Immune complex-mediated glomerulonephritis is ameliorated by thrombin-activatable fibrinolysis inhibitor deficiency

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Summary
The activity of plasmin plays a critical role in the development of chronic glomerulonephritis. Thrombin-activatable fibrinolysis inhibitor (TAFI) is a potent inhibitor of plasmin generation. We hypothesized that TAFI is involved in the pathogenesis of glomerulonephritis because it inhibits plasmin generation. To demonstrate this hypothesis, we compared the development of immune complex-mediated glomerulonephritis in wild-type and TAFI-deficient mice. After six weeks of treatment with horse spleen apoferritin and lipopolysaccharide to induce glomerulonephritis, mice deficient in TAFI had significantly better renal function as shown by lower concentrations of albumin in urine and blood urea nitrogen compared to wild-type mice. In addition, the activity of plasmin and matrix metalloproteinases was significantly increased, and mesangial matrix expansion and the deposition of collagen and fibrin in kidney tissues were significantly decreased in TAFI-knockout mice as compared to their wild-type counterparts. Depletion of fibrinogen by batroxobin (Defibrase) treatment led to equalization of the renal function and the amount of collagen deposition in the kidneys of TAFI-knockout and wild-type mice with immune complex-mediated glomerulonephritis. Together these observations suggest that TAFI-mediated inhibition of plasmin generation plays a role in the pathogenesis of glomerulonephritis, and that it may constitute a novel molecular target for the therapy of this disease.

Keywords
Knockout mouse, glomerulonephritis, coagulation, fibrin, cytokines, plasmin, inflammation

Introduction
The deposition of immune complex in the kidneys is a frequent cause of chronic glomerulonephritis and subsequent renal failure (1). The presence of immune complexes in the glomerular structures causes renal cell injury by activating the complement system, by stimulating the infiltration of inflammatory cells and by inducing the secretion of vasoactive substances, adhesion molecules, cytokines and pro-coagulant factors from resident cells (1–2). This initial inflammatory response may ultimately evolve to an end-stage renal fibrosis, which is characterized by increased accumulation of myofibroblasts, excessive deposition of extracellular matrix proteins (e.g. collagen), progressive destruction of glomeruli and renal failure (3–4). Glomerulonephritis caused by immune complex in humans are serum sickness disease and autoimmune diseases such as systemic lupus erythematosus, subacute bacterial endocarditis and hepatitis C-related cryoglobulinemia (1). The mechanistic pathways involved in the transition from tissue injury to fibrosis in the kidneys are not understood but decreased local activation of extracellular matrix-degrading enzymes may play a critical role (5–7). An important physiological activator of matrix degradation in the kidneys is the plasminogen-plasmin system (8–9). A balance between the processes of degradation and deposition of extracellular matrix is a fundamental requisite for maintaining the structural and functional integrity of the glomerulus (5). Thus, decreased activ-

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ity of the plasminogen-plasmin system may tilt the balance towards major matrix deposition leading to glomerular scarring and renal dysfunction (5, 10).

Activation of the plasminogen-plasmin system occurs when urokinase or tissue plasminogen activator cleaves the zymogen, plasminogen, yielding plasmin (8–9). Plasmin, the effector enzyme of the system, stimulates degradation of extracellular matrix by directly affecting several matrix components including laminin, fibronectin, tenasin C and proteoglycans (11). In addition, plasmin promotes extracellular matrix degradation by its ability to activate the pro-enzyme forms of matrix metalloproteinas (MMP), particularly MMP-1 (collagenase-1), MMP-3 (stromelysin-1) and MMP-9 (gelatinase B) (8–9). Intraglomerular fibrin formation may contribute to renal scarring by serving as a matrix upon which fibroblasts can proliferate and secrete connective tissue components (12–13). Thus, plasmin may also indirectly influence extracellular matrix turnover through its well-known potent fibrinolytic activity. Reduced plasmin generation in transgenic mice overexpressing plasminogen activator inhibitor-1 (PAI-1) is associated with more severe forms of glomerulonephritis compared with mice with physiological levels of plasmin, supporting the hypothesis that plasmin protects against fibrosis in the kidneys (14). In addition, decreased fibrinolytic activity has also been reported in most patients with nephrosclerosis secondary to systemic diseases including diabetes mellitus and arterial hypertension, suggesting that it is also implicated in the pathogenesis of renal scarring in humans (15–19).

