Localization and Production of Plasminogen Activator Inhibitor–1 in Human Healthy and Atherosclerotic Arteries

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High plasma levels of plasminogen activator inhibitor type-1 (PAI-1), the principal inhibitor of the fibrinolytic system, have been associated with thrombotic and arterial disease. To study PAI-1 expression in healthy and atherosclerotic human arteries, a detailed analysis was made by light and electron microscopy immunocytochemistry and by in situ hybridization. In healthy arteries PAI-1 was found both at the level of endothelial cells and of smooth muscle cells (SMCs) of the arterial media. In early atherosclerotic lesions PAI-1 was also detected in intimal SMCs and in extracellular areas in association with vitronectin. Immunogold analysis by electron microscopy revealed PAI-1 in vesicular structures in endothelial cells and in SMCs with normal or foam cell characteristics. In advanced atheromatous plaques, PAI-1 mRNA expression in SMCs within the fibrous cap was increased compared with SMCs located in the adjacent media or in normal arterial tissue. PAI-1 mRNA was also detected in macrophages located at the periphery of the necrotic core. The increased synthesis of PAI-1 by cellular components of the atherosclerotic plaque and the extracellular accumulation of PAI-1 may contribute to the thrombotic complications associated with plaque rupture and possibly play a role in the accumulation of extracellular matrix deposits. (Arteriosclerosis and Thrombosis 1993;13:1090-1100)

Key Words • plasminogen activator inhibitor type-1 • atherosclerosis • vitronectin • arterial wall • endothelial cells • smooth muscle cells

The endothelium covers the luminal surface of the entire vascular system and normally provides a thromboresistant interface between flowing blood and the thrombogenic subendothelial tissues. Endothelial cells (ECs) express many anti-thrombotic factors, such as glycosaminoglycans, thrombomodulin, prostacyclin, and tissue-type plasminogen activator (t-PA), but may also express thrombogenic factors such as tissue factor and increased amounts of an inhibitor of the fibrinolytic system, plasminogen activator inhibitor type-1 (PAI-1). The fibrinolytic system is an important protective mechanism against thrombosis. The central reaction of this system is the activation of plasminogen to plasmin through the action of PAs. Fibrinolytic activity within and at the surface of the vessel wall results from the local concentration of active (free) t-PA, which is determined, in part, by the local concentrations of its primary inhibitor, PAI-1. Impaired fibrinolysis due to overexpression of PAI-1 from endothelial cells may contribute to occlusive thrombus formation. Several clinicopathological conditions have been associated with high plasma levels of PAI-1 and thrombotic complications. A causal relation between high PAI-1 levels and the occurrence of thrombosis may be inferred from the observations that mice transgenic for the PAI-1 gene suffer from spontaneous thrombosis of tail and hind leg veins and that circulating anti-PAI-1 antibodies enhance endogenous thrombolysis and partially prevent thrombus extension in rabbits.

Several factors associated with inflammatory and atherosclerotic processes increase the expression of PAI-1 by cultured ECs. Of particular importance in the context of vascular disease are the inflammatory mediators tumor necrosis factor–α, interleukin-1, and transforming growth factor–β, which are released from activated platelets and macrophages. Furthermore, lipoprotein[a], a well-established risk factor for coronary artery disease, increases the thrombogenic phenotype of ECs by increasing PAI-1 mRNA. This pattern of expression of PAI-1 by ECs in culture suggests that PAI-1 may participate in atherosclerotic processes.

The potential importance of PAI-1 in atherogenesis has led us to study the expression of PAI-1 in normal and atherosclerotic tissue by immunocytochemistry at the level of light and electron microscopy and by in situ hybridization. We observed that (1) PAI-1 is produced both by ECs and smooth muscle cells (SMCs) and is present in intracellular vesicular structures; (2) in advanced atherosclerotic lesions PAI-1 is also expressed by macrophages; and (3) in extracellular matrix deposits PAI-1 colocalizes with vitronectin.
Methods

Preparation of Vascular Tissue

For immunohistochemistry and in situ hybridization, human tissue specimens obtained during autopsy, performed 8 to 12 hours after death, were fixed in 4% formaldehyde (E. Merck, Darmstadt, FRG), embedded in paraaffin (Merck), sectioned into 7- to 10-μm slices, mounted on microscope slides, and stored at room temperature until further use. Samples obtained during cardiovascular surgery were immediately placed in 4% formaldehyde and processed as described above or embedded within 1 hour of removal in optimal cutting temperature compound (OCT compound, Miles Scientific, Elkhart, Ind), frozen in cold methylbutane (Merck), and stored at −70°C. Sections for immunohistochemical purposes were cut at 7 μm, fixed with cold acetone, air dried, and used within 24 hours. For in situ hybridization, sections were cryosectioned at 7 μm, placed on poly-L-lysine-coated (Fluka, Buchs, Switzerland) slides, air dried, fixed in 4% paraformaldehyde (Sigma, St Louis, Mo) in phosphate-buffered saline (PBS), dehydrated, and stored at −20°C. Arterial segments were obtained from 17 patients, 12 during surgery and 5 after autopsy.

