosporine in addition. Thirteen of the bacteriuric males, 16 of the nonbacteriuric males, 18 of the bacteriuric females and 11 of the nonbacteriuric females received mycophenolate mofetil (MMF). Of the bacteriuric males, one patient was treated with azathioprine, one with tacrolimus, and one with sirolimus. Of nonbacteriuric males, one patient received azathioprine and one sirolimus, and six patients were treated with tacrolimus. Of the bacteriuric females, four patients received azathioprine and three patients tacrolimus. Of nonbacteriuric females, four patients received azathioprine and four patients tacrolimus. Levels of leukocyturia were analyzed in relation to daily immunosuppressive dose, plasma cytokines and urine cytokines. More than five white blood cells per hpf in the urine was considered as leukocyturia and patients were treated with antibiotics. Blood leukocyte counts of 52 bacteriuric (27 male and 25 female) and 52 nonbacteriuric patients (30 male and 22 female), and plasma levels of C-reactive protein (CRP) of 19 bacteriuric (seven male and 12 female) and 31 nonbacteriuric patients (16 male and 15 female) were available for analysis. Blood leukocyte counts and plasma levels of CRP were similar in the four groups (P = NS, Table 1).

Determination of plasma cytokines, soluble cytokine receptors, and soluble cytokine receptor antagonists

Values obtained in 40 healthy controls (mean ± 1 SD) are indicated in parentheses. Plasma sIL-1RA (x ± 1 SD: 670 ± 1164 pg/ml), IL-2 (x ± 1 SD: 24 ± 38 pg/ml), sIL-2R (x ± 1 SD: 2085 ± 2126 pg/ml), IL-3 (x ± 1 SD: 27 ± 50 pg/ml), IL-4 (x ± 1 SD: 6 ± 17 pg/ml), IL-6 (x ± 1 SD: 2 ± 12 pg/ml), sIL-6R (x ± 1 SD: 28 212 ± 7836 pg/ml), IL-10 (x ± 1 SD: 0 ± 1 pg/ml), TGF-β_2 (x ± 1 SD: 4 ± 7 pg/ml), IFN-γ (x ± 1 SD: 383 ± 859 pg/ml), and TNF-α (x ± 1 SD: 12 ± 55 pg/ml) were determined by enzyme-linked immunosorbent assay (ELISA). sIL-1RA, IL-2, sIL-2R, IL-3, IL-4, IL-6, sIL-6R, IL-10, TGF-β_2 and TNF-α were measured with Quantikine kits (R&D Systems, Wiesbaden, Germany), and IFN-γ with HBT kits (Holland Biotechnology BV, Firma Biermann,

Table 1. Demographic data of bacteriuric and nonbacteriuric patients.

Parameter	Bacteriuric		Nonbacteriuric		*P*-value	
	Male ($n = 33$)	Female ($n = 33$)	Male ($n = 33$)	Female ($n = 33$)	Male bacteriuric versus nonbacteriuric	Female bacteriuric versus nonbacteriuric
Age (years)	45 ± 15	47 ± 12	44 ± 13	48 ± 17	NS	NS
Range of age (years)	19–66	15–68	19–67	22–64	NS	NS
Post-transplant (month)	14 ± 3	12 ± 9	16 ± 2	21 ± 3	NS	NS
Plasma creatinine (mg/dl)	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.2	1.3 ± 0.3	NS	NS
Leukocyturic patients (*n*)	7	12	0	0	NS	NS
Blood leukocyte count ($\times 10^9$/l)*	9.4 ± 2.5	9.8 ± 3.7	8.2 ± 3.7	8.3 ± 2.4	NS	NS
C-reactive protein (mg/l)*	29 ± 50	8 ± 7	7 ± 4	5 ± 4	NS	NS
Immunosuppression						
Prednisolone (*n*)	33	33	33	33	NS	NS
Cyclosporin A (*n*)	30	29	27	26	NS	NS
Mycophenolate mofetil (*n*)	13	18	16	11	NS	NS
Azathioprine (*n*)	1	4	1	4	NS	NS
Tacrolimus (*n*)	1	3	6	4	≤0.050	NS
Sirolimus (*n*)	1	0	1	0	NS	NS

Data are given as mean ± 1 SD.