The mechanism of decreased plasmin generation in glomerulonephritis is still debated. Enhanced levels of PAI-1 in injured kidney tissues is one attractive explanation but deficiency of PAI-1 has not always been related with increased activation of the plasmin, suggesting that other factors are implicated in the pathogenesis of the disease (20). A potential candidate is thrombin-activatable fibrinolysis inhibitor (TAFI). TAFI is a glycoprotein with molecular weight of 55 kD also known as procarboxypeptidase R, or proplasmin. TAFI is secreted as a zymogen and is converted to its active form, TAFIa, by thrombin, plasmin, thrombin-thrombomodulin complex or trypsin (21). TAFIa decreases plasmin generation by cleaving the carboxyterminal lysine residues from fibrin necessary for plasmin activation or by directly inhibiting the activity of plasmin (22). We have previously reported that deficiency of TAFI protects mice from pulmonary fibrosis suggesting the importance of

Figure 1: Horse spleen apoferritin (HSA)-induced glomerulonephritis mouse model. Plasma levels of anti-HSA IgG expressed as % of control pool plasma (A), the level of IgG2a in renal tissue homogenates (B) and C3a in plasma (C) were measured by EIA as described in Materials and methods. The ratio of urine protein to creatinine in freshly voided urine (D). The concentration of albumin in urine collected over 24 h (E) and blood urea nitrogen (BUN) (F). Bars indicate the mean ± SEM (n=3–5) mice in each group. Statistical differences between groups were analyzed by ANOVA and post hoc Tukey’s test. No significant difference was found between WT/SAL and KO/SAL mice. WT/SAL, wild-type mice treated with saline. WT/HSA, wild-type mice treated with HSA. KO/SAL, TAFI-knockout mice treated with saline. KO/HSA, TAFI-knockout mice treated with HSA.
TAFI in the mechanism of fibrogenesis (23). In the present study, we hypothesized that TAFI-mediated decreased plasmin generation is involved in the pathogenesis of glomerulosclerosis. To demonstrate this hypothesis, we compared the development of glomerulonephritis in wild-type and TAFI-deficient mice.

Materials and methods
For a more detailed description of methods see the supplementary material online at www.thrombosis-online.com.

Animals
TAFI knockout mice were generated and characterized by Nagashima et al. (24). The Mie University’s Committee on animal investigation approved the experimental protocol, and the experiments were performed according to the guidelines for animal experiments of the National Institute of Health.

Animal model of glomerulonephritis
Immune complex-mediated glomerulonephritis was induced as described by Welch et al. (25). There were four groups of animals: wild-type mice (WT/SAL, n=7) or knockout (KO/SAL, n=7) mice treated with intraperitoneal sterile saline (SAL) and wild-type mice (WT/HSA, n=7) or knockout (KO/HSA, n=7) mice treated with intraperitoneal injection of horse spleen apoferritin plus lipopolysaccharide (HSA). Briefly, treated animals received HSA (10 mg, 5 days/week) and lipopolysaccharide (LPS) (0.1 mg, 3 days/week) by intraperitoneal injection (IP) for six weeks while saline control WT animals received 0.1 ml of 0.15 M NaCl five days per week for six weeks. After six weeks, the animals were sacrificed by CO₂ narcosis, blood was obtained by cardiac puncture, and kidneys were harvested for histological analyses.

To deplete fibrinogen, batroxobin (Tobishi Co, Tokyo, Japan) was administered by subcutaneous injection to mice in the WT/HSA (n=4) and KO/HSA (n=4) groups at a dose of 4.5 BU per...
kilogram of body weight every 48 hours (h) from day 0 for six weeks. Horse spleen apoferritin and LPS were administered as described above. Wild-type (WT/SAL, n=4) and TAFI-deficient (KO/SAL, n=4) mice treated with intraperitoneal saline served as controls.