For electron microscopy immunogold labeling. Vessel segments obtained at cardiovascular surgery were fixed by immersion in 4% paraformaldehyde, plus 0.1% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.4, for 30 minutes and diced into 1-mm cubes. The fixation was continued for up to 2 hours at room temperature. Postfixation in osmium tetroxide was not employed because preliminary experiments showed that this treatment leads to a complete loss of antigenicity. After fixation, the samples were dehydrated in 50% and 70% ethanol and transferred first to 1:1 (vol/vol) then 2:1 (vol/vol) LR White (Polyscience Inc, Warrington, Pa) resin/70% ethanol for 60 minutes. Blocks were placed in fresh resin and infiltrated overnight at 4°C. The samples were then embedded in gelatin capsules containing fresh LR White resin and placed in a vacuum oven at 40°C to 50°C for 24 hours. Blocks were trimmed, and semithin sections were cut with a glass knife and stained with toluidine blue. Ultrathin sections (70 to 110 nm) were cut using a diamond knife and picked up on uncoated or Formvar-coated 200-mesh nickel grids.

Antibodies

The following polyclonal and monoclonal (MAb) antibodies were used to detect the proteins and cell types listed. For PAI-1, MBs 380 and 3785 (subclass IgG1, American Diagnostica Inc, Greenwich, Conn), MAB 3 PAI (a gift from Prof Binder, TechnoClone Ltd, Vienna, Austria), and MAB 7F5 (a gift from Dr P. DeClerck, Leuven, Belgium) were all used at a final concentration of 20 μg/mL. The polyclonal rabbit anti-human PAI-1 IgG 395R (American Diagnostica), used for double immunogold labeling studies, was used at an IgG concentration of 10 μg/mL. For α-actin, MAB (subclass IgG2a, catalogue No. 1148818, Boehringer Mannheim, Mannheim, FRG) was used at a final concentration of 1 μg/mL as an SMC marker. For von Willebrand factor, the polyclonal rabbit anti-human von Willebrand factor (catalogue No. A 082, Dako, Glostrup, Denmark) was used at a final IgG concentration of 25 μg/mL as an EC marker. For vitronectin, MAB (subclass IgG1, catalogue No. 1087738, Boehringer Mannheim) was used at a final concentration of 15 μg/mL. For macrophages, MABs 25F9 and 27E10 (subclass IgG1, BMA, Augst, Switzerland) were used as macrophage-specific markers at a dilution of 1:10 or 1:100. Goat anti-mouse IgG coupled with 15-nm colloidal gold particles (GAM-10 or GAM-15, respectively) and goat anti-rabbit IgG coupled with 10-nm gold particles (GARb-10) were obtained from BioCell Research Laboratories (Cardiff, UK) for use as immunogold probes.

To establish the specificity of immunolabeling using the anti–PAI-1 MAbs, three different types of negative controls were used: (1) 20 μg/mL of anti–PAI-1 MAb preincubated for 1 hour at 37°C with 80 μg/mL PAI-1 (American Diagnostica); (2) an isotype-matched MAB not reacting with human tissue or serum proteins (MsIgG1, catalogue No. 6602872, Coulter clone, Hialeah, Fla), used at a final concentration of 10 μg/mL; and (3) nonimmune mouse serum or nonimmune mouse ascitic fluid.

Immunohistochemistry

For immunohistochemical staining either the alkaline phosphatase–anti-alkaline phosphatase double-bridge technique11 (Dako APAAP kit) or the avidin-biotinylated peroxidase complex (Vectastain ABC kit, Vector Laboratories, Burlingame, Calif) method was used. Before staining, paraaffin was removed from tissue sections by heating the slides at 56°C for 30 minutes followed by progressive rehydration, whereas the cryosections were slowly thawed and fixed in precooled acetone for 10 minutes at 4°C. All further steps were performed according to the manufacturer’s instructions. Incubation with the first antibody was done for 45 to 60 minutes. A 3,3′-diaminobenzidine solution (DAB; Sigma) was used as the peroxidase substrate. After immunoreaction, the slides were washed three times in distilled water and counterstained for 10 minutes with hematoxylin (Merck). In some experiments the DAB reaction product was silver-enhanced using the IntenSE kit (Amer sham Int, Amersham, UK).

