PAI-1 donor polymorphism influences long-term kidney graft survival

Jean-Philippe Rérolle, Elisa Munteanu, Mireille Drouet, Jean-Christophe Szelag, Béatrice Champtiaux, Fatima Yagoubi, Pierre-Marie Preux, Jean-Claude Aldigier and Yann Le Meur

1 Transplantation, 2 Immunology and 3 Biostatistics units, CHU Dupuytren, Limoges, France

Abstract

Background. The type 1 plasminogen activator inhibitor (PAI-1) is involved in the development of fibrosis, and its intrarenal expression is increased in interstitial fibrosis and tubular atrophy (IFTA). Moreover, a 4G/5G polymorphism of the PAI-1 gene has been described associating 4G haplotype with higher PAI-1 plasma activity. We investigated the relationship between the donor and recipient PAI-1 polymorphism and kidney graft survival.

Methods. The PAI-1 genotype was determined for both the 304 donors and the 337 corresponding recipients. In recipients, PAI-1 antigen levels were also determined. We compared 4G/4G donors versus donors with other genotypes.

Results. Donor or recipient genotype did not influence the PAI-1 plasma level in recipients. Actuarial kidney graft survival was significantly reduced in the 4G/4G donor group (107 months versus 147.5 months, \( P = 0.013 \)), while recipient PAI-1 genotype did not show any influence on graft survival. Moreover, graft loss due to IFTA proved significantly higher in the 4G/4G donor group (13% versus 6%, \( P = 0.03 \)). Multivariate analysis showed that the significant independent variables associated with graft loss were the donor 4G/4G genotype, acute clinical rejection and donor age.

Conclusion. Our study suggests that donor PAI-1 polymorphism influences kidney graft survival and that the donor 4G/4G genotype is an independent risk factor for graft loss. Prospective studies are needed to confirm these results.

Keywords: chronic allograft nephropathy; gene polymorphism; PAI-1; renal transplantation

Introduction

Interstitial fibrosis and tubular atrophy (IFTA) is a clinically-pathological entity characterized by fibrosclerosis of the different renal structures leading to progressive decline of renal function after kidney transplantation [1]. The molecular mechanisms that underlie the pathophysiology of IFTA remain far from clear. It seems to result from combined effects of immunological and non-immunological factors [2].

Degradating proteases are important participants in tissue remodelling and repair and, therefore, their activation may play a role in the morphological changes observed in IFTA. Plasmin is a potent serine protease responsible for fibrinolysis but is also involved in matrix metalloproteinase activation, growth-factor release and direct degradation of extra-cellular matrix proteins [3]. The type 1 plasminogen activator inhibitor (PAI-1) controls plasmin formation and acts as an inhibitor of fibrinolysis and matrix degradation and seems to be involved in the development of tissue fibrosis and IFTA [3].

Upregulation of PAI-1 has been demonstrated in human IFTA accompanied by persistent fibrin deposition in the graft [4,5]. Furthermore, the glomerular PAI-1 mRNA level has been shown to be predictive of long-term graft function [5] and is correlated with the intensity of interstitial fibrosis on a kidney graft biopsy [6]. PAI-1 activity is increased in renal allograft recipients receiving ciclosporin [7], and PAI-1 levels are related to the rate of renal failure progression after kidney transplantation [8]. In the other hand, PAI-1 synthesis is inhibited by angiotensin converting enzyme inhibitor (ACEI) or angiotensin II type 1 receptor antagonist (ARB) therapies [9,10].

A common 4G/5G polymorphism in the promoter region of the human PAI-1 gene has been described and is associated with different levels of serum PAI-1 activity. The 5G variant binds the E2F transcription repressor, whereas 4G fails to do so and is associated with the higher PAI-1 plasma level [11]. Studies on the influence of recipient PAI-1 gene polymorphism on IFTA are controversial. Lahlou et al. [8] did not find any influence of this polymorphism on the rate of decline of renal function after transplantation, whereas Reis et al. [12] found that transplant recipients with IFTA had significantly lower frequencies of the 5G/5G genotype and 5G allele. All these studies focused on kidney graft recipients. However, a substantial part of the PAI-1 synthesis is probably assumed by renal graft cells [5,6] such as mesangial cells, endothelial cells or epithelial cells.
PAI-1 production of these cells depends on the donor genetic background. We then hypothesized that donor PAI-1 polymorphism could influence the outcome of renal transplantation. To test this hypothesis we determined the donor PAI-1 genotype and investigated the relationship between this genotype and kidney graft survival after renal transplantation. At the same time, the role of the recipient PAI-1 genotype and recipient plasma PAI-1 antigen level was studied.

