Plasminogen Activator Inhibitor-1 Is a Significant Determinant of Renal Injury in Experimental Crescentic Glomerulonephritis

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Abstract. Crescentic glomerulonephritis is characterized by glomerular fibrin deposition, and experimental crescentic glomerulonephritis has been shown to be fibrin-dependent. Net fibrin deposition is a balance between activation of the coagulation system causing glomerular fibrin deposition and fibrin removal by the plasminogen-plasmin (fibrinolytic) system. Plasminogen activator inhibitor-1 (PAI-1) inhibits fibrinolysis by inhibiting plasminogen activators and has effects on leukocyte recruitment and matrix deposition. To test the hypothesis that the presence of PAI-1 and its levels were a determinant of injury in crescentic glomerulonephritis, accelerated anti-glomerular basement membrane glomerulonephritis was induced in mice genetically deficient in PAI-1 (PAI-1−/−), PAI-1 heterozygotes (PAI-1 +/−), and mice engineered to overexpress PAI-1 (PAI-1 tg). Compared with strain-matched genetically normal animals, PAI-1−/− mice with glomerulonephritis developed fewer glomerular crescents, less glomerular fibrin deposition, fewer infiltrating leukocytes, and less renal collagen accumulation at day 14 of disease. The reduction in disease persisted at day 28, when injury had become more established. In contrast, mice overexpressing the PAI-1 gene (PAI-1 tg), that have basal plasma and renal PAI-1 levels several times, normal developed increased glomerular crescent formation, more glomerular fibrin deposition, increased numbers of infiltrating leukocytes, and more renal collagen at both time points. These studies demonstrate that PAI-1 is a determinant of glomerular fibrin deposition and renal injury in crescentic glomerulonephritis.

Crescentic glomerulonephritis (GN) is characterized by glomerular fibrin deposition (1,2). Glomerular fibrin deposition is a feature of rapidly progressive GN in both humans and in experimental models (3,4). In experimental systems, this lesion is fibrin-dependent (5,6). Net glomerular fibrin deposition is a balance between activation of the coagulation system and fibrin deposition and fibrin removal by the plasminogen-plasmin system (7,8). Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor that inhibits the activity of both tissue type plasminogen activator (tPA - the predominant glomerular plasminogen activator (9)) and urokinase type PA (uPA) (reviewed in references 10 and 11). PAI-1 is upregulated early in the course of experimental immune renal injury (12,13). Inhibition of tPA and uPA by PAI-1 leads to multiple events that favor deposition of fibrin and other matrix proteins, by inhibiting plasmin generation and the direct effects of uPA. PAI-1 has other effects on cell migration and leukocytes that seem to favor recruitment of leukocytes to inflammatory lesions (11). There is some evidence, including studies of in vivo fibrin-associated immune glomerular injury, PAI-1 has a pathogenic role in renal disease. Recent reviews have highlighted both the pathogenetic potential for PAI-1 in renal disease and its potential role as a therapeutic target (10,11).

Functional studies of the role of fibrin in crescentic GN have shown that this fibrin deposition is an important mediator of injury (4–6,15). The net amount of fibrin accumulation in glomeruli results from pathogenetic procoagulant effects driven by tissue factor (7,16) and the protective effects of the plasminogen plasmin system (8,12). Studies in mice deficient in components of this system have demonstrated a protective role for endogenous plasmin (generated by the conversion of plasminogen to plasmin by tPA) in experimental crescentic GN (8). Increased glomerular PAI-1 expression has been found in both human and experimental crescentic GN (12,17–19). Given these findings, it is logical that molecules that inhibit plasmin generation, such as PAI-1, would be pathogenetic in crescentic GN. However, this hypothesis has not been tested in vivo.

These studies address the hypothesis that in fibrin-associated immune glomerular injury, PAI-1 is an important determinant.
of injury, crescent formation, and matrix accumulation. They use mice that have been genetically manipulated to be PAI-1 deficient (PAI-1−/− mice) or PAI-1 overexpressing (PAI-1 tg mice) in which experimental crescentic GN, a fibrin associated form of immune glomerular injury, had been induced. In addition to studying disease at 14 d, experimental crescentic GN was studied at 28 d to determine whether any effect of PAI-1 on fibrin/matrix deposition and injury could be overcome by other inhibitors of plasmin.

