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Modulation of matrix gelatinases and metalloproteinase-activating process in acute kidney rejection

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Abstract Changes in matrix metalloproteinase (MMP) activities would contribute to the accumulation of extracellular matrix during acute kidney allograft rejection. MMP-2 and MMP-9 and other gelatinolytic activities were examined in the rejected graft and the urine of a rat model of acute kidney rejection (orthotopic allotransplantation from a Buffalo donor to a Wistar-Furth recipient) by either zymography or fluorescence assay. MMP-2, membrane type 1 (MT1)-MMP, and tissue inhibitor of metalloproteinase (TIMP)-2 were also examined by immunodetection. The proMMP-2 activity and protein level increased in the graft during rejection when compared with normal Buffalo kidney, whereas activated MMP-2 decreased. TIMP-2 protein levels were markedly decreased and MT1-MMP proteolytic fragments (44–40 kDa) were undetectable. This suggests an altered MT1-MMP-dependent processing of proMMP-2 into active MMP-2 due to a diminished TIMP-2 level in acute kidney rejection. In the urine the overall gelatinolytic activity decreased considerably, although activity associated with an as yet unidentified 78-kDa protein appeared 6 days after transplantation.

Keywords Kidney transplantation · Acute rejection · Extracellular matrix · MMP-2 · TIMP-2 · Urine

Introduction

Matrix metalloproteinases MMP-2 and MMP-9, also known as gelatinases A and B, are the most important members of the MMP family, as together they can degrade the major components of the extracellular matrix (ECM) [10]. Substrates degraded by MMP-2 and MMP-9 include laminin, gelatin, fibronectin, collagens I, II, IV, V, VII, X, and XI, aggrecan, elastin, and vitronectin. MMPs are soluble enzymes that are secreted into the extracellular milieu in a latent form (proMMPs), where they may be proteolytically processed into active MMPs by cleavage of their *N*-terminal prodomains [10]. Their activity is regulated by tissue inhibitors of metalloproteinases (TIMPs) [7, 32], which are also present in most tissues and body fluids and which bind to MMPs to inhibit their action [3, 14]. To be activated, proMMP-2 requires a ternary complex with membrane type 1 (MT1)-MMP [25] and TIMP-2 [13, 17, 18, 21] at the cell surface.

During both acute and chronic rejection of kidney transplants, accumulation of ECM proteins is observed in different areas of the graft [1, 8, 16, 22, 24]. Since MMP-2 and MMP-9 are important regulators of ECM integrity, they may well be involved in graft rejection processes. The regulation of ECM remodeling in the

kidney by MMPs has previously been studied by use of models of interstitial and glomerular fibrosis [6, 11, 14], but never with graft rejection models. To determine whether MMP-2 and MMP-9 could be involved in tissue rejection, we investigated their activities in a well-established model of acute rat kidney rejection [31]. We also performed ischemia/reperfusion and isotransplantation in order to differentiate the effects related to surgery from those related to rejection of the graft by the host. We also monitored the MMPs' gelatinolytic activity in urine to determine whether this could be correlated with any changes that occur during acute rejection.

Materials and methods

Animals

For this study, an established model of acute kidney rejection was used [31]. Buffalo (BUF, $RT1^b$) and Wistar-Furth (WFu, $RT1^n$) rats (Harlan Sprague-Dawley, Indianapolis, Ind., USA) were used as donor and recipient, respectively, for the allograft rejection model. Six animals were given allotransplants. Two other groups of six animals each were used as controls: group 1 was composed of ischemic/reperfused WFu rats, and group 2 comprised isograft (WFu → WFu) rats. Urine was collected from each animal immediately prior to surgery (day 0) and postoperatively on days 3 and 6. Six days after surgery, the rats were killed, and both the transplanted or ischemic/reperfused left kidney (T) and the contralateral right kidney (C) were collected. Kidneys from separate BUF donor rats were also used as a control in this study.