Material and methods

Patients
Three hundred and thirty-seven consecutive patients transplanted between 1990 and 2005, routinely followed as outpatients in our centre, were enrolled in the study. These patients received 367 grafts from 304 donors. The ethics committee of Limoges hospital approved the protocol. Informed consent was obtained from each recipient. Consent for the use of donor material was obtained from the French ‘agence de la biomedecine’. The following inclusion criteria were used: recipient age > 18 years, functioning graft of > 1 year after transplantation and kidney graft from a cadaveric donor. Exclusion criteria were as follows: patient age > 18 years, pregnancy, graft survival < 1 year and combined kidney and pancreas or heart or liver transplantation.

For each patient, the following clinical parameters were recorded from the medical file: the date of birth and sex of both donor and recipient, the weight and height of recipients, preformed reactive antibodies (historical maximum), HLA mismatches between donor and recipient, the duration of cold ischaemia, induction therapy, immunosuppressive treatment, occurrence of clinical acute rejection within the first year posttransplantation, the presence of hypertension and the use of either ACEI or ARB for ≥3 months prior to the study, and finally the annual plasma creatinine level.

IFTA was the proposed diagnostic for graft loss if (i) IFTA was present on a previous biopsy in the follow-up of the patient, (ii) renal function gradually decreased over several months and (iii) all other causes of graft loss were excluded (recurrence of initial nephropathy, sepsis, lymphoma, myeloma, neoplasia).

Plasminogen activator inhibitor-1 4G/5G polymorphism
Genomic DNA was extracted from peripheral blood leukocytes for recipient, and lymph nodes or spleen lymphocytes for donor by salting out extraction as described [13]. PAI 4G/5G promoter genotype polymorphism was determined by a PCR amplification of genomic DNA followed by electrophoresis of the capillaries. The PAI-1-promoter was amplified by the following primer:

5′-AGCCACGTTGATGTCTAGGT-3′ in combination with a downstream primer 5′-TCCAACCTCACGCAGACAG-3′ labelled with a fluorescent label (6-FAM). A nonpolymorphic gene, the growth factor hormone (HGH), was amplified as an internal control PCR product with the antisens primer 5′-GCCTTCCAACCAACCCCATCCCTTA-

3′ and the 5′-Fam-TCCAGCGGGGAAATCC-4′. The protocol allowed the obetration of amlicons of 188 base pair (bp) for the 4G allele, 189 bp for the 5G allele and 159 bp for the internal control. Briefly, the PCR reactions were performed in a final volume of 25 µl on a 9700 PCR thermocycler (Perlin Elmer, Courtaboeuf, France). 2.5 ng of genomic DNA was added to 0.2 mM/l of each dNTP (Pharmacia, Charbonnière, France) 0.4 µM/l of each primer; Taq polymerase buffer with 2 mM MgCl2, 1.25 Taq polymerase (Applied Biosystems, Courtaboeuf, France). The cycle condition was 94°C for 2 min, followed by 28 cycles at 94°C for 30 s, 57°C for 30 s and 72°C for 30 s with a final elongation step of 10 min at 72°C. The detection of the fluorescent amplicons was performed on an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems, Courtaboeuf, France). Thus 1 µl of the PCR reaction product was added to 24 µl of deionized formamide (Sigma, France), and 1 µl of ROX-500 size standard was denaturized by heatin 3 min at 94°C, and then placed on ice until used. Electrophoresis was performed using the Performance Optimized Polymer 6 (Applied Biosystems) with a 47 cm/50 µm capillary.

Plasmonic PAI quantification
Plasma PAI-1 antigen levels were determined using a TinElize kit (Trinity Biotech, Wicklow, Ireland) following the instructions from the manufacturer. The plasma of 275 recipients was collected between March 2005 and April 2006 at the time of transplantation, and immediately frozen at −80°C until used. Each assay was performed in duplicate.

