Intramural Plasminogen Activator Inhibitor Type-1 and Coronary Atherosclerosis

Burton E. Sobel, Douglas J. Taatjes, David J. Schneider

Abstract—Altered expression of plasminogen activator inhibitor type-1 in vessel walls, reviewed here, might affect coronary atherogenesis. Upregulation might exacerbate vasculopathy by potentiating thrombosis and by inhibiting vascular smooth muscle cell migration, resulting in attenuation of thickness of elaborated fibrous caps implicated in the vulnerability of atheroma to rupture. (Arterioscler Thromb Vasc Biol. 2003;23:●●●●●●●.)

Key Words: vulnerable plaques ■ diabetes ■ fibrinolysis ■ atherothrombosis ■ coronary artery disease

Plasminogen activator inhibitor type-1 (PAI-1) is a member of the serpin superfamily of proteinase inhibitors. Its name pertains to its capacity to inhibit plasminogen activators (PAs), including tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). Their inhibition limits the dissolution of fibrin and consequently, clots, by the fibrinolytic system in the blood.

Regulation of the activity of proteolytic enzymes in tissues by endogenous inhibitors such as serpins is pivotal in the maintenance of homeostasis.1 Numerous serpins have been identified and characterized. Most are single-chain proteins containing a conserved domain of between 370 and 390 residues. Glycosylation typifies those serpins present in blood.1

Names can, of course, be deceiving. The term “serpin” is short-hand for serine proteinase inhibitor. However, serpins are multifunctional. For example, α1-antitrypsin, also called α1-proteinase inhibitor, blocks the cytotoxicity of neutrophil defensins and might act as an antioxidant.3 Most serpins inhibit diverse proteinases to differential extents. Thus, although α1-antitrypsin is named because it inhibits trypsin, it also inhibits elastase, protease 3, cathepsin G, PAs, and other proteinases.2 PAI-1, named because it inhibits PAs, is also an inhibitor of α-thrombin.3

Serpins form irreversible complexes with their target proteinases that can be dissociated only under denaturing conditions. When acting as proteinase inhibitors, they are cleaved at the proteinase recognition site, resulting in their own irreversible inactivation. Among the serpins, PAI-1 is unique. Under physiologic conditions, it contains a strained loop, maintaining its inhibitory conformation. In the absence of vitronectin, with which it is associated and protected in blood, it undergoes spontaneous conversion to a latent, inactive form, resulting from a reversible conformational change in the strained-loop region.4,5 Thus, whether in the blood or in tissues, PAI-1 exhibits changes in the ratios of proteinase inhibitory activity with respect to the concentration of PAI-1 protein.6,7

Functions of PAI-1 in Blood and Tissues
Degradation of fibrin and consequently, clots, within the vascular system is initiated by activation of the zymogen plasminogen and t-PA. Cleavage of the zymogen forms plasmin, a serine proteinase responsible for the dissolution of the clots. t-PA is the predominant PA in blood, and u-PA, the predominant 1 in tissues. The inhibition of unleashed PA activity in blood by PAI-1 is essential for maintenance of homeostasis and preclusion of induction of a bleeding diathesis.8,9

We use the term “proteo(fibrino)lytic” system to refer to the analogous activations within tissues and their susceptibility to inhibition by PAI-1. Matrix metalloproteinases (MMPs), including stromelysin and collagenase, are activated by u-PA in tissues. This results in dissolution of extracellular matrix,10 exacerbation of rupture of vulnerable atherosclerotic plaques,11–14 and modification of the rate of migration of specific cellular components, such as vascular smooth muscle cells (VSMCs). The lattermost process reflects inhibition of cell surface u-PA, a component that is critical to migration of VSMCs and numerous other types of cells.15 As judged from results in studies of cells in culture and the association of increased u-PA cell surface expression by tumor cells with metastatic potential, it appears that overexpression of PAI-1 reduces migration of these cells in vitro and also in vivo. Mechanisms implicated include inhibition of cell surface expression of u-PA activity and competition for an integrin binding site on vitronectin, sometimes called PAI-1 binding protein.16

Conversely, PAI-1 might exert promigratory effects. Such effects might reflect the limitation of excessive proteolysis in...
extracellular matrix, where PAI-1 is colocalized with vitronectin, because matrix can provide traction for migrating cells. This dichotomy of possibilities is obviously relevant to the effects of PAI-1 on neointimal formation and the composition of atheroma (please see the following section).