**Evaluation of animal model**
The concentration of albumin in urine was measured using a commercial kit from Wako Pure Chemicals Industries (Chu-ku, Osaka, Japan). Total protein in urine and homogenized renal tissue was measured using the BCA protein assay kit from Pierce (Rockford, IL, USA). The blood concentration of urea nitrogen was measured by the urease indophenol method, and the plasma anti-HSA antibodies were measured by enzyme immunoassay as described in supplementary materials (available online at www.thrombosis-online.com).

**Immunohistochemistry**
 Immunohistochemical staining of collagen type I and fibrin was performed using rabbit anti-mouse collagen I (Bethyl Laboratories, Montgomery, TX, USA) antibody and rabbit anti-fibrinogen (Dako, Glostrup, Denmark) antibody as described (22). Smooth muscle cells and complement C3 deposits were stained by using monoclonal anti-caldesmon and anti-mouse C3 (Hycult Biotechnology) antibodies. All staining was performed after appropriate blocking of endogenous renal peroxidase and biotin.

**Evaluation of histological findings**
For quantification of pathological findings and renal deposition of C3, fibrin and collagen, tissue preparations of each group of mice were selected at random. Digitized images of 20 high power fields were then taken from the WT/SAL, KO/SAL, WT/HSA and KO/HSA groups by an investigator blinded to the experimental group. A second blinded investigator then carried out a densitometric analysis of collagen I, fibrin/fibrinogen, C3 deposition and periodic acid Schiff-positive areas using the WinRoof image processing software. Computer-assisted densitometric analysis of histological findings was performed using an Olympus BX50 microscope with a Plan objective, combined with an Olympus DP70 Digital Camera (Tokyo, Japan) and the WinRoof image processing software (Mitani Corp., Fukui, Japan) for Windows.

**Gelatin zymography and measurement of hydroxyproline**
Gelatin zymography to detect MMP-1 and MMP-9 was carried out using a commercial kit from Invitrogen Life Technology (Carlsbad, CA, USA), and the hydroxyproline content in the renal tissues was measured by a colorimetric method as described (26).

**Biochemical analysis**
The concentration of total protein was measured with the BCA protein assay kit (Pierce) and the concentrations of active transforming growth factor-B1 (TGF-B1), osteopontin, plasminogen activator inhibitor-1 (PAI-1) and C3a-desArg were measured by commercial enzyme immunoassay kits as described in supplementary materials (see online at www.thrombosis-online.com). Fibrinogen was determined by enzyme immunoassay using rabbit anti-mouse fibrinogen (Dako, Glostrup, Denmark) and biotin-labeled antifibrinogen antibodies. The activity of plasmin was determined using the synthetic substrate S-2251 (Chromogenix, Mölndal, Sweden) as described (26).

**Statistical analysis**
Data are expressed as the mean ± standard error of the mean (SEM). Statistical differences between groups were evaluated by one-way analysis of variance with Tukey’s test as post hoc analysis. P<0.05 was considered as statistically significant. All statistical analysis was performed using Graph pad software package (Graph pad, San Diego, CA, USA).
Results

Animal model
We measured the circulating level of anti-HSA IgG in all groups of mice and found that mice (WT/HSA and KO/HSA) treated with daily injections of the antigen (apoferitin) had a significantly elevated plasma level of anti-apoferritin compared to mice treated with saline alone (WT/SAL and KO/SAL). Significant renal deposition of IgG2a was also found in both WT/HSA and KO/HSA mice compared to saline-treated control mice (WT/SAL and KO/SAL). The activation of C3 was described to play an important, though not necessarily indispensable, role in immune complex-mediated renal diseases; thus we measured the plasma level of the complement fraction C3a-desArg as marker of C3 activation, and found that both WT/HSA and KO/HSA treated mice had a significantly lower plasma level of C3a compared to saline treated mice (WT/SAL and KO/SAL) (27). However, none of these parameters was significantly different between WT/HSA and KO/HSA mice (Fig. 1A-C). Renal function was assessed by the ratio of protein to creatinine in fresh voided urine, the concentration of albumin in 24-h urine, and by the plasma level of blood urea nitrogen. The urinary ratio of total protein to creatinine, the concentration of urine albumin, and the level of blood urea nitrogen were markedly increased in WT/HSA mice as compared to KO/HSA mice in the 6th week after starting HSA injections, suggesting the occurrence of a remarkable renal dysfunction in WT/HSA mice as compared to KO/HSA mice. Neither the urinary ratio of protein to creatinine nor the blood urea nitrogen level was significantly different between WT/SAL and KO/SAL groups (Fig. 1D-F).