Statistical analysis
Qualitative variables were described by raw numbers and proportions and quantitative variables by means and standard deviations. Comparisons between several groups of patients with different genotypes were done using the chi-square test for qualitative variables and the Student t-test for quantitative variables. Survival curves were estimated by the Kaplan–Meier method, and several survival curves were compared using log rank tests. The date of origin was the graft date for each patient. Multivariate analysis was done using the Cox model. The initial model included all variables with a P-value of <0.25, and the final model was obtained using the step-by-step descending method. The Cox model was also used to estimate the relative risks of the significant prognostic factors at 95% confidence intervals. All the statistical analyses were done using a significant level for the P-value fixed at 0.05.

Results

PAI-1 genotype
The PAI-1 genotype was determined for 304 donors (leading to 367 kidney grafts between 1990 and 2005) and for 337 recipients. The plasma level of PAI-1 antigen was obtained for 275 recipients. Twenty-nine patients had a second
transplantation, 6 in the 4G/4G group and 23 in the other groups.

Among the 304 genotyped donors for the PAI-1 promoter polymorphism, 71 (23.3%) were 4G/4G, 164 (53.9%) were 4G/5G and 69 (22.7%) were 5G/5G. For the recipients, 78 (23%) were 4G/4G, 165 (48.7%) were 4G/5G and 96 (28.3%) were 5G/5G. Distribution of the PAI-1 allele was not different from expected values based on the Hardy-Weinberg postulate.

We chose to compare the donor 4G homozygous genotype with other donor PAI-1 gene promoter polymorphisms (4G/5G and 5G/5G). Baseline characteristics did not differ significantly between the groups (Table 1) with regard to kidney donor and recipient age or sex, preformed reactive antibodies, degree of HLA mismatching, cold ischaemic time, occurrence of clinical acute rejection within the first year posttransplantation, hypertension, use of ACEI or ARB, initial nephropathy or duration of follow-up. The mean follow-up period was 69.7 ± 45.5 months for the 4G group and 72 ± 46.8 months in the other group (P = ns).

### Table 1. Baseline demographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>Donors 4G/4G (N = 283)</th>
<th>Donors 4G/5G or 5G/5G (N = 342)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor age (year)</td>
<td>37.8 ± 14.8</td>
<td>41.2 ± 15.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Donor sex (% of men)</td>
<td>67.8%</td>
<td>67.5%</td>
<td>0.99</td>
</tr>
<tr>
<td>Recipient age (year)</td>
<td>47 ± 14.5</td>
<td>49.4 ± 13.6</td>
<td>0.16</td>
</tr>
<tr>
<td>Recipient sex (% of men)</td>
<td>63.1%</td>
<td>56.9%</td>
<td>0.37</td>
</tr>
<tr>
<td>PRA &gt; 80%</td>
<td>3.7%</td>
<td>3.9%</td>
<td>0.99</td>
</tr>
<tr>
<td>HLA mismatches</td>
<td>3.7 ± 1.2</td>
<td>3.6 ± 1.3</td>
<td>0.68</td>
</tr>
<tr>
<td>Cold ischaemic time (min)</td>
<td>1170 ± 300</td>
<td>1194 ± 342</td>
<td>0.55</td>
</tr>
<tr>
<td>Clinical acute rejection</td>
<td>29.7%</td>
<td>22.4%</td>
<td>0.14</td>
</tr>
<tr>
<td>Hypertension</td>
<td>50.6%</td>
<td>54.1%</td>
<td>0.66</td>
</tr>
<tr>
<td>ACEI or ARA II treatment</td>
<td>60.5%</td>
<td>57.4%</td>
<td>0.71</td>
</tr>
<tr>
<td>Calcineurin inhibitors</td>
<td>93.8%</td>
<td>96%</td>
<td>0.59</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>69.7</td>
<td>72</td>
<td>0.68</td>
</tr>
<tr>
<td>Primary renal disease</td>
<td></td>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td>Glomerular</td>
<td>45.2%</td>
<td>47%</td>
<td></td>
</tr>
<tr>
<td>Vascular</td>
<td>8.3%</td>
<td>4.9%</td>
<td></td>
</tr>
<tr>
<td>Interstitial</td>
<td>3.6%</td>
<td>8.8%</td>
<td></td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>19%</td>
<td>19.1%</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>4.8%</td>
<td>4.9%</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>19%</td>
<td>15.2%</td>
<td></td>
</tr>
</tbody>
</table>