Surgical procedure

The allotransplant animal model (BUF → WFu) experienced orthotopic kidney transplantation performed by a modified method of Fisher and Lee [9]. The left kidney from the donor was perfused through the aorta with chilled heparinized saline solution and harvested intact, after transection of the ureter, the renal artery close to the aorta, and the renal vein near the vena cava. The kidney was maintained in chilled Ringer's lactate solution for fewer than 30 min. The recipient was anesthetized, and a left nephrectomy was performed after transection of the ureter, renal artery, and vein. End-to-end anastomoses of donor and recipient renal arteries and veins were performed, with 10–0 nylon sutures being used. After releasing the vascular clamps, we made an end-to-end anastomosis of donor and recipient ureters, using 10–0 nylon sutures, and the abdomen was closed. Animals did not receive any immunosuppressive therapy. The same procedure was used for isotransplanted (WFu → WFu) kidneys. For the ischemic/reperfused group, the renal artery of the left kidney in WFu rats was clamped for 30 min. The principles of laboratory animal care (NIH publication 86–23) were followed.

Preparation of tissues and urine collection

Kidneys were removed from the various animals. The cortices and medullae were separated surgically, and each tissue was pooled into two groups of three animals per group, for preparation of cortex or medulla homogenates and glomeruli. Cortices and medullae were homogenized in 8 ml of 250-mM sucrose and 10-mM Hepes/Tris pH 7.5 buffer per gram of tissue. The resulting homogenates were frozen at −80 °C until required for use. For preparation of glomeruli, minced cortices were passed sequentially through sieves of 180 and 130 μm by being washed with ice-cold Hank's balanced saline solution (HBSS), pH 7.4. Glomeruli were collected on an 85-μm mesh, washed with HBSS, and re-suspended in 20-mM Hepes/Tris, pH 7.0, then lysed by several passages through a fine needle (26G1/2) before being stored at −80 °C. Urine was centrifuged at 10,000 *g* for 10 min at 4 °C; the pellet was discarded and the supernatant was frozen until required for testing. For tissue homogenates and urine, protein concentration was measured by the Coomassie Plus protein assay reagent from Pierce (Rockfort, Ill., USA).

Gelatin zymography

MMP activity was monitored by gelatin zymography in cortices, medullae, and glomeruli from contralateral (C) and treated (T) kidneys [19]. Briefly, proteins (20 μg sample/well) were resolved on 7.5% polyacrylamide gels containing 0.1% gelatin and colored with Coomassie blue to visualize the gelatin digested where MMPs had migrated. Purified recombinant MMP-2 and MMP-9 were used as controls, as was phenanthroline (0.5 mM), a zinc-chelating agent that specifically inhibits MMPs. For gelatinolytic activity in urine, creatinine concentration was determined in every sample with a kit from Sigma-Aldrich (Oakville, Ont., Canada), the manufacturer's recommendations being followed. Urine samples containing 0.5 μg creatinine were processed for zymography as described above for tissue samples.

Immunodetection of MMP-2, MT1-MMP, and TIMP-2

Proteins (40 μg) from cortex and medulla homogenates and from glomeruli were resolved by SDS-polyacrylamide gel electrophoresis (PAGE) on 7.5% acrylamide gels with a Mini-Protean II apparatus (Bio-Rad, Mississauga, Ont.). The proteins were transferred electrophoretically onto a 0.45-μm pore size polyvinylidene difluoride (PVDF) membrane (Millipore, Mississauga, Ont.) with a semi-dry electroblotter apparatus (Millipore). The transfer was carried out at 1 mA/cm^2 for 90 min in 96-mM glycine, 10-mM Tris buffer, pH 8.8, and 10% methanol. PVDF membranes were incubated for 120 min at room temperature in Tris-buffered saline (150-mM NaCl, 50-mM Tris, pH 7.0) containing 0.1% Tween-20 (TBS-T) and either a mouse anti-MMP-2 mAb diluted 1:2500, a mouse anti-human-MT1-MMP mAb diluted 1:1000, or a rabbit anti-TIMP-2 pAb (Chemicon, Temecula, Calif., USA) diluted 1:1000. The membranes were washed three times for 15 min in TBS-T and incubated for 60 min with horseradish peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG (Amersham, Oakville, Ont.) diluted 1:1000 in TBS-T containing 5% powdered milk. The membranes were finally washed three times in TBS-T for 15 min each, and the immune complex was revealed with ECL reagents, the manufacturer's protocol being followed (Amersham).