Immunogold Labeling

After the aldehyde quenching (0.02 mol/L glycine in PBS for 5 minutes) and blocking (1% bovine serum albumin, 0.1% fish gelatin, and 5% nonimmune goat/ rabbit serum in PBS for 5 minutes) steps, the “on grid” immunolabelings were done by incubation of the sections with the primary anti–PAI-1 antibody (7F5 at 10 μg/mL) overnight at 4°C. The immunogold probe GAM-10 was used at a dilution of 1:50. Nonimmune mouse serum or control mouse ascitic fluid, clone NS-1 (Sigma), was used in place of the primary antibody as a control. For simultaneous localization of PAI-1 and vitronectin a double-labeling procedure12 was performed by incubation of the sections with a mixture of rabbit anti–PAI-1 and anti-vitronectin MAbs, and subsequent detection was performed with a mixture of GAM-15 and GARb-10.

PAI-1 cRNA Probe Preparation for In Situ Hybridization

A 1100-bp fragment derived from human PAI-1 cDNA13 was subcloned in the Bluescript M13+ vector...
Fig 1. Photomicrographs showing immunohistochemical localization of plasminogen activator inhibitor type-1 (PAI-1) in human normal arteries. Normal arterial samples that were fixed in formaldehyde and embedded in paraffin were immunostained using (panels a through c) avidin-biotinylated peroxidase complex (brown; normal aorta) and (panels d through f) alkaline phosphatase-anti-alkaline phosphatase (bright red; normal carotid artery). Panels a and d: An isotype-matched monoclonal antibody unreactive with human tissue or serum proteins (negative control); panels b and e: immunostaining for PAI-1 using a murine monoclonal antibody; panels c and f: immunostaining for α-actin to the internal elastic lamina. Note the positive staining for PAI-1 and α-actin in the vasa vasorum (arrows). I, tunica intima; M, tunica media; A, adventitia. Magnification ×110 (panels a through c), ×69 (panels d through f).
FIG 2. Photomicrographs showing immunohistochemical localization of plasminogen activator inhibitor type-1 (PAI-1) in human atherosclerotic arteries. Arteries with intimal thickening, obtained after autopsy, were analyzed using (panels a through c) avidin-biotinylated peroxidase complex (popliteal artery) or (panels d through f) alkaline phosphatase-anti-alkaline phosphatase (carotid artery). Panels a and d: An isotype-matched monoclonal antibody unreactive with human tissue or serum proteins (negative control); panels b and e: immunostaining for PAI-1; panels c and f: immunostaining for α-actin. The arrowheads point to the internal elastic lamina. I, tunica intima; M, tunica media. Bars=100 μm. Magnification ×110 (panels a through c), ×168 (panels d through f).
FIG 3. (Facing page) Photomicrographs showing immunohistochemical localization of plasminogen activator inhibitor type-1 (PAI-1) in human advanced atheromatous plaques. Human arteries presenting advanced atheromatous plaques were analyzed using the alkaline phosphatase--anti-alkaline phosphatase technique. Panels a through d: Popliteal artery obtained after autopsy; panels e through h: circumflex coronary artery obtained during a bypass operation. Panels a and e: an isotype-matched monoclonal antibody unreactive with human tissue or serum proteins (negative control); panels b and f: immunostaining for \( \alpha \)-actin; panels c and g: immunostaining for PAI-1; panels d and h: immunostaining for vitronectin. Note the presence of PAI-1 in smooth muscle cell--containing areas and in acellular areas in association with vitronectin. The arrowheads point to the internal elastic lamina. I, tunica intima; M, tunica media. Bars=100 \( \mu \)m. Magnification \( \times 135 \) (panels a through d), \( \times 54 \) (panels e through h).

