PERSPECTIVES IN BASIC SCIENCE

Plasminogen activator inhibitor type 1 is a potential target in renal fibrogenesis

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Plasminogen activator inhibitor type 1 is a potential target in renal fibrogenesis. The progression of renal lesions to fibrosis involves several mechanisms, among which the inhibition of extracellular matrix (ECM) degradation appears to play an important role. Two interrelated proteolytic systems are involved in matrix degradation: the plasminogen activation system and the matrix metalloproteinase system. The plasminogen activator inhibitor type 1 (PAI-1), as the main inhibitor of plasminogen activation, regulates fibrinolysis and the plasmin-mediated matrix metalloproteinase activation. PAI-1 is also a component of the ECM, where it binds to vitronectin. PAI-1 is not expressed in the normal human kidney but is strongly induced in various forms of kidney diseases, leading to renal fibrosis and terminal renal failure. Thrombin, angiotensin II, and transforming growth factor-β are potent in vitro and in vivo agonists in increasing PAI-1 synthesis. Several experimental and clinical studies support a role for PAI-1 in the renal fibrogenic process occurring in chronic glomerulonephritis, diabetic nephropathy, focal segmental glomerulosclerosis, and other fibrotic renal diseases. Experimental models of renal diseases in PAI-1–deficient animals are in progress, and preliminary results indicate a role for PAI-1 in renal fibrogenesis. Inhibition of PAI-1 activity or of PAI-1 synthesis by specific antibodies, peptidic antagonists, antisense oligonucleotides, or decoy oligonucleotides has been obtained in vitro, but needs to be evaluated in vivo for the prevention or the treatment of renal fibrosis.

The hallmark of terminal renal failure is the irreversible destruction of the normal renal parenchyma, more or less rapidly invaded and replaced by fibrosis. To date, although numerous studies have been reported on the various factors that may promote, or conversely delay, the progression of chronic renal failure, we are still looking for an integrated and unifying understanding of the molecular and cellular mechanisms implicated in the renal fibrogenic process. Fibrosis is due to the abnormal accumulation of extracellular matrix (ECM) in basement membranes and interstitial tissues and is combined, at the same time, with the elimination of normal parenchymal cells by necrosis, lack of regeneration, and/or apoptosis [1]. The abnormal ECM in fibrosis is made of an excess of normal components of ECM such as fibronectin, laminin, proteoglycans, and collagen type IV, but also of an accumulation of proteins that is not found in the normal ECM such as collagen type I and type III in the glomerular mesangium [2]. These latter proteins characterize the scarring process and are usually irreversibly deposited in the fibrotic tissues. The renal parenchymal cells themselves may undergo a fibroblastic transdifferentiation and overproduce the ECM components. Fibroblasts and myofibroblasts proliferating within the kidney are also involved in the fibrogenic process. On the other hand, the ECM can be degraded, and it is likely that the fibrogenic process may also result from a deficit in ECM degradation. However, the relationship between ECM degradation and fibrogenesis is more complex than initially suspected, since abnormal ECM accumulation is often preceded or combined with an increased expression of ECM-degrading enzymes [3]. This increased proteolytic activity is presumably required for degradation of the normal ECM by infiltrating inflammatory and fibroblastic cells and its replacement by abnormal ECM. Two main degrading systems are known to play a role: the matrix metalloproteinases (MMPs) and the plasminogen activation system (PAS) [4]. The relationship between these systems is numerous and has been reviewed elsewhere recently [5–7]. The aim of the present review is to analyze the available experimental and clinical data supporting a role for the main inhibitor of plasminogen activation, the plasminogen activator inhibitor type 1 (PAI-1), as a key player in renal fibrogenesis.

PAI-1 IS AN INHIBITOR OF FIBRINOLYSIS AND OF MATRIX METALLOPROTEINASE ACTIVATION

The plasminogen/plasmin system was first recognized for its fibrin-degrading activity and is the main, if not
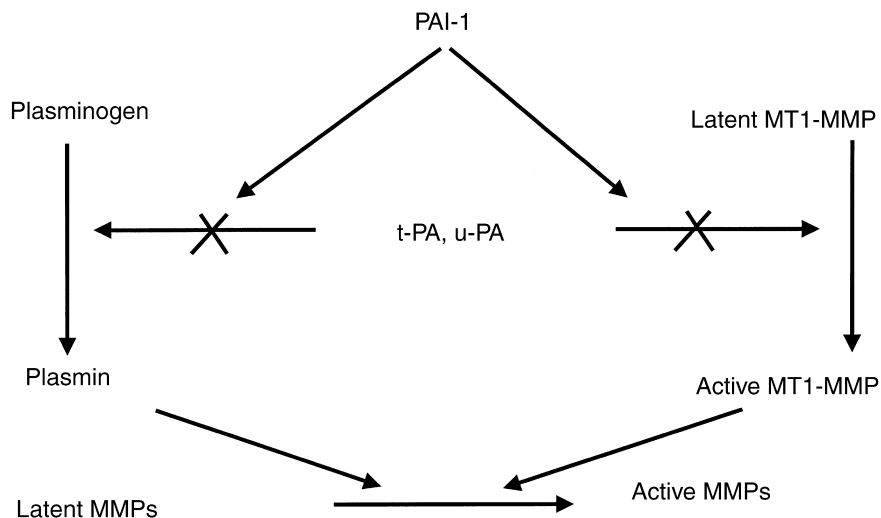
Fig. 1. Schematic diagram of plasminogen activation and matrix metalloproteinase activation. Abbreviations are: PAI, plasminogen activator inhibitor; MMP, matrix metalloproteinase; MT-MMP, membrane type matrix metalloproteinase; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator.

