

Pleiotropic functions of plasminogen activator inhibitor-1

H. R. LIJNEN

Center for Molecular and Vascular Biology, K.U., Leuven, Belgium

To cite this article: Lijnen HR. Pleiotropic functions of plasminogen activator inhibitor-1. *J Thromb Haemost* 2005; 3: 35–45.

Summary. Plasminogen activator inhibitor-1 (PAI-1), a 45-kDa serine proteinase inhibitor with reactive site peptide bond Arg345-Met346, is the main physiological plasminogen activator inhibitor. It occurs in human plasma at an antigen concentration of about 20 ng mL⁻¹. Besides the active inhibitory form of PAI-1 that spontaneously converts to a latent form, also a substrate form exists that is cleaved at the P₁-P₁' site by its target enzymes, but does not form stable complexes. Besides its role in regulating hemostasis, PAI-1 plays a role in several biological processes dependent on plasminogen activator or plasmin activity. Studies with transgenic mice have revealed a functional role for PAI-1 in wound healing, atherosclerosis, metabolic disturbances such as obesity and insulin resistance, tumor angiogenesis, chronic stress, bone remodeling, asthma, rheumatoid arthritis, fibrosis, glomerulonephritis and sepsis. It is not always clear if these functions depend on the antiproteolytic activity of PAI-1, on its binding to vitronectin or on its interference with cellular migration or matrix binding.

Keywords: PAI-1, fibrinolysis, thrombosis.

Introduction

Mammalian blood contains an enzymatic system, the fibrinolytic (or plasminogen/plasmin) system, that is capable of dissolving blood clots. This system comprises an inactive proenzyme (plasminogen) that can be converted to the active enzyme (plasmin) that degrades fibrin into soluble fibrin-degradation products. Two immunologically distinct types of physiological plasminogen activators have been identified: tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). t-PA-mediated plasminogen activation is mainly involved in the dissolution of fibrin in the circulation. u-PA binds to a specific cellular receptor (u-PAR or

CD87) resulting in enhanced activation of cell-bound plasminogen. Inhibition of the fibrinolytic system may occur either at the level of plasmin by α_2 -antiplasmin or α_2 -macroglobulin, or at the level of the plasminogen activators, mainly by plasminogen activator inhibitor-1 (PAI-1) [1].

Besides its role in maintaining normal hemostasis by regulating the fibrinolytic system, PAI-1 plays a role in many other (patho)physiological processes. Several reviews on PAI-1 have recently been published [2–5]. This contribution will focus on some of the functions of PAI-1 that have emerged mainly in studies with transgenic mice. It is not always clearly established whether these processes depend on the antiproteolytic activity of PAI-1, on its binding to vitronectin or on interference with cellular migration/matrix binding (see below).

Structure–function analysis of PAI-1

Production and secretion

PAI-1 was first identified in conditioned media of cultured human endothelial cells and subsequently in plasma, platelets, placenta and conditioned media of fibrosarcoma cells and hepatocytes [6]. It is also produced by vascular smooth muscle cells, mesangial cells, fibroblasts, monocytes/macrophages and by stroma cells from adipose tissue (see below). In healthy individuals, highly variable plasma levels of both PAI activity and PAI-1 antigen have been observed. PAI activity ranges from 0.5 to 47 U mL⁻¹ (t-PA neutralizing units, 1 mg active PAI-1 corresponds to 700 000 U) with 80% of the values below 6 U mL⁻¹. PAI-1 antigen ranges between 6 and 85 ng mL⁻¹ (geometric mean 24 ng mL⁻¹). PAI-1 levels are strongly elevated in several thromboembolic disease states (see below). PAI-1 has been demonstrated in a large variety of tissues, suggesting that a cell common to these tissues, such as endothelial or smooth muscle cells, may be the main site of production. Except for platelets, which contain an inactive form of PAI-1, PAI-1 is not stored within cells, but is rapidly and constitutively secreted after synthesis. PAI-1 exhibits a circadian variation; its plasma concentration is highest in the morning and lowest in the late afternoon and evening, whereas t-PA activity exhibits an opposite diurnal variation [7]. Circadian clock components such as BMAL1 and BMAL2 with different spatiotemporal distribution appear

Correspondence: H. R. Lijnen, Center for Molecular and Vascular Biology, K.U. Leuven, Campus Gasthuisberg, O & N, Herestraat 49, B-3000 Leuven, Belgium.
Tel.: +32 1634 5771; fax: +32 1634 5990; e-mail: roger.lijnen@med.kuleuven.ac.be

to drive circadian variation of PAI-1 by activating its promoter [8].