Fluorescent assay of gelatinase activity

The gelatinolytic activity in urine samples (2 μg of creatinine) was measured with the EnzChek Gelatinase/Collagenase Assay Kit from Molecular Probes (Eugene, Ore., USA) as previously described [5]. In the presence of gelatinases, the substrate is cleaved and the fluorescence released is followed at an excitation wavelength of 480 nm and an emission wavelength of 520 nm. Fluorescence was read for 15 min at 37 °C, in the presence or absence of 0.4-mM phenanthroline, with a fluorometer (Molecular Devices, Sunnyvale, Calif.).

Densitometric and statistical analyses

The intensity of the bands obtained by zymography or by immunodetection was measured with a Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA). Significant differences in

the gelatinolytic activities measured in urine were determined by Student's *t*-test. *P* values of less than 0.05 were considered statistically significant.

Results

Gelatinolytic activity examined in transplanted kidney

In the model used, each WFu rat received the left kidney of a BUF donor in orthotopic allotransplantation (BUF → WFu). Ischemia/reperfusion and isotransplantation were performed; kidneys obtained under these conditions and from BUF rats were used as controls. Animals were maintained for 6 days without immunosuppression, which resulted in rejection of the allotransplanted grafts. At the time of biopsy, the rejected kidneys presented altered coloration, and their sizes were enlarged approximately threefold when compared with the size of either contralateral, ischemic/reperfused, or isotransplanted kidneys.

The activities of MMP-2 and MMP-9 were monitored by gelatin zymography (Fig. 1), and their specific activities (relative density units/h per μg) determined in rejected and BUF control kidneys by laser densitometry of the zymogram (Table 1); the ratios of these activities have been compared (Allog T/BUF in Table 1). BUF

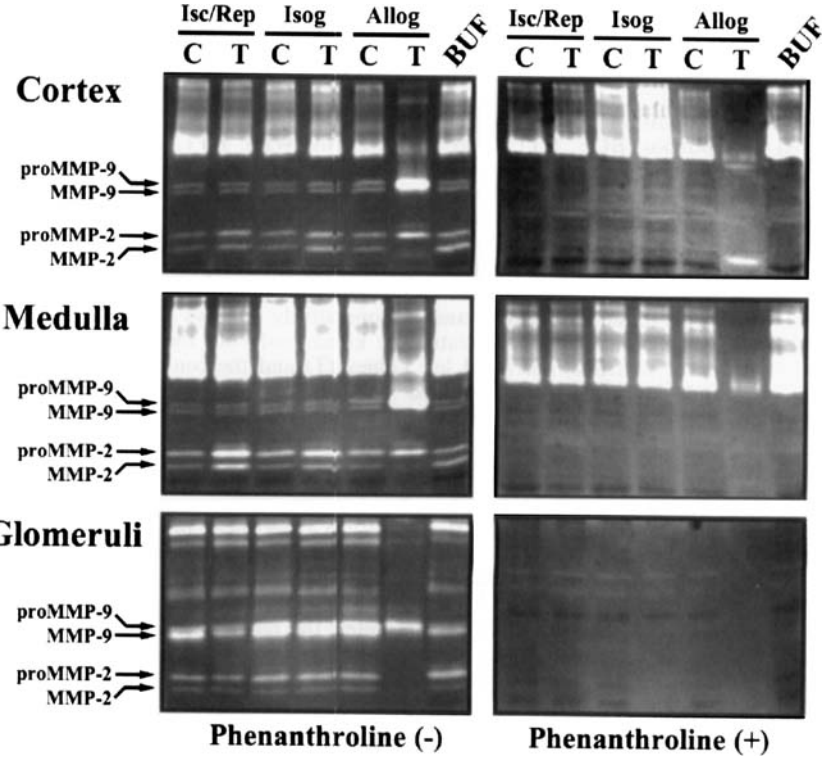

Fig. 1 Zymography examination of MMP activity in the cortices, medullae, and glomeruli from ischemic/reperfused animals (*Isc/Rep*) and isotransplant (*Isog*), and allotransplant (*Allog*) recipients. Contralateral (*C*) and treated (*T*) kidneys from each condition were examined, as well as BUF control kidneys. Samples were subjected to zymography in the absence or presence of phenanthroline