Eighty-one patients (96.4%) in the 4G/4G donor group and 277 (97.8%) in the other donor group (P = ns) had an induction therapy with antithymocyte globulins or anti IL2 receptor monoclonal antibodies. Seventy-six patients (93.8%) were treated with cyclosporine or tacrolimus in the 4G donor group versus 266 (96%) in the other group. The majority of patients received ciclosporin therapy, which was adapted according to their ciclosporin trough level (83%), and for the remaining patients (17%) therapy was adapted according to C2 concentration. For the patients under ciclosporin therapy, mean C0 blood levels during the period of follow-up were 141.4 ng/ml in the 4G/4G group versus 136.6 ng/ml in the other group (P = 0.12), and mean C2 blood levels were 822.38 ng/ml versus 857.8 ng/ml (P = 0.59). For the patients under tacrolimus therapy, mean C0 blood levels during the period of follow-up were 8.3 ng/ml in the 4G/4G group versus 8.7 ng/ml in the other group (P = 0.55).

### PAI-1 donor or recipient genotype do not influence patients’ survival

Actuarial patient survival curve in different donor or recipient genotype groups are shown in Figure 1. We did not find any influence of these different genotypes on patient survival.

### PAI-1 donor genotype but not the recipient genotype influences kidney graft survival

Actuarial graft survival curves, censored for death in different donor or recipient genotype groups, are shown in Figure 2. Kidney graft survival was significantly reduced in the 4G/4G donor group with an average of 109 months versus 147.5 months for the 4G/5G or 5G/5G donor group, P = 0.013. Kidney graft survival was not different between 4G/5G or 5G/5G donor group. In contrast, the recipient genotype had no effect on graft survival (133.3 months for the 4G/4G recipient group versus 150.5 months for the 4G/5G or 5G/5G recipient group, P = ns). There is no synergistic effect of the 4G/4G genotype in the recipient in the 4G/4G donors (data not shown).

### Causes of graft loss

Twenty-three patients died of cardiovascular events or cancer with a functioning graft. Thirty-eight patients lost their graft during the follow-up period, 15 in the 4G/4G donor group and 23 in the 4G/5G or 5G/5G donor group. When we separate graft loss due to IFTA confirmed by the biopsy with graft loss due to other causes (recurrence of initial nephropathy, myeloma, lymphoma, septic shock) (Table 2), we found that graft loss by IFTA was significantly higher in the 4G/4G donor group (13% versus 6%, P = 0.03). There was no difference in graft loss for other reasons.

Among the population of patients losing their graft by IFTA, 63.5% in the 4G donor group and 47% in the other donor group have a history of acute rejection (P = 0.42).

### Factors associated with kidney graft loss

A simple linear regression analysis revealed that only three factors were significantly correlated with kidney graft loss (donor 4G/4G genotype, clinical acute rejection, donor age) (Table 3). The other parameters (donor and recipient age or sex, initial nephropathy, cold ischaemia time, number of HLA mismatches, preformed reactive antibodies, induction therapy, recipient PAI-1 genotype, plasma PAI-1 antigen level, calcineurin inhibitor therapy, presence of hypertension and the use of ACEI or ARB > 3 months prior to the study) had no influence. Using step-by-step backward multiple regression analysis the significant independent variables associated with kidney graft loss were identified as the donor genotype 4G/4G, the occurrence of clinical acute rejection and donor age (Table 3).
Influence of the PAI-1 donor genotype on kidney graft function

We tested the hypothesis that PAI-1 polymorphism may influence renal function deterioration after renal transplantation. When the patients were divided into two significantly different groups ($P < 0.001$) according to the decline of their renal function: non-progressors group ($n = 226; -0.512 \pm 3.7\text{ ml/mn/year}$); progressors groups ($n = 129; -11.6 \pm 8.6\text{ ml/mn/year}$), we found a significantly higher proportion of 4G/4G donor in the progressors group versus non-progressors group (30% versus 18%; $P = 0.02$). We also used a 30% increase of creatinine level from the 1-year baseline as a marker. By Kaplan–Meier analysis, we showed that patients in the 4G group tended to have a faster decrease in renal function even if not significant (Figure 3).