Synthesis and secretion of PAI-1 can be modulated by various agonists such as hormones, growth factors, endotoxin, cytokines and phorbol esters [1]. Evidence has also been provided for transcriptional regulation of PAI-1 synthesis. Genetic variation at a polymorphic locus of the *PAI-1* gene is associated with differences in plasma PAI-1 levels. A single guanosine insertion/deletion (4G/5G) polymorphism in the PAI-1 promoter region may play an important role in the regulation of *PAI-1* gene expression; the 4G allele, which occurs at a frequency of about 0.56, is associated with higher PAI-1 levels [9]. Four other polymorphisms were investigated in relation to PAI-1 levels in healthy men, aged 50–59 years, from France and Northern Ireland. Two G/A substitutions were detected at positions –844 and +9785. The former is in strong positive linkage disequilibrium with the 4G/5G polymorphism at position –675. Two polymorphisms in the 3' untranslated region were identified. One corresponds to a T/G substitution at position +11 053 and is in negative linkage disequilibrium with the G/A substitution (+9785). The other is a 9-nucleotide insertion/deletion located between nucleotides +11 320 and 11 345 in a 3-fold-repeated sequence. This polymorphism is in strong positive linkage disequilibrium with the G/A substitution (+9785). The overall heterozygosity provided by the five PAI-1 polymorphisms (including the four new variants and the 4G/5G polymorphism) is 0.77. No significant association was found between PAI-1 activity and genotypes; furthermore, the well-known associations between PAI activity and body mass index, serum triglycerides, or insulin were homogeneous according to PAI-1 genotypes [10]. Possibly gene–environment interactions complicate this association, as suggested by different correlations of PAI-1 activity and triglyceride levels among the 4G/5G genotypes in patients with non-insulin-dependent diabetes mellitus [11], or in dyslipidemic patients [12]. Interestingly, activation of the renin–angiotensin–aldosterone system by salt intake has a more pronounced effect on PAI-1 antigen in subjects with the 4G/4G genotype [13]. However, the Stockholm Heart Epidemiology Program (SHEEP) failed to detect synergistic interactions between PAI-1 4G/5G polymorphism and environmental factors such as smoking, physical inactivity, hypertriglyceridemia, hypertension, high C-reactive protein, overweight, diabetes mellitus and hypercholesterolemia [14].

In vascular endothelial cells PAI-1 is induced by hypoxia, via a tyrosine kinase pathway [15]. Angiotensin II upregulates PAI-1 expression via the angiotensin type 1 (AT1) receptor [16]. This effect is enhanced by aldosterone, and combined AT1 receptor and aldosterone receptor antagonism in man reduces PAI-1 levels [17]. Inhibition of angiotensin-converting enzyme (ACE) in man also reduces (morning) PAI-1 levels. A comparison of ACE inhibition and AT1 receptor antagonism revealed that short-lasting interruption of the renin–angiotensin–aldosterone system by both approaches reduces PAI-1 antigen levels, whereas the duration of this effect is longer for ACE inhibition [18]. A study in patients after myocardial

infarction indicated that a greater early reduction of PAI-1 was achieved with the high-tissue-penetrating ACE inhibitor quinapril than with the low-tissue-penetrating inhibitor enalapril [19]. It remains to be investigated whether such approaches to reduce PAI-1 levels have the potential to reduce the incidence of thrombotic cardiovascular events.

Physicochemical properties

PAI-1 is a single-chain glycoprotein of about 45 kDa consisting of 379 or 381 amino acids (NH₂-terminal heterogeneity). It is a member of the serpin family with reactive site peptide bond Arg345–Met346 [2]. PAI-1 has three potential glycosylation sites of which only two are used (Asn209 and Asn265), but no cysteines [20]. The *PAI-1* gene, located on chromosome 7, bands q21.3–q22, is approximately 12.2 kb and consists of nine exons [21]. As a result of alternative polyadenylation yielding an additional 3' untranslated region, a mRNA species of 3.2 kb occurs in addition to one of 2.4 kb.

