Expression of urokinase plasminogen activator and its receptor during acute renal allograft rejection

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Background. In inflammation, urokinase plasminogen activator (uPA) and its receptor (uPAR) play an important role in fibrinolysis and in activation and chemotaxis of neutrophils and lymphocytes. Moreover, the uPA/uPAR system is involved in processes that affect turnover of the extracellular matrix (ECM). The aim of this study was to determine the local and systemic release of uPAR, and the expression of uPA and uPAR in renal tissues during acute renal allograft rejection.

Methods. Blood, urine, and tissue samples were collected from 33 patients diagnosed with acute allograft rejection and from 14 transplant patients without rejection. From 10 healthy volunteers, blood and urine were collected as a control. In urine and blood samples, the levels of uPAR were determined by enzyme-linked immunosorbent assay (ELISA). Immunostaining and in situ hybridization for uPA and uPAR were performed on renal biopsies.

Results. uPAR was detectable at low levels in serum and urine of healthy volunteers and was increased in nonrejecting allograft recipients. Serum and urine levels of uPAR were higher in transplant recipients with rejection compared to non-rejectors. The urine and serum levels of uPAR correlated with the renal function. Immunostaining and in situ hybridization showed an up-regulation of both uPA and uPAR in rejection biopsies. Nonrejected grafts displayed no expression of uPA and uPAR by immunostaining, or of uPAR by in situ hybridization. uPA was detected in a limited number of tubular epithelial cells by in situ hybridization. During rejection, lymphocytes as well as tubular epithelial cells showed uPA and uPAR expression. In the vascular types of rejection, strong expression of uPA was also seen in the entire vessel wall, while uPAR was expressed by the endothelium.

Conclusion. This study shows that (1) uPA and uPAR are up-regulated during acute renal allograft rejection; (2) uPAR levels in urine and serum correlate with serum creatinine levels, and (3) uPA and uPAR are produced by inflammatory cells, tubular epithelium, and vascular endothelium during acute renal allograft rejection.

Despite the introduction of successful immunosuppressive drug therapies, acute renal allograft rejection still occurs in 40% to 60% of patients after cadaveric renal transplantation and causes graft loss in up to 4% in the first year after transplantation [1, 2]. Histopathologically, acute rejection is characterized by an inflammatory infiltrate, consisting mainly of T lymphocytes, located in the interstitium, tubular epithelium, and, as type and severity of rejection change, vascular endothelium [3]. In recent years, the complex molecular mechanisms underlying the activation and extravasation of lymphocytes into the allograft have been largely unraveled [4, 5].

Components of the fibrinolytic system have recently been recognized as major players in the recruitment of inflammatory cells. The fibrinolytic system consists of two serine proteases, the tissue-type plasminogen activator (tPA) and the urokinase-type plasminogen activator (uPA), that both facilitate the conversion of plasminogen into plasmin, which is the crucial protease in fibrinolysis [6]. tPA is mainly involved in intravascular thrombolysis, whereas uPA mediates a number of other functions, including cell migration, pericellular proteolysis, tissue remodeling, and fibrosis. The activity of both PAs is inhibited by plasminogen activator inhibitors (PAI) type 1 and, to a lesser extent, type 2 [7]. uPA exerts most of its effects via interaction with the urokinase PA receptor (uPAR, CD87). uPAR, a glycosyl-phosphatidylinositol (GPI)-anchored molecule that was first described in 1985 [8], is present at the cell surface of a wide variety of different cell types, such as monocytes, macrophages, neutrophils, T cells, endothelial cells, smooth muscle cells, and renal tubular epithelial cells [9–14].

Beside their contribution to fibrinolysis, uPA and its receptor play an important role in the activation and...
chemotaxis of neutrophils, macrophages, and lymphocytes through interaction with several integrins [15–18].

Furthermore, the uPA/uPAR system is involved in a variety of processes that are associated with turnover of the extracellular matrix (ECM), such as tumor cell invasion and dissemination, angiogenesis, wound healing, and embryogenesis [16, 18–21]. This effect is partly due to the generation of matrix metalloproteinases (MMPs) by plasmin [22, 23]. So, activation of the uPA/uPAR system not only initiates chemotaxis and extravasation of inflammatory cells, but also leads to pericellular proteolysis, thus facilitating the penetration of these cells into the inflamed tissue.