the only, efficient fibrinolytic system in vivo (Fig. 1). The limiting rate of plasmin generation is the amount of tissue-type (t-PA) or urokinase-type (u-PA) plasminogen activators (PAs) in their active forms [4]. This amount is tightly regulated through the control of the synthesis and release of the proenzyme forms of PA, the extracellular activation of these proenzymes, and their inhibition by specific inhibitors such as PAI-1, PAI-2, and PAI-3 [5]. These inhibitors belong to the SERPIN family, after the SERine Protease INhibitor. PAI-1, a 50 kD glycoprotein, is the main PAI secreted in vivo and is a potent fast-acting and irreversible inhibitor of t-PA and u-PA but not of plasmin. It forms stochiometric complexes with active PAs, which are subsequently endocytozed and degraded. Membrane receptors for u-PA and t-PA have been identified that facilitate the activation of plasminogen at the cell surface and the activation of the PA proenzymes by trace amounts of plasmin [8]. In addition, u-PA receptor (u-PAR) plays a role in binding and internalization of u-PA/PAI complexes and in cell adhesion and migration through an interaction with vitronectin and integrins [8].

The other main extracellular proteolytic system, the MMPs and their inhibitors (TIMP-1, TIMP-2, and TIMP-3), exist in a number of different metalloproteinases that contain several conserved motifs and a zinc binding site, which is required for full enzymatic activity. To date, at least 20 MMPs have been identified that belong to four different groups according to their specificity: (*1*) the collagenases, which cleave preferentially interstitial collagens type I and III; (*2*) the gelatinases, which degrade the collagens type IV and V; (*3*) the stromelysins, which degrade gelatin, fibronectin, laminin, and elastin; and (*4*) the membrane-type MMPs (MT-MMPs) [7]. The MMPs are secreted in the extracellular space in catalytically latent forms because of the binding

of the active site zink atom to an unpaired cystein of the propeptide domain. Disruption of the cystein-zink bound by conformational change or by limited proteolysis, as produced by plasmin, leads to the opening of the switch. Then the autocatalytic cleavage of the propeptide yields the active enzyme [7]. The activation of MMPs may also occur through the cleavage by membrane-bound MMPs, which are called MT-MMPs. The activation of MMP2 at the cell surface is due to MT1-MMP, which binds TIMP-2 and forms a ternary complex with MMP2. Active MMP2 is then released into the extracellular space, but may also remain at the cell surface, where it has been shown to bind to the integrin $\alpha_v\beta_3$ [9]. Interestingly, in our laboratory, u-PA was shown to promote MMP2 activation when purified u-PA was added to the culture medium conditioned by human mesangial cells, but not when added to purified pro-MMP2. We were able to demonstrate that mesangial cells release a soluble form of MT1-MMP that was activated by u-PA and then was responsible for MMP2 activation [10]. PAI-1, as a potent inhibitor of u-PA, inhibits u-PA-induced, MT1-MMP–mediated MMP2 activation [11]. Thus, PAI-1 regulates plasmin formation and fibrinolysis and, through several different mechanisms, plays a role in the control of MMP activation. Its role in matrix remodeling derives from these effects. Moreover, PAI-1 is also a component of the ECM, where it seems to bind tightly to the somatomedin B domain of vitronectin [12]. This interaction stabilizes PAI-1 in an active conformation, still able to inhibit extracellular PA activity, and thus plasmin formation and MMP activation. In addition, PAI-1 binding to vitronectin has been shown to disrupt the interaction between uPAR and vitronectin, preventing adhesion of the cell to the ECM. uPAR may bind u-PA and promote ECM degradation either directly by u-PA through the proteolytic cleavage of fibronectin, laminin, and other
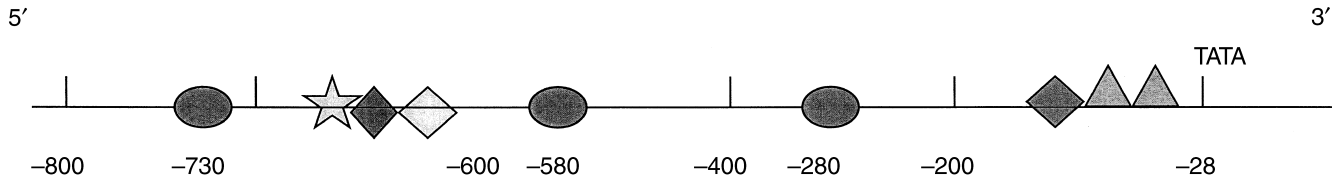
Fig. 2. Schematic representation of the human PAI-1 gene promoter. The following sequences are represented: (▲) Sp1-like binding sites (between nucleotides −85 and −42; from Chen et al [15]), (◆) AP1-like binding sites (between nucleotides −674 and −650 and nucleotides −87 −66; from Keeton et al [16]), (☆) NF-κB–like binding site (position −674; from Dawson et al [17]; and (●) SMAD binding sites (position −280, −580, and −730; Dennler et al [18]). In addition, the promoter regions −800, −549 and −100 + 75 have been shown to be required for the PAI-1 gene response to glucocorticoids (van Zonneveld et al [19]).