Influence of donor 4G/4G polymorphism on kidney graft survival after stratification of recipients for acute rejection

Twenty-five patients in the 4G/4G donor group and 63 in the other donor group had a history of one or more episode of acute rejection. Among this population, actuarial kidney graft survival was not different between the 4G/4G donor group and the other donor group (107.5 months $\pm$ 30.1 versus 141 months $\pm$ 52; $P = 0.19$).

In the population of patients losing their graft by IFTA, 7/11 (63.5%) in the 4G/4G donor group and 8/17 (47%) in the other group ($P = 0.42$) had a history of acute rejection.

Influence of PAI-1 polymorphism on the plasma PAI-1 antigen level

The plasma PAI-1 antigen level in the recipient was not influenced by the donor (52.1 ng/ml $\pm$ 25.9 for 4G/4G versus 51.2 ng/ml $\pm$ 28 for 4G/5G and 5G/5G; $P = \text{ns}$) or the recipient (52.2 ng/ml $\pm$ 25.7 for 4G/4G versus 51.1 ng/ml $\pm$ 28.1 for 4G/5G and 5G/5G; $P = \text{ns}$) PAI-1 genotype (Figure 4).

Factors associated with the plasma PAI-1 antigen level

Using the Spearman test, the PAI-1 antigen level was significantly correlated with the following variables: glycaemia, triglycerides level, protidaemia, platelet and white blood cells counts, haemoglobin level and delay since transplantation (Table 4).
PAI-1 donor polymorphism influences long-term kidney graft survival

Fig. 2. Cumulative graft survival according to the donor (A) and recipient (B) PAI-1 genotype.

Table 2. Percentage of kidney graft loss due to IFTA and to other causes according to donor PAI-1 genotype

<table>
<thead>
<tr>
<th></th>
<th>4G/4G donor genotype n = 84</th>
<th>Other donor group n = 283</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft loss by IFTA</td>
<td>11 (13%)</td>
<td>17 (6%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Graft loss for other</td>
<td>4 (4%)</td>
<td>6 (2%)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Conversely, the decrease in renal function was inversely correlated with the PAI-1 antigen level (Table 4).

**Influence of treatment with ACEI or ARB on the plasma PAI-1 antigen level**

Influence of treatment with ACEI or ARB on the plasma PAI-1 antigen level was studied in the whole patient group and in the subsets of each donor genotype. We did not find any difference in all these categories of recipients. In the whole patient group, 169/275 (61.4%) patients were under ACEI or ARB therapy. The PAI-1 antigen level was 55.9 ng/ml in the patients treated versus 51.8 ng/ml in the patients not treated (p = 0.97). In the 4G/4G donor group, 35/59 (59.9%) patients were under ACEI or ARB therapy. The PAI-1 antigen level was 55.9 ng/ml in the patients treated versus 53.3 ng/ml in the patients not treated (p = 0.73). In the other donor group, 130/216 (60.2%) patients were under ACEI or ARB therapy. The PAI-1 antigen level was 50.3 ng/ml in the patients treated versus 51.1 ng/ml in the patients not treated (p = 0.84).

**Discussion**

The improvement in immunosuppressive therapy over the last 10 years has dramatically reduced the incidence of acute rejection and the relevance of this event on renal graft loss. At the same time, the role of IFTA in renal graft survival has grown exponentially. Although the pathogenesis of chronic vascular, interstitial and glomerular damage in the transplanted kidney is still largely unclear, both immune and nonimmune mechanisms may participate in the development of IFTA [2].

The genetic regulation of cytokine and chemokine gene expression is now increasingly recognized as a significant
Table 3. Independent factors correlated with kidney graft loss by simple and multiple linear regression analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Crude Relative Risk (95% CI)</th>
<th>P</th>
<th>Adjusted Relative Risk (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor genotype 4G/4G</td>
<td>2.24 (1.17–4.31)</td>
<td>0.015</td>
<td>2.34 (1.12–4.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Clinical acute rejection</td>
<td>2.22 (1.07–4.59)</td>
<td>0.032</td>
<td>1.52 (1.12–1.72)</td>
<td>0.04</td>
</tr>
<tr>
<td>Donor age</td>
<td>1.04 (1.01–1.06)</td>
<td>0.0058</td>
<td>1.04 (1.01–1.07)</td>
<td>0.01</td>
</tr>
<tr>
<td>IEC/AA2</td>
<td>1.18 (0.57–2.45)</td>
<td>0.6551</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Recipient sex</td>
<td>1.14 (0.60–2.15)</td>
<td>0.693</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Recipient age</td>
<td>1.02 (0.99–1.04)</td>
<td>0.1836</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Donor sex</td>
<td>0.72 (0.34–1.52)</td>
<td>0.3876</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cold ischaemic time</td>
<td>1.00 (0.99–1.001)</td>
<td>0.5339</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HLA mismatch</td>
<td>0.90 (0.71–1.16)</td>
<td>0.43</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Recipient genotype</td>
<td>1.04 (0.38–2.83)</td>
<td>0.9453</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Recipient hypertension</td>
<td>1.06 (0.52–2.17)</td>
<td>0.875</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