Table 1 Specific gelatinolytic activities of MMP-2 and MMP-9, and expression of MMP-2 determined in allotransplanted kidney (*Allog T*) compared with BUF rat kidney. The bands corresponding to both forms of MMP-2 and MMP-9 in zymography and to both forms of MMP-2 in immunoblot analysis were quantitated by laser densitometry (*NA* not available)

Kidney region	MMP	Specific activity (relative units/h per μg)		Ratios of specific activities	Ratios of proteins immunodetected
		Allog T	BUF	(Allog T/BUF)	(Allog T/BUF)
Cortex	proMMP-9	1050	147	7.1	NA
	MMP-9	152	190	0.8	NA
	proMMP-2	831	450	1.8	2.1
	MMP-2	0	571	0	0.4
Medulla	proMMP-9	2007	59	34.0	NA
	MMP-9	0	64	0.0	NA
	proMMP-2	657	421	1.6	1.2
	MMP-2	100	576	0.2	0.5
Glomeruli	proMMP-9	638	92	6.9	NA
	MMP-9	102	390	0.3	NA
	proMMP-2	33	678	0.1	0
	MMP-2	47	202	0.2	0

kidneys were chosen as control because they represent the transplanted kidneys before the rejection process. In addition, the activities quantified in the contralateral kidneys of the allotransplant recipients were not much different from those measured in the BUF kidneys (data not shown). In renal cortex samples, similar patterns of gelatin degradation were obtained for contralateral and ischemic/reperfused kidneys. Both showed a considerable degradation of gelatin by proteins above proMMP-9 (> 100 kDa). Phenanthroline has very little effect on the activity in the bands above proMMP-9, although it has very clear effects on MMP-9 and MMP-2 (pro- and mature forms), confirming their identity (Fig. 1, right panels). In the ischemic/reperfused kidney, a slight increase in both forms of MMP-2 was observed when compared with the contralateral kidney, whereas the levels of proMMP-9 and MMP-9 remained unchanged. Renal isotransplantation did not alter the pattern of gelatin degradation by proteins from the renal cortex, with the exception of very slight increases in the activities of MMP-2 and proMMP-2. Renal cortex homogenate from a normal BUF donor kidney was also used as a control for the activity of MMPs in the allotransplanted kidney before the process of acute rejection. The gelatin degradation pattern of cortex from BUF kidney was similar to that from the contralateral kidneys of the different groups. However, considerable changes were observed in cortex from allotransplanted kidneys, as it had the weakest gelatinolytic activity above proMMP-9, suggesting an important proteinase turnover. This turnover might be the source of the new activity that appeared right under the normal position of MMP-2, which is not sensitive to phenanthroline (Fig. 1, right panels). MMP-9 and MMP-2 levels were also changed considerably in the cortices of rejected kidneys. The proMMP-9- and proMMP-2-specific activities (Table 1) increased sevenfold and twofold, respectively, when compared with those in the BUF kidney. MMP-9 activity decreased (ratio of 0.8) and MMP-2 activity disappeared. In the medulla, the patterns of gelatinolytic activities were generally the same as those in the cortex, particularly for MMP-9 and MMP-2. Similarly to what was seen with the cortex, MMP-2 and proMMP-2 activities showed a slight increase in the medullae of the treated kidneys from ischemic/reperfused and isotransplant groups. The proMMP-9 activity increased 34-fold in the rejected kidneys (Table 1), whereas that of MMP-9 was dramatically diminished. ProMMP-2 activity increased 1.6-fold whereas that of MMP-2 diminished (ratio of 0.2) in the medullae of rejected kidneys. The glomeruli presented a more clearly defined gelatinolytic pattern above proMMP-9 (> 100 kDa) than did the renal cortices from which they were isolated. Most of these activities disappeared in the presence of phenanthroline. These gelatinases were not significantly modulated by the different treatments, aside from the glomeruli of allotransplanted kidneys, from which they disappeared. Among the various treatment groups, the strongest effects observed were in the rejected kidney glomeruli where all MMP activities decreased except for proMMP-9, whose activity increased 7-fold, when compared with the BUF control kidney.