Renal pathological findings
Renal histological sections stained with periodic acid Schiff (PAS), were photographed, and the densitometry of renal structural changes and cellularity were evaluated by a blinded observer using the WinRoof image analyzer software (Fig. 2A-D). Renal histology showed significantly increased tubular atrophy (arrow), increased mesangial hypercellularity, defined as the
number of cell nuclei per equatorial cross-section, and increased mesangial matrix expansion with enhanced deposition of periodic acid Schiff-positive material in mice treated with HSA compared to control saline-treated mice. In addition, the glomerular and tubular changes in WT/HSA mice were significantly more conspicuous than in KO/HSA mice. However, total glomerular cellularity was not significantly different in WT/HSA mice compared with KO/HSA mice.

**Renal deposition of C3 and immune complex**
Deposition or formation of immune complexes in kidney tissues triggers activation of the complement system, which is followed by enhanced deposition of complement products in glomerular structures. Here we investigated the presence of C3 deposits in the kidneys of the animals by immunohistochemistry. Significantly more immunoreactivity of C3 was observed in tubulointerstitial spaces, basement membrane of glomerular capillaries and along the base of the Bowman capsule parietal layer from WT/HSA mice compared with KO/HSA and WT/SAL mice. C3 deposition was also significantly enhanced in KO/HSA mice compared to KO/SAL mice and in WT/SAL mice (p=0.01) compared to KO/SAL mice (Fig. 3A, B). Similar glomerular deposition of apoferritin was detected in WT/HSA (see supplementary Fig. 1 online at www.thrombosis-online.com) compared to saline-treated mice.

**Renal deposition of fibrin**
To investigate activation of the coagulation system in the kidneys of our mouse model, fibrin was evaluated by immunohistochemistry and EIA. Fibrin deposition was significantly increased in both WT/HSA and KO/HSA groups compared to WT/SAL and KO/SAL groups but the fibrin/fibrinogen accumulation in the mesangial and tubulointerstitial spaces was significantly higher in WT/HSA than in KO/HSA mice. The deposition of fibrin was not significantly different between the WT/SAL and KO/SAL groups (Fig. 4A, B). The concentration of fibrin(ogen) in renal tissue homogenates was significantly increased in WT/HSA and KO/HSA groups compared to WT/SAL and KO/SAL groups; the level of fibrin(ogen) in tissue homogenates from WT/HSA mice was significantly augmented compared to KO/HSA mice (Fig. 4C).

**Renal fibrosis**
The grade of renal fibrosis was investigated by Masson’s trichrome staining (see supplementary Fig. 2A, B online at www.

![Figure 5: Collagen I immunostaining and renal content of hydroxyproline.](image-url)
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thrombosis-online.com), immunostaining of collagen type I, and by measuring the content of hydroxyproline in the kidneys (Fig. 5A-C). Both trichrome and collagen stain showed increased deposition of collagen in the mesangium and tubulointerstitial spaces in the WT/HSA group compared to WT/SAL and KO/HSA groups. No difference was detected between KO/HSA and KO/SAL mice. The content of hydroxyproline was also significantly higher in WT/HSA mice than in the WT/SAL and KO/HSA groups. No difference was observed between KO/HSA and KO/SAL mice.

Caldesmon is an actin-binding protein and a molecular marker of fibrotic differentiation in smooth muscle cells. Immunoreactivity of caldesmon in renal tissues was significantly stronger in WT/HSA mice than in WT/SAL and KO/HSA mice (see supplementary Fig. 3A, B online at www.thrombosis-online.com).

**Plasmin activity**

Since levels of plasmin might account for the observed differences in fibrin and extracellular matrix deposition in our glomerulonephritis model, we compared plasmin activity in renal tissues from both WT/HSA and KO/HSA mice. In addition, the activity of MMP-1 and MMP-9, both of which are activated by plasmin, was also measured. The activity of plasmin, MMP-1 and MMP-9 was significantly enhanced in KO/HSA mice as compared to that in WT/HSA mice (Fig. 6A-C) while MMP2 was undetectable (data not shown). Interestingly, a significant inverse correlation was found between plasmin activity in kidney tissues and the content of hydroxyproline (Fig. 6D).