variable affecting allograft outcome, particularly genes participating in the fibrosis process [14,15,16]. PAI-1 is a multifunctional glycoprotein with impressive fibrosis promoting effects in the kidney. High renal PAI-1 levels seem to predict a bad long-term outcome [3,17]. Genetic variation in the PAI-1 gene is associated with varying levels of PAI-1 activity in healthy individuals as well as in patients with coronary artery disease [3] or diabetes mellitus [3]. In renal transplantation, the recipient has been the focus of most transplantation genomics studies [8,12,16,18]. Recently, however, the importance of the donor genotype in kidney transplantations has been noted [14,15,18].

The presence of the increased PAI-1 level in renal transplant recipients exhibiting IFTA [19] and the positive correlation found by Delarue et al. between the degradation of renal function and the PAI-1 mRNA level in the renal biopsy suggest that in situ production of PAI-1 by the graft cells could play an important role in the pathogenesis of IFTA. Nevertheless, the influence of donor PAI-1 gene polymorphism on kidney graft survival had never been studied.
We standardized a method based on capillary electrophoresis of fluorescent PCR product on a 9100 ABI PRISM sequencer. The use of 28 cycles was found to be the optimal PCR condition. POP 6 was the most discriminating gel and allowed a discrimination of 1 bp. The validity of the techniques to attribute 4G/5G genotypes was ascertained by sequencing 10 PCR products of the 3 patterns according to the dye terminator protocol. The technique was then intensively validated on those DNA of certified profiles and 104 DNA of healthy Caucasian subjects. The frequency of the three genotypes did not differ for the donors, the recipients and healthy subjects. In each batch we included a negative control, 4G/4G DNA, 5G/5G DNA and 4G/5G DNA. In each test we had two internal control of fragments size. The first was a PCR product of a nonpolymorphic gene (HGH) of 159 bp, and the second consisted of the addition of internal size marker (ROX) before running the electrophoresis. These internal controls ascertained the reproducibility of intra- and intertests. Homozygote 4G/4G had a single peak at 188 bp, homozygote 5G/5G a single peak at 189 bp and heterozygote 4G/5G two peaks, one at 188 bp and the other at 189 bp.

We showed, for the first time, in a large cohort of patients (304 donors for 367 renal transplantations) that the donor 4G/4G genotype significantly reduces kidney graft survival by increasing graft loss due to IFTA. We chose to compare the 4G homozgygote genotype with other PAI-1 gene promoter polymorphisms (4G/5G and 5G/5G) because homozgyosity for PAI-1 4G allele is an established risk factor of renal injury [17].

This study confirms the importance of PAI-1 in the pathogenesis of IFTA [5] and completes the results of Grandalliano et al. and Delarue et al. suggesting that increased production of PAI-1 in IFTA is mediated by kidney graft cells themselves under the control of donor genome [5,6]. Mesangial, endothelial and interstitial cells have been characterized as the main sources of PAI-1 [6].

In our study, recipient plasma levels of the PAI-1 antigen were not significantly influenced by the donor or recipient PAI-1 gene polymorphism as previously described by Lahiu et al. [8]. Furthermore, the PAI-1 plasma antigen level is strongly correlated with metabolic, inflammatory and prothrombotic markers such as platelet counts, triglycerides, glycemia, etc. (Table 4). These results have already been reported by other authors in a population of kidney graft recipients [8]. All these factors are extremely variable from 1 day to the next; therefore, dosage of the PAI-1 plasma antigen level on any one day is not particularly relevant. So, the PAI-1 antigen level cannot be considered as a risk factor of graft loss or a marker of IFTA.