On the other hand, the fibrinolytic system is also supposed to play an important role in the prevention of both glomerular and tubulointerstitial fibrosis, by diminishing the accumulation of tubulointerstitial matrix through the induction of plasmin and MMPs [24, 25]. Several studies on components of the fibrinolytic system in the diseased kidney have been performed. An up-regulation of glomerular uPAR expression has been described in renal thrombotic microangiopathy and in a murine model of nephrotoxic nephritis [26, 27]. Also, in crescentic glomerulonephritis, an up-regulation of uPA was shown [28]. Studies using tPA knockout mice have shown a protective effect of plasminogen and tPA against acute inflammatory injury in a model of crescentic glomerulonephritis [29]. Recently, we described a local and systemic release of uPAR in kidneys from patients with pyelonephritis [30].

A limited number of studies deal with the involvement of the fibrinolytic system in renal allograft rejection, the majority of which concern chronic rejection. A correlation between renal allograft function and uPA protein expression was first described by Bukovsky et al [31], but a possible relationship between uPA/uPAR expression and acute rejection has not been studied in detail up to now. However, up-regulation of uPA, uPAR, and PAI-1 in chronically rejected kidneys has been reported [32–34], suggesting that the fibrinolytic system plays an important role in tissue remodeling within the chronically rejected graft and the development of fibrosis, one of the major histopathologic features of chronic rejection.

The aim of the present study was to determine the local and systemic release of uPAR, the expression of uPA and uPAR in renal tissues during acute renal allograft rejection, and to correlate these data with renal transplant function and with severity of rejection.

METHODS

Patients

Thirty-three patients with biopsy-proven acute renal allograft rejection were selected randomly from the patient population of the Academic Medical Center of the University of Amsterdam. These patients were divided into three groups in accordance with the severity of the rejection, using the Banff criteria for acute renal allograft rejection [3] (type 1, 14 patients; type 2, 9 patients; and type 3, 10 patients). All biopsies contained at least seven glomeruli and two arteries. As control, 14 transplant patients who showed no clinical or histopathologic evidence of acute or chronic allograft rejection were selected at random from a total population of 200 transplant patients, undergoing protocol graft biopsy several months after transplantation according to a research protocol. The protocol had been approved by the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam, and each patient had given written informed consent.

Urine and serum samples from all patients were collected at the time of biopsy. The main clinical data of all patients are summarized in Table 1. Urine and serum samples were also obtained from a control group of 10 nontransplanted healthy volunteers.

Enzyme-linked immunosorbent assay (ELISA)

uPAR concentrations in urine and serum were measured by ELISA according to the manufacturer using purified mouse antihuman uPAR monoclonal antibody (mAb) (4 µg/mL) (R&D systems, Abingdon, UK) as coating antibody, biotinylated goat-antihuman uPAR antibody (100 ng/mL) (R&D systems) as detecting antibody, and recombinant human uPAR (R&D systems) as standard. The detection limit of the assay was 0.2 ng/mL. Urine concentrations are expressed as µg/mmol creatinine in order to correct for dilution and renal function.

Immunohistochemical studies

Immunohistochemical studies were performed on cryostat sections (4 µm thickness). After fixation in acetone for 15 minutes at 4°C, slides were washed, preincubated first in 10% normal goat serum for 15 minutes, and then incubated for 16 hours at 4°C with mouse IgG2 antihuman uPAR mAb (CD87) (American Diagnostica Inc., Greenwich, CT, USA) or IgG1 anti-uPA mAb (American Diagnostica). Endogenous peroxidase activity was then blocked using 0.1% NaNO2 and 0.3% H2O2 in phosphate-buffered saline (PBS) for 10 minutes at room temperature. Sections were washed and incubated with polymerized horseradish peroxidase (HRP)-conjugated goat antimouse IgG antibody (Powervision™; Immunovision Technology, Daly City, CA, USA). Enzyme activity of HRP was finally detected using 3-amino-9-ethylcarbazol (Sigma, Zwijndrecht, The Netherlands) and counterstained with hematoxylin.