PAI-1 is stabilized by binding to a PAI binding protein identified as S-protein or vitronectin [22]. The PAI-1 binding motif is localized to residues 12–30 of the somatomedin B domain of vitronectin [23]. The active inhibitory form of PAI-1 spontaneously converts to a latent conformation that can be partially reactivated by denaturing agents. The structural basis of the latency in PAI-1 has been resolved by determination of the structure by single-crystal X-ray diffraction. Part of the reactive center loop is inserted in the major β -sheet of PAI-1 and is therefore not accessible to the target enzyme (locked conformation). Reactivation of latent PAI-1 by denaturants results in partial elimination of this insertion [24]. Studies with a monoclonal antibody that rapidly converts PAI-1 to the latent conformation confirmed the existence of a specific intermediate structure compatible with a partial insertion of the reactive center loop into β -sheet A [25]. The reactive center loop may indeed be inserted up to residue P13 in active PAI-1 [26]. Another molecular form of intact PAI-1 has been isolated that does not form stable complexes with t-PA but is cleaved at the P1–P1' peptide bond ('substrate PAI-1') [27]. The X-ray structure of the cleaved substrate variant shows that it has a new β -strand (s4A) formed by insertion of the NH₂-terminal portion of the reactive site loop into β -sheet A subsequent to cleavage [28]. Thus, inhibitory PAI-1 may not only convert to latent PAI-1, which can be reactivated, but also to substrate PAI-1, which is irreversibly degraded by its target proteinases. This may have implications for the regulation of the fibrinolytic system.

Mechanism of action

PAI-1 reacts very rapidly with single-chain and two-chain t-PA and with two-chain u-PA, with second-order inhibition rate constants of the order of $10^7 \text{ M}^{-1} \text{ s}^{-1}$, but it does not react with single-chain u-PA [6]. Like other serpins, PAI-1 inhibits its target proteinases by formation of a 1 : 1 stoichiometric reversible complex, followed by covalent binding between the

hydroxyl group of the active site serine residue of the proteinase and the carboxyl group of the P1 residue at the P1-P1' reactive center ('bait region') of the serpin. The rapid inhibition of both t-PA and u-PA by PAI-1 involves a reversible high-affinity second-site interaction between sequence 350–355 of PAI-1, which contains three negatively charged amino acids, and highly positively charged regions in t-PA (residues 296–304) or in u-PA (residues 179–184). PAI-1 also binds to fibrin and fibrin-bound PAI-1 may inhibit t-PA-mediated clot lysis [29].

In the presence of vitronectin, PAI-1 displays a 200-fold accelerated thrombin inhibition, as a result of a conformational effect of vitronectin (VN) binding on the reactive site loop of PAI-1 [30]. Furthermore, by high-affinity binding to VN, PAI-1 may compete with uPAR-dependent or integrin-dependent binding of cells to the extracellular matrix [31]. Thereby, PAI-1 may play a role in cell adhesion and/or migration via a mechanism independent of its antiproteolytic activity. PAI-1 inhibits u-PA-induced chemotaxis by internalizing the uPAR, thus regulating cell migration [32]. Active u-PA bound to uPAR on the cell surface can indeed be inhibited by PAI-1, and the resulting inactive PAI-1–u-PA–uPAR complexes are rapidly internalized by the low-density lipoprotein (LDL) receptor-related protein (LRP) [33,34]. Thus, PAI-1 initiates an LRP-dependent decrease in cell surface uPAR. The binding of u-PA to uPAR stimulates intracellular signaling [35] and also induces conformational changes in uPAR [36,37] which increase its affinity for VN and promote its interaction with a variety of integrins [35,38]. PAI-1 can detach cells from extracellular matrices by disrupting uPAR–VN and integrin–VN interactions as a result of its binding to u-PA present in u-PA–uPAR–integrin complexes [31,39]. VN is quite unique among adhesive proteins not only because PAI-1 binds to it with high affinity and specificity but also because cells can attach to it through integrins, uPAR or both. The high-affinity binding sites for both PAI-1 and uPAR reside in two overlapping but distinct regions of the NH₂-terminal somatomedin B (SMB) domain of VN [40], and are immediately adjacent to the single RGD sequence in the molecule. Because the affinity of PAI-1 for the SMB domain is much higher than that of uPAR [31], PAI-1 can competitively inhibit the uPAR-dependent attachment of cells to VN. Binding of PAI-1 to SMB also inhibits integrin-mediated cell adhesion, presumably by sterically blocking the adjacent RGD site [40,41]. The anti-adhesive effect of PAI-1 on vascular cells adherent to VN correlates with apoptosis, mediated through the caspase 3 pathway [42].