Association of PAI-1 With Atherosclerosis
It was learned many years ago that elevated PAI-1 in blood was a risk factor and perhaps a determinant of an initial acute and a recurrent acute myocardial infarction (MI). The discoverers of this possibility recognized that inhibition of fibrinolysis present before an index MI could contribute to the development of a state in which the balance between thrombosis and fibrinolysis was shifted toward thrombosis. Such an imbalance was implicated as a determinant of increased risk of infarction. Because thrombosis begets thrombosis (with thrombin and other constituents within thrombi being powerful agonists of platelet activation and the coagulation cascade), increased PAI-1 in blood might facilitate persistence of microthrombi and a prothrombotic state.

Persistence of fibrin can potentiate fibrosis and atherogenesis as well as arterial thrombosis. It can augment neointimal formation in response to oxidative injury. These conclusions have been drawn from results of studies in which augmentation of prevailing concentrations of PAI-1 was induced in experimental animals, with consequent augmentation of persistence of deposition of fibrin.

Although increased PAI-1 in blood has not been established to be associated with increased coronary events in epidemiologic studies, it has been implicated in prospective and case-control studies and other types of clinical investigations. In the Physicians' Health Study, it appeared that increased PAI-1 activity, as judged from concentrations of t-PA antigen, was associated with increased coronary events. However, the association might have been dependent on the association of increased PAI-1 with other phenomena, such as insulin resistance.

The Evolution of Vulnerable Coronary Atheroma
Atherogenesis entails many processes that have been reviewed extensively elsewhere. A common atherogenic phenotype has been identified in transgenic mice that includes not only increased PAI-1 but also osteopontin and decreased active (as opposed to active plus latent) transforming growth factor-\(\beta\). In 7 different mouse lines, increased PAI-1 was present “wherever lesions developed.”

Until relatively recently, the evolution of coronary artery disease was thought to be tantamount to progressive obstruction by plaques, largely attributable to the migration and proliferation of VSMCs. Such lesions were thought to account not only for stable angina pectoris but also precipitation of acute coronary syndromes. This paradigm was overturned by the seminal work of Falk and Davies et al, who found that acute coronary syndromes were associated with plaques vulnerable to rupture that differed markedly from plaques typically associated with stable angina, particularly by being lipid laden and remarkably acellular with respect to VSMCs, and being covered by thin rather than thick fibrous caps. Plaques rupture, often mediated by activation of MMPs in the shoulder regions of the plaques, and can precipitate intramural hemorrhage and thrombosis, potentially catastrophic luminal compression, and intraluminal obstruction, with consequent manifestations of acute coronary syndromes including unstable angina, MI, and sudden cardiac death.

The classic paradigm of evolution of slowly progressive and slowly obstructive atheroma richly populated by VSMCs would imply that increased PAI-1 in the tissues might be protective by inhibiting VSMC migration. In fact, VSMC migration is increased in PAI-1-deficient mice and leads to a cellular response characterized by robust VSMC accumulation in the neointima after electrically induced intraluminal injury. Increased PAI-1 might decrease cell migration into the neointima and hence, luminal obstruction, as well as diminish the propensity to plaque rupture by limiting activation of MMPs by otherwise-uninhibited PAs within the vessel wall.

By contrast, overexpression of PAI-1 within vessel walls has opposite implications in the context of the more recent paradigm as schematized in Figure 1. We have hypothesized that it inhibits VSMC migration into the neointima as a component of the atherosclerotic process, thereby predisposing the developing atheroma to be relatively devoid of VSMCs. Because VSMCs are known to produce collagen and elastin, contributing to thick (and potentially protective against rupture), fibrous caps, PAI-1 overproduction could therefore potentiate development of VSMC-poor plaques with thin fibrous caps, necrotic cores, and predominant inflammatory and phagocytic cells as opposed to predominant VSMCs, rendering the plaques prone to rupture. Persistence and propagation of microthrombi, known to contain clot-associated mitogens exacerbating “atherothrombosis,” would be favored by PAI-1 overproduction. In fact, mice expressing a stable form of PAI-1 in blood develop robust coronary arterial clots without typical antecedent atherosclerosis.

