Colocalization of Thrombin, PAI-1, and Vitronectin in the Atherosclerotic Vessel Wall

A Potential Regulatory Mechanism of Thrombin Activity by PAI-1/Vitronectin Complexes

A. Allart Stoop, Florea Lupu, Hans Pannekoek

Abstract—The serine protease thrombin is a mitogen for vascular smooth muscle cells. To that end, thrombin cleaves the surface-exposed, protease-activated receptor type 1 (PAR-1), resulting in signal transduction and ultimately, proliferation of these cells. Regulation of thrombin activity in the human atherosclerotic vessel wall has not been studied in great detail, conceivably because the traditional plasma thrombin inhibitor, anti–thrombin III, is not encountered at this location. By using immunofluorescence confocal microscopy, we demonstrate that the antigens of thrombin, plasminogen activator inhibitor 1 (PAI-1), and vitronectin (Vn) colocalize in human neointimal atherosclerotic arterial tissue. Furthermore, it is shown by in situ reverse zymography that these specimens harbor the active form of PAI-1, which is the only configuration of PAI-1 capable of complexing with Vn and inhibiting serine proteases, eg, thrombin.

Two different criteria were used to establish that neointimal atherosclerotic material contains active α-thrombin, namely, its ability to bind to the thrombin inhibitor hirudin and to convert the thrombin-specific chromogenic substrate S2238. The latter activity could be fully prevented by preincubation with the thrombin-specific inhibitor, phenyl-prolyl-arginyl-chloromethyl ketone. The thrombin concentration measured by conversion of the chromogenic substrate was 7 to 12 nmol/L in the vascular specimens studied. This concentration range suffices to activate the PAR-1 receptor on vascular smooth muscle cells and to cause neointimal proliferation. It is concluded that the human atherosclerotic arterial vessel wall provides conditions that favor a regulatory mechanism of thrombin activity by PAI-1/Vn complexes. (Arterioscler Thromb Vasc Biol. 2000;20:1143-1149.)

Key Words: atherosclerosis ▪ smooth muscle cells ▪ thrombin ▪ PAI-1 ▪ vitronectin

Plasminogen activator inhibitor type 1 (PAI-1) is a multifunctional glycoprotein that belongs to the serine protease inhibitor (serpin) superfamily.1 It regulates the fibrinolytic system by rapid formation of inactive, equimolar complexes with its target serine proteases, tissue-type (t-PA) and urokinase-type (u-PA) plasminogen activators (reviewed in References 2 and 3). The spectrum of PAI-1 target specificity is expanded by defined cofactors. Particularly in the presence of the glycoprotein vitronectin (Vn) or the glycosaminoglycan heparin, PAI-1 efficiently inhibits the serine protease thrombin.4,7 In vitro, the presence of either 1 of these cofactors accelerates the rate of thrombin inhibition by PAI-1 by at least 2 orders of magnitude.4,8 The interplay between PAI-1, thrombin, and Vn has been studied in detail, and these interactions are typified by a so-called “branched suicide substrate” mechanism.9 According to this mechanism, the initial enzyme/inhibitor Michaelis complex is converted, presumably via a number of intermediates, into either an inactive thrombin/PAI-1 complex (suicide pathway) or a mixture of cleaved PAI-1 and regenerated active thrombin (substrate pathway). The partition ratio for these pathways depends on the nature of the reactants, their relative concentration, and, in defined cases, the presence of cofactors that may specifically promote 1 of these 2 reactions.9