**Circulating levels of pro-fibrotic cytokines and fibrinolysis markers**

The renal expression of pro-fibrotic cytokine TGF-β1 plays a key role in the pathogenesis of glomerulosclerosis by stimulating the secretion of extracellular matrix proteins from fibroblasts.
and by its role in the transition of epithelial cells to myofibroblasts. We measured the renal and plasma concentration of active TGF-β1 and found that it was markedly increased in both WT/HSA and KO/HSA mice compared to WT/SAL and KO/SAL, respectively. Elevated concentrations of TGF-β1 in plasma have been associated with induction and aggravation of renal sclerosis (28). The WT/HSA group had a significantly higher renal and circulating level of TGF-β1 than KO/HSA mice (Fig. 7A, B). Osteopontin is another pro-fibrotic cytokine that may stimulate tissue scarring by promoting epithelial-to-mesenchymal transition and by stimulating the proliferation and survival of fibroblasts. The plasma concentration of osteopontin was significantly elevated in WT/HSA mice compared with WT/SAL and KO/HSA mice but no difference was found between KO/HSA and KO/SAL (Fig. 7C). PAI-1 was found to be elevated in virtually all forms of glomerulonephritis, and it was demonstrated to contribute to renal fibrosis by inhibiting fibrinolysis and by facilitating inflammatory cell infiltration into the kidneys. We measured the circulating levels of PAI-1 and found that it was significantly elevated in both WT/HSA and KO/HSA mice in comparison to WT/SAL and KO/SAL controls, respectively. No significant difference was found between KO/HSA and WT/HSA mice (Fig. 7D). Fibrin/fibrinogen accumulation is believed to play a crucial role in renal scarring after longstanding immune-mediated glomerular damage. We measured circulating levels of fibrinogen and found that it was significantly more elevated in both WT/HSA and the KO/HSA group compared to WT/SAL and KO/SAL, but it was significantly more elevated in WT/HSA mice than in KO/HSA mice (Fig. 7E).

**Effect of fibrinogen depletion on immune-mediated glomerulonephritis**

Batroxobin therapy was used to test whether the protective effect of TAFI deficiency on immune-mediated glomerulonephritis was mediated via an increase in activity of the fibrinolytic system due to the absence of TAFI with consequently less renal deposition of fibrin. After six weeks, the concentration of plasma fibrinogen was significantly decreased in WT/HSA and KO/HSA mice treated with batroxobin compared to untreated mice (Fig. 8A), demonstrating that our protocol led to a significant de-

![Graph A: Renal tissue active TGF-β1](image1)

![Graph B: Plasma active TGF-β1](image2)

![Graph C: Plasma osteopontin](image3)

![Graph D: Plasma PAI-1](image4)

![Graph E: Plasma fibrinogen](image5)

**Figure 7**: Renal and plasma concentrations of TGF-β1, plasma osteopontin, PAI-1 and fibrinogen. TGF-β1 (A-B), osteopontin (C), PAI-1 (D) and fibrinogen (E) were measured using specific EIA kits (n=5–7). Data are expressed as the mean ± SEM. Statistical differences between groups were analyzed by ANOVA and post hoc using Tukey’s test.
pletion of circulating fibrinogen. In addition, WT/HSA and KO/HSA mice treated with batroxobin showed no difference in renal function (Fig. 8B, C), in the renal level of the active forms of TGF-β1 (Fig. 8D) and in the renal content of hydroxyproline (Fig. 8E). The values of each parameter were not significantly different between HSA-treated (WT/HSA, KO/HSA) and saline-treated (WT/SAL, KO/SAL) groups. The fact that batroxobin treatment prevented the development of the disease suggests that the protective effect of TAFI deficiency depends on either regulation of fibrinolysis or some other fibrin-dependent mechanism. No bleeding complications or death was associated with batroxobin treatment.