In situ hybridization

uPA and uPAR-specific digoxigenin (DIG)-labeled riboprobes for in situ hybridization were prepared by
T7 RNA polymerase driven in vitro transcription, from cloned-specific polymerase chain reaction (PCR) products as template. Briefly, cDNA was prepared on total RNA extracted from human placenta using random hexamer priming. Primers used in the PCR reaction were 5'-GAGAATTCCACCAACATCGAGAA-3' and 5'-TAATACGACTCATTAGGGCCAGTGATCTCACAGTTGTG-3' for uPA, yielding a 426 bp product; and 5'-TACATCGAGTACGGTGAGAAGG-3' and 5'-TAATACGACTCATAAGGGTGACTGGAACATCCAGGTCTGG-3' for uPAR, yielding a 563 bp product. The underlined primer regions encode the T7-promoter element. PCR products were cloned and clones were identified by sequencing using an ABI sequencer (Perkin Elmer Corp., Norwalk, CT, USA) with a dye-terminator cycle-sequencing kit (Perkin Elmer Corp.). After purification by phenol-extraction, isolated inserts were used as a template in an in vitro transcription reaction using T7 polymerase (DIG-RNA Labeling Kit; Roche Diagnostics, Mannheim, Germany).

Using these probes, in situ hybridization was performed as follows: 7 μm thick sections were deparaffinized, digested with 20 μg/mL proteinase K (Invitrogen, Life Technologies, Breda, The Netherlands) for 15 minutes at 37°C, treated with 0.2% glycine (Merck, Amsterdam, The Netherlands) for 5 minutes, and postfixed with 2% paraformaldehyde (Merck) and 0.1% glutaraldehyde (Merck) in PBS for 20 minutes. After pretreatment with hybridization solution containing 50% formamide (Merck), 5× standard sodium citrate (SSC) (Invitrogen, Life Technologies), 1% Blocking Reagent (Roche, Almmere, The Netherlands), 5 mmol/L ethylenediaminetetraacetic acid (EDTA) (Sigma, Zwijndrecht, The Netherlands), 0.1% Tween-20 (Sigma), 0.1% CHAPS (Sigma), 0.1 mg/mL heparin (BD Biosciences, Alphen aan den Rijn, The Netherlands), and 1 mg/mL yeast RNA (Invitrogen, Life Technologies), the sections were subjected to hybridization. The DIG-labeled riboprobes (final concentrations 250 ng/mL uPA and 400 ng/mL uPAR) were added to above-mentioned hybridization solution. Hybridization was performed in a stove for 18 hours at 70°C. After hybridization, the slides were washed with 2× SSC containing 50% formamide at 65°C. After washing with 0.1% Tween-20 in PBS, they were treated with 2% Blocking Reagent (Roche), 10% normal goat serum, and 0.1% Tween-20 in PBS for 30 minutes. Hereafter, they were incubated with alkaline phosphatase-conjugated F(ab) fragments of sheep anti-DIG antibody (Roche); bound alkaline phosphatase activity was visualized with nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate, toluidine salt (NBT/BCIP) (Roche). Subsequently, a picro sirius red counterstaining was performed. Slides were rinsed in tap water and mounted with glycergel (Dako, Glostrup, Denmark). All slides were coded and judged by two independent pathologists who were unaware of the codes.

### Statistical analysis

Data were analyzed by analysis of variance (ANOVA), followed by a Bonferroni post hoc test or Dunnett T3 post hoc test.
RESULTS

Association between clinical data and histologic diagnosis

The clinical and biochemical characteristics of the patients are summarized in Table 1. The patients were divided into four groups, according to the histopathologic diagnosis: (1) no rejection (N = 14 patients), (2) acute rejection type 1 (N = 14 patients), (3) acute rejection type 2 (N = 9 patients), and (4) acute rejection type 3 (N = 10 patients). No statistically significant differences were observed among the four groups for age, gender, age of kidney donor, time of first rejection episode (when applicable), and time of biopsy. The mean serum creatinine value at the time of biopsy was not significantly different, except in the type 3 group vs. the nonrejecting group. Both values were determined by analysis of variance (ANOVA), followed by a Dunnett’s T3 post hoc test.

As summarized in Table 1, all patients received immunosuppressive therapy based on prednisone and cyclosporine. Three patients in every rejection group received azathioprine. All nonrejectors and two patients in the type 3 rejection group received mycophenolate mofetil. Further, all nonrejectors were treated with basiliximab, an interleukin-2 (IL-2) receptor antagonist. Six of the nonrejectors, one patient from the type 1 rejection group, and two from the type 3 rejection group received tacrolimus.

The histologic findings are summarized in Table 2.