components or indirectly through plasmin generation and MMP activation [8]. As a result, PAI-1 has been shown to promote cell migration at least in vitro [13, 14].

PAI-1 IS STRONGLY UP-REGULATED IN THE KIDNEY DURING EXPERIMENTAL AND HUMAN RENAL DISEASES

Regulation of PAI-1 gene expression

The PAI-1 gene has been cloned and sequenced and contains at its 5′ regulatory end several known consensus *cis* regulatory elements, which bind *trans*activating factors such as Sp1, activated protein-1 (AP-1), nuclear factor-κB (NF-κB), Smad3 and Smad4, and others (Fig. 2) [15–19]. The PAI-1 gene transcription is activated in many different renal cell types by inflammatory cytokines, especially interleukin-1β (IL-1β) [20] and tumor necrosis factor-α (TNF-α) [21, 22], and by transforming growth factor-β (TGF-β) [20, 23], glucocorticoids, and nonspecific protein kinase C (PKC) activators such as phorbol myristate acetate (PMA) [24]. Many cell types have been shown to produce PAI-1, such as endothelial cells, smooth muscle cells, hepatocytes, fibroblasts, or inflammatory cells [4, 5]. Renal cells themselves also produce PAI-1 in culture conditions [21–23] and in vivo in pathological conditions (Table 1). In physiological conditions, the main source of circulating PAI-1 is the platelets, where it is stored and released upon activation. Three different forms of circulating PAI-1 can be detected: an active free form representing up to 80% of total PAI-1, a latent inactive form, and a form complexed to t-PA. The plasma level of circulating PAI-1 has been shown to be genetically controlled, and a polymorphism in the 5′ gene promoter has been described. Two alleles, 4G and 5G, at position −674 in the promoter region, are encountered, and the plasma level of PAI-1 has been shown to be higher in patients with the 4G/4G genotype than in those with the 5G/5G genotype, while the heterozygotes 4G/5G have intermediate values [40]. The genotype determines not only the plasma level of PAI-1, but it has been shown also to be associated with an increased risk of myocardial infarction in adult male patients. The molecular mechanisms involved in the increased synthe-

Table 1. Experimental and human kidney diseases in which plasminogen inhibitor type 1 (PAI-1) accumulation has been demonstrated

Type of disease	Host	Reference
I. Fibrin-associated nephropathies		
Crescentic glomerulonephritis	man	Rondeau [25]
	rat	Feng [26]
	rabbit	Malliaros [27]
Thrombotic microangiopathy	man	Rondeau [25]
	man	Xu [28]
Lupus nephritis	mouse	Moll [29]
Mixed cryoglobulinemia	mouse	Moll [29]
Endotoxinemia	mouse	Moll [29]
	mouse	Yamamoto [30]
Radiation nephropathy	rat	Oikawa [31]
Acute renal graft rejection	man	Wang [32]
II. Fibrin-free nephropathies		
Cobra venom nephritis	rat	Barnes [33]
Anti-Thy1.1 nephritis	rat	Tomooka [34]
Hypertensive nephropathy	rat	Tamaki [35]
Diabetic nephropathy	man	Yamamoto [36]
Focal segmental glomerulosclerosis	man	Yamamoto [37]
Cyclosporine toxicity	rat	Shihab [38]
	rat	Duymelink [39]

sis of PAI-1 by the 4G allele as compared with the 5G allele are related to the binding of NF-κB to the *cis* regulatory region −680 to −670, which is partially inhibited by a regulatory protein, binding to the 5G sequence but not, or to a lesser extent, to the 4G sequence. Under IL-1 stimulation of the cells, the PAI-1 gene transcription rate is higher with the 4G allele than with the 5G allele [41].