Materials and Methods

Experimental Design

Mice with a genetic deletion of PAI-1 (20,21) on a C57BL/6 × 129/SvJ background, backcrossed once onto a C57BL/6 background (75% C57BL/6, 25% 129/SvJ) were bred in a specific pathogen-free facility (Monash Medical Center, Melbourne, Australia). Mice of an identical genetic background (i.e., 75% C57BL/6, 25% 129/SvJ) bred from the backcross were used as controls. PAI-1+/− mice were generated by interbreeding PAI-1−/− and PAI-1+/− mice. PAI-1 transgenic mice on a pure C57BL/6 background expressing a murine PAI-1 minigene under a CMV promoter were generously provided by Dr. D. Ginsburg (Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, Michigan). Genetically normal pure C57BL/6 mice were used as controls. Male mice aged 8 to 12 wk were used for experiments. Anti-mouse glomerular basement membrane (GBM) globulin was prepared as described previously (23). Mice were sensitized by subcutaneous injection of a total of 2 mg of sheep globulin in 100 μl of Freund complete adjuvant in divided doses in each flank 10 d before challenge with 5 mg of sheep anti-mouse GBM globulin. Renal injury was studied at two time points: 14 d and 28 d after challenge with sheep anti-mouse GBM globulin. Results are expressed as the mean ± SEM. The significance of differences between groups was determined by ANOVA, followed by Bonferroni Multiple Comparison Test for paired comparisons (GraphPad Prism; GraphPad Software Inc., San Diego, CA). For studies involving PAI-1 tg mice (two groups only) the unpaired t test was used.

For studies using mice genetically deficient in PAI-1, the following groups of mice were studied:

- PAI-1+/− mice: baseline (n = 6), GN day 14 (n = 6), GN day 28 (n = 6)
- PAI-1+/− mice: baseline (n = 6), GN day 14 (n = 6), GN day 28 (n = 6)
- PAI-1−/− mice: baseline (n = 7), GN day 14 (n = 7), GN day 28 (n = 7)

For studies using mice genetically engineered to overexpress PAI-1, the following groups of mice were studied:

- C57BL/6 mice: baseline (n = 8), GN day 14 (n = 8), GN day 28 (n = 8)
- PAI-1 tg mice (C57BL/6): baseline (n = 6), GN day 14 (n = 6), GN day 28 (n = 6)

Assessment of PAI-1 Genotype and Plasma and Renal PAI-1 Levels

PCR based protocols were used to determine the genotype of PAI-1+/+, PAI-1+/−, PAI-1−/−, and PAI-1 tg mice. The PAI-1 transgene was detected as described previously (22), PAI-1−/−, PAI-1+/−, and PAI-1−/− mice were genotyped by using primers for the wild-type PAI-1 gene (22) and by detecting the presence of the neomycin resistance gene as a marker for the mutant allele (20). The absence of the PAI-1 was confirmed by measurement of PAI-1 protein in plasma via ELISA using monoclonal antibodies generated by immunizing genetically deficient mice as described previously (24). Results are expressed as ng of PAI-1 protein per ml of plasma. For measurement of PAI-1 in renal tissue by ELISA, tissue from mice was homogenized and extracted as described previously by Lijnen et al. (25) and results expressed as pg of PAI-1 per mg of wet weight.

Assessment of Glomerular Crescent Formation and Glomerular Fibrin Deposition

Kidney tissue was fixed in Bouin fixative and embedded in paraffin, and 3-μm tissue sections were cut and stained with periodic acid–Schiff (PAS). A glomerulus was considered to exhibit crescent formation if two or more layers of cells were observed in Bowman’s space. A minimum of 50 glomeruli was assessed to determine the crescent score for each animal. For detection of fibrin in glomeruli, tissue was embedded in Optimal Cutting Temperature Compound, frozen in liquid nitrogen, and stored at −70°C. Immunofluorescence was performed on 4-μm cryostat cut tissue. Glomerular fibrin deposition was assessed on a minimum of 30 glomeruli per mouse using FITC-goat anti-rabbit fibrin/fibrinogen serum (Nordic Immunological Laboratories, Berks, UK), which cross-reacts with mouse fibrin at a dilution of 1:100 and scored semiquantitatively (0 to 3+) using two different scales. First, the proportion of glomeruli staining positive for fibrin in each mouse was assessed. Second, the overall intensity of fluorescence present in each mouse was scored.