*Blood leukocyte count was available on 52 bacteriuric (27 male and 25 female) and 52 nonbacteriuric (30 male and 22 female) patients, and plasma levels of C-reactive protein were available on 19 bacteriuric (seven male and 12 female) and 31 nonbacteriuric (16 male and 15 female) patients.

Bad Nauheim, Germany). Plasma was snap frozen within 2 h after the blood was drawn and stored at −30 °C until testing.

Determination of urine cytokines, soluble cytokine receptors, and soluble cytokine receptor antagonists

Values obtained in 14 healthy controls (mean ± 1 SD) are indicated in parentheses. Urine sIL-1RA ($x \pm 1$ SD: 1955 ± 956 pg/ml), IL-2 ($x \pm 1$ SD: 17 ± 9 pg/ml), sIL-2R ($x \pm 1$ SD: 1339 ± 961 pg/ml), IL-3 ($x \pm 1$ SD: 20 ± 7 pg/ml), IL-4 ($x \pm 1$ SD: 2 ± 2 pg/ml), IL-6 ($x \pm 1$ SD: 2 ± 2 pg/ml), sIL-6R ($x \pm 1$ SD: 1739 ± 1096 pg/ml), IL-10 ($x \pm 1$ SD: 0.5 ± 0.5 pg/ml), TGF-β_2 ($x \pm 1$ SD: 13 ± 14 pg/ml), IFN-γ ($x \pm 1$ SD: 11 ± 14 pg/ml), and TNF-α ($x \pm 1$ SD: 0 ± 0 pg/ml) were determined by ELISA. sIL-1RA, IL-2, sIL-2R, IL-3, IL-4, IL-6, sIL-6R, IL-10, TGF-β_2, and TNF-α were measured with Quantikine kits (R&D Systems), and IFN-γ with HBT kits (Holland Biotechnology BV). All transplant recipients were outpatients at the time of testing. Urine samples were freshly obtained in the morning, snap frozen within 2 h, and stored at −30 °C until testing.

Statistical analysis

Mann–Whitney *U*-test was applied using the STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES (SPSS, Chicago, IL, USA). Adjustment for multiple testing ($n = 23$) was done according to the method of Bonferroni. *P*-values of <0.002 were considered significant and were bold printed in Table 2.

Results

Although we studied 12 different cytokines in plasma and urine, only the urine levels of sIL-1RA, IL-6, IL-8, and sIL-2R were strongly associated with UTI.

Plasma and urine cytokine levels in male versus female patients

Bacteriuric

Female bacteriuric patients ($n = 33$) had significantly higher urine sIL-1RA (3395 ± 1564 pg/ml vs. 2391 ± 1176 pg/ml: $P < 0.0001$; Fig. 1a) and a significantly higher urine/plasma ratio of sIL-1RA (4.97 ± 3.70 vs. 3.59 ± 2.10: $P = 0.040$) than bacteriuric male patients ($n = 33$). The individual daily doses of immunosuppressive drugs were similar in the two groups (data not shown).

Nonbacteriuric

Female nonbacteriuric patients ($n = 33$) had higher urine sIL-1RA (2380 ± 1173 pg/ml vs. 1461 ± 1327 pg/ml: $P = 0.001$; Fig. 1b), higher urine IL-1α (17.9 ± 24.6 pg/ml vs. 2.1 ± 3.4 pg/ml: $P = 0.05$), and a higher urine/plasma ratio of sIL-1RA (3.59 ± 2.09 vs. 2.47 ± 2.29: $P = 0.002$) than male nonbacteriuric patients ($n = 33$). The individual daily dose of immunosuppressive drugs was similar in the two groups (data not shown).

Table 2. Plasma and urine cytokines in bacteriuric versus nonbacteriuric males.