Renal expression of PAI-1

Plasminogen activator inhibitor type 1 expression is almost undetectable in the normal mouse, rat, and human kidney by immunohistochemistry and in situ hybridization [28, 29, 42]. Conversely, it has been shown that t-PA is expressed by endothelial cells in the kidney and by epithelial cells of the inner medulla collecting duct, while u-PA synthesis has been localized in epithelial cells of the proximal tubule, especially in the S3 segment, and in the large ascending limb of the Henle's loop. More recently, the binding of u-PA at the apical membrane of epithelial cells of the collecting duct has been observed. Interestingly, MMP9 binding was also demonstrated at
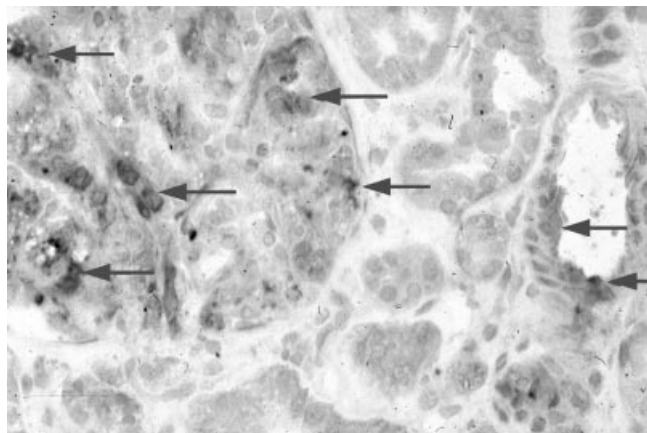
Fig. 3. Immunohistolocalization of PAI-1 in the kidney during thrombotic microangiopathy. While no PAI-1 can be detected in the normal human kidney, PAI-1 antigen is demonstrated in endothelial cells of glomerular capillaries and intrarenal arterioles (arrows). Some mesangial cells are also stained [28].

the same place, suggesting a role and perhaps an interaction of luminal proteinases in the collecting duct functions [43]. Such a role for luminal proteinases has been recently described for the regulation of the epithelial sodium channel activity [44].

Several studies have shown that PAI-1 synthesis and deposition are promoted in the kidney during experimental and human nephropathies (Table 1). PAI-1 deposition may occur along with an inflammatory reaction associated with an activation of coagulation and fibrin deposition in the intravascular or the extravascular spaces [42, 30]. In thrombotic microangiopathy [25, 28], which characterized the pathological pattern of hemolytic uremic syndrome, and in inflammatory glomerulonephritis with extracapillary crescent formation [25–27, 29], we and others were able to demonstrate the presence of PAI-1 antigen by immunohistochemistry, usually colocalizing with fibrin deposits and suggesting that fibrin-trapped PAI-1 may inhibit local plasminogen activation and thus prevent fibrinolysis (Fig. 3). Glomerular cells, mainly endothelial and mesangial cells, and vascular cells, were shown to express PAI-1 by in situ hybridization (Fig. 3) [28]. Similarly, in fibrin-independent kidney injuries such as diabetes and focal segmental sclerosis [36], and cyclosporine A nephropathy [38, 39] and in the aging kidney, PAI-1 synthesis by endothelial, mesangial, and even tubular epithelial cells has been shown to be up-regulated. PAI-1 deposition is associated with ECM expansion and fibrosis, showing that PAI-1 may be a component of the ECM and suggesting that it could play a role in the turnover of the ECM.

The in vivo regulation of PAI-1 synthesis is not yet well understood. In the normal human kidney, mesangial cells do not produce PAI-1 nor express smooth muscle

α actin, whereas they do produce it in large amounts when they are seeded in two-dimensional culture on plastic. We have shown that PAI-1 produced by mesangial cells in culture is released in the culture medium or incorporated in the ECM, as demonstrated by immunocytochemistry and in situ hybridization (Fig. 4) [45, 46]. Similarly, cultured mesangial cells express smooth muscle cell α actin, and both PAI-1 and α actin have been recognized as in vivo and in vitro markers of mesangial cell activation [47]. In contrast, when mesangial cells are embedded in three-dimensional matrix or when they formed hillocks, they ceased proliferating to express smooth muscle specific α actin and to synthesize PAI-1. This suggests that in the normal kidney, a tonic inhibition of PAI-1 gene transcription prevents PAI-1 synthesis and deposition in the ECM by mesangial cells, and that this inhibition involves cell–matrix interactions. Heparan sulfate proteoglycans may be responsible for such an inhibition since we demonstrated that heparin and non-anticoagulant heparan sulfate derivatives were able to inhibit PAI-1 accumulation in the matrix of cultured human mesangial cells [48]. In diseased conditions in vivo, human mesangial cells express PAI-1, and this up-regulation may be related to the stimulation by various cytokines or growth factors, such as TNF-α or IL-1β [21], TGF-β, and thrombin [49]. On the other hand, the up-regulation of PAI-1 synthesis in vivo may result from the abolition of the tonic inhibition of PAI-1 gene transcription by the surrounding normal mesangial matrix. Actually, integrin-mediated ECM interactions with various cell types have been shown to regulate gene expression. The signaling pathways involve intracellular integrin-associated proteins such paxillin, β catenin, vinculin, and talin, which then recruit cytoskeleton proteins and mitogen-activated protein kinases. In vitro, these pathways have been shown to stimulate PAI-1 gene transcription strongly [50]. However, in vivo, less is known about the role of ECM on *PAI-1* gene regulation. One can speculate that alterations in either the amount or the composition of ECM may alter the PAI-1 synthesis and accumulation in the surrounding matrix. This may apply to mesangial cells but also to any other renal parenchymal cells.