Glomerular Leukocyte Accumulation

Kidney tissue was fixed in periodate lysine paraformaldehyde (PLP) for 4 h, washed in 7% sucrose solution, and then frozen in liquid nitrogen. Tissue sections (6 μm) were stained to demonstrate CD3+ cells and macrophages using a three-layer immunoperoxidase technique, as described previously (26,27). The primary monoclonal antibodies were KT3, anti-mouse CD3, which recognizes T cells (American Type Culture Collection [ATCC], Manassas, VA), and M1/70, anti-CD11b, which recognizes macrophages and neutrophils (ATCC). A minimum of 20 glomeruli were assessed per animal, and results were expressed as cells per glomerular cross section (c/gcs).

Assessment of Proteinuria and Serum Creatinine

Urinary protein concentrations were determined by the Bradford method on timed urine collections. Urine was collected for 24 h at baseline and over the last 24 h of an experiment. Serum creatinine concentrations at the completion of experiments (day 14 or day 28) were measured by the alkaline picric acid method using an autoanalyser.

Renal Collagen and Matrix Accumulation

Renal collagen content was assessed by determining total hydroxyproline according to the method of Bergam and Loxley (28). Samples were hydrolyzed in 6 N HCL by incubation at 110°C overnight. The hydrolysate was neutralized with 2.5 M NaOH. Hydrolysates in isopropanol were oxidized by chloramine T then mixed with p-dimethylaminobenzaldehyde (25 min, 60°C), and the absorbance was measured at 558 nm. Total collagen was calculated using the assumption that collagen contains 12.7% hydroxyproline by weight. Results were expressed as μg/mg kidney wet weight.
Results

PAI-1 Protein Levels are Undetectable in PAI-1 −/− Mice and Increased in PAI-1 tg Mice

Plasma PAI-1 levels in normal (without GN) PAI-1 +/+ mice (C57BL/6 × 129SvJ background) were 9.7 ± 1.5 ng/ml (Table 1). PAI-1 protein was undetectable in all PAI-1 −/− mice. PAI-1 +/+ mice had intermediate levels of PAI-1 protein (reduced to 30 to 40% of normal). Normal C57BL/6 mice had PAI-1 levels falling between those of PAI-1 +/+ and PAI-1 −/− mice. Mice engineered to genetically overexpress PAI-1 (PAI-1 tg mice) produced significantly more PAI-1 than all other mice (72 ± 8.1 ng/ml).

Glomerulonephritis is Less Severe in Mice Lacking PAI-1

Endogenous PAI-1 Mediates Glomerular Crescent Formation and Fibrin Deposition. Genetically normal PAI-1 +/+ mice developed proliferative GN with glomerular crescent formation and fibrin deposition 14 d after challenge with sheep anti-mouse GBM globulin (Figures 1, B and E; Figure 2). Glomerular crescent formation and fibrin deposition persisted to day 28. PAI-1 −/− mice were significantly protected from glomerular crescent formation at both the day 14 and day 28 time points (Figures 1C and 2A). Renal PAI-1 protein levels progressively increased in PAI-1 +/+ mice but remained undetectable in PAI-1 −/− mice (Table 2). A modest reduction in crescent formation was observed in PAI-1 +/+ mice at day 14, but not at day 28. Fibrin deposition was detected in most glomeruli of PAI-1 +/+ mice, mainly in the glomerular tuft. However, fibrin deposition was absent in over half of glomeruli in PAI-1 −/− mice (Figures 1F and 2B). At the later time point (day 28), there had been no progressive increase in glomeruli affected in PAI-1 −/− mice. In addition to there being fewer glomeruli in which fibrin was detected, overall intensity of fluorescence was decreased in PAI-1−-deficient mice (0 to 3+, per mouse) (day 14: PAI-1 +/+ 2.8 ± 0.2, PAI-1 −/− 1.4 ± 0.2; day 28: PAI-1 +/+ 1.8 ± 0.3, PAI-1 −/− mice all a score of 1).