A number of arguments can be advanced suggesting that regulation of thrombin activity by PAI-1/Vn complexes might be relevant in the context of the (diseased) vessel wall. First, administration of thrombin to endothelial cell matrixes results in the release of SDS-stable thrombin/PAI-1 complexes, depending on the availability of Vn.10 Apparently these matrixes are devoid of other thrombin-binding serpins, since only SDS-stable thrombin/PAI-1 complexes are encountered. Second, the apparent concentration of the reactants in the vessel wall is sufficiently elevated for an efficient interaction among the 3 components. Notably, it has been reported that the concentration of active PAI-1 in the atherosclerotic vessel wall is 10 to 23 nmol/L,11,12 which is similar to the concentration of active thrombin as determined in this
study. It is expected that the concentration of Vn in the vessel wall exceeds that of PAI-1 and thrombin, since the plasma concentration of Vn is relatively high (3 to 6 μmol/L).13 Most plasma proteins are encountered in both normal and atherosclerotic intima, and their concentration in interstitial fluid is usually proportional to their concentration and size.14 Third, different cell types, among which are the monocyte/macrophage and the smooth muscle cell that are present in the atherosclerotic vessel wall, are equipped to remove thrombin/PAI-1 complexes by surface exposure of the LDL receptor–related protein (LRP).15 Interestingly, LRP-mediated clearance of thrombin/PAI-1 complexes is promoted by Vn.16 This finding agrees with our in vitro study showing that PAI-1, thrombin, and Vn form a ternary complex.17 Taken together, these observations support the hypothesis that thrombin activity in the vessel wall is controlled by PAI-1/Vn complexes.

The presence and generation of PAI-118–20 and Vn21 in the vessel wall, particularly in atherosclerotic lesions, have been reported in several studies. Specifically, an increase of PAI-1 mRNA and antigen in human vascular specimens has been correlated with the progression of atherosclerosis. In those studies, it was assumed that endothelial cells and vascular smooth muscle cells were the primary source of PAI-1 biosynthesis. In addition, evidence has recently been presented that vascular smooth muscle cells in the intima and media of human atherosclerotic plaques from carotid arteries synthesize Vn mRNA.22

The presence of active thrombin in the vessel wall has been demonstrated by preparing extracts of atherosclerotic lesions.14 In addition, its presence can also be inferred from several independent observations. Specifically, vascular smooth muscle cells in human atherosclerotic arteries express the protease-activated receptor type 1 (PAR-1) on their surfaces,23 a receptor that is preferentially activated by thrombin. Accordingly, thrombin stimulates proliferation of cultured smooth muscle cells24 and induces PAR-1–mediated production of cytokines.25 Presumably these effects result in the formation of neointima in vivo. Direct evidence for the importance of thrombin-promoted neointima formation comes from experiments in the rat carotid artery balloon injury model.26 Infection with an adenoviral vector encoding the thrombin inhibitor hirudin demonstrated that thrombin-induced neointima formation could be significantly reduced. In aggregate, these observations indicate that thrombin, PAI-1, and Vn are present in the vessel wall, especially under atherosclerotic conditions.

The issue of regulation of thrombin activity in the vessel wall has not been addressed in great detail. In this respect, it is interesting to note that although low levels of antithrombin III (ATIII) have been localized in the subendothelium,14,27 neither ATIII nor thrombin/ATIII complexes are present in the deeper regions of the lesion.14 In this article, data are presented that support the hypothesis that PAI-1/Vn complexes may act as physiological inhibitors of active thrombin in the atherosclerotic vessel wall. To that end, we examined whether the PAI-1, Vn, and thrombin proteins colocalize in human atherosclerotic specimens by using immunofluorescence microscopy. Furthermore, we established by in situ activity assays that thrombin and PAI-1 occur in an active configuration in these specimens. On the basis of these results, we postulate that PAI-1/Vn complexes regulate thrombin-induced neointima formation.

Methods

Vascular Tissue

Human vascular specimens were acquired from 4 different anatomic locations, namely, coronary arteries obtained after heart transplantation, carotid arteries after endarterectomies, saphenous veins that had been used as a coronary bypass conduit, and aortas obtained after vascular surgery. Each of these specimens displayed atherosclerotic lesions, although neither necrotic cores nor massive lipid deposits were present. The coronary and carotid arteries were used for immunofluorescent staining. For that purpose, the tissues were fixed for 2 hours at 4°C by immersion in 4% (vol/vol) paraformaldehyde in PBS. The tissues were then embedded in OCT medium (Miles Scientific) and frozen by immersion in LN2-cooled isopentane. All human specimens were obtained after informed consent was obtained from the patients or relatives, according to a protocol approved by the institutional ethics committee.