EXPERIMENTAL AND CLINICAL EVIDENCE FOR A ROLE OF PAI-1 IN THE FIBROGENIC PROCESS

In two different studies, it has been shown that PAI-1 is a key player in the occurrence of postinflammatory pulmonary fibrosis. After a fibrinous alveolitis has been induced in mice either by intratracheal administration of bleomycin [51] or by hyperoxic atmosphere [52], a progressive pulmonary fibrosis appeared. Disruption of the *PAI-1* gene was shown to decrease the accumulation
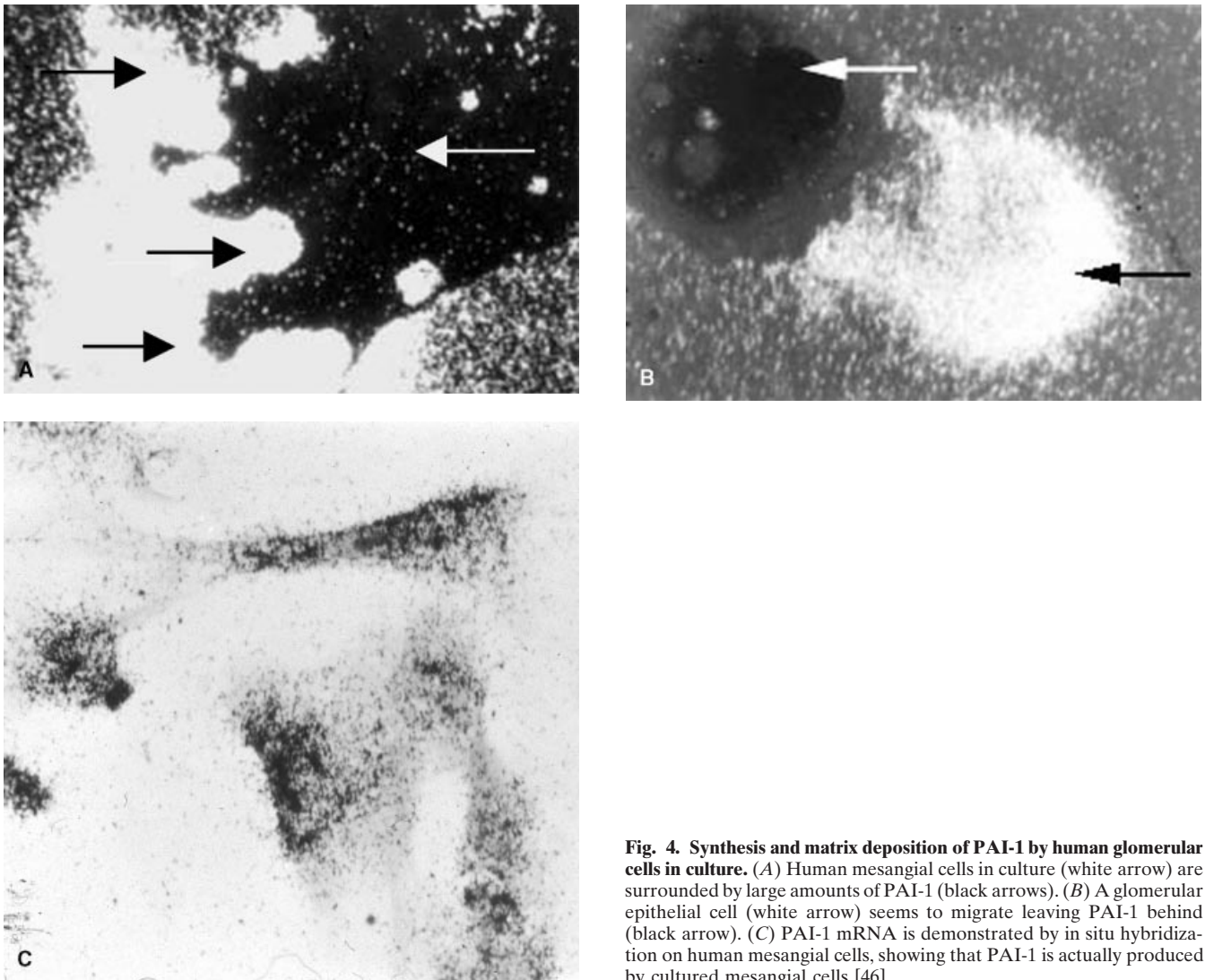
Fig. 4. Synthesis and matrix deposition of PAI-1 by human glomerular cells in culture. (*A*) Human mesangial cells in culture (white arrow) are surrounded by large amounts of PAI-1 (black arrows). (*B*) A glomerular epithelial cell (white arrow) seems to migrate leaving PAI-1 behind (black arrow). (*C*) PAI-1 mRNA is demonstrated by in situ hybridization on human mesangial cells, showing that PAI-1 is actually produced by cultured mesangial cells [46].

of fibrin in the lungs of experimental animals and to prevent most of the pulmonary fibrosis. Conversely, overexpression of PAI-1 in transgenic mice increased both fibrin accumulation and the severity of the subsequent fibrosis. In our opinion, the best scenario that can be proposed is that PAI-1 accumulation at sites of fibrin formation inhibits fibrinolysis and MMP activation. The fibrin matrix has been shown to be progressively invaded by fibroblasts, which produce large amounts of collagens, and make the fibrin matrix more resistant to digestion. Local inhibition of MMP activation may also explain the progressive accumulation of ECM proteins and the development of fibrosis (Fig. 5).