Overall, both proMMP-2 and proMMP-9 activities increased during rejection in the cortex and the medulla. In contrast, the activities of MMP-2 and MMP-9 decreased. Furthermore, the sums of the activities for both MMP-2 and proMMP-2 in the cortex and the medulla give similar total activities for both the rejected kidney and the BUF control kidney, indicating that there exists a balance between the two forms of MMP-2. However, the total activity for both forms of MMP-9 in the cortex and in the medulla was much higher in the rejected kidney than in the BUF kidney.

ProMMP-2 activation is altered in acute kidney rejection

The levels of expression of the latent and active MMP-2 forms were further determined by Western blotting analysis in the three kidney compartments (cortex, medulla, and glomeruli) from the rejected (Allog) and the BUF kidneys (Fig. 2). Both forms of MMP-2 were detected in the three compartments of the BUF kidneys. However, proMMP-2 was the only form seen in the cortex and medulla from the rejected graft. The combined proMMP-2 and MMP-2 protein level in the BUF kidneys was similar to that of proMMP-2 in the rejected kidneys, as determined by laser densitometry (data not shown). This suggests that no changes in the synthesis or degradation of the protein occurred in the medulla and cortex during acute rejection. In the glomeruli, proMMP-2 and MMP-2 were undetectable, suggesting that they may have been degraded during rejection.

Comparative ratios of expression levels for both forms of MMP-2 in Allograft and BUF kidneys (Allog T/BUF) were determined from immunodetection using laser densitometry; these ratios of immunodetected proteins are shown in Table 1. The ratios indicated that

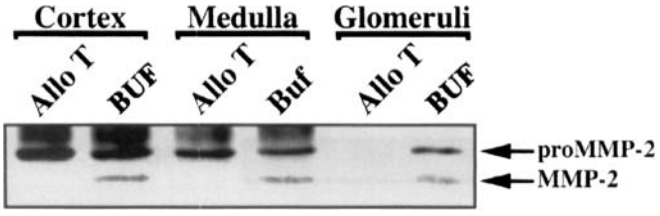

Fig. 2 Detection of latent and active forms of MMP-2 in the rejected kidney. Samples of renal-cortex and medulla homogenates, or isolated glomeruli, from rejected kidneys (*Allo T*) or from BUF-rat kidneys were subjected to Western blot analysis, and MMP-2 was immunodetected with a specific monoclonal antibody

proMMP-2 levels increased in both the cortex (2.1-fold) and the medulla (1.2-fold). In contrast, the rejected kidney had less MMP-2 than did BUF kidney in either cortex or medulla. The comparison of these ratios indicates a correlation between the activity and the expression of each form of MMP-2 and suggests the diminished processing of proMMP-2 into its active MMP-2 form during acute kidney rejection. Accumulation of proMMP-9 in the rejected kidney is also possible, since a large increase in the activity of proMMP-9 but not of MMP-9 activity was observed. Attempts were made to detect both forms of MMP-9 as well as TIMP-1 (data not shown). However, these experiments were unsuccessful because of lack of cross-reactivity with rat tissues.

TIMP-2 protein levels and MT1-MMP proteolytic processing are diminished in kidney acute rejection

Since the activation process of MMP-2 requires the formation of a ternary complex composed of proMMP-2, MT1-MMP, and TIMP-2, immunodetection of these proteins was performed on the renal samples to investigate any changes in their expression that could explain the diminished processing of proMMP-2 into MMP-2. The antibody directed against MT1-MMP recognized at least three bands either in the cortex (Fig. 3, upper panel) or in the medulla (data not shown). These were identified as the proMT1-MMP (66 kDa), the active form of MT1-MMP (60 kDa) confirmed by the active recombinant MT1-MMP loaded as control, and the degraded inactive forms of MT1-MMP (44–40 kDa). During acute rejection, the proMT1-MMP and, even more so, MT1-MMP, increased significantly. In contrast, no inactive fragments (44–40 kDa) were detected in the rejected kidney. TIMP-2 immunodetection was also performed with kidney cortex samples; TIMP-2 was present under most conditions but was undetectable during rejection, both in the cortex (Fig. 3, lower panel) and in the medulla (data not shown). These results indicate that crucial proteins involved in proMMP-2 activation are strongly affected by acute graft rejection.