Role of PAI-1 in hemostasis

PAI-1 and thrombosis

Several studies in animal models suggest that increased PAI-1 levels may promote fibrin deposition *in vivo* (reviewed in [1]). When endotoxin-treated rabbits, with a markedly increased plasma PAI-1 level, are infused with the defibrinogenating snake venom Ancrod, renal fibrin deposits are produced,

whereas in normal rabbits Ancrod infusion causes hypofibrinogenemia without fibrin deposition [43]. In an experimental rabbit model of jugular vein thrombosis, inhibition of PAI-1 with the use of a monoclonal antibody resulted in promotion of endogenous thrombolysis and inhibition of thrombus extension [44]. Mice, transgenic for the human *PAI-1* gene, develop venous thrombosis at the tip of the tail within 3 days after birth, but no arterial thrombosis [45]. Furthermore, transgenic mice which totally lack functional PAI-1 lyse experimental pulmonary emboli at a faster rate than controls. Homozygous PAI-1-deficient mice also develop venous thrombi significantly less frequently than wild-type mice after local injection of endotoxin in the footpad, although inflammation was similar [46]. These findings support a causal role for PAI-1 in the development of venous thrombosis. Studies in PAI-1-deficient mice also indicated a major role of PAI-1 in the resistance to lysis of platelet-rich arterial thrombi [47]. Several other recent studies in transgenic mice have addressed the relation between PAI-1 levels and thrombosis. Thus, transgenic mice overexpressing murine PAI-1 or stable human PAI-1 do not show evidence of arterial or venous thrombosis up to 4 months of age, whereas 90% of transgenic mice older than 6 months develop spontaneous occlusions [48,49]. Chronically elevated PAI-1 levels thus appear to be associated with age-dependent coronary arterial thrombosis in mice. In the *klotho* mouse, increased PAI-1 expression associated with fibrin deposition was observed with aging, which may contribute to development of thrombosis [50]. In a mouse model of myocardial infarction induced by coronary ligation, cardiomyocytes and mast cells contribute to enhanced PAI-1 expression resulting in development of interstitial and perivascular fibrosis in the heart [51]. Furthermore, inhibition of plasminogen activators in mice prevents cardiac rupture, but impairs therapeutic angiogenesis and causes heart failure [52]. A chronic coronary artery ligation model in myeloperoxidase-deficient mice revealed markedly delayed myocardial rupture, due to decreased oxidative inactivation of PAI-1 leading to decreased tissue plasmin activity [53].

In man, increased levels of PAI activity resulting in a decreased fibrinolytic capacity have been reported in several thrombotic disease states, including venous thromboembolism, coronary artery disease and acute myocardial infarction. In patients with venous thrombosis defective fibrinolysis may be due to a low concentration of t-PA or to an increased level of PAI-1. In 35% of patients with spontaneous or recurrent deep vein thrombosis a poor fibrinolytic response to venous occlusion was observed, which was due to deficient t-PA release in 25% and to increased PAI-1 levels in 75% of these cases [54]. In some but not all studies, an impaired fibrinolytic capacity after venous occlusion was observed in patients with thrombotic episodes [55,56]. In the Physician's Health Study [57], PAI-1 levels in patients who developed venous thrombosis during a 5-year follow-up were not different from controls. The association between enhanced PAI-1 levels and symptomatic venous thrombosis is not consistent and requires further study.

High plasma PAI-1 levels in patients with acute myocardial infarction or unstable angina were found to be predictive for

recurrent (within 3 years) myocardial infarction in some studies [58] but not in others [59]. In a prospective study in patients with angina pectoris, high basal levels of t-PA antigen but not of PAI activity were associated with an increased risk of myocardial infarction [60,61]. In the Physician's Health Study [62], increased t-PA antigen levels were predictive of myocardial infarction within the 5-year follow-up period, but this association disappeared after adjustment for body mass index, HDL-cholesterol, and blood pressure. High PAI-1 levels, associated with the 4G allele of the 4G/5G polymorphism, were observed in asymptomatic young adults, and in young patients with myocardial infarction [12,63]. The 4G allele of this polymorphism was claimed to be a risk factor for myocardial infarction [63], although this was not confirmed in the ECTIM Study [64]. In the Stockholm Heart Epidemiology Program (men and women with first-time myocardial infarction vs. controls) the 4G allele was slightly associated with an increased risk of myocardial infarction in women, but not in men [14]. Thus, the homozygous form of the 4G allele is associated with increased PAI-1 antigen levels, but the relation to thrombotic disease requires further investigation. It was recently reported that the PAI-1 4G/4G genotype constitutes a risk factor for myocardial infarction in patients with elevated insulin levels [65].