To date, similar proofs for PAI-1 involvement in renal fibrosis are lacking. Indirect evidence for a role of PAI-1 was provided when Kitching et al demonstrated that plasminogen and plasminogen activators protect against renal injury in crescentic glomerulonephritis [53]. However, in this study the late fibrotic phase of the disease was not studied. It has also been shown that in the anti-Thy 1.1 glomerulonephritis in the rat, glomerular matrix accumulation is linked to inhibition of the plasminogen system by PAI-1 [54]. A recent study indicates that PAI-1 may be involved in the pathogenesis of glomerular and vascular sclerosis after irradiation [31]. In this model, a strong activation of the renin-angiotensin system has been demonstrated as well as a strong induction of PAI-1 synthesis in the kidney. The inhibition of angiotensin I-converting enzyme (ACE) by enalapril or of angiotensin II (Ang II) receptors by losartan prevented PAI-1 up-regulation and the late development of renal fibrosis and renal failure. These results also suggest that Ang II stimulates PAI-1 synthesis in vivo, which in turn inhibits ECM degradation and promotes renal fibrosis. In vitro experiments have shown that Ang II increases PAI-1 synthesis by mesangial cells [54]. However, in some cell types such as endo-
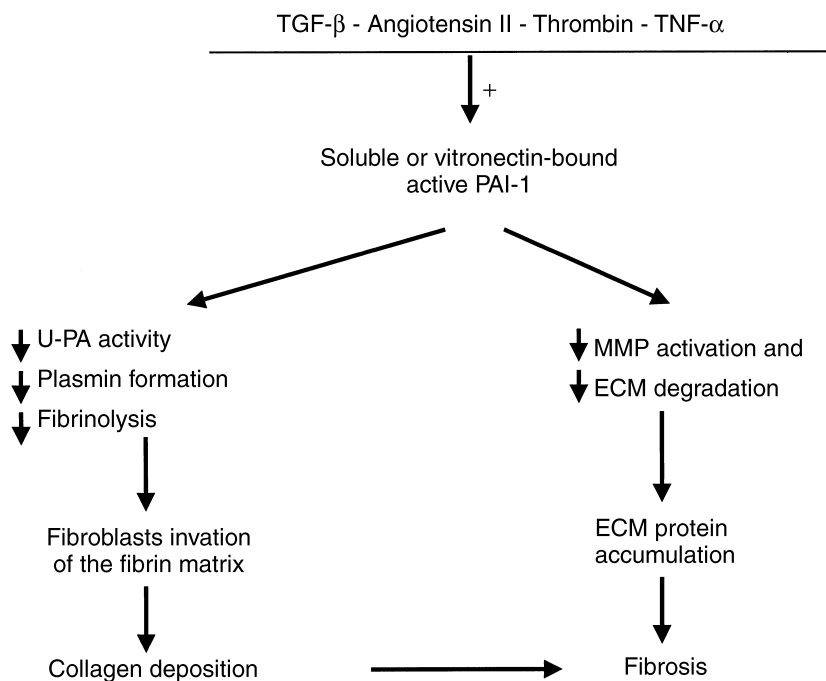
TGF-β - Angiotensin II - Thrombin - TNF-α

Fig. 5. Schematic diagram of PAI-1 actions as a fibrogenic molecule. Potent agonists such as TGF-β, angiotensin II, thrombin, and TNF-α may promote fibrosis through an increase in PAI-1 synthesis. Active PAI-1 inhibits plasmin formation and MMP activation and thus inhibits fibrinolysis and ECM degradation, resulting in tissue fibrosis.

thelial cells [55] and proximal tubule epithelial cells [56], angiotensin IV but not Ang II activates PAI-1 synthesis. This effect seems mediated by specific angiotensin IV receptors different from angiotensin II type 1 or 2 (AT1 or AT2) receptors. In addition, the short-term effects of Ang II on matrix protein synthesis and on PAI-1 are prolonged by a long-term effect of TGF-β, in which the synthesis is also induced by Ang II [57, 58]. More recently, a reduced angiotensinogen expression has been shown to attenuate renal interstitial fibrosis in obstructive nephropathy in mice [59]. In this study, the Ang II-mediated TGF-β expression seemed involved in the renal fibrogenic process independently of the systemic blood pressure.