Parameter (pg/ml)	Bacteriuric ($N = 33$)	Nonbacteriuric ($N = 33$)	*P*-value
TNF-α	12 ± 20	14 ± 31	0.406
sIL-2R	2429 ± 1379	2196 ± 1954	0.145
IL-2	10 ± 13	7 ± 11	0.169
IL-3	8 ± 17	6 ± 12	0.580
IL-4	4 ± 9	15 ± 55	0.557
IL-6	18 ± 35	17 ± 40	0.389
sIL-6R	42 474 ± 14 344	39 740 ± 13 465	0.569
IL-10	6 ± 8	3 ± 3	0.315
TGF-β2	14 ± 67	3 ± 10	0.434
IL-1RA	745 ± 448	738 ± 372	0.879
IFN-γ	1278 ± 1851	1008 ± 1211	0.807
U_TNF-α	9 ± 16	4 ± 6	0.308
U_sIL-2R	1373 ± 1926	1048 ± 945	0.314
U_IL-2	13 ± 28	11 ± 16	0.865
U_IL-3	16 ± 40	8 ± 11	0.393
U_IL-4	27 ± 81	13 ± 31	0.747
U_IL-6	55 ± 193	13 ± 43	**0.001**
U_IL-8*	179 ± 394	11 ± 14	0.007
U_sIL-6R	971 ± 795	640 ± 533	0.062
U_IL-10	25 ± 98	27 ± 107	0.884
U_TGF-β2	11 ± 18	6 ± 8	0.875
U_IL-1RA	2034 ± 1561	1461 ± 1327	0.030
U_IFN-γ	64 ± 109	40 ± 85	0.401

All data are given as mean ± 1 SD.

U_, urine levels; TNF-α, tumor necrosis factor-α; sIL-2R, soluble interleukin-2 receptor; sIL-6R, soluble interleukin-6 receptor; TGF-β2, transforming growth factor-β2; sIL-1RA, soluble interleukin-1 receptor antagonist; IFN-γ, interferon-γ.

*IL-8 was measured only in nine bacteriuric and eight nonbacteriuric patients.

P-values were calculated using the Mann–Whitney *U*-test. Adjustment for multiple testing ($n = 23$) was done according to the method of Bonferroni. *P*-values of <0.002 were considered significant and are bold printed.

Because of these gender-related associations, we analyzed gender-specific cytokines and UTI symptoms.

Bacteriuric versus nonbacteriuric

Bacteriuric male patients ($n = 33$) had higher urine IL-6 ($x \pm 1$ SD: 55 ± 193 pg/ml vs. 13 ± 43 pg/ml: $P = 0.001$; Fig. 2a), higher urine IL-8 (179 ± 394 pg/ml vs. 11 ± 14 pg/ml: $P = 0.007$; Fig. 2b), and higher urine sIL-1RA (2034 ± 1561 pg/ml vs. 1461 ± 1327 pg/ml: $P = 0.030$) than nonbacteriuric male patients ($n = 33$; Table 1). The IL-6 urine/plasma ratio was higher in bacteriuric patients than in nonbacteriuric patients (2.91 ± 3.04 vs. 2.43 ± 5.57: $P = 0.020$).

In contrast to males, bacteriuric female patients ($n = 33$) had similar urine IL-6 (82 ± 293 pg/ml vs. 15 ± 36 pg/ml; $P = 0.162$) and IL-8 (79 ± 94 pg/ml vs. 114 ± 254 pg/ml; $P = 0.492$), slightly higher urine sIL-1RA (3395 ± 1564 pg/ml vs. 2391 ± 1176 pg/ml; $P = 0.020$) and a slightly higher IL-6 urine/plasma ratio (5.04 ± 13.06 vs. 1.50 ± 1.95; $P = 0.050$) than nonbacteriuric female patients.