In Situ Hybridization

The procedure was performed according to Holland.\(^{10} \) The slides were brought to room temperature at least 2 hours before use. They were acid treated in 0.2 mol/L HCl for 20 minutes, washed, and fixed in 4% (v/v) paraformaldehyde for 10 minutes. This was followed by two wash steps in PBS and 10 minutes' incubation in 0.25% acetic anhydride in 0.1 mol/L tris(hydroxymethyl)aminomethane (Tris)–HCl, pH 8.0. After three wash steps in PBS, slides were dehydrated and air dried. The cRNA probe (in 50% formamide [GIBCO/BRL, AG, Basel, Switzerland], 0.3 mol/L NaCl, Denhardt's solution, 0.02 mol/L Tris-HCl, pH 8.0, 5 mmol/L EDTA, 5% dextran sulfate, 50 mmol/L dithiothreitol [DTT; Sigma], and 500 \( \mu \)g/mL yeast total RNA [Boehringer Mannheim]) was used at \( 10^6 \) cpm per slide. Sections were covered with siliconized coverslips and hybridized at 50°C overnight (12 hours) in a chamber humidified with a mixture similar to the hybridization solution except for probe, dextran sulfate, DTT, and yeast total RNA. To remove coverslips, sections were immersed in 4x standard saline–sodium citrate buffer (SSC) (1 × SSC is 0.15 mol/L NaCl and 0.015 mol/L trisodium citrate, pH 7.0) at 37°C and then washed in 4x SSC at 37°C. After RNase A (Boehringer Mannheim) treatment (20 \( \mu \)g/mL) for 30 minutes at 37°C, the slides were washed in 0.5 mol/L NaCl, 10 mmol/L Tris-HCl, pH 7.5, and 1 mmol/L EDTA followed by a 1-hour incubation at 50°C in 1x SSC and two incubations for 1 hour each at 50°C in 0.1x SSC. All solutions of the posthybridization wash steps contained 2 mmol/L DTT. The graded alcohol series for the final dehydration contained 300 mmol/L ammonium acetate. The sections were air dried and covered with an autoradiographic emulsion (NTB-2, Kodak) following the manufacturer's instructions, stored in black air-tight boxes at 4°C, and developed after 10 to 20 days.

Results

Immunohistochemical Localization of PAI-1

PAI-1 in healthy human arteries. The presence of PAI-1 and \( \alpha \)-actin in normal human arterial tissue was investigated by the alkaline phosphatase--anti-alkaline phosphatase technique in successive tissue sections using specific antibodies. Fig 1 shows the immunoreactions in an aorta (a through c) and in a carotid artery (d through f). As illustrated in Fig 1b, the ECs stained positive for PAI-1. Serial sections were also reacted with anti–von Willebrand factor antibodies to ascertain the identity of the ECs (not shown). Strikingly, a strong PAI-1–specific staining was observed in the tunica media of the normal arteries (Fig 1b and 1e), whereas no immunostaining was observed with the isotype-matched MAb used as a negative control (Fig 1a and 1d) nor with antibodies preincubated with pure PAI-1 (not shown).

The distribution of PAI-1 within the tunica media was similar to that of \( \alpha \)-actin, an SMC marker (Fig 1c and 1f). The vasa vasorum (arteries and veins) within the adventitia also showed positive PAI-1 and \( \alpha \)-actin immunostaining (Fig 1e and 1f).

PAI-1 in arteries with early atherosclerotic lesions. Successive sections of human popliteal artery and carotid arteries presenting early atherosclerotic lesions were analyzed. The popliteal artery shown in Fig 2 (a through c) and the carotid artery shown in Fig 2 (d through f) exhibit a thickening of the tunica intima characteristic of an atherosclerotic process. These arteries are representative of the other arteries analyzed. No staining was observed when using the control antibody (Fig 2a and 2d). As was observed in normal arteries, strong PAI-1 (Fig 2b and 2e) as well as \( \alpha \)-actin (Fig 2c and 2f) specific immunostainings were detected within the tunica media. \( \alpha \)-Actin was also found within the tunica intima of arteries presenting early atherosclerotic lesions, which indicated that a significant proportion of the cells, including "foam cells" present in the thickened intima, were SMCs. The strongly positive PAI-1 staining in the intima was associated with some foam cells and with spindlelike cells harboring a centrally located, elongated nucleus characteristic of SMCs. Moreover, several intimal cells were simultaneously positive for \( \alpha \)-actin and for PAI-1 in the serial sections studied.

PAI-1 in advanced atheromatous plaques. Advanced atheromatous plaques were analyzed in detail. Fig 3 (a through d) shows a fibrofatty lesion with foam cell–rich regions and extracellular matrix accumulation in a popliteal artery; Fig 3 (e through h) exhibits a characteristic fibrous plaque in a circumflex coronary artery. The necrotic core contains cholesterol clefts and has disrupted the internal elastic lamina. In the popliteal artery, \( \alpha \)-actin (Fig 3b) and PAI-1 (Fig 3c) were present in the media and in the superficial region of the lesion, but PAI-1 was also detected in regions surrounding the foam cell–rich areas and adjacent to the internal elastic lamina, where it appeared to be localized in extracellular matrix structures not associated with any cells.