Table 1. Plasma levels of PAI-1 protein measured by ELISA in mice without glomerulonephritis

<table>
<thead>
<tr>
<th>Plasma PAI-1a (ng/ml)</th>
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<tr>
<td>PAI-1 −/−</td>
<td>ND</td>
</tr>
<tr>
<td>PAI-1 +/+</td>
<td>3.7 ± 0.2b</td>
</tr>
<tr>
<td>PAI-1 +/+</td>
<td>9.7 ± 1.5</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>C57BL/6 - PAI-1 tg</td>
<td>72.7 ± 8.1d</td>
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a PAI-1, plasminogen activator inhibitor-1; +/+ , wildtype mice; +/− heterozygous mice; −/− knockout mice; ND not detected.
b Results are expressed in ng of protein per ml of plasma, as the mean ± SEM.
c PAI-1 −/−, +/−, and +/+ mice had a mixed genetic background (C57BL/6 75%, 129SvJ 25%).
d Significant at a level of P < 0.001 versus all other groups (ANOVA, Bonferroni Multiple Comparison Test).

Leukocyte Recruitment Is Diminished in the Absence of PAI-1. CD3+ T cells and macrophages were detected in glomeruli of genetically normal PAI-1 +/+ mice (Figure 3).
Table 2. Renal PAI-1 protein levels measured by ELISA in PAI-1 intact and deficient mice with GN

<table>
<thead>
<tr>
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<th>Baseline no GN*</th>
<th>GN day 14</th>
<th>GN day 28</th>
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<tr>
<td>PAI-1 +/+</td>
<td>40.2 ± 3.1b (8)</td>
<td>175.3 ± 117.8 (7)</td>
<td>1000 ± 173.2 (6)</td>
</tr>
<tr>
<td>PAI-1 +/-</td>
<td>NP</td>
<td>166.2 ± 81.0 (4)</td>
<td>189.4, 115.5 (2)</td>
</tr>
<tr>
<td>PAI-1 -/-</td>
<td>NP</td>
<td>ND</td>
<td>ND</td>
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</table>

*GN, glomerulonephritis; PAI-1, plasminogen activator inhibitor-1; +/+, wildtype mice; +/-, heterozygous mice; -/-, knockout mice; NP, test not performed, ND, not detected.

**Results are expressed in pg of PAI-1 per mg of renal tissue (wet weight), as the mean ± SEM. Numbers in parentheses indicate number of mice tested.

While leukocyte numbers were unchanged in PAI-1 heterozygotes, complete absence of PAI-1 resulted in a substantial reduction in both T cells and macrophages at both time points.

**Functional Indices of Renal Injury in the Absence of PAI-1.** Baseline urinary protein and renal function (measured by serum creatinine) were similar in the three experimental groups (urinary protein: 0.4 ± 0.1 mg/d in all groups; serum creatinine: PAI-1 +/+ 17 ± 0.4, PAI-1 +/- 17 ± 0.5, PAI-1 -/- 19 ± 0.4 μmol/L). PAI-1 +/+ and PAI-1 +/- mice had developed significant proteinuria and renal impairment by day 14 of disease. Urinary protein excretion in PAI-1 -/- mice with GN was significantly lower than the proteinuria observed in either PAI-1 +/+ or PAI-1 +/- mice at day 14 and lower than proteinuria in PAI-1 +/- mice at day 28 (Figure 4A). Serum creatinine levels in PAI-1 -/- mice at either day 14 or day 28 were not different from baseline values (Figure 4B).

Renal Collagen Content in GN in the Absence of Endogenous PAI-1. Genetic deletion of PAI-1 did not affect baseline renal collagen levels (Table 3). By day 14 of disease, PAI-1 +/+ mice had developed a 100% increase in renal collagen content, with levels remaining elevated at day 28. Renal collagen content in PAI-1 -/- mice was significantly less that that in PAI-1 +/+ mice at day 14, but this decrease failed to reach statistical significance at day 28. Collagen accumulation in PAI-1 +/- mice was more than in PAI-1 -/- mice but less than PAI-1 +/+ mice at day 14 of GN.

Glomerulonephritis is More Severe in Mice Overexpressing PAI-1

**Overexpression of PAI-1 Enhances Glomerular Crescent Formation and Glomerular Fibrin Deposition.** Normal C57BL/6 mice developed proliferative and crescentic GN 14 d
after challenge with sheep anti-mouse GBM globulin, with glomerular fibrin deposition (Figures 5A, 5C, and 6). Overall, disease in these mice was marginally less than disease in PAI-1 \(+/\+)