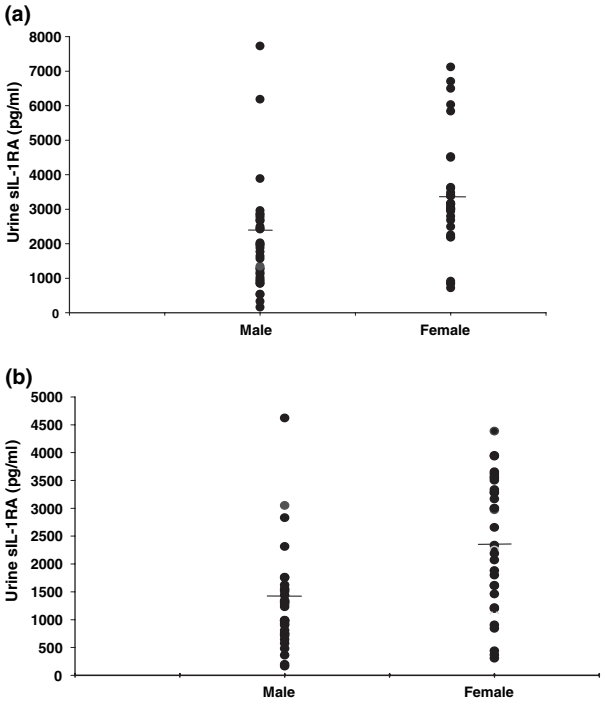

Figure 1 Anti-inflammatory cytokine soluble interleukin-1 receptor antagonist (sIL-1RA) in urine of male ($n = 33$) and female ($n = 33$) kidney recipients. There was a highly significant gender-related difference which was more pronounced in bacteriuric ($P < 0.0001$; a) than in nonbacteriuric ($P = 0.001$; b) patients. Mean values are depicted by horizontal bars.

Bacteriuria and dose of immunosuppressive drugs

Bacteriuria in male patients was associated with higher doses of immunosuppressants. All male bacteriuric and nonbacteriuric patients received prednisolone, 13 bacteriuric and 16 nonbacteriuric were treated in addition with MMF, one patient in each group received azathioprine or sirolimus, and one bacteriuric and six nonbacteriuric patients received tacrolimus during the investigation period. Bacteriuric male patients were treated with higher daily doses of prednisolone (0.11 ± 0.09 vs. 0.07 ± 0.06 mg/kg body weight: $P = 0.02$) and MMF (24 ± 9 vs. 18 ± 7 mg/kg body weight: $P = 0.03$) than nonbacteriuric male patients. Interestingly, IL-6 and IL-8 secretion were not inhibited by the high doses of prednisolone and MMF, suggesting that IL-6 and IL-8 were produced outside the blood circulation.

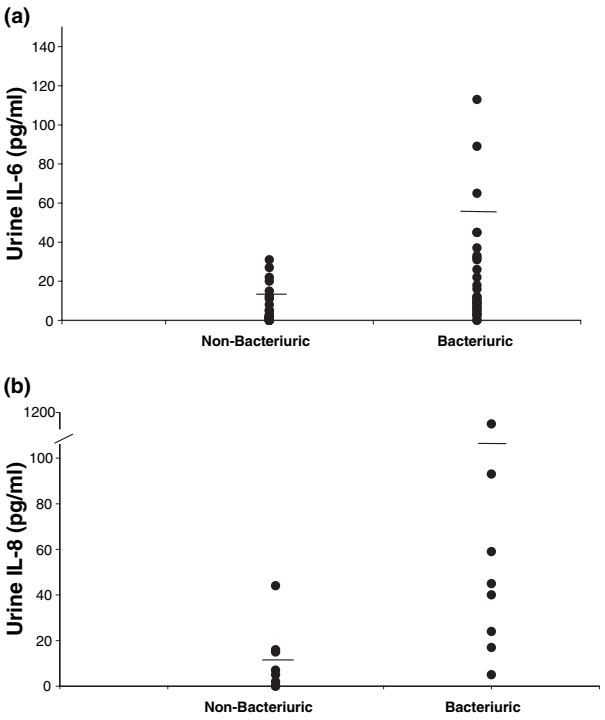

Figure 2 Urine proinflammatory cytokines interleukin (IL)-6 and IL-8 in male transplant recipients with or without bacteriuria. IL-6 ($P = 0.001$; a) and IL-8 ($P = 0.007$; b) were significantly higher in male patients with bacteriuria ($n = 33$) than in males without bacteriuria ($n = 33$). IL-8 was studied only in nine males with and eight males without bacteriuria. One bacteriuric patient had a urine IL-8 of 1152 pg/ml. Mean values are depicted by horizontal bars.