Antibodies

Two different antibody preparations were used for the detection of thrombin antigen, namely, a sheep polyclonal anti-human thrombin immunoglobulin (IgG) preparation that had been affinity purified (SAHT-AP, Kordia), and a murine monoclonal anti-human thrombin antibody (EST-4, American Diagnostica Inc). The polyclonal antiserum SAHT-AP binds both free thrombin and thrombin–thrombin inhibitor complexes and was used at a 25-fold dilution. For the detection of PAI-1 antigen, 2 murine monoclonal anti-human PAI-1 antibodies, 7F5 and 12H15 (kind gifts from Dr P. Declerck, University of Leuven, Leuven, Belgium), and a rabbit polyclonal anti-human PAI-1 antisemur (American Diagnostica Inc) were used. Each of these antibodies binds to PAI-1 and to serine protease/PAI-1 complexes and was used at a 50- to 100-fold dilution. For the detection of Vn antigen, a rabbit polyclonal anti-human Vn antiserum and 3 murine monoclonal anti-human Vn antibodies (VN7, VN9, and 13H1, generously donated by Dr K.T. Preissner, Max-Planck-Institute, Bad Nauheim, Germany) were used. These monoclonal and polyclonal antibodies bind to human Vn and were used at a 50- to 100-fold dilution. For the detection of hirudin antigen, a rabbit polyclonal anti-hirudin antisemur (American Diagnostica Inc) was used at a 50-fold dilution. Three different fluorescently labeled, secondary antibodies were used, namely, horse anti-mouse IgG antisemur conjugated to FITC, porcine anti-rabbit IgG antisemur conjugated to Texas red and donkey anti-goat IgG antisemur conjugated to Cy5. All secondary antibodies were purchased from Vector Laboratories and were used at a 50-fold dilution.

Immunofluorescent Staining

Cryosections (8 μm) of fixed tissue were cut and stained with the antibodies as described in the previous paragraph. To accurately detect and localize thrombin, PAI-1, and Vn, we used 2 or 3 different monoclonal and polyclonal antibodies that were specific for each antigen. To further establish the specificity of staining, we performed the following negative-control experiments: (1) omission of the primary antibody, (2) replacement of the primary antibody with equivalent amounts of isotype-matched nonimmune IgG or nonimmune serum, and (3) incubation with an antibody preparation that had been preabsorbed with the purified antigens. Initially, the staining pattern for each antigen was established in single-labeling experiments. Immunofluorescent staining was essentially performed as described before.26 For simultaneous staining for thrombin, Vn, and PAI-1, the incubations were done as follows: (1) with a mixture of rabbit anti-human Vn antisemur and murine monoclonal anti-human PAI-1 antibody 7F5, (2) with sheep anti-human thrombin antisemur, (3) with a mixture of porcine anti-rabbit IgG conjugated to Texas red and horse anti-mouse IgG conjugated to FITC, and
(4) with donkey anti-goat IgG conjugated to Cy5. Each incubation was done for 1 hour at room temperature, followed by extensive washing with PBS.

Confocal Microscopy
Sections stained by using immunofluorescence methods were analyzed with a Bio-Rad MRC 600 confocal laser scanning unit attached to a Nikon Diaphot inverted microscope (Bio-Rad Microscience Ltd). The light source was a krypton/argon laser (Ion Laser Technology) with principal lines at 488, 568, and 674 nm. In the triple-labeling procedure, we used the K1, K2, and RHS filters for visualization of FITC, rhodamine/Texas red, and Cy5 staining. The signals of the 3 fluorophores were sequentially collected by using appropriate excitation filters. FITC staining appears in green, Texas red in red, and Cy5 in dark red light. For convenience, Cy5 was pseudocolored in blue. The three 8-bit images were merged by using confocal assistant software (Bio-Rad Microscience), resulting in a 24-bit color image.