Discussion

The present study demonstrated for the first time that TAFI deficiency protects against renal dysfunction in a mouse model of glomerulonephritis. This study also showed that diseased mice with TAFI deficiency have significantly increased activity of both plasmin and matrix metalloproteinases, but significantly less mesangial matrix expansion and deposition of fibrin and collagen in the kidneys compared with their wild-type counterparts with the disease. Similarly to previous reports (24), in the experiments reported here, in the absence of a challenge there were very few differences observed between wild-type and the TAFI-deficient mice even in parameters such as the level of plasmin in plasma.

As was previously reported, repeated injections for six weeks of HSA and LPS induced an immune complex-mediated glomerulonephritis associated with strong tubulo-interstitial inflammation (25). Pathological examination of the kidneys in the mouse model showed focal areas of expansion and increased cellularularity of the mesangium and enhanced number of atrophic tubules in the renal interstitium from HSA-treated mice compared to animals treated with saline alone (Fig. 2). The renal disease in this model has been shown to occur as a consequence of apoferritin-IgG complex deposition in the kidneys, and in agreement with this, we found enhanced circulating level of anti-apoferritin IgG associated and elevated level of IgG2a in renal tissue homogenates in HSA-treated mice (25). Comparative evaluation

![Figure 8: Effect of fibrinogen depletion on immune complex-mediated glomerulonephritis.](image)
showed no difference in the levels of plasma anti-apoferritin IgG or renal IgG2a between wild-type and TAFI-deficient mice, suggesting the occurrence of similar grade of formation and renal deposition of immune complex in both mouse genotypes. In contrast, renal functional studies as evaluated by the urinary protein to creatinine ratio and the level of blood urea nitrogen demonstrated dysfunction in wild-type mice but not in TAFI-knockout mice. This difference in function was especially reflected in pathological findings which showed more prominent mesangial matrix expansion, tubular atrophy and tubulo-interstitial inflammation in wild-type mice compared with TAFI-deficient mice. Overall, these observations underscore the importance of TAFI in the pathogenesis of immune complex-mediated nephropathy in the mouse. In 2002 Ikeguchi et al. showed that recombinant thrombomodulin treatment of a rat acute lethal model of thrombotic glomerulonephritis led to an increase in TAFIα and a decrease in glomerular infiltration of leukocytes and neutrophils but they did not report an effect on disease outcome of TAFI inhibition (29).

Since activated TAFI reduces fibrinolysis, we evaluated the degree of fibrin deposition in the kidney tissues to determine whether this affects the marked inflammatory changes in wild-type mice compared with TAFI-knockout mice. Fibrin is formed when fibrinogen is cleaved by thrombin and plays a critical role in the reparative process of injured tissues (13). When there is excessive production of fibrin or it is not resolved for a prolonged time it may induce or exacerbate the inflammatory response by stimulating the local influx of monocytes and macrophages, the expression of adhesion molecules, cytokines and chemokines from resident cells and by increasing vascular permeability and angiogenesis (12–13). TAFI is an important suppressor of fibrin degradation (30), and the fact that diseased TAFI-deficient mice had significantly increased plasmin activity and reduced fibrin deposition in the kidneys compared with their wild-type counterparts suggests that TAFI plays a detrimental role in our disease model by promoting fibrin-mediated inflammatory responses. Equalization of renal function, circulating levels of active TGF-β1 and in the total hydroxyproline content between WT/HSA and KO/HSA after treatment with batroxobin, which decreases the circulating concentration of fibrinogen, supports the assumption that fibrin plays a detrimental role in the pathogenesis of our model of glomerulonephritis.

Complement activation following renal deposition of immune complexes may be an alternate mechanism for the enhanced inflammatory responses in immune complex-mediated nephropathy (1). The role of C3 deposits is unclear as shown by one study utilizing C3-deficient mice that concluded that, “although complement is required for the normal glomerular metabolism of immune complexes, other, complement-independent, factors are involved in the generation of glomerular injury in this model” (31). Peptides released during activation of C3 and C5 are potent chemotactrants for mononuclear cells, macrophages, neutrophils and assembly of the membrane attack complex on renal epithelial cells. In addition, it may induce the expression of several pro-inflammatory cytokines (3). Interestingly, TAFI has been shown to cleave the C-terminal residue of C3a and C5a inactivating them (32). However, unexpectedly we found that TAFI-deficient mice had significantly less total C3 deposition in the kidneys compared with the wild-type mice. Previous data, however, had suggested that glomerular disease in this type of glomerulonephritis model is independent of C3 deposition (25).