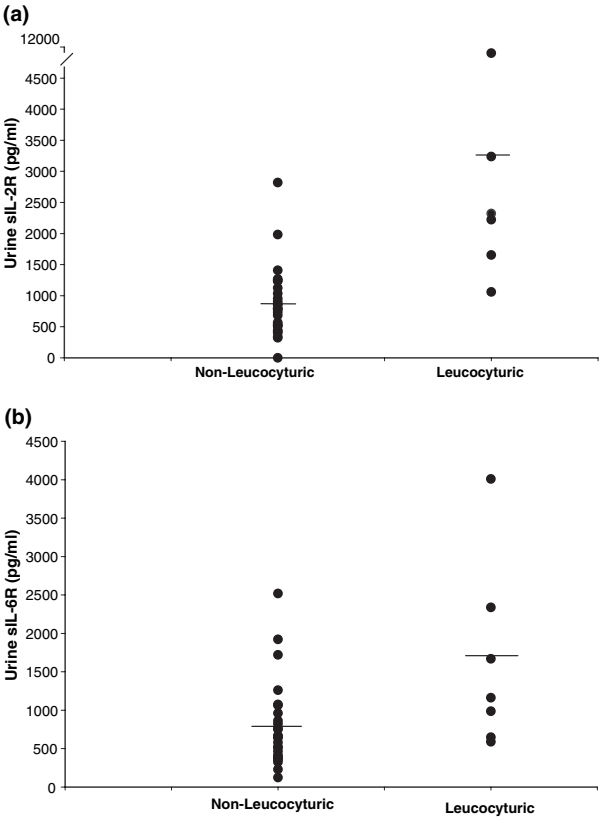

Figure 3 Urine soluble interleukin-2 receptor (sIL-2R) and soluble interleukin-6 receptor (sIL-6R) in male transplant recipients with or without leukocyturia. sIL-2R ($P = 0.0003$; a) and sIL-6R ($P = 0.03$; b) were significantly higher in male patients with leukocyturia ($n = 7$) compared with male patients without leukocyturia ($n = 26$). One leukocyturic patient had a urine sIL-2R level of 11 291 pg/ml. Mean values are depicted by horizontal bars.

In contrast to male patients, the individual daily doses of immunosuppressive drugs in female bacteriuric and nonbacteriuric patients were similar (data not shown).

Gram− versus gram+ bacteriuria

The gram− bacteria appeared to stimulate the immune system somewhat stronger than gram+ bacteria, and male patients had a more local-type response whereas female patients showed systemic cytokine stimulation.

The gram− bacteriuric male patients ($n = 13$) had a higher urine sIL-6R level (1175 ± 728 pg/ml vs. 839 ± 827 pg/ml: $P = 0.05$) and a higher urine/plasma ratio of sIL-6R (0.033 ± 0.022 vs. 0.016 ± 0.008: $P = 0.05$) than gram+ bacteriuric male patients ($n = 20$). The individual daily dose of immunosuppressive drugs was similar in the two groups (data not shown).

In contrast to male patients, gram− bacteriuric female patients ($n = 16$) had higher *plasma* sIL-2R (4028 ± 4399 pg/ml vs. 1554 ± 1536 pg/ml: $P = 0.02$) and higher *plasma* sIL-1RA (1475 ± 1303 pg/ml vs. 718 ± 393 pg/ml: $P = 0.03$) levels than gram+ bacteriuric female patients ($n = 17$) suggesting a systemic response. The individual daily doses of immunosuppressive drugs were similar in the two groups (data not shown).