In Situ Reverse Zymography
For in situ reverse zymography, cryosections (8 μm) were cut from unfixed tissue and air dried for 30 minutes. The sections were preincubated for 30 minutes at room temperature with 0.1 U/mL of unfixed tissue and air dried for 30 minutes. These sections were incubated at 37°C, containing 1.8% (wt/vol) casein, 0.2% (wt/vol) casein-FITC, 1% (wt/vol) low-melting agarose, and 40 μg/mL plasminogen in PBS. Each of these reagents was purchased from Sigma Chemical Co. For the localization of nuclei, 50 ng/mL ethidium bromide was included in the casein overlay. A coverslip was mounted on the section, and the section was incubated for 10 minutes at 4°C to polymerize the casein overlay. Progression of caseinolysis was then observed for 60 minutes at room temperature by confocal microscopy. A consecutive section of the same tissue was cut, air dried, and fixed for 10 minutes in ice-cold acetone. Subsequently, this section was used for the localization of PAI-1 antigen by immunofluorescence, according to the protocol described above.

Localization of Active Thrombin
For the localization of active thrombin, cryosections (8 μm) of unfixed tissue were air dried for 30 minutes. These sections were incubated for 60 minutes at room temperature with 0.02 IU/mL hirudin (Sigma) in blocking solution and then fixed in ice-cold acetone. Subsequent immunofluorescent staining was done as described above.

Thrombin Activity Assay
For thrombin activity measurements, cryosections (8 μm) of unfixed saphenous vein or aortic tissue was preincubated with u-PA. Subsequently, an overlay was added that contained plasminogen and casein, conjugated to FITC. Caseinolysis, visualized as dark areas due to diffusion of released FITC, is mediated by plasmin that is generated by limited proteolysis of plasminogen by u-PA. Inhibition of u-PA by active PAI-1 results in opaque fluorescent areas due to the lysis resistance of FITC-conjugated casein. In the representative section displayed, we observed a large, lysis-resistant neointimal area, while other parts of the section displayed smaller, lysis-resistant spots. Clearly, the presence of PAI-1 antigen colocalizes with zones of lysis resistance, conceivably due to active PAI-1. Because most of the PAI-1 antigen colocalizes with this inhibitory activity, we deduced that the majority of PAI-1 in the vessel wall was present in an active configuration. Furthermore, we concluded that active PAI-1 is cell associated, as suggested by the distinct lysis-resistant entities, present in predominantly lysed areas (eg, the left part of Figure 2B), and by colocalization with ethidium bromide–stained nuclei (data not shown).

Results
Colocalization of PAI-1, Vn, and Thrombin in Vascular Specimens
To determine the localization of PAI-1, Vn, and thrombin in human carotid arteries, we used immunofluorescence confocal microscopy. To simultaneously localize these 3 components, sections were incubated with primary antibodies directed against PAI-1 (monoclonal anti–PAI-1, 7F5), Vn (polyclonal anti-Vn antiserum), and thrombin (polyclonal anti-thrombin antiserum, SHAT-AP), followed by incubation with the respective secondary antibodies, each of which was coupled to a different fluorescent label. This triple-labeling analysis revealed that PAI-1 was present throughout the neointima, whereas the most intense staining was encountered in the subendothelium (Figure 1A). The most intense staining for Vn (Figure 1B) and thrombin (Figure 1C) was observed in distinct areas within the neointima. When all 3 staining patterns were superimposed, colocalization of the 3 antigens, visualized by the appearance of a white to pink color, was observed (Figure 1D). Extensive colocalization studies were also performed on consecutive sections by using double labeling for Vn/PAI-1 and Vn/thrombin. A considerable extent of colocalization of the 3 proteins was found. To confirm the validity of these observations, a series of different antibodies was used against PAI-1, Vn, and thrombin as indicated in the Methods section. In control experiments, either omitting the primary antibody or replacing the primary antibody by an isotope-matched, nonimmune IgG, we observed only autofluorescence of the internal elastic lamina. In the normal vessel wall, colocalization of PAI-1, Vn, and thrombin was not observed. This is explained by the lower levels of PAI-1 in the normal vessel wall and the lack of thrombin generation, although high amounts of prothrombin were found (F.L., unpublished observations, 1999).