Transforming growth factor-β is a potent inducer of PAI-1 synthesis through the activation of Smad3 and Smad4, two transcription factors that have been shown recently to mediate almost all, if not all, of the effects of TGF-β on PAI-1 gene transcription [23]. TGF-β is also a potent cytokine stimulating collagen IV, fibronectin, and laminin production in the kidney [60]. In addition, it increases the synthesis of MMP2 and MMP9 and of TIMP-1 and TIMP-2, at least in vitro. The resulting effect on ECM turnover and accumulation is hardly predictable from the analysis of such elementary effects. In addition, it has been demonstrated in vitro that the latent form of TGF-β is secreted and binds to the ECM. Plasmin, and to a lesser extent u-PA, is able to cleave and promote the release of active TGF-β from this latent form. In turn, TGF-β stimulates ECM proteins synthesis and, at the same time, increases PAI-1 production, which

inhibits further plasmin generation and thus TGF-β activation. The plasmin–TGF-β interaction could therefore appear as an autolimited process that will allow transient and timely regulated degradation and synthesis of ECM proteins. Interestingly, in this potential scenario, PAI-1 would be required to prevent prolonged TGF-β activation and further development of fibrosis. However, TGF-β is considered to be a major fibrogenic molecule, at least in many experimental [61, 62] and human kidney diseases, such as chronic glomerulonephritis [37], diabetic nephropathy [36], focal segmental glomerulosclerosis [37], HIV-associated nephropathy, and chronic allograft nephropathy and the associated interstitial fibrosis [63]. The effects of TGF-β on ECM protein synthesis and on inhibitors of ECM degradation in the kidney may overcome the stimulating effect on MMP synthesis. Among the inhibitors, PAI-1 plays a central role as a component of the ECM and as an inhibitor of plasminogen activation [64]. It has recently been shown that the interstitial fibrosis associated with protein-overload proteinuria was less pronounced in PAI-1–deficient mice than in their wild-type controls, further supporting a role for PAI-1 in renal fibrogenesis (abstract; Oda et al, *J Am Soc Nephrol* 10:578A, 1999).

Transforming growth factor-β has been shown to be involved in the pathogenesis of diabetic glomerulosclerosis [36]. Both TGF-β and PAI-1 have been demonstrated by immunohistochemistry on renal biopsy of diabetic patients. In vitro, high-glucose medium increases the synthesis of TGF-β through the glucosamine 6-phosphate pathway and the production of ECM proteins and of

PAI-1 by cultured mesangial cells [65]. Furthermore, when cultured on nonenzymatically glycated collagen I, endothelial cells have been shown to up-regulate their synthesis of PAI-1.

The PAI-1 4G/5G polymorphism, which correlates with the PAI-1 plasma level, has been investigated in diabetic patients. Controversial results were reported since in Japanese patients with type II diabetes, the 4G/4G genotype was associated with more frequent vascular complications than the 5G/4G or the 5G/5G genotypes, but the incidence of diabetic nephropathy was the same in the three groups [66]. Similarly, in Caucasian patients with type I diabetes, the 4G/4G genotype was significantly associated with diabetic retinopathy but not with diabetic nephropathy (abstract; Pucci et al, *J Am Soc Nephrol* 10:132, 1999). In contrast, in Chinese patients with type II diabetes, the 4G/4G genotype was significantly associated with diabetic nephropathy, and this effect was even more pronounced in patients with the DD genotype for ACE [67]. Further large scale studies are required to determine the role of PAI-1 in human diabetic nephropathy. To our knowledge, no study has reported on experimental diabetic nephropathy in PAI-1–deficient or PAI-1–overexpressing mice, but this experimental approach is worth testing.

MODULATION OF PAI-1 SYNTHESIS OR OF PAI-1 ACTIVITY AS FUTURE THERAPEUTIC INTERVENTIONS

From animal studies using genetically modified mice overexpressing or deficient in u-PA, t-PA, plasminogen, or PAI-1, it has been possible to evaluate the physiological role of each of these molecules [6]. The isolated deficit in t-PA, u-PA, or PAI-1 is not associated with an obvious abnormal phenotype, but there is a tendency to thrombosis for t-PA and u-PA and to bleeding for PAI-1 deficit. In contrast, mice deficient in plasminogen or in both t-PA and u-PA exhibit a reduced growth rate, a tendency to thrombosis, and a delay in wound healing. PAI-1–deficient mice are protected from thrombosis and fibrosis after fibrinous alveolitis [51]. Transgenic mice overexpressing PAI-1 have been reported to be prone to thrombosis and tissue fibrin deposition and to late development of fibrosis. It has been suggested that PAI-1 inhibition would thus be beneficial for the prevention of thrombosis and of fibrin-derived fibrosis.

Different methods of inhibition are available: neutralizing anti-PAI-1 antibodies, synthetic antagonists of PAI-1, and inhibitors of PAI-1 synthesis. These approaches are still experimental, but could be useful in evaluating the therapeutic effect of PAI-1 inhibition in vivo. Anti–PAI-1 antibodies have been proposed to prevent rethrombosis shortly after myocardial infarction, since high plasma levels of PAI-1 are frequently encoun-

tered in these cases, which seem to favor thrombosis and extension of myocardial ischemia. Obviously, this is a short-term treatment that is associated with a risk of immunization against heterologous antibodies and a progressive lost of efficacy. In addition, it requires intravenous injection and would not be easy to administer. Renal diseases that may need such PAI-1 inhibition have a prolonged course and would require a long-term blockade of PAI-1 action.