Leukocyturia and bacteriuria

Not all bacteriuric patients developed leukocyturia. Only seven of the 33 male bacteriuric patients had more than five WBC/hpf in the urine (=significant leukocyturia). Leukocyturic male patients had higher urine sIL-2R (3235 ± 3643 pg/ml vs. 872 ± 577 pg/ml: $P = 0.001$; Fig. 3a), higher urine sIL-6R (1629 ± 1214 pg/ml vs. 794 ± 550 pg/ml: $P = 0.03$; Fig. 3b) and a higher urine/plasma ratio of sIL-2R (0.788 ± 0.371 vs. 0.410 ± 0.233; $P = 0.007$) than nonleukocyturic male patients. The individual daily dose of immunosuppressive drugs was similar in the two groups (data not shown).

Only 12 of 33 bacteriuric female patients had more than five WBC/hpf in the urine. Leukocyturic female patients had marginally higher plasma sIL-6R (50 500 ± 18 326 pg/ml vs. 36 348 ± 15 640 pg/ml: $P = 0.050$) than nonleukocyturic female patients. The individual daily dose of immunosuppressive drugs was similar in the two groups (data not shown).

Discussion

Urinary tract infection is the most common bacterial complication in renal transplant recipients [1–3]. Early recognition and management of UTI may improve the outcome of transplant patients. The incidence of UTI in patients not receiving prophylaxis was reported as high as 98% [1]. 57% (81 of 141) of the female and 21% (53 of 247) of the male patients suffered from bacteriuria. The rate of bacteriuria in our patients is similar to that reported in other studies [2,8], which also showed higher rates of bacteriuria and UTI in females than in males.

The aim of this study was to identify urine and plasma cytokine patterns that might be associated with bacteriuria, in the hope that this may aid the development of early effective treatment strategies of UTI. Plasma and urine cytokines were determined in blood and urine samples of 132 patients. Because UTI is an inflammatory process, evaluation of plasma and urine levels of proinflammatory cytokines are candidate indicators of UTI [12–15]. Cytokines and cytokine receptors, such as IL-1, IL-6 and sIL-6R, which are released primarily from macrophages, as well as anti-inflammatory cytokine antagonists, such as sIL-1RA, are likely to be involved in the pathogenesis of UTI. Lipopolysaccharide (LPS) derived from the outer membrane of gram− bacteria acts as a chemoattractant for monocytes and induces the release of free radicals, IL-1β, IL-6, IL-8, and TNF-α [16–18]. The cell wall of gram+ bacteria also induces the production of proinflammatory cytokines [19,20]. Recently, it was reported that mucosal pathogens trigger a local innate host response by activating epithelial cells [21]. Samuelsson *et al.* reported on Toll-like receptor 4 expression and cytokine responses in the human urinary tract mucosa after *in vitro* challenge of urinary tract biopsies with *Escherichia coli* bacteria. They observed a rapid cytokine response with production of IL-1β, IL-6, and IL-8 but not of IL-4 or IFN-γ [21]. Interestingly, however in the present study, there was no difference between plasma cytokine levels measured in patients with or without bacteriuria. It is possible that transplanted patients cannot present the classical symptoms of infection because of the effects of immunosuppressive therapy. All female and male patients studied in the present series were free of clinical symptoms of UTI. They came to the hospital for routine checkups. It is well-known that systemic IL-1 and TNF increases induce clinical symptoms such as fever and pain. Because blood leukocyte counts, plasma CRP levels, and plasma cytokine levels were similar in bacteriuric and nonbacteriuric male and female patients, we speculate that the absence of inflammation markers in the blood might be the reason for the absence in our patients of the typical clinical symptoms of UTI, such as fever or pain. Prio *et al.* [22] suggested that in frail, elderly humans asymptomatic urinary infections are associated with low-grade immune activity. The age of these patients was over 70 years. The mean age of our patients in the four groups was 44–48 years, suggesting that most of our patients should not show age-related low-grade immune activity.