Localization of PAI-1 Activity
To determine PAI-1 activity, we used immunofluorescent staining for PAI-1 in combination with in situ reverse zymography. The distribution of PAI-1 antigen was detected by immunofluorescence (Figure 2A) and was comparable to that found in other single and triple PAI-1 immunostaining experiments (eg, Figure 1A). In a consecutive section of the same specimen, PAI-1 activity was detected by in situ reverse zymography (Figure 2B). For the latter purpose, a cryosection of unfixed saphenous vein or aortic tissue was preincubated with u-PA. Subsequently, an overlay was added that contained plasminogen and casein, conjugated to FITC. Caseinolysis, visualized as dark areas due to diffusion of released FITC, is mediated by plasmin that is generated by limited proteolysis of plasminogen by u-PA. Inhibition of u-PA by active PAI-1 results in opaque fluorescent areas due to the lysis resistance of FITC-conjugated casein. In the representative section displayed, we observed a large, lysis-resistant neointimal area, while other parts of the section displayed smaller, lysis-resistant spots. Clearly, the presence of PAI-1 antigen colocalizes with zones of lysis resistance, conceivably due to active PAI-1. Because most of the PAI-1 antigen colocalizes with this inhibitory activity, we deduced that the majority of PAI-1 in the vessel wall was present in an active configuration. Furthermore, we concluded that active PAI-1 is cell associated, as suggested by the distinct lysis-resistant entities, present in predominantly lysed areas (eg, the left part of Figure 2B), and by colocalization with ethidium bromide–stained nuclei (data not shown).
serum, followed by an incubation with a fluorescently labeled secondary antibody. Our results demonstrate that thrombin activity was encountered at the center of the neointima, whereas only minor staining of active thrombin was found in the media (Figure 3). Significantly, the distribution of thrombin activity was comparable to that of thrombin antigen found in single- and triple-stained sections (eg, Figure 1C). No staining of active thrombin was observed when the incubation with hirudin was omitted.

Next, we established whether the amount of active thrombin generated in the vessel wall would be adequate for the activation of PAR-1, which is present on the surface of

![Figure 1](image1.png)

**Figure 1.** Colocalization of PAI-1, Vn, and thrombin in atherosclerotic arteries visualized by triple immunofluorescent staining. An 8-μm cryosection of a carotid artery was simultaneously incubated with the murine anti–PAI-1 monoclonal antibody 7F5 (a), a rabbit polyclonal anti-Vn antiserum (b), and the sheep polyclonal anti-thrombin antiserum SAHT-AP (c); subsequently, the section was incubated with the matching secondary antibodies conjugated to different fluorescent labels as described in Methods. To visualize colocalization of all 3 antigens, the separate images were superimposed (d). White and pink areas represent colocalization of all 3 antigens and are indicated by arrows. Each immunofluorescent staining was performed with at least 5 different cryosections. Autofluorescence is indicated with an asterisk. Magnification is ×120.

![Figure 2](image2.png)