In the prospective ECAT study, 10 fibrinolytic variables were measured in 3043 patients with angina pectoris recruited from 18 European centers [66]. A first analysis after adjustment for other non-fibrinolytic coronary risk factors (body mass index, triglyceride levels, diabetes, systolic blood pressure) revealed that an increased risk of coronary events within 2 years was associated with higher baseline concentrations of t-PA antigen but not of PAI-1 activity and antigen levels. However, several studies, including the ECAT study itself, have reported strong correlations between t-PA and PAI-1 antigen levels, as much in healthy populations as in patients with coronary heart disease [67]. Therefore, it was surprising that in the ECAT study only t-PA antigen and not PAI-1 was identified as a predictive risk factor for coronary heart disease. Re-examination of the prognostic value of these fibrinolytic variables after separate adjustment for clusters of markers of insulin resistance, inflammation or endothelial cell damage, affected the prognostic value of PAI-1 and t-PA levels differently. Factors involved in the insulin resistance syndrome strongly affected PAI-1, and to a lesser extent, t-PA antigen. The latter was primarily influenced by inflammation and endothelial cell damage. Thus, t-PA antigen levels may constitute a biological marker of coronary heart disease, influenced by a variety of pathophysiological pathways including inflammation. In contrast, PAI-1 levels, which are mainly dependent on the metabolic status, emerge as a risk factor predictive of the future development of atherothrombosis [68].

PAI-1 and bleeding

Excessive fibrinolysis due to decreased PAI-1 levels has been reported in a few cases and was associated with bleeding complications [69,70]. A complete deficiency of PAI-1 has been

reported in a 9-year-old girl who had several episodes of major hemorrhage, all in response to trauma or surgery [71]. DNA sequence analysis revealed a 2-bp insertion at the 3' end of exon 4. This mutation results in a shift in the reading frame after the codon for amino acids 210, resulting in a new stop codon. The predicted protein thus lacks the 169 COOH-terminal amino acid region of mature PAI-1, which includes the Arg-Met reactive site peptide bond.

The effect of *PAI-1* gene disruption on organ development and reproduction, hemostasis, thrombosis and thrombolysis has been investigated in mice. Surprisingly, PAI-1-deficient mice are viable and fertile, and have no significant organ abnormalities, demonstrating that reproduction and development can proceed normally in the absence of PAI-1. Possibly, other proteinase inhibitors, which are able to reduce plasminogen activation and/or plasmin activity, might compensate for PAI-1 deficiency, or PAI-1 deficiency in mice may induce only a mild hyperfibrinolytic state [46]. Spontaneous bleeding or delayed rebleeding was not observed after partial amputation of the tail or of the cecum in PAI-1-deficient mice. This is in contrast to the delayed rebleeding observed after trauma or surgery in patients with reduced or absent PAI-1 levels. This difference in phenotype between mice and man may be due to the about 5-fold lower basal plasma levels of active PAI-1 in wild-type mice than in man, but species-dependent differences in the plasma levels of t-PA and possibly of other components of the fibrinolytic system might also play a role. Nevertheless, mouse models have been extensively used to study the role of PAI-1 in a variety of biological processes (see below).

PAI-1 and wound healing

Luminal stenosis as a result of arterial neointima formation limits the success of vascular reconstructions for treatment of atherothrombosis. A study with PAI-1-deficient and wild-type control mice suggested that PAI-1 inhibits vascular wound healing and arterial neointima formation after mechanical or electrical injury, mainly by affecting cellular migration. Adenoviral-mediated overexpression of human PAI-1 suppressed arterial neointima formation in this model [72]. In contrast, in murine models of vascular injury induced by ferric chloride, rose bengal or copper, a positive correlation was observed between PAI-1 levels and neointima formation [73,74]. In the ferric chloride model interaction of PAI-1 with VN may play a role in the observed thrombotic response by preventing premature thrombus dissolution and embolization [73]. Furthermore, in the presence of VN, PAI-1 is a more efficient inhibitor of thrombin, which may explain the fact that PAI-1 and VN protect against stenosis in a murine carotid artery ligation model, possibly by inhibition of thrombin-mediated smooth muscle cell (SMC) proliferation [75]. In contrast, Peng *et al.* reported that endogenous VN and PAI-1 enhance neointima formation in response to vascular occlusion or injury in mice, possibly by promoting intravascular fibrin deposition and by the capacity of VN to enhance SMC-fibrin interactions [76]. A critical feature in these studies appears to be