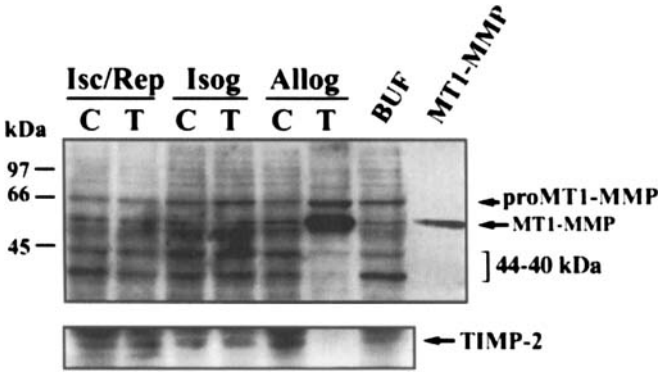

Fig. 3 Detection of MT1-MMP and TIMP-2 in the rejected cortex. Samples of renal cortices from the controls and from the rejected kidneys (*Allog*) were subjected to Western blot analysis. MT1–MMP (*upper panel*) and TIMP-2 (*lower panel*) were immunodetected with specific monoclonal antibodies. Recombinant MT1-MMP was loaded as a control (*right side* of the gel in *upper panel*)

Gelatinolytic activity in urine measured by zymography and fluorescence

Urine was collected from each rat before surgery (day 0), 3 days after surgery (day 3), and before the rat was killed (day 6), and gelatinolytic activity was measured by gelatin zymography (Fig. 4). Urine samples were assessed based on equal amounts of creatinine. Since changes in urinary creatinine may occur during

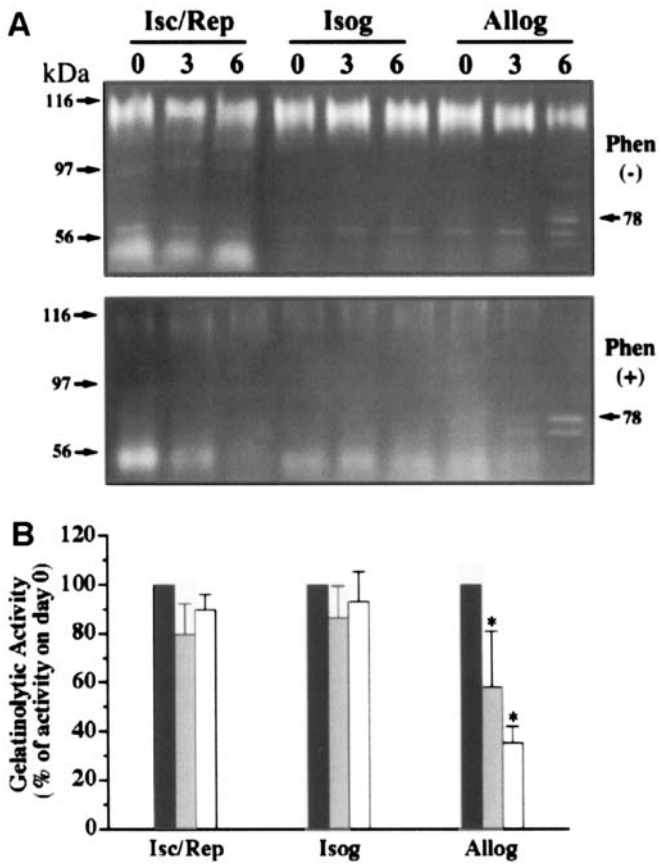

Fig. 4A, B Gelatinolytic activity measured by zymography in urine from animals following ischemia/reperfusion (*Isc/Rep*; $n=3$), isotransplantation (*Isog*; $n=3$), and allotransplantation (*Allog*; $n=4$). Urine was collected on days 0 (before surgery), 3, and 6 and subjected to zymography with or without phenanthroline (**A**). The total activities for days 0 (*black*), day 3 (*gray*), and day 6 (*white*) were quantified (**B**). *Significant difference from the activity measured on day 0 ($P<0.05$)