Ciclosporin-treated kidney transplant recipients have shown evidence of impairment in fibrinolysis with a high PAI-1 antigen and activity level in plasma when compared to the control patient [20], probably by means of TGF-β, which is one of the transcriptional factors that activate PAI-1 gene transcription. However, both our patients, in the 4G/4G group and the ‘control’ group, were treated equally with ciclosporin, and all had similar serum levels of ciclosporin; thus the results observed in our study are independent of any effect of ciclosporin on PAI-1 production and the differences observed are exclusively due to the influence of the PAI-1 donor genotype.

We did not find a significant difference between the two groups in terms of decreased renal function, even though a tendency for a worse renal function was seen in the 4G/4G donor group (Figure 3), and patients in the progressor-group were more likely to have received a kidney from the 4G/4G donor. This can probably be explained by the fact that the creatinine level remained stable for a long period despite the development of IFTA, and that decreased renal function was delayed with graft loss following a few months after the onset of increase in the creatinine level. Chow et al. [17] found that the recipient PAI-1 genotype was associated with progressive renal dysfunction after acute rejection in renal transplant patients, suggesting that histologic IFTA lesions following an episode of acute rejection are promoted by the donor-PAI-1 genotype. We were not able to confirm these results. In our study, neither the donor nor recipient PAI-1 genotype influenced kidney graft survival in patients with a history of acute rejection. Moreover, the proportion of patients with a history of acute rejection was not different between the 4G/4G group and other donor groups, among patients who lost their graft by IFTA.

Many factors can regulate PAI-1 synthesis, including angiotensin [9]. In different animal models of renal sclerosis, treatment with ACEI or ARB could achieve regression of sclerosis by decreasing intrarenal PAI-1 expression [9,10]. We did not find any effect of these treatments on PAI-1 antigen plasma levels in our patients, consolidating the idea that local expression of PAI-1 is more important in the induction of sclerosis by decreasing intrarenal PAI-1 expression [9,10]. In different animal models of renal sclerosis, treatment with ACEI or ARB could achieve regression of sclerosis by decreasing intrarenal PAI-1 expression [9,10]. We did not find any effect of these treatments on PAI-1 antigen plasma levels in our patients, consolidating the idea that local expression of PAI-1 is more important in the induction of sclerosis by decreasing intrarenal PAI-1 expression [9,10].

This study suggests that select donor genes could be incorporated as a donor-specific variable that could influence IFTA, emphasizing the importance of the donor genetic makeup beyond traditional HLA matching. Having determined that the donor PAI-1 genotype plays a role in the decline of a kidney graft, screening of the donor prior to transplantation can be proposed to adjust the therapy. For example, drugs known to inhibit PAI-1 synthesis such as angiotensin-converting enzyme inhibitors could be given systematically to recipients of kidneys from 4G/4G donors. Furthermore, for these recipients, immunosuppressive therapy could also be modified by avoidance of corticosteroids or ciclosporin, which are known to induce PAI-1 synthesis [20,21]. Interestingly, a study of Sartori et al. demonstrated...
a reduction in the PAI-1 antigen and activity in patients with
steroid-free immunosuppression [22].

Several questions remain unanswered by this study. PAI-1 local synthesis has been shown to be up-regulated in acute and chronic rejection [3,5,6] and donor genotype probably influenced this local production. It would thus be interesting to evaluate the potential correlation between the pathology of the graft, the intrarenal expression of PAI-1 and donor genotype. Because biopsies were not performed at inclusion, the present study cannot answer this question.

Moreover, the results of this genotype association study are interesting and important but need to be replicated in an independent population. Therefore, this work opens the way to further studies.

Conclusion

In conclusion, our study confirms the role of PAI-1 in the pathogenesis of IFTA, in a large cohort of unselected renal transplant recipients, and suggests that increased production of PAI-1 in IFTA is mediated by kidney graft cells themselves under the control of the donor genome. It underlines the importance of the donor and of his genetic makeup for the long-term graft survival. The identification of a gene as a marker of individual susceptibility to IFTA is unravelling new opportunities to optimize and introduces new therapeutic choices for renal transplant recipients.

Conflict of interest statement. None declared.

Reference


Accepted in revised form: 7.4.08