Osteopontin is a pro-inflammatory factor that has been linked to excessive inflammatory response in the kidneys (33). Osteopontin is a widely distributed matricellular glycoprotein whose expression is increased in tubular epithelial cells during glomerular and tubulointerstitial diseases (33). Osteopontin may exacerbate renal inflammation by stimulating the recruitment of monocytes and macrophages at sites of tissue injury and by inducing the polarization of T-helper type I cells. Apart from its critical role in inflammation, osteopontin may also promote the fibrotic process in the kidneys by stimulating the proliferation and survival of fibroblasts (34). In the present study, we found that mice deficient in TAFI had lower circulating levels of osteopontin compared with wild-type mice, suggesting that osteopontin is involved in the pathogenesis of glomerular and tubulointerstitial inflammation in our mouse model.

The end-stage of glomerulonephritis is the development of glomerular and tubulointerstitial sclerosis and renal failure. The hallmark of renal fibrosis is the extensive deposition of extracellular matrix proteins (e.g. collagen I, fibronectin, proteoglycans) in glomerular and tubulointerstitial spaces (3). Synthesis and deposition of matrix proteins occur in the early stages of glomerular injury and renal fibrosis ensues when a concomitant degradative process does not counterbalance this productive phase (5, 10). Degradation of extracellular matrix (ECM) is mainly mediated by MMPs, which are produced and secreted in inactive forms called pro-MMPs. Since pro-MMPs are activated by plasmin, conditions associated with decreased plasmin generation generally cause reduced MMP activity leading to impaired collagen degradation (8, 9, 11, 34). In the present study, we found that the kidneys from wild-type mice had decreased plasmin activity with concomitant reduction in the activity of metalloproteinases (MMP-1, MMP-9), and increased renal collagen deposition compared with TAFI-deficient mice, indicating that TAFI promotes glomerulosclerosis by decreasing the availability of plasmin in the kidneys. Another explanation for the protection from renal fibrosis of TAFI deficiency may be the higher fibrinolytic activity. Fibrin itself can also promote fibrosis by providing a matrix onto which myofibroblasts can migrate proliferate and further produce matrix proteins (12). The significant inverse correlation observed in our cohorts of wild-type mice between renal tissue plasmin activity and renal content of hydroxyproline underscores the pathophysiological importance of plasmin activity in the process of renal fibrosis (Fig. 6D). It is worth noting that in contrast to the protective action of plasmin in chronic glomerulonephritis, excessive plasmin activity causes enhanced interstitial renal fibrosis in obstructive nephropathy through a fibrin-independent mechanism (35); in this regard, we have recently reported that TAFI-deficient mice with obstructive nephropathy have more interstitial fibrosis than their wild-type counterparts (26). Excessive secretion of growth factors may also disrupt the balance between deposition and degradation of matrix proteins and cause renal fibrosis. TGF-β1 is a growth factor that is critical in renal fibrosis for its ability to stimulate the secretion of extracellular matrix proteins (37). TGF-β1 may also
decrease the degradation of matrix proteins by promoting the secretion of plasminogen activator inhibitor-1, which decreases the generation of plasmin. In addition, TGF-β1 has also been reported to favor epithelial-to-mesenchymal transition leading to an increased number of collagen secreting myofibroblasts. In the present study, wild-type mice had significantly increased circulating level of active TGF-β1 compared with TAFI-knockout mice, suggesting that TGF-β1 downregulation could explain the decreased renal fibrosis in TAFI-deficient mice. Interestingly, immunoreactivity of caldesmon in renal tissues was more intensively positive in wild-type mice than in TAFI-knockout mice, suggesting that TAFI-deficient mice had a decreased number of cells able to secrete collagen in the kidneys.

In brief, our results showed that TAFI-deficient mice with immune-complex-related glomerulonephritis have better renal function, significantly increased activity of plasmin and matrix metalloproteinases and significantly less mesangial expansion and deposition of collagen and fibrin in the kidneys compared with their wild-type counterparts. Together these observations suggest that TAFI-mediated inhibition of plasmin generation plays a key role in the pathogenesis of glomerulonephritis, and that it may be a novel molecular target for the therapy of this disease.

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