Assessment of Composition of Plaques in Experimental Animals
Elucidation of effects of PAI-1 on the evolution of plaques requires methods for characterization of the composition and dimensions of lesions as well as development of experimental animal preparations that simulate critical aspects of human atherogenesis, elusive goals. Genetically altered mice have been used extensively for such purposes and to evaluate the effects of diverse pharmacologic agents and interventions.

Assessment of the composition of lesions can be performed with a procedure based on 1 developed by Paigen and coworkers in 1987, designed originally for analysis of lesion size. We and others have modified the approach to focus on compositional analysis (Figure 2). Using a combination of fluorescence microscopy, bright-field histology, polarized-light microscopy, and computer-assisted image analysis, we have observed age- and sex-dependent compositional differences in lesions in apolipoprotein E (apoE)–deficient mice, including an increase in the colla-
gen content by 2.5-fold in 20-week-old female compared with male animals. Others have observed intraplaque hemorrhage and plaque rupture in apoE-knockout and other strains of transgenic mice.71–74

Figure 1. Diagrammatic representation of the hypothesized effects of increased PAI-1 in the vessel wall and in blood on the evolution of atheroma. VSMC migration is dependent on cell surface PA activity and might be diminished by increased PAI-1. Several MMPs are activated by plasmin generated by PAs cleaving plasminogen. Inhibition of their activation would potentiate accumulation of matrix. Increased PAI-1 in blood might exacerbate atherothrombosis by facilitating the persistence of microthrombi and the deleterious effects of clot-associated mitogens.

Figure 2. A and B, Demonstration of a computer-assisted image-analysis technique used to semiquantify the composition of atherosclerotic lesions. A, Section of aorta from a 20-week-old, male apoE-knockout mouse was stained with SYTOX green and imaged with a 10× objective lens and epifluorescence microscopy. Cell nuclei in this portion of the lesion are easily recognizable at this magnification. The image was cropped to isolate the lesion and thresholded, and an overlay color (yellow) was assigned to the SYTOX green-positive pixels falling within the threshold range (B). Published with permission from Wadsworth et al70 (Histochem Cell Biol. 2002;118:59–68).
Assessment of Composition of Plaques in Humans

Effects of PAI-1 expression on neointima formation have been explored after diverse interventions, in diverse sites, and with diverse experimental animal species and strains. Unfortunately, results have often appeared to be contradictory. Zhu and coworkers observed diminished mean intima-media ratios in PAI-1–knockout animals compared with PAI-1–overexpressing apoE-knockout mice subjected to common carotid arterial ferric chloride–induced injury. They attributed the change to diminished persistence of mural thrombi. Sjoland and coworkers found that PAI-1 expression had no effect on neointimalization in the aorta but that PAI-1 deficiency was protective against lesion formation in either PAI-1 overproducers or PAI-1–deficient mice that were fed a high-fat diet. They found that PAI-1 expression had no effect on neointimalization in the aorta but that PAI-1 deficiency was protective against lesion formation in the carotid arteries from the PAI-1–/apoE– double-knockout mice. Luttun and coworkers found that PAI-1 deficiency led to larger, more matrix-rich, advanced atherosclerotic plaques in apoE-deficient mice. Anatomic location did not appear to influence the results. Of note, increased numbers of macrophages without a change in neointimal VSMC content were seen in the PAI-1–deficient mice. With copper-induced carotid artery injury, lesion development was attenuated in PAI-1–deficient transgenic mice compared with wild-type mice.

The diversity of results from these studies might reflect differences in strains of mice studied, the types of injury induced, location of lesions characterized, variables measured, the extent of expression of transgenes provided, and the tissue and organ specificity of the transgene’s expression, as well as the sex and ages of the animals studied, among numerous other factors. However, these efforts have sharpened the means of characterization of composition of lesions and helped to focus on its importance in understanding the determinants of the evolution of vulnerable plaques.

Assessment of Composition of Plaques in Humans

In addition to applying methods analogous to those used with experimental animal preparations in characterization of human vessels in biopsies, surgically excised tissue, or material obtained at necropsy, several approaches are being developed to assess the composition of atheroma. Techniques developed to do so include intravascular ultrasound, optical coherence tomography, thermography, spectroscopy, magnetic resonance imaging (MRI), computed tomography (CT), nuclear immunoscintigraphy, and positron emission tomography.