the presence or absence of thrombus/fibrin, raising the hypothesis that PAI-1 may inhibit neointima formation in the absence of fibrin (mechanical or electrical injury models with only transient thrombosis), but enhance it in the presence of fibrin (injury models induced with ferric chloride, rose bengal or copper, with persistent thrombus [77]). However, in a vascular injury model that induces persistent fibrin deposition in femoral arteries of mice, overexpression of murine PAI-1 did not affect neointima formation [48]. Differential interaction of PAI-1 with VN during different phases of wound healing or in different parts of the vasculature may further complicate this issue (reviewed in [77]). PAI-1 also promotes neointima formation after oxidative vascular injury in atherosclerosis-prone mice [78] and in balloon-injured rat carotid arteries [79]. PAI-1-deficient mice also exhibit accelerated skin wound healing [80].

In a mouse model of allograft vascular disease (AVD), PAI-1 deficiency greatly aggravated the extent of intimal proliferation after allogeneic transplantation, suggesting that PAI-1 is important in limiting the early phase of AVD [81].

PAI-1 and atherosclerosis

Elevated plasma PAI-1 levels have been associated with the progression of atherosclerosis [82], supposedly by inhibiting the clearance of fibrin incorporated into atherosclerotic plaques. In the ARIC (Atherosclerosis Risk in Communities) study [83], high baseline PAI-1 levels were correlated with vessel wall thickness, suggesting a relationship between PAI-1 levels and the severity of vessel wall damage. Local high concentrations of PAI-1 have also been observed in coronary arteries with atherogenic lesions and may contribute to the development of vessel wall damage [84]. However, enhanced local expression of plasminogen activators was also observed within the atherosclerotic plaque, which may contribute to destabilization and rupture of the plaque [85].

Progression of atherosclerosis in LDL receptor and apolipoprotein E (apoE)-deficient mice was claimed to be independent of genetic alterations in PAI-1 [86]. Eitzman *et al.* reported that plaque growth in mice was not affected by PAI-1 deficiency in the aortic root, but was reduced in the carotid bifurcation [87]. In contrast, Luttun *et al.* [88] reported that PAI-1 deficiency in apoE-deficient mice resulted in larger plaques at all sites of the vasculature (due to enhanced extracellular matrix (ECM) deposition), but only at advanced stages of atherosclerosis. This study suggests that PAI-1 may promote plaque growth because of its antifibrinolytic properties, but may also have a protective role by limiting plaque growth and preventing abnormal matrix remodeling. Atherosclerosis-prone apoE-deficient mice do show enhanced thrombosis in the ferric chloride model, associated with increased arterial expression of PAI-1 [89].

An orally administered low-Mr PAI-1 inhibitor was shown to protect apoE-deficient mice against atherosclerosis [90]. Furthermore, it reduced the pro-atherosclerotic effects of angiotensin II (which induces PAI-1 expression) in

ApoE-deficient mice [91]. These recent studies support the hypothesis that PAI-1 is pro-atherogenic and suggest that it may be a therapeutic target in treatment or prevention of atherosclerosis.

PAI-1 and metabolic disturbances

Increased levels of PAI-1 were observed in the insulin resistance syndrome, and a significant correlation was found between plasma PAI-1 levels and body mass index, triglyceride levels, insulin levels and systolic blood pressure [92]. Improving insulin resistance by diet, exercise, or oral antidiabetic drugs results in a decrease in plasma PAI-1. In patients with insulin resistance syndrome, improving the lipid profile indeed enhanced fibrinolytic activity as a result of reduced PAI-1 levels [93].