Secretion of uPAR in serum and urine during acute rejection

The urokinase receptor uPAR was detectable at low levels in serum (2.6 ± 0.2 ng/mL) and urine (679 ± 80 ng/mmol creatinine) of healthy volunteers. As shown in Table 3, both serum and urine levels of uPAR were significantly higher in transplant recipients with stable renal function in comparison with healthy controls. A further increase in uPAR level was measured in serum and urine of patients with acute rejection. As shown in Figure 1, the urine and serum levels of uPAR correlated with the renal function, determined by mean creatinine levels in the blood (serum R = 0.68, P < 0.01; urine R = 0.50, P < 0.01; Spearman rho test). Furthermore, a correlation was found between the urine and serum levels of uPAR in all groups (R = 0.71, P < 0.01; Spearman rho test), as is shown in Figure 2.

Further, we attempted to correlate the serum and urine uPAR levels with the severity of renal rejection as defined by the Banff classification. As shown in Figure 3, serum and urine uPAR levels seemed to correlate with the severity of the rejection, although only for type 3 significant differences could be measured.

These data indicate that uPAR production is up-regulated after renal transplantation and is significantly further up-regulated during acute rejection episodes.

Immunostaining for uPA and uPAR

In order to localize the expression of uPA and uPAR, all kidney biopsies (N = 47) were stained for both molecules by immunohistochemistry. Subsequently, all slides

<table>
<thead>
<tr>
<th>Banff classification</th>
<th>No rejection</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
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<tr>
<td>Number of glomeruli (mean ± SD)</td>
<td>16 ± 9</td>
<td>11 ± 6</td>
<td>14 ± 8</td>
<td>13 ± 6</td>
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<td>Score</td>
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<td>2</td>
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</tr>
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<td>Interstitial inflammation</td>
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<td>0</td>
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<td>0</td>
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<td>Intimal arteritis</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>Interstitial fibrosis</td>
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<td>3</td>
<td>0</td>
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<td>Tubular atrophy</td>
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<td>0</td>
<td>0</td>
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<td>Intimal thickening</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Mesangial matrix increase</td>
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<td>1</td>
<td>0</td>
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*Kidney biopsies were scored according to the Banff classification [3].

<table>
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<th>Table 2. Histologic characteristics of the biopsies</th>
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<td>Number of glomeruli (mean ± SD)</td>
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<td>Score</td>
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<table>
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<th>Table 3. Levels of urokinase plasminogen activator receptor (uPAR) in serum and urine</th>
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<tr>
<td>No Rejection</td>
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<td>Acute rejection, all types</td>
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</table>

Levels of uPAR in serum and urine. Data are displayed as mean ± SEM of the individual serum and urine concentrations of uPAR in healthy subjects (N = 10), transplant patients with adequate graft function and no evidence of rejection (N = 14), and all pooled rejection patients (N = 33). 

*P ≤ 0.05 compared to healthy subjects; **P < 0.05 compared to the non rejection group. Both P values were determined by analysis of variance (ANOVA), followed by a Dunnett’s T3 post hoc test.
Fig. 1. Correlation between urokinase plasminogen activator receptor (uPAR) level in serum (A), in urine (B), and the serum creatinine concentration. Serum and urine levels of uPAR were determined by enzyme-linked immunosorbent assay (ELISA). The urine and serum levels of uPAR correlate with the renal function, determined by mean creatinine levels in the blood (serum $R^2 = 0.68$, $P < 0.01$; urine $R^2 = 0.50$, $P < 0.01$; Spearman rho test).

were judged by two independent pathologists who were blinded for diagnosis. Representative images are shown in Figure 4A to F. In the renal biopsies from the group of patients without rejection no positive immunostaining for uPA (Fig. 4A) and uPAR (Fig. 4D) was observed.

In biopsies from patients with rejection, the mononuclear infiltrate was strongly positive for uPAR (Fig. 4E) and weakly for uPA (Fig. 4B). The damaged tubular epithelium was also strongly positive for uPA (Fig. 4B). In these biopsies, the staining was predominantly cytoplasmic. In the biopsies with acute rejection type 2 and type 3, the endothelium strongly expressed both uPA (Fig. 4C) and uPAR (Fig. 4F). uPA was predominantly expressed by the smooth muscle cells within the vessel wall and uPAR was preferentially expressed by endothelial cells.

**In situ hybridization for uPA and uPAR mRNA**

Next, in situ hybridization for uPA and uPAR mRNA was performed on all biopsies ($N = 47$) to confirm the cellular source of uPA and uPAR (Fig. 4G to L). Control biopsies (obtained from transplant patients without rejection) showed positive staining for uPA in only a limited amount of proximal tubular cells as expected from the literature (Fig. 4G) [14]. These biopsies were negative for uPAR (Fig. 4I).