Technological advances with 1 or more of these modalities should ultimately permit clinical identification of plaques that are vulnerable and assessment of the efficacy of prevention or amelioration of vulnerability with therapeutic interventions. Several of the techniques developed are invasive. Intravascular ultrasound can demonstrate plaque rupture in an acute setting. It can also characterize the thickness of caps and areas of a plaque’s necrotic core (Figure 3). Because of high axial resolution, optical coherence tomography might be more effective in defining the presence of a thin cap. Intravascular thermography takes advantage of the increased temperature associated with vulnerable plaques that might reflect active inflammation.

Near-infrared spectroscopy has been employed with specimens ex vivo to identify plaque vulnerability. It has a sensitivity of 90% for identification of a large lipid pool and a sensitivity of 77% to 84% for detection of the presence of a thin, fibrous cap and inflammatory cell infiltrate. Noninvasive techniques permit assessment of plaque vulnerability with less risk to patients. MRI has been used to monitor changes in composition of atherosclerotic plaques in animal preparations and in humans. Delineation of composition of coronary atherosclerotic plaques with respect to the presence of calcium or conversely, detection of “soft” lesions, has been accomplished with fast spiral, multigated CT. A good correlation between the presence of “soft” lesions, defined by CT, and plaques with a large lipid core, defined by intravascular ultrasound, has been reported.

Nuclear imaging of vulnerable plaques has been performed by tagging antibodies generated against oxidized LDL. Such tagged antibodies selectively immunostain human atherosclerotic lesions and have been used to delineate changes in the
content of oxidized lipid in murine atheroma. Recent studies have used positron emission tomography to characterize the thickness of intimal caps. Refinement of 1 or more of these techniques should facilitate both assessment of plaque composition and its response to treatment in relatively large numbers of patients.

**Associations Between Activity of the Fibrinolytic System in the Blood and the Proteo(fibrino)lytic System in Vessel Walls**

PAI-1 and other proteo(fibrino)lytic system constituents are expressed ubiquitously in diverse cell types under the control of diverse promoters. The extent to which an imbalance in fibrinolysis is accompanied by a corresponding imbalance in proteo(fibrinolysis) is difficult to ascertain because of the difficulty of directly assessing activity of the proteo(fibrinolytic) system in specific tissues in human subjects. However, pathophysiologic condition sheds considerable light on this question. Insulin resistance with or without frank type 2 diabetes has long been known to be associated with impaired fibrinolysis in blood. Both oxidized and glycated LDL (increased with diabetes) can induce PAI-1 synthesis in endothelial cells, as can lipoprotein(a) (Lp(a)). PAI-1 content in coronary atheroma from patients parallels Lp(a) content. Free fatty acids (FFAs) and triglycerides (increased with diabetes) can augment PAI-1 expression through apparently independent signaling pathways. Conversely, expression of PAI-1 by HepG2 and endothelial cells in culture is reduced by diverse lipid-lowering agents as well. Obesity (often associated with insulin resistance), insulin resistance per se, type 2 diabetes, and the elevated triglycerides and VLDL typical of type 2 diabetes are associated with impairment of fibrinolysis. The impairment is attributable to augmented concentrations of PAI-1 in blood. Even in response to transitory venous occlusion, a physiologic stimulus of elaboration of PAI-1 locally, augmentation of fibrinolysis is attenuated in people who are obese (and often insulin resistant) and whose who have type 2 diabetes (also typified by insulin resistance) as a result of the increased activity of PAI-1. Insulin resistance is associated with compensatory hyperinsulinemia in many obese people, in prediabetic subjects, and in the early phases of type 2 diabetes preceding pancreatic beta cell failure. Human HepG2 cells (an immortal hepatoma cell line) elaborate increased PAI-1 when exposed to insulin or pro-insulin in vitro. Both agonists increase PAI-1 expression in vivo as well. In experimental animals subjected to infusions of insulin or pro-insulin under euglycemic clamp conditions, increased PAI-1 expression is evident not only in blood but also in the aortic wall. In healthy human subjects in whom lipids and endogenous insulin are increased in blood in response to infusions of glucose and liposyn, PAI-1 concentrations in blood increase. In human subjects with type 2 diabetes, concentrations of PAI-1 in atheroma are increased compared with those in age- and sex-matched patients with comparably obstructive coronary atheroma. Furthermore, PAI-1 expression in internal mammary artery segments used for coronary artery bypass grafting is increased as well, even though the macroscopic changes indicative of atherosclerosis are absent. Thus, increased vessel wall PAI-1 expression appears to precede formation of grossly evident atheroma under conditions of insulin resistance. Taken together, these observations indicate that at least in 1 common condition, namely, a state of insulin resistance, parallel shifts in the balance of fibrinolysis and thrombosis favoring thrombosis in blood and inhibition of proteo(fibrinolysis) in vessel walls are evident.