To determine whether PAI-1 in the extracellular matrix is associated with vitronectin, an extracellular matrix and plasma protein that binds and stabilizes...
Arteries obtained from cardiovascular surgery were immediately fixed in aldehydes, embedded in LR White resin, sectioned, and immunostained "on grid" for PAI-1 (panels b and c) or double labeled for PAI-1 and vitronectin (panels a and d). Panel a: Simultaneous localization of PAI-1 and vitronectin at the endothelial cell (EC) level in a healthy mammary artery. PAI-1 (revealed by 10-nm gold particles, smaller arrows) was distributed mostly in clusters, probably in vesicular structures and, in lower amounts, in the subendothelial space. Vitronectin (15-nm gold particles, bigger arrows) was located mainly close to the EC surface or intraacellularly in some vesicles. Occasionally an association of 10- and 15-nm gold particles was observed (arrowheads). Panels b and c: The spindle-shaped cells, with ultrastructural characteristics of lipid-laden smooth muscle cells (SMCs), showed large amounts of intracellular gold-labeled PAI-1, disposed in small aggregates in an atherosclerotic aorta. In lipid-laden SMCs ("foam cells," FC) the antigen was located at the periphery of the lipid droplets (ld). Panel d: Double immunogold staining of an extracellular area of an advanced atherosclerotic plaque for PAI-1 (10-nm particles, smaller arrows) and vitronectin (15-nm particles, bigger arrows). Both antigens localized in close association with elastic laminae (el) and collagen bundles (cb). L, lumen; N, nucleus. Bars=200 nm. Magnification ×43,000 (panel a), ×36,000 (panel b), ×41,000 (panel c), ×35,000 (panel d).  

PAI-1,15,16 we also performed an immunohistochemical analysis for vitronectin. In the popliteal artery, vitronectin (Fig 3d) as well as PAI-1 (Fig 3c) were found in the region adjacent to the internal elastic lamina and surrounding the foam cell–rich areas. In the coronary artery, PAI-1 (Fig 3g) colocalized with α-actin (Fig 3f) in the tunica media and in some regions of the plaque where SMCs were present. In addition, strong PAI-1 and vitronectin staining was observed in acellular regions underneath foam cell–rich areas (Fig 3g and 3h).

Electron Microscopy Studies

To identify the subcellular structures associated with PAI-1, we performed electron microscopic analysis after immunogold labeling using specific anti-PAI-1 antibodies or double labeling for PAI-1 and vitronectin. PAI-1 labeling–gold particles in a healthy mammary artery (Fig 4a) were detected mainly in clusters in vesicle-like structures. Some gold particles were also detected in the subendothelial space. Vitronectin was found mainly in association with the surface and in the basal lamina of ECs; rarely, it was intracellularly located in some vesicles (Fig 4a). Large amounts of PAI-1 were detected in a granular distribution in SMCs in an aorta presenting an atherosclerotic lesion (Fig 4b) and in foam cells with ultrastructural characteristics of SMCs (Fig 4c), particularly at the periphery of the lipid droplets.

Double immunogold labeling of PAI-1 and vitronectin showed that in atherosclerotic arteries both antigens colocalized in extracellular areas in close association with elastic laminae and collagen bundles (Fig 4d). These results suggest that in advanced atherosclerotic lesions PAI-1 is present in the extracellular matrix associated with vitronectin.

Identification of PAI-1 Producer Cells

In situ hybridization was performed to identify the PAI-1 producer cells. These studies revealed a strong labeling of individual ECs and a diffuse pattern of PAI-1 expression in SMCs throughout the normal arterial media (Fig 5a) and in early atherosclerotic lesions (not shown). When the sense probe was used as a negative control (Fig 5b) the preparation was practically devoid of silver grains. Analysis of serial sections by in situ hybridization and immunohistochemistry using cell type–specific antibodies of a freshly obtained popliteal artery with an advanced lesion showed that the gene for PAI-1 is actively expressed both in SMCs (Fig 5e and 5f) and in macrophages (Fig 5g and 5h) situated in the fibrous cap of the necrotic core. The intensity of labeling of the SMCs in the fibrous cap (Fig 5e) was higher than that of SMCs in the media of the same arterial segment (not shown) and in the media of a normal artery (Fig 5a).