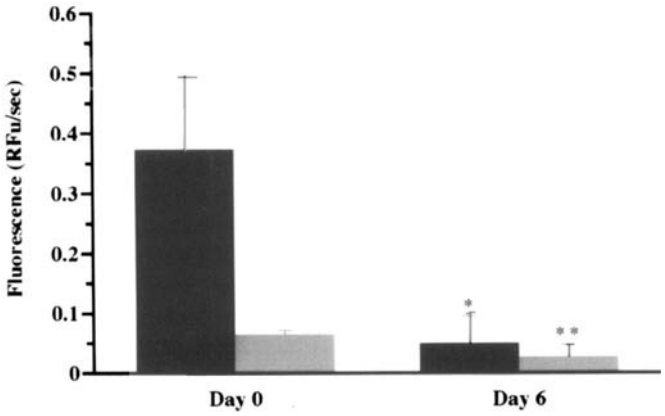

Fig. 5 Gelatinolytic activity in the urine before allotransplantation (day 0) and 6 days after was measured by a fluorescein-conjugated gelatin with (*black*) or without (*gray*) phenanthroline. Activity is presented as relative units of fluorescence per second ($n=4$). *Significant difference ($P<0.05$) compared with the activity measured on day 0 without phenanthroline, **difference compared with the activities on day 0, both with and without phenanthroline

acute rejection [16], zymography was also performed with equal amounts of proteins; similar results were obtained. A hydrolytic activity near 78 kDa (indicated by an arrow in Fig. 4) was observed on day 6 after surgery in the urine of the rats given allotransplants. This activity was not inhibited by phenanthroline, indicating that this gelatinase is not related to MMPs. A diminution of the total amount of gelatinolytic activity was also observed via zymography. The total activity under each condition was measured by laser densitometry (Fig. 4B). The activity was decreased by 40% (day 3) and by 65% (day 6) in the urine of the allotransplant recipients. The gelatinolytic activity in the urine from two other groups (ischemic/reperfused and isotransplant) did not show any significant changes. The diminution of gelatinolytic activity in urine from allograft animals was also studied by fluorescence assay (Fig. 5). From days 0, 3, and 6, urine from the allotransplant recipients was incubated in the presence of a quenched fluorescein-conjugated gelatin. To distinguish the activity of MMPs from that of other gelatinases, we performed measurements in the presence or absence of phenanthroline. As in zymography, the gelatinolytic activity measured by fluorescence showed a significant decrease in the urine of animals experiencing acute rejection on day 6. It decreased to very low but detectable levels both in the absence and in the presence of phenanthroline.

Discussion

In the present study the activity of MMPs involved in the degradation of the ECM was studied in an established model of acute kidney rejection. The activities of both the latent and the active forms of MMP-2 and MMP-9 in rejected kidney, ischemic/reperfused, or isograft tissues, as well as the activities of other unknown gelatinases in the urine of the animals, were evaluated by gelatin zymography. Ischemia and reperfusion are recognized as being involved in both acute and chronic rejection [20]. Recent studies have reported that ischemia and reperfusion increase the expression and activity of MMP-2 and MMP-9 in rat muscle and lung, but not in the kidney [23, 33]. Thus, our results are in agreement with these studies and indicate that ischemia/reperfusion did not contribute to the changes in MMP activity measured during acute rejection.