The impact of insulin on PAI-1 expression appears to be mediated by stabilization of PAI-1 mRNA. Results in studies of diverse cell types, HepG2 cells and vascular wall cellular elements, intact vessels in vivo, and vessels in patients with insulin resistance are consistent with agonistic effects of insulin resistance, hyperinsulinemia, and hyperpro-insulinemia. Results in epidemiologic studies are consistent with this association as well. Hyperglycemia itself, typical of impaired glucose tolerance or type 2 diabetes, and insulin resistance per se might increase endothelial cell expression of PAI-1. Thus, the combination of hyperinsulinemia, hyperglycemia, increased FFAs, and increased triglycerides typical of type 2 diabetes and insulin resistance, even without diabetes, promotes increased expression of PAI-1.

**The Impact of Increased PAI-1 Expression in Vessel Walls on Atherosclerosis**

Type 2 diabetes is clearly associated with an increased incidence of acute coronary syndromes and increased PAI-1 in blood and vessel walls. As Glagov et al hypothesized, acute coronary syndromes are often associated with abluminal atherosclerosis without profound obstruction but typified by plaques vulnerable to rupture. Coronary atherosclerosis in association with the insulin resistance typical of type 2 diabetes is characterized by a paucity of VSMCs, with augmentation of macrophage infiltration as well as an increased lipid content in lesions. Thus, type 2 diabetes is associated with coronary atherosclerosis that is not only accelerated but also characterized by plaques vulnerable to rupture. The paucity of VSMC content appears to reflect, at least in part, the concomitant overexpression of PAI-1. This interpretation is supported by analysis of coronary atheroma from patients without diabetes or insulin resistance who have had acute coronary syndromes. In such atheroma, the content of macrophages parallels the concentration of PAI-1, and the plaques are remarkably devoid of VSMCs. It is supported also by the increased incidence of sudden cardiac death associated with insulin resistance in nondiabetic subjects in view of the well-established increase in PAI-1 expression seen with insulin resistance. Much confusion regarding the impact of PAI-1 on vasculopathy has resulted from the conflation of results of studies of restenosis with those of studies on the evolution of atherosclerosis. It is known that the increased VSMC migration in response to vessel wall injury depends in part on cell surface u-PA activity. Thus, increased PAI-1 attenuates the VSMC migratory response to injury. Restenosis and the vascular response to injury appear to reflect predominantly increased proliferation of VSMCs. By contrast, mechanisms
involved in the evolution of vulnerable as opposed to less-vulnerable atherosclerotic plaques appear to differ. Development of vulnerable atheroma associated with decreased numbers of VSMCs in the cap regions of vulnerable atheroma appears to reflect decreased migration (as opposed to proliferation) of VSMCs into the neointima and possibly increased apoptosis.19,74,120

PAI-1 expression is increased in atherosclerotic lesions.109,121 It might or might not be increased in lesions, accounting for restenosis. However, when oxidative vascular injury is the insult precipitating vascular injury, increased expression of PAI-1 appears to occur and augment neointimal formation, perhaps by providing a fibrin scaffold for fibrous tissue deposition and VSMC proliferation.19 This observation is consistent with the possibility that increased PAI-1 in patients with type 2 diabetes subjected to percutaneous coronary interventions, another form of vessel wall injury, might contribute to augmented restenosis.122,123 Because proliferation of diverse cell types rather than increased migration might be pivotal in restenosis, limitation of migration of VSMCs by PAI-1, implicated in the evolution of vulnerable atheroma, would not necessarily be expected to attenuate the restenotic process. This interpretation is consistent with the observation that “collagen-rich sclerotic content is increased in restenotic lesions from patients with diabetes.”107 This suggests that an accelerated fibrotic process consistent with limited migration of VSMCs is typical of the evolution of restenotic lesions after angioplasty in patients with diabetes. Neointimal tissue proliferation is accentuated in the presence of insulin resistance and coronary stenting.124 However, the impact of PAI-1 on the evolution of vulnerable plaques and on restenosis is not necessarily the same.