Among male recipients, we found that urine IL-6 levels were higher in patients with bacteriuria than in patients without bacteriuria. Moreover, male patients with significant bacteriuria had higher levels of urine chemokine IL-8, proinflammatory cytokine receptor sIL-6R, and anti-inflammatory cytokine antagonist sIL-1RA than nonbacteriuric patients. Because the corresponding plasma levels were not increased, these data suggest that patients with bacteriuria produced IL-6, sIL-6R, IL-8, and sIL-1RA locally in the urinary tract. Soluble IL-1RA increased in the urine during culture-confirmed UTI to a level characteristic for the response to inflammation. Other authors reported significantly elevated urine IL-8 and IL-6 concentration of patients with UTI whereas plasma levels were in the normal range [13,23–25]. Several previous studies have documented elevated urine IL-6 levels in immunocompetent adults and children with UTI [26,27]. Hedges *et al.* suggested that bacteriuria is accompanied by elevated urinary IL-6 levels and that IL-6 is locally produced without any apparent relationship with bacteremia [23]. Cell types that can produce IL-6 within the urinary tract include renal fibroblasts, macrophages, and endothelial as well as epithelial cells [28–31]. To our knowledge, this is the first study to suggest that IL-6 is released into the urine of renal transplant recipients during bacteriuria. It is interesting that IL-6, sIL-6R, IL-8, and sIL-1RA cytokine levels were elevated in the urine but not in the plasma. Most plausibly these cytokines are produced in the urinary tract by tissue cells and macrophages, possibly as a result of the lower concentration of immunosuppressive drugs in the urine than in the blood. Only minor quantities of intact ester or intact prednisolone are excreted in the urine [32,33]. The concentration of prednisolone in the urine is <4% of the applicated dose. The main metabolite of MMF secreted into the urine is the glucoronide (MPGA) form of mycophenolic acid (MPA). Whereas most of MPAG is eliminated from the circulation into the urine via tubular secretion and its clearance correlates well with the glomerular filtration rate, only a negligible amount (<1%) of the active form of

MMF (MPA) is excreted into the urine [34]. Therefore, we believe that cells in the urinary tract are not affected by immunosuppressive drugs administered to the patients. The higher urine/plasma ratio of cytokines in bacteriuric female and male patients when compared with nonbacteriuric patients further suggests that cytokines were produced in the urinary tract.