In type I rejection, the mononuclear infiltrate showed strong positive staining for both uPA (Fig. 4H) and uPAR (Fig. 4K). Also, the tubular epithelium displayed strong uPA and uPAR expression during rejection. In addition, in the vascular types of rejection, strong expression of uPA was seen in the entire wall of the involved vessels (Fig. 4I), while uPAR was expressed by the endothelium (Fig. 4L).

**DISCUSSION**

Beside their well-described role in fibrinolysis, uPA and its receptor uPAR are involved in other important biologic processes such as inflammation, tissue remodeling, angiogenesis, and tumor metastasis. In this study, we demonstrate a clear systemic and local production of uPAR associated with high expression of uPA and uPAR in the kidney in patients with acute renal allograft rejection.
Roelofs et al: uPA and uPAR in acute rejection

Fig. 3. Levels of urokinase plasminogen activator receptor (uPAR) in serum (A) and urine (B). Serum and urine levels of uPAR were determined by enzyme-linked immunosorbent assay (ELISA). Results are displayed as mean ± SEM of the individual serum (A) and urine (B) concentrations of uPAR in transplant patients without rejection, transplant patients with acute rejection type 1, 2, and 3, and healthy subjects. Serum and urine levels of uPAR correspond with severity of rejection. *P < 0.05 compared to the nonrejection group; **P < 0.05 compared to the control group of healthy volunteers. Both P values were determined by analysis of variance (ANOVA), followed by a Dunnett T3 post hoc test.

High serum levels of soluble uPAR have been reported in patients with sepsis [35], advanced malignancies [19], and in healthy volunteers after lipopolysaccharide (LPS) administration [36]. As far as the systemic production of uPAR is concerned, the cellular source of uPAR in patients with acute renal rejection is unknown. Beside leukocytes, endothelial cells are likely candidates. Indeed, activation of T cells by phorbol ester, IL-2, or via the TCR/CD3 complex causes a rapid up-regulation of uPAR in vitro. In contrast, tumor necrosis factor-α (TNF-α) has no effect and transforming growth factor-β1 (TGF-β1) substantially decreased uPAR expression in activated T cells [11]. An up-regulation of uPAR has been also reported on monocytes of healthy volunteers receiving LPS injection and in vitro in whole blood stimulation with LPS or TNF-α [36]. Endothelial cells also synthesize and release uPAR upon inflammation. Indeed, after stimulation with phorbol 12-myristate 13-acetate (PMA), endothelial cells produce appreciable quantities of soluble uPAR in vitro [37] and we found a distinct synthesis and expression of uPAR by endothelial cells in the kidney during vascular rejection.

Variations in immunosuppressive regimens are unlikely to account for the differences in uPA and uPAR secretion and expression because no differences were found between patients from the same group that were treated with different immunosuppressive agents. Moreover, renal biopsies obtained from patients with acute rejection treated with basiliximab, prednisone, and mycophenolate mofetil displayed similar uPA and uPAR expression as acute rejection patients who were treated with cyclosporine, prednisone, and mycophenolate mofetil (data not shown).

Transplant patients without rejection also have significantly higher levels of uPAR in blood compared to healthy volunteers, although uPAR mRNA was not detected by in situ hybridization in renal biopsies of these patients. On the one hand, this might reflect an increased systemic synthesis and release of uPAR due to higher circulating levels of inflammatory cytokines in patients with renal transplantation compared to healthy volunteers [38, 39]. On the other hand, renal filtration of soluble uPAR might be impaired in transplant patients without rejection. This can contribute to increased serum levels of soluble uPAR. Along this line, we found a significant correlation between serum levels of uPAR and serum creatinine. This might reflect a higher synthesis of uPAR due to the severe inflammatory state (acute rejection) combined with a decreased filtration of uPAR.