PAI-1 and Neointimalization

Much debate surrounds the potential impact of PAI-1 on neointimal formation and atherogenesis. Under conditions in which thrombosis is the major determinant of atherothrombotic lesion formation, decreased PAI-1 activity promotes thrombosis and exacerbates development of lesions.125 The impact of PAI-1 on vascular remodeling might depend on the extent to which PAI-1 is associated with vitronectin and the extent to which the insult generating a specific type of lesion is mediated by thrombosis.126–128

As judged from the clinical characteristics of coronary artery disease in patients with insulin resistance, including those with type 2 diabetes, potentiation of evolution of vulnerable plaques might be the dominant feature. At least 2 studies have shown that attenuation of PAI-1 expression pharmacologically or in transgenic PAI-1–knockout mice also attenuates perivascular fibrosis, presumably through a reduction in the generation and persistence of the fibrin scaffolding thought to augment it.129,130 As so well stated in a recent editorial, “the composite effect of PAI-1 in VSMC migration likely depends on multiple factors, including cellular phenotype, the chemotactic stimulus, and the composition of matrix in which they reside and subsequently migrate . . . PAI-1 might indeed ‘play a molecular’ Jekyll and Hyde when it comes to SMC migration,”131 depending on the stimuli underlying lesion formation; the milieu in which it occurs; the functional properties, conformation, and association of PAI-1 with vitronectin; the extent of concomitant fibrillar and fragmented collagen deposition;131,132; and the phenotypes of diverse cells participating in the evolution of lesion formation.

Attempts to elucidate the impact of modulation of PAI-1 expression in vessel walls on the evolution of atheroma have involved studies in apoE-deficient mice. In animals rendered deficient in PAI-1 as well, intensified dissolution of fibrin clots in areas of turbulent flow was seen, without a change in the extent of atherosclerosis in the aortic arch.60 However, increased PAI-1 expression within the aortic wall was not demonstrated in the PAI-1–overproducing animals studied.64 By contrast, in preliminary results with transgenic mice that we have generated that overproduce PAI-1 in the vessel wall and are apoE-deficient, vessel wall PAI-1 expression was increased and plaque VSMC content was diminished.133

Factors in Addition to Insulin Resistance That Can Affect PAI-1 Expression

As judged from concentrations of PAI-1 in blood, amelioration of insulin resistance and compensatory hyperinsulinemia diminishes PAI-1 expression.119,134–138 Derangements in lipid metabolism might contribute to the association between increased PAI-1 in blood and insulin resistance, in view of the well-established, agonistic effects of FFAs, triglycerides, and VLDL on PAI-1 expression.139–143 Pharmacologic modification of factors other than lipids in insulin resistance that increase PAI-1 expression might decrease PAI-1 expression as well. Activation of the renin-angiotensin system (RAS) increases PAI-1 expression. Thus, exposure to angiotensin II induces PAI-1 expression in vascular endothelial cells. The induction is precluded by angiotensin type-1 receptor blockade.144 Conversely, inhibition of generation of angiotensin in vivo attenuates inhibition of fibrinolysis in genetically obese mice.129

In cultured endothelium, stimulation of the angiotensin-4 receptor by a metabolite of angiotensin II augments PAI-1 expression as well.145 However, in vivo in human subjects, the receptor apparently predominantly responsible for the relationship between activation of the RAS and PAI-1 expression is the angiotensin type-1 receptor.

Administration of angiotensin-converting enzyme (ACE) inhibitors to patients leads to reductions in PAI-1 concentrations in blood and a decreased incidence of coronary events.146 Polymorphisms of the PAI-1 gene might influence the response of PAI-1 expression to stimulation with angiotensin and a reduction of expression by ACE inhibition,147 as well as potentiation of coronary risk. Polymorphisms of the ACE gene (insertion/deletion polymorphisms) might influence the interaction between the fibrinolytic and RAS systems with respect to their impact on the fibrinolytic system.148 Thus, the 4G allele of the 4G/5G PAI-1 promoter polymorphism appears to augment risk.149,150 However, the clinical impact of this and other polymorphisms has not yet been determined.