A synthetic inhibitor of PAI-1 has been produced, derived from the reactive center loop of PAI-1 and which induces the inactive conformation of PAI-1 [68]. This 14 amino acid residue inhibitor has been shown to rapidly inhibit PAI-1 function and the formation of t-PA/PAI-1 complexes in vitro. This peptide has a poor inhibitory effect of vitronectin-bound PAI-1, but is able to enhance in vitro lysis of platelet-rich clots and platelet-poor clots containing recombinant PAI-1. Clearly, this approach may be used to increase the intravascular fibrinolytic activity in vivo, while the tissue vitronectin-bound PAI-1 remains fully active. Experimental studies of thrombotic microangiopathy, as recently described after endotoxin and ricin administration in the rat [69], are required to evaluate the effect of such a circulating PAI-1 inhibitor, given either before disease induction or later, when microthrombosis is already present. In the future, the design of other PAI-1 inhibitors would be useful if these compounds can be administered per os on a long-term basis.

The blockade of PAI-1 synthesis in vivo has not been reported but is theoretically a potential way of treatment. Specific inhibition of PAI-1 gene expression may be achieved using the antisense oligonucleotide strategy [70, 71]. This strategy has been shown to block PAI-1 synthesis efficiently in cultured cells in vitro. However, no report is available concerning the in vivo efficacy of these oligonucleotides. Transfection of renal cells in vivo has been reported but remains difficult and requires appropriate vectors. Similarly, PAI-1 synthesis could be inhibited by decoy oligonucleotides directed to the one or two main transcription factors, which are activated in diseased conditions and which increase PAI-1 gene transcription. These transcription factors may very likely vary according to the underlying renal diseases. For example, transcription factors activated by thrombin such as AP-1, NF-κB, and thrombin-induced nuclear factor (TINF) may be adequate targets of decoy oligonucleotides when coagulation and fibrin formation play an important role in the pathogenesis of the renal diseases, that is thrombotic microangiopathy and extracapillary glomerulonephritis. On the other hand, Smad3 and Smad4 would be the potential targets of decoy oligonucleotides to prevent TGF-β–mediated renal PAI-1 synthesis and fibrosis in hypertensive, diabetic, or autoimmune nephropathies [23]. This strategy would block most of the effects of

the TGF-β pathway and may be efficient to lessen the severity of renal diseases, since inhibition of TGF-β1 expression by antisense oligonucleotides has been shown to suppress ECM accumulation in experimental glomerulonephritis induced by anti-Thy 1.1 antibody [72].

Although the inhibition of PAI-1 synthesis or activity may prove useful in fibrogenic renal injuries, it has to be emphasized that PAI-1 may also have a protective role. For example, in the arterial stenosis model after intimal injury in mice, it has been shown that u-PA deficiency delays re-endothelialization of the vessel but that PAI-1 deficiency is associated with an increased intimal hyperplasia when compared with wild-type controls. PAI-1 administration inhibited the excessive neointima formation that was observed in PAI-1–deficient mice [6]. Similarly, the transplant arteriosclerosis, which constitutes a major cause of solid organ graft failure, has been shown to be reduced in mice lacking plasminogen as compared with their normal counterparts [73]. Leukocyte infiltration in the transplant media, which preceded smooth muscle cell proliferation and migration, was significantly reduced in Plg−/− animals, indicating that plasmin plays a role in the infiltration by inflammatory cells, either by degrading ECM proteins in concerted action with MMPs or by activating or releasing chemotactic growth factors sequestered within the matrix, such as basic fibroblast growth factor, vascular endothelial growth factor, or TGF-β1. Finally, recent evidence has been provided that PAI-1 may protect from cardiac rupture after acute myocardial infarction in mice. Interestingly, the administration of TIMP-1 in this model has the same protective effect as PAI-1 administration, further supporting the strong relationship between the plasminogen activation system and the MMPs [74]. Therefore, although it is a potent inhibitor of plasminogen generation and ECM degradation, PAI-1 may also protect tissues from excessive remodeling. Experimental studies evaluating the in vivo effects of PAI-1 inhibition on the course of kidney diseases are thus needed before clinical applications in humans can be proposed.

CONCLUSIONS AND PERSPECTIVES

Plasminogen activator inhibitor type 1 appears to be a potential interesting target in the treatment or the prevention of renal fibrosis. It is not expressed in the normal kidney, but its synthesis is up-regulated in several renal diseases leading to renal fibrosis and terminal renal failure. Experimental evidence is accumulating that PAI-1, by blocking plasmin generation and, in turn, MMP activation, is a profibrogenic molecule. Its role as an inhibitor of ECM degradation is further suggested by its strong interaction with vitronectin, which concentrates PAI-1 in the ECM. It has to be noted, however, that PAI-1, as TIMP-1, may have beneficial effects in some

diseases, especially in vascular remodeling after intimal injury or in chronic transplant arteriopathy. Transgenic mice deficient in PAI-1 or overexpressing it are potent tools to dissect the respective contribution of PAI-1 in the different models of renal fibrogenesis. Several ways of PAI-1 inhibition are proposed but need to be evaluated in the various forms of renal diseases.

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