The mechanisms by which enhanced PAI-1 levels are linked with the insulin resistance syndrome are not well understood. *In-vitro* data indicate that PAI-1 synthesis by endothelial cells and hepatocytes could be affected by insulin, proinsulin and atherogenic lipoproteins. VLDL (very low density lipoproteins) induce transcription by the human PAI-1 promoter in endothelial cells. A VLDL responsive element (VLDLRE) is located in the promoter region, and its activity is influenced by the common 4G/5G polymorphism located adjacent to and upstream of the binding site of the VLDL-inducible transcription factor. These findings may provide a molecular explanation for the link between VLDL and enhanced plasma PAI-1 activity and for the interaction between the 4G/5G polymorphism and plasma levels of triglycerides [94]. However, analysis of the Stanislas Cohort indicated that metabolic parameters involved in the insulin resistance syndrome explained the major part of PAI-1 variability, whereas different polymorphisms (see above) had only a minor contribution [95].

A recent review of the effect of PAI-1 in diabetes mellitus and cardiovascular disease concluded that control of PAI-1 levels may be of great importance in at-risk populations, such as patients with insulin resistance, impaired glucose tolerance or diabetes. Among other factors, enhanced adipose tissue mass and elevated insulin levels contribute to elevated plasma PAI-1 levels with associated prothrombotic and proinflammatory effects. Lowering PAI-1 levels may be achieved by diet and/or exercise or by treatment with peroxisome proliferator-activated receptor- γ agonists (e.g. thiazolidinediones) or inhibitors of the renin-angiotensin system [96]. Plasma PAI-1 levels are elevated in patients with Type 2 diabetes or with metabolic syndrome; obese adipose tissue contributes significantly to PAI-1 levels [97,98]. In the Insulin Resistance Atherosclerosis Study (IRAS), plasma C-reactive protein and PAI-1 levels were higher in insulin-resistant subjects who later developed diabetes than in subjects who did not, and PAI-1 levels predicted diabetes independently from other known risk factors [99].

PAI is expressed in murine as well as in human adipose tissue [97,100–102] and its expression in human adipose tissue is positively correlated with body mass index [103,104]. Human visceral adipose tissue expresses more PAI-1 than subcutaneous abdominal adipose tissue [101,105], whereas only the former is

associated with insulin resistance. Glucocorticoids and insulin promote PAI-1 production by human adipose tissue [106]. PAI-1 inhibits insulin signaling by competing with $\alpha_v\beta_3$ for binding to VN [107]. Recently, it was shown that plasma PAI-1 levels are more closely associated with fat accumulation in the liver than in adipose tissue [108]. In human and murine fat, PAI-1 production was also observed in stroma cells [109,110]. Tumor necrosis factor (TNF)- α is elevated in obesity and in acute inflammatory states and contributes to the associated elevated PAI-1 levels. Studies in mice deficient in the p55 and p75 TNF- α receptors suggested that the binding of TNF- α to the p75 receptor may attenuate induction of PAI-1 [111].

A potential role of PAI-1 in development of obesity is supported by the findings that PAI-1-deficient mice kept on a high-fat diet develop adipose tissue more rapidly than their lean counterparts [112] and that transgenic mice overexpressing murine PAI-1 have lower body weight and lower adipose tissue mass [113]. Similarly, transgenic mice overexpressing a stable human PAI-1 variant had virtually no intraperitoneal fat [114]. In contrast to these studies, disruption of the *PAI-1* gene in genetically obese and diabetic ob/ob mice reduced adiposity and improved the metabolic profile [115]. Another study recently reported that PAI-1-deficient mice on a high-fat diet developed less obesity and insulin resistance than wild-type controls, and downregulation of PAI-1 by an angiotensin type 1 receptor antagonist in wild-type mice ameliorated diet-induced obesity [116]. Some of these differences may be explained by the different genetic background of the mice used in these studies.

PAI-1 and tumor angiogenesis

The contribution of PAI-1 to tumor development has long been controversial. Recently, a pro-angiogenic role of PAI-1 has emerged. Indeed, PAI-1 deficiency in mice led to reduced angiogenesis in two models of tumor transplantation [117,118], in the mouse aortic ring assay [119,120] and in the laser-induced choroidal neoangiogenesis assay [121]. These findings are in agreement with clinical data that high expression of PAI-1 in tumors correlates with poor prognosis [2]. Further studies showed that tumor growth is inhibited in PAI-1-deficient mice and stimulated in mice which overexpress PAI-1 [122]. However, in a transgenic mouse model of metastasizing breast cancer primary tumor growth and vascular density were not different in PAI-1-deficient and wild-type mice [123]. Recent studies have highlighted the importance of host-derived PAI-1 compared with tumor-derived PAI-1 for tumor growth and tumor angiogenesis [124,125]. It was demonstrated that PAI-1 at physiological concentrations in mice promotes tumor development, but inhibits invasion and vascularization at pharmacological concentrations [119]. PAI-1 can contribute to angiogenesis by regulating plasmin-mediated proteolysis, or by modulating cell migration by affecting cell-matrix interactions [126]. Using adenoviral gene transfer of PAI-1 mutants in mice revealed that PAI-1 promoted tumor angiogenesis not by interacting

with VN but rather by inhibiting proteolytic activity [118]. The roles of u-PA and PAI-1 in tumor growth and malignancy have recently been reviewed in detail [2,127].