The gram− bacteria appeared to stimulate the immune system somewhat stronger than gram+ bacteria. Others reported on lower IL-8 urine levels in patients infected by gram+ cocci when compared with patients infected by gram− organisms [13]. We found that in male patients, gram− bacteria induced inflammatory cytokine production mainly in the urinary tract, whereas in female patients the inflammatory responses were systematic, with high sIL-2R and sIL-1RA levels in the plasma. Our data suggest that increased *plasma* sIL-1RA is a natural response to inflammation in female patients. *Urine* slL-1RA was also higher in female recipients than in male recipients, both in the bacteriuric and nonbacteriuric group. However, the difference was much more pronounced in the bacteriuric group because of the fact that bacteriuric females had significantly higher urine sIL-1RA than nonbacteriuric females. Soluble IL-1RA is a potent inhibitor of IL-1 and inhibits specifically the bioactivity of IL-1 on T cells and endothelial cells [35,36]. Female gonadal steroids in normal physiologic amounts also can induce expression of sIL-1RA [37]. The high levels of urine sIL-1RA in bacteriuric and nonbacteriuric females may be the result of (i) continuous stimulation in the bladder by insignificant bacteriuria, or (ii) the effect of female gonadal steroids that induce the production of sIL-1RA [37,38]. Lynch *et al.* reported of gender-related differences in IL-1α, IL-1β, and IL-1RA secretion from mononuclear cells and urinary excretion of IL-1β and IL-1RA [39]. The results suggest that the regulation of IL-1 secretion is fundamentally different in women than in men and alludes to the possibility that IL-1 may serve different biologic functions [39]. Cells of women isolated during the luteal phase secreted five to 10 times more IL-1α, IL-1β, and IL-1RA than cells of men, and cells of women isolated during the follicular phase secreted 13–28 times more of the three cytokines. In addition, urinary excretion correlated with cellular secretion of IL-1β and IL-1RA. Constant stimulation with endotoxin by low-grade bacteriuria in women can induce endotoxin tolerance. However, the results of studies on endotoxin tolerance and cytokine responses are controversial. *In vivo* and *in vitro* studies indicated the down-regulation of IL-1 gene expression and protein production after repeated exposure of whole blood to endotoxin [40,41], whereas other studies showed significant increases in serum IL-1 in endotoxin-tolerant individuals and human peripheral blood monocytes following a repeated LPS stimulus [42–45]. Expression and production of sIL-1RA usually rose after restimulation with endotoxin in cells of endotoxin-tolerant individuals. Lemaire *et al.* showed that minimally invasive surgery induces endotoxin tolerance in the absence of detectable endotoxemia and that *ex vivo* stimulation of whole blood cells causes an increase of IL-1RA production [40]. Henricson *et al.* showed that sIL-1RA blocks LPS-induced colony-stimulating factor (a mediator of inflammatory response) production as well as the induction of early endotoxin tolerance by LPS [46]. The results of our study are in agreement with the aforementioned references. Repeated exposure to endotoxin of low-grade bacteriuria may cause an increase of IL-1 and IL-1RA production in local urinary tract epithelial cells and inflammatory cells that infiltrated the urinary tract. We cannot rule out that gonadal steroids affect the local production of IL-1RA in the female urinary tract and result in higher IL-1RA production in infected women compared with infected men. When we analyzed plasma and urine cytokine levels in patients under and over 50 years separately for male, female, bacteriuric, and nonbacteriuric patients, we did not find any significant difference between younger and older women with or without bacteriuria. Because concentrations of gonadal steroids should be different in females before and after menopause, this finding argues against a major influence of gonadal steroids on IL-1RA secretion in female renal transplant recipients with UTI. Obviously, continuous stimulation of low-grade bacteriuria in females induces an inflammatory and also an anti-inflammatory response [47]. Because females are more prone to UTI and have a consistent stimulation with bacterial antigens in the lower urinary tract they are likely to develop a stronger anti-inflammatory response than male patients.

Only a small number of male and female bacteriuric patients had significant leukocyturia, suggesting that leukocyturia *per se* is not a precise marker of UTI in renal transplant recipients. Previous studies showed that, in complicated cases of UTI occurring during pregnancy, recurrent UTI, or pyelonephritis, simple urinalysis is not sufficient for the diagnosis of UTI. Additional steps including manual diagnostic microscopy and semiquantitative urine cultures are necessary. In renal transplant recipients, immunosuppressive drugs inhibit the inflammatory response to UTI and, as a result, the patients cannot recruit leukocytes in the urinary tract.

We did not calculate urine cytokine/urine creatinine ratios because we suppose that the cytokines are not filtered by the glomeruli but rather secreted postglomerular into the urine by monocytes and epithelial cells of the urinary tract. Urine levels of postglomerular secreted cytokines can increase independently of urine creatinine levels. Urine cytokine/urine creatinine ratios would

therefore not reflect the actual stimulation of monocytes and epithelial cells in the urinary tract.

Our data show gender-related urine cytokine patterns. Male renal transplant recipients with UTI have a strong inflammatory cytokine response, with activation of IL-6, IL-8, sIL-2R, and sIL-6R producing cells, whereas female patients with UTI block the inflammatory response to UTI by production of sIL-1RA. The higher sIL-1RA plasma and urine levels in females compared with males may be (i) the result of an anti-inflammatory response to frequent UTI in females or (ii) due to the effect of female gonadal steroids that induce the production of sIL-1RA. We hypothesize that the higher urine levels of IL-6, IL-8, sIL-1RA, sIL-2R, and sIL-6R in patients with UTI might originate from local cytokine production in epithelial cells and inflammatory cell infiltrates of the urinary tract of the renal transplant. Our data support strategies for active treatment of asymptomatic bacteriuria in immunosuppressed renal transplant recipients.

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