uPAR was also dramatically increased in urine during renal rejection and this increase was significantly correlated with the severity of the rejection. The release of uPAR in the urine might be caused by leakage of uPAR by the tubuli that are damaged during rejection. Indeed, as shown by in situ hybridization and immunostaining, uPAR was strongly expressed by inflammatory cells and damaged tubuli during rejection. In vitro, tubular epithelial cells secrete uPAR upon stimulation with inflammatory cytokines such as TNF-α and IL-1β [30]. During vascular rejection episodes, endothelial cells also synthesize and express uPAR. A similar up-regulation of uPAR expression by immunostaining has been reported in several renal diseases such as chronic allograft nephropathy [32], thrombotic microangiopathy [26], chronic pyelonephritis [30], and nephrototoxic serum sickness [27]. The pattern of uPA expression during renal rejection was comparable to that observed for uPAR. This colocalization of uPA and uPAR suggests interactions between both molecules during rejection. Indeed, it has been shown that uPA and uPAR are able to up-regulate their
Fig. 4. Immunostaining and in situ hybridization for urokinase plasminogen activator (uPA) and urokinase plasminogen activator receptor (uPAR). Immunostaining (A to F) (red staining with blue hematoxylin counterstaining) and mRNA in situ hybridization (G to L) (blue staining with picrosirius red counterstaining) results for uPA (A, B, C, G, H, and I) and uPAR (D, E, F, J, K, and L) in nonrejected transplants (A, D, G, and J), type I rejection (B, E, H, and K) and vascular rejection (C, F, I, and L). Nonrejected grafts show no expression of uPA/uPAR with immunostaining (A and D). In situ hybridization shows mRNA expression of uPA in a limited number of tubular epithelial cells (G, arrows), and no mRNA expression of uPAR (J) in nonrejected grafts. In type I rejection, the activated lymphocytes show strong positive staining for both uPA and uPAR protein with immunostaining and mRNA using in situ hybridization (B, E, H, and K). Since the overall staining intensity of the in situ hybridization for uPAR was less than for uPA, an inset was placed in (K). Furthermore, the tubular epithelial cells show positive staining for uPA in type I rejection (B and H). In the vascular types of rejection, strong expression of uPA is seen in the entire wall of the involved vessels (C and I), while uPAR is expressed by the endothelium (F and L) [original magnifications 200× and 400× (C, F, I, and L)].
expression reciprocally in human kidney cells, monocytes, and endothelial cells [40, 41].

Whether activation of the uPA/uPAR system exerts detrimental or beneficial effects during renal allograft rejection remains to be elucidated. The possible detrimental effects of uPA and uPAR during rejection are related to their proinflammatory properties. Indeed, binding of uPA to uPAR results in the generation of cell surface-associated plasmin, which is critical for pericellular proteolysis, an essential step in the migration of inflammatory cells [42]. Independently of plasmin generation, uPAR exerts a crucial role in transendothelial migration of monocytes through the formation of complexes with the β1 integrin CD11b/CD18 [43]. Moreover, soluble uPAR specifically binds αβ, αβ, αβ, and αβ, and those uPAR-integrin interaction may mediate cell-cell interaction [15]. Finally, uPA exerts chemotactic activities [44].

Apart from its proinflammatory role, the uPA/uPAR system may to some extent protect the kidney from injury. Indeed, through the generation of plasmin, uPA and uPAR participate in the digestion of fibrin, which is known to accumulate during renal injury and to be deleterious for the kidney [29, 45, 46]. Moreover, uPA is able to directly degrade some matrix components such as fibronectin [47] and to activate latent MMPs, which promote degradation of ECM [48]. In addition, urokinase is implicated in the posttranslational activation of several growth factors, including TGF-β1 and heparocyte growth factor (HGF) [49, 50]. Studies involving a mouse model of heart transplantation have shown that cardiac fibroblasts mediate ECM accumulation with the β1 integrin CD11b/CD18 [43]. Moreover, soluble uPAR specifically binds αβ, αβ, αβ, and αβ, and those uPAR-integrin interaction may mediate cell-cell interaction [15]. Finally, uPA exerts chemotactic activities [44].

Since TGF-β1 and HGF are critical regulators of ECM accumulation that function in an opposite manner, the precise effect of urokinase in tissue remodeling and fibrosis remains unclear. Indeed, it has been recently reported that uPAR deficiency accelerates renal fibrosis [abstract: Zhang G et al, J Am Soc Nephrol 12:722, 2001] but tPA deficiency reduces renal fibrosis [52] in the same mouse model of chronic obstructive nephropathy. Moreover, tPA-based therapy has been reported to reduce the glomerular matrix accumulation in an experimental model of glomerulonephritis [53].

CONCLUSION

The present study shows strong local up-regulation of the uPA/uPAR system during acute renal allograft rejection. Future studies in animal models will be required to determine the pathophysiologic significance of uPA and uPAR in renal inflammation and fibrosis, before the fibrinolytic system could serve as a target for therapeutic interventions during acute renal rejection and eventually for the prevention of chronic allograft nephropathy.

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REFERENCES

17. Gyetko MR, Todd RF, Wilkinson CC, Strain RG: The urokinase