PAI-1 in other pathophysiological processes

Chronic stress is positively associated with increased plasma concentrations of PAI-1 in healthy middle-aged men [128]; high job stress is also associated with enhanced PAI-1 levels and may contribute to development of cardiovascular disease [129]. In mice, restraint stress leads to a dramatic induction of plasma PAI-1 antigen and maximal induction of PAI-1 mRNA in adipose tissue; in PAI-1-deficient mice restraint stress induced less tissue thrombosis compared with age-matched wild-type mice. The authors suggest that PAI-1 induction by stress (adipose-tissue origin) increases the risk for thrombosis, especially in older subjects [130].

A potential role of PAI-1 in bone remodeling is suggested by the finding that osteoblastic cells from PAI-1-deficient mice have a significantly enhanced potential to degrade non-mineralized bone-like matrix [131]. Studies in PAI-1-deficient mice indicated a specific role for PAI-1 in regulation of trabecular bone turnover during estrogen deficiency [132].

The pathology of asthma involves chronic airway inflammation and expansion of the subepithelial ECM. Altered PAI-1 expression may predispose to asthma in humans. In patients with severe asthma, massive infiltration of PAI-1-producing mast cells in the airways is observed. Furthermore, the 4G allele frequency (associated with elevated plasma PAI-1) was significantly higher in asthmatic patients than in controls [133]. PAI-1-deficient mice are protected against the accumulation of ECM and fibrosis in the lung after bleomycin treatment [134] and hyperoxia [135]. Rapid removal of a provisional fibrin-rich matrix contributes to this effect [136]. After chronic lipopolysaccharide challenge, subepithelial fibrin deposition was diminished in PAI-1-deficient mice, whereas airway hyperreactivity and expansion of the subepithelial area persisted longer in wild-type mice [137].

Intra-articular fibrin deposition is a striking feature of rheumatoid arthritis. Studies in PAI-1-deficient mice revealed that deficiency of PAI-1 results in increased synovial fibrinolysis, leading to reduced fibrin accumulation in arthritic joints and reduced severity of antigen-induced arthritis [138].

Long-term inhibition of nitric oxide synthase (NOS) induces expression of PAI-1 in vascular tissues, hypertension and perivascular fibrosis. PAI-1-deficient mice are protected against the structural vascular changes (fibrosis) that accompany hypertension in the setting of long-term NOS inhibition [139]. In hypertensive patients, aldosterone may be an important factor contributing to the variability of PAI-1 levels in individual subjects [140]. In animal models, aldosterone receptor antagonism decreases PAI-1 expression and fibrosis [141].

Crescentic glomerulonephritis is characterized by glomerular fibrin deposition. PAI-1-deficient mice with experimental glomerulonephritis developed fewer glomerular crescents, less

fibrin deposition, fewer infiltrating leukocytes and less renal collagen accumulation, whereas mice with overexpression of PAI-1 showed the opposite pattern [142]. In another study it was shown that PAI-1 deficiency aggravates the course of experimental glomerulonephritis in mice through overactivation of transforming growth factor (TGF)- β [143]. Treatment of rats with experimental glomerulonephritis with a PAI-1 mutant that binds to matrix VN but does not inhibit plasminogen activators, reduced glomerulosclerosis, probably by competing with endogenous PAI-1 and restoring plasmin generation, inhibiting inflammatory cell infiltration, decreasing local TGF- β concentration and reducing matrix accumulation [144].

Elevated PAI-1 levels have been associated with an unfavorable outcome in sepsis [145]. The 4G/5G polymorphism may play a role, since the 4G allele results in a higher transcription level than the 5G allele in response to interleukin-1 [146]. This overproduction of PAI-1 may contribute to disseminated intravascular coagulation in patients suffering from septic shock.

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