Zymography experiments indicated that the latent proMMP-2 and proMMP-9 forms generally remained unprocessed during acute kidney rejection. Moreover, there is a correlation between the enzymatic activity of both forms of MMP-2 and their expression in the different anatomical regions of the kidney. Since previous studies have reported that, in acute rejection, the equilibrium between the synthesis and the degradation of the ECM is displaced towards accumulation of ECM [30], our results indicate that this accumulation could be a consequence of the non-processing of both MMP-2 and MMP-9. Immunodetection of the heterotrimeric complex formed by MMP-2, MT1-MMP, and TIMP-2, suggests that the disappearance of TIMP-2 from the graft may result in the non-processing of proMMP-2 [13, 17, 18]. Moreover, our results indicate that MT1-MMP is present in its activated form in the rejected kidney. Since the 44–40 kDa form represents inactive by-products, reflecting the consumption of MT1-MMP in the processing and release activity of MMP-2, the decreased presence of the 44–40 kDa fragment is in good agreement with the non-processing of proMMP-2 into MMP-2 [21].

Another finding regarding gelatinases in acute kidney rejection was the large increase in proMMP-9 activity in cortex and medulla from the rejected graft. The total combined activity measured for both forms of MMP-9 was not similar in the rejected and the BUF kidneys, indicating that part of this activity did not originate from the graft itself. Previous studies have shown that infiltration of the graft by immune cells has generally occurred by day 5 after transplantation in rat models [15]. Since neutrophils and macrophages are important producers of MMP-9 [12, 26, 27], the increase in proMMP-9 activity measured in the cortex and the medulla could thus be the result of immune cell infiltration. Active MMP-9 produced by neutrophils may be rapidly degraded by proteinases, under some circumstances, to limit further tissue damage [27]. It has been suggested that MMP-9 becomes decreased as a consequence of a higher turnover (degradation) of the active form of MMP. Although this could explain the decrease in MMP-9 activity, it does not explain the accumulation

of proMMP-9 observed in the rejected graft, and the non-processing of proMMP-9 should also be considered. Further studies are needed for us to understand the molecular events involved in the processing of proMMP-9. Within the glomeruli, endothelial and mesangial cells are sources of MMP-9 [2] that may explain why the activity of MMP-9 is the only one remaining in the glomeruli of rejected kidneys.

Many non-invasive or less-invasive procedures were proposed for the diagnosis of kidney rejection before, such as duplex Doppler ultrasound assessment, fine-needle aspiration biopsy, urine cytology, urine-cytokine analysis, serum-cytokine analysis, and cytokine analysis of biopsy material [4]. However, only a few means of diagnosing acute kidney rejection are sufficiently accurate as to warrant their routine use. It was documented that gelatinolytic activities such as MMP-2 and MMP-9 are present in the urine of healthy patients [29] and that they could be used as diagnostic tools, since pathological conditions such as bladder carcinoma, for example, may enhance the level of MMP-2 and MMP-9 [28]. Thus, additional indicators of rejection, involving enzymatic activity towards the ECM, were sought in this study.

In the urine collected from animals with acute kidney rejection, a specific activity appeared at 78 kDa, corresponding to a zinc-independent gelatinase, which could be used as a prognostic marker in clinical use. The total gelatinolytic activity was shown to be strongly decreased in the urine of animals experiencing acute rejection, in a time-dependent manner. This reduction could be the result of the considerable decrease of gelatinolytic activity observed in the graft tissue. The degradation of the high-molecular-weight gelatinase in the rejected kidney could produce the 78-kDa activity that appeared in the urine. These results showed that measurement by fluorescence assay could also allow the rapid detection of any variation in urinary gelatinolytic activity and, thus, the rapid diagnosis of acute kidney rejection.

Few studies have considered the importance of MMPs as key players in the process of ECM accumulation in interstitial-fibrosis models. This is the first evidence of inhibition of proMMP activation in an acute kidney-rejection model. The disappearance of TIMP-2, which is necessary for the proteolytic cleavage of proMMP-2 into MMP-2, and the altered MT1-MMP proteolytic processing in acute rejection, constitute the major observations made in this study. The non-processing of proMMPs may contribute to the accumulation of ECM in the graft. Further studies are needed to determine whether the disappearance of TIMP-2 is a cause or a consequence of acute rejection. Neither does proMMP-9 seem to be processed, which could also contribute to the accumulation of ECM. In addition, fluorescent measurement of the gelatinolytic activity in urine from animals could eventually present a new, sensitive, and non-invasive method for the diagnosis of kidney rejection.

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