In addition to angiotensin, another component of the RAS might influence PAI-1 expression in tissues, particularly the kidney, namely, aldosterone. In rodents subjected to radiation...
to induce renal injury, an aldosterone antagonist diminished PAI-1 expression that was otherwise increased by 8-fold compared with expression in nonirradiated controls.\textsuperscript{151} Other pharmacologic agents besides inhibitors of the RAS used in the treatment of patients with cardiovascular disease have been shown unexpectedly to lower PAI-1 expression, as judged from concentrations in blood. An example is pravastatin.\textsuperscript{152}

Recently, much consideration has been given to relationships between inflammatory processes and accelerated atherosclerosis, particularly in association with diabetes and insulin resistance. Increased PAI-1 expression is associated with inflammation, in part, because of stimulation of PAI-1 expression by cytokines such as interleukins -1 and -6,\textsuperscript{153} tumor necrosis factor, and oxygen-centered free radicals\textsuperscript{154} and in part because markers of inflammation such as C-reactive protein can themselves increase PAI-1 expression.\textsuperscript{155}

Pharmacologic modulation of expression of growth factors\textsuperscript{99,156,157} or of nitric oxide\textsuperscript{158–160} might affect PAI-1 expression as well. Nitric oxide suppresses expression of PAI-1 in VSMCs in vitro.\textsuperscript{158} However, it does not alter expression of PAI-1 by endothelial cells.\textsuperscript{159} Infusion of L-N\textsuperscript{6}-monomethyl arginine, an agent that decreases elaboration of nitric oxide in vivo, does not change the concentration of PAI-1 in blood of healthy human subjects.\textsuperscript{160}

**Clinical Implications**

To the extent that increased vessel wall PAI-1 is demonstrated ultimately to be a causative factor in the evolution of plaques vulnerable to rupture, diminution of PAI-1 overexpression might be clinically beneficial. It will, of course, not be appropriate to modify vascular PAI-1 expression specifically, eg, with antagonists of PAI-1 being developed, in the absence of proof that overexpression in the vessel wall is indeed deleterious. There exists the theoretical risk that antagonism of PAI-1 activity might potentiate cell migration, facilitating metastasis of occult tumor cells as a result of enhanced migratory capacity. However, just as has been the case in recognition of the linkage between hypercholesterolemia and coronary artery disease, long-term studies might ultimately demonstrate benefit from such an approach. Hypercholesterolemia was implicated as a risk factor for coronary artery disease first in patients with essential hypercholesterolemia (now known to be a consequence of LDL receptor deficiency). Subsequently, epidemiologic data demonstrated striking associations between increased LDL cholesterol and the risk of coronary events. Initially, it was feared that pharmacologic modulation of cholesterol synthesis might be deleterious in view of the essentiality and ubiquitous nature of sterol metabolism in numerous, fundamental biochemical processes. Ultimately, however, benefits of ameliorating hypercholesterolemia became unequivocal, and the public health impact of this favorable modulation became immense. Similarly, early studies of hypertension treated pharmacologically demonstrated only the capacity of the administered agents to reduce blood pressure. Acceptance of pharmacologic treatment required demonstration of a mortality benefit that could only be inferred from early mechanistic and clinical trial assessments.

Perhaps fortuitously, many pharmacologic agents and lifestyle modifications utilized in patients at risk for coronary artery disease might lower vessel wall PAI-1 expression, as judged from effects in cells in culture, experimental animals, and patients. These include administration of lipid-lowering agents, in view of the potentiation of PAI-1 expression by fatty acids and triglycerides, inhibition of the RAS with angiotensin receptor blockers or ACE inhibitors, augmentation of insulin sensitivity with thiazolidinediones, diminution of compensatory hyperinsulinemia in patients with syndromes of insulin resistance (including type 2 diabetes) by improvement of glycemic control with metformin and sulfonlureas, enhancement of insulin sensitivity with exercise and caloric restriction, and the use of statins. The development of vessel wall–specific antagonists of PAI-1, being undertaken by several pharmaceutical corporations, might provide an even more powerful approach for modulating PAI-1 activity within the vessel wall and attenuating any deleterious effects proved to be a consequence of it.

The possibility that attenuation of overexpression of PAI-1 in vessel walls might be beneficial to patients at risk for coronary events is suggested by the observations that have been acquired in mechanistic and observational studies. What remains to be seen is whether its implementation will ultimately impact favorably on the evolution of coronary atherosclerosis in a fashion that confers genuine protection against the evolution of vulnerable plaques and their consequences.

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