Discussion

In this article we present a detailed analysis of PAI-1 expression in normal and atherosclerotic arteries by immunohistochemistry and in situ hybridization. Both ECs and SMCs of normal arteries showed strong immunostaining for PAI-1 and were positive for PAI-1 mRNA as analyzed by in situ hybridization. We were able to show by electron microscopy that in both cell types PAI-1 is present in vesicular structures and also in the subendothelial space. These data as well as the recent findings of Schneiderman et al17 show that at least part of the ECs in human arteries express PAI-1, but they do not yet allow the determination of whether all arterial ECs produce PAI-1. Indeed, perfusion fixation is not possible for human tissue, and in tissue obtained at autopsy or during surgery the endothelium is often damaged, as revealed by electron microscopy. Immunohistochemical staining for von Willebrand factor was not a reliable marker of intact endothelium, since we found this protein also associated with damaged endothelium or denuded areas. In arterial tissues most of the PAI-1 appeared to colocalize with the SMC marker α-actin, suggesting that large amounts of PAI-1 are associated with SMCs in the arterial wall. In situ hybridization showed that SMCs of a healthy arterial wall indeed express PAI-1 mRNA. In contrast to our findings, Schneiderman et al17 were unable to detect PAI-1 mRNA in the media of a normal artery.

The analysis of atherosclerotic human arteries revealed the presence of PAI-1 within the plaque. Most of the PAI-1 in atherosclerotic lesions colocalized with α-actin. Examination by electron microscopy of immunogold–labeled PAI-1 showed that PAI-1 is intracellularly located within SMCs. Whether PAI-1 is stored or is on its secretion pathway could not be determined. The observation of a positive PAI-1 mRNA signal in SMCs of the thickened intima supports the hypothesis that these cells are responsible, at least in part, for the presence of high PAI-1 levels in the plaque.

In advanced atherosclerotic lesions, synthesis by SMCs of extracellular matrix components leads to accumulation of collagen, elastic fibers, and proteoglycans.
in the intima. Our data show that SMCs in these lesions also synthesize PAI-1. The increased expression of PAI-1 by SMCs in the fibrous cap of the necrotic core, as compared with SMCs in the adjacent media or in a normal media, is in accord with the recent observations of Schneiderman et al and may be the result of the local release of cytokines such as tumor necrosis factor-α, which is produced by the activated macro-
phages that are also present in these areas.\(^{19,20}\) Interestingly, some of the macrophages located in the necrotic core also exhibited a strong expression of PAI-1 mRNA, which suggests that these cells may contribute to PAI-1 accumulation in the atherosclerotic plaque. In vitro data suggest that human monocytes\(^ {21}\) are indeed able to produce PAI-1.

In advanced atheromatous plaques large quantities of PAI-1 were present in the extracellular matrix; these colocalized with vitronectin, a PAI-1 binding protein.\(^ {22}\) Previous reports demonstrated that vitronectin protects PAI-1 against inactivation\(^ {16}\) and maintains PAI-1 in the active form in matrices of ECs and lung tissue extracts.\(^ {15}\) The colocalization of PAI-1 and vitronectin in the extracellular matrix of advanced atherosclerotic plaques, as shown by electron microscopy, suggests that vitronectin may help to immobilize PAI-1 in an active form. Niculescu et al\(^ {23}\) have found vitronectin in atherosclerotic lesions in similar locations, in association with collagen, elastin, and cell debris, and have suggested possible connections with the activation of the complement system. Seiffert et al\(^ {24}\) showed by in situ hybridization that SMCs produce PAI-1, which may contribute to the increased levels of PAI-1 observed within the plaque. The most frequent and critical event that converts an asymptomatic atherosclerotic plaque into a symptomatic one is a superimposed thrombosis on fissured lesions.\(^ {27}\) Plaque rupture may result in the release of procoagulant substances, such as tissue factor,\(^ {28}\) thus favoring occlusive thrombi formation. While such thrombi are efficiently removed by a normal fibrinolytic system, the presence of excess PAI-1 in the vessel wall may promote the persistence of intravascular fibrin. Furthermore, plasmin cleaves many extracellular matrix proteins either directly or indirectly through the activation of metalloproteinases.\(^ {29}\) The presence of high levels of PAI-1 within the plaque may inhibit plasmin formation and therefore extracellular matrix breakdown. This may then well lead to the stabilization of the lesion and the excessive accumulation of extracellular matrix, favoring the formation of obstructive plaques.

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