mice on a mixed 75% C57BL/6 \(\times\) 25% 129Sv/J background. Crescent formation and glomerular fibrin deposition persisted at day 28. Renal PAI-1 protein was increased in PAI-1 \(tg\) mice, most evident at day 14 (Table 4). At day 14, crescentic GN was more severe in PAI-1 \(tg\) mice compared with normal C57BL/6 mice (Figures 5B, 5D, and 6). An increased proportion of glomeruli from PAI-1 \(tg\) mice was affected by crescent formation and more glomeruli exhibited fibrin deposition. This increased severity of disease persisted at day 28. Overall intensity of fibrin deposition was also increased in PAI-1 \(tg\) mice. C57BL/6 mice with GN had moderate fluorescence intensity for fibrin (0 to 3+ [mean \(\pm\) SEM]; day 14, 1.5 \(\pm\) 0.2; day 28, 0.6 \(\pm\) 0.1). Sections from all PAI-1 \(tg\) mice developed strong immunofluorescence for fibrin (all 3+) at day 14, which was still moderate in intensity at day 28 (1.6 \(\pm\) 0.3).

Leukocyte Recruitment Is Enhanced in PAI-1 \(tg\) Mice. Mice that overexpressed PAI-1 demonstrated increased recruitment of T cells and CD11b+ leukocytes into glomeruli compared with genetically normal C57BL/6 mice with GN (Figure 7). This increased leukocyte influx in PAI-1 \(tg\) mice persisted at day 28 of disease.

Functional Renal Injury Is Enhanced by PAI-1 Overexpression. C57BL/6 mice with GN developed significant proteinuria, measured at both day 14 and day 28, with only minor renal impairment, consistent with the degree of glomerular crescent formation in these mice (Figure 8). As with glomerular crescent, formation, fibrin deposition, and leukocyte recruitment, PAI-1 \(tg\) mice with GN developed increased urinary protein excretion and renal impairment, evident at both day 14 and day 28.

Renal Collagen Accumulation Is Accelerated in PAI-1 \(tg\) Mice with GN. Normal C57BL/6 mice and PAI-1 \(tg\) mice had similar baseline renal collagen (Table 5), indicating that transgenic overexpression of PAI-1 in the absence of pathologic stimuli does not lead to increased renal collagen content. Compared with C57BL/6 mice, PAI-1 \(tg\) mice developed increased renal collagen accumulation at both 14 and 28 d.
PAI-1 has major potential pathogenetic effects in inflammatory renal disease, potentially acting on several different mediator systems (10,11). By inhibiting the activity of plasminogen activators and therefore the generation of plasmin, it can inhibit fibrin removal, resulting in enhanced fibrin accumulation. In addition to its capacity to enhance net fibrin deposition, PAI-1 has the capacity to contribute to the accumulation of collagens and other matrix proteins seen in progressive renal disease. PAI-1 limits conversion of plasminogen to plasmin, the active enzyme that may activate matrix metalloproteinases and act directly on matrix to limit collagen matrix accumulation. The increased PAI-1 levels observed in GN may result in increased leukocyte recruitment in GN both directly (14) and via the chemotactic effects of fibrin (29).

The alterations in glomerular crescent formation fibrin deposition and leukocyte accumulation in PAI-1 −/− and PAI-1 tg mice demonstrate an important role for PAI-1 in experimental crescentic GN. Findings of alterations in renal collagen content in PAI-1 −/− and PAI-1 tg mice support recent studies implicating PAI-1 in renal fibrosis (14). Collectively, the current studies suggest that PAI-1 is important in determining the outcome of renal injury. They support the hypothesis that inhibition of PAI-1 is a potential strategy in the therapy of both rapidly progressive GN and other progressive renal diseases.

In the first section of this study, mice genetically deficient in PAI-1 were significantly protected from renal injury in experimental crescentic GN compared with genetically matched PAI-1+/+ mice on a C57BL/6 background. The relatively mild injury in PAI-1 −/− mice did not show a significant "catch up" effect when anti-GBM GN was prolonged to 28 d. The protection afforded PAI-1 +/+ mice was most significant in crescent formation, fibrin deposition, leukocyte recruitment, and the accumulation of collagen. A secondary finding in these studies was that PAI-1 +/+ mice,

| Table 4. Renal PAI-1 protein levels measured by ELISA in genetically normal C57BL/6 mice and in mice overexpressing PAI-1 (PAI-1 tg mice) with GN |
|-------------------------------|-----------------|-----------------|
| Baseline no GN*                | GN day 14       | GN day 28       |
| C57BL/6                       | 29.4 ± 7.1 (8)b | 150.5 ± 75.2 (4)|
| PAI-1 tg                      | 961.4 ± 117.8 (6)c | 2480 ± 677.3 (6)d | 382.4 ± 93.5 (6) |

*GN, glomerulonephritis; PAI-1 tg, plasminogen activator inhibitor-1 overexpressing transgenic mouse on a C57BL/6 background.
Results are expressed in pg of PAI per mg of renal tissue (wet weight), as the mean ± SEM. Numbers in parentheses indicate number of mice tested.

**p < 0.0001 versus baseline values for C57BL/6 mice (unpaired t test).

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**p = 0.02 versus day 14 values for C57BL/6 mice (unpaired t test).
The results of the current studies seem at first glance to be discordant from those of Dewerchin et al. (36), who found that renal fibrin deposition was not significantly reduced in the absence of PAI-1. However, in the model of acute LPS induced renal injury used in those studies, fibrin deposition was extraglomerular. In the model used in our current studies, fibrin deposition is predominantly glomerular. Analysis of renal fibrinolytic activity after endotoxin injection, as well as renal tPA and uPA activity (by Dewerchin et al. (36)), showed increased renal fibrinolytic activity in PAI-1−/− mice and preservation of renal tPA activity in PAI-1−/− mice (compared with PAI-1+/+ mice, when renal tPA activity fell to undetectable levels). These results are consistent with PAI-1 having an important role in protective fibrinolytic activity in murine “anti-GBM GN” as (1) tPA is the major PA in the glomerulus (9); (2) we have demonstrated tPA to be the more important protective PA in glomerular fibrin deposition in murine crescentic GN (8); and (3) PAI-1−/− mice have preserved renal tPA activity and increased total renal fibrinolytic activity (36).

The current studies do not address the relative importance of the potential sources of PAI-1 in crescentic GN. PAI-1 is present in plasma and is produced by a variety of renal cells, including mesangial cells (37,38), endothelial cells (38), and tubular cells (39). It is likely that both tissue-derived and plasma-derived PAI-1 are important in this disease, though it is probable that tubular cell–derived PAI-1 is not particularly important in the glomerular fibrin deposition observed in experimental anti-GBM GN.

There is evidence in human GN that expression of PAI-1 may help determine disease severity. Lupus nephritis, WHO classes III and IV, is more severe in humans homozygous for the PAI-1 5′ gene promoter 4G polymorphism (4G/4G) (40), which, compared with the 5G/5G phenotype, results in increased PAI-1 expression, particularly in response to inflammatory stimuli. Our experimental data that disease severity is

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**Table 5. Renal collagen content in genetically normal C57BL/6 mice and in mice overexpressing PAI-1 (PAI-1 tg mice) with GN**

<table>
<thead>
<tr>
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<th>Baseline no GN</th>
<th>GN day 14</th>
<th>GN day 28</th>
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<tbody>
<tr>
<td>C57BL/6</td>
<td>4.6 ± 0.2b</td>
<td>7.1 ± 0.4</td>
<td>7.7 ± 0.7</td>
</tr>
<tr>
<td>PAI-1 tg</td>
<td>3.9 ± 0.3</td>
<td>9.5 ± 0.6c</td>
<td>10.1 ± 0.7d</td>
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</table>

*a* GN, glomerulonephritis; *b* PAI-1 tg, plasminogen activator inhibitor-1 overexpressing transgenic mouse on a C57BL/6 background.

*b* Results are expressed in mg of collagen per mg of renal tissue (dry weight), as the mean ± SEM.

*P* < 0.01 versus day 14 C57BL/6 values (unpaired t test).

*P* = 0.04 versus day 28 C57BL/6 values (unpaired t test).
increased in PAI-1 tg mice and decreased in PAI-1 $^{-/-}$ mice (both strain-matched) support these human observational studies. In summary, the current studies demonstrate that PAI-1 is an important determinant of crescent formation, injury, fibrin deposition, and collagen accumulation in experimental crescentic GN.

Acknowledgments

Dr. Ginsburg is thanked for providing the PAI-1 tg mice bred and used in these studies. The assistance of Ms. Janelle Sharkey and Ms. Alice Wright is acknowledged. These studies were supported by grants from the National Health and Medical Research Council of Australia (NH&MRC), the FWO, the Interuniversitary Attraction Poles, the Research Fund K.U.Leuven, and European Union.

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