**Figure 2.** Localization of PAI-1 activity in atherosclerotic specimens. In a, a section was studied by immunofluorescent staining for PAI-1 with the anti–PAI-1 monoclonal antibody 7F5 and the corresponding secondary antibody conjugated to FITC as described in Methods. The area with the largest extent of PAI-1 staining is indicated with an arrow, the lumen of the section with an L, and a fold in the tissue with an asterisk. In b, a representative example is given of in situ reverse zymography to detect PAI-1 activity. Experimental details and analysis by confocal microscopy are described in Methods. The area that displays the most pronounced lysis resistance is designated by an arrow. The image is displayed with a pseudocolor banding approach to emphasize the differences in the extent of lysis. The 256-gray-level scale of an 8-bit image was divided into 9 color-encoded bands (top left-hand corner). The lowest fluorescence levels, equivalent to total lysis of the fluorescent substrate, are shown in dark blue, while the highest fluorescence representing the lysis-resistant areas is shown in white. Panels a and b are consecutive sections of the same specimen. Magnification is ×145.
vascular smooth muscle cells in atherosclerotic lesions. For that purpose, cryosections of unfixed saphenous vein or aortic tissue were preincubated in either the presence or absence of 10 nmol/L of the thrombin inhibitor PPACK. Subsequently, the tissue sections were incubated with the thrombin-specific chromogenic substrate S2238, and the optical density at 405 nm was measured at different intervals. It should be mentioned that at the relatively low concentration of PPACK used (10 nmol/L), thrombin is virtually the only serine protease present in the arterial specimens that will be effectively inhibited. Preincubation with PPACK of specimens derived either from the saphenous vein or aorta resulted in virtually no thrombin activity (change in absorbance per 3 hours was $1.3 \times 10^{-3} \pm 5.8 \times 10^{-4}$ and $1.7 \times 10^{-3} \pm 5.8 \times 10^{-4}$, respectively; Figure 4). A preincubation in the absence of PPACK resulted in an increase of the optical density at 405 nm in 3 hours of $0.111 \pm 0.021$ and $0.062 \pm 0.014$, respectively. We verified that the increase in optical density developed linearly over time. Thrombin activity was quantified by calibration with a standard $\alpha$-thrombin preparation and calculated per unit volume of tissue. Consequently, it was determined that the saphenous vein tissue used contained a thrombin concentration of $\approx 7$ to 12 nmol/L, whereas the aortic tissue harbored $\approx 8$ to 12 nmol/L thrombin. Clearly, the determined $\alpha$-thrombin concentrations coincided with the range of concentrations that is required (1 to 10 nmol/L) to optimally activate PAR-1.

**Discussion**

We have previously reported that thrombin is efficiently inhibited in vitro by PAI-1, provided that the inhibitor is complexed to Vn. In general, the in vivo relevance of interactions between serine proteases and serpins is governed by a number of criteria, namely, (1) the presence of the target protease, its cognate inhibitor, and cofactor in the same compartment; (2) the rate of inhibition; and (3) the concentration of the reactants. According to these criteria, an efficient interaction between thrombin and PAI-1/Vn complexes in the plasma compartment is unlikely due to the low concentration of biologically active PAI-1, in contrast to that of the traditional thrombin inhibitor ATIII. Instead, we hypothesized that regulation of thrombin activity by PAI-1/Vn complexes might be a relevant mechanism within the arterial vessel wall. The presence of active thrombin is anticipated in the neointima, in view of its assumed role as a potent mitogen for smooth muscle cells and the occurrence of fibrin deposits within atherosclerotic plaques. Accordingly, by using 2 different assays, we show here that active thrombin can be easily demonstrated in atherosclerotic tissue. Actually, most plasma (precursor) proteins are present in both normal and atherosclerotic intima, and usually their concentrations in interstitial fluid are related to their plasma concentration and size. The generation of thrombin is thought to be initiated by coagulation factor VII/VIIa bound to tissue factor, which is exposed on the surfaces of infiltrating cells. Our findings on active thrombin in the vessel wall confirm a previous study on the presence of free $\alpha$-thrombin in extracts of human atherosclerotic lesions. Interestingly, thrombin activity is predominantly detected in the neointima. This observation is consistent with a role for thrombin in the proliferation of smooth muscle cells, which are preferentially present in the neointima.

In this study, we analyzed human arterial specimens by immunofluorescence to provide support for the regulatory...
scheme deduced from in vitro experiments, indicating that thrombin activity is regulated by PAI-1/Vn complexes. As contingent aid for this proposal, it should be mentioned that although low levels of the thrombin inhibitor ATIII have been localized in the subendothelial space,27 no thrombin/ATIII complexes could be detected in the vessel wall.14 These observations virtually exclude ATIII as a potential regulator of thrombin inhibition by PAI-1/Vn complexes. Obviously, these observations do not constitute direct evidence for the actual presence of inactive ternary complexes that are typical for thrombin inhibition.17 We have not attempted to demonstrate the occurrence of ternary complexes in these tissues, since it cannot be excluded that complex formation is generated during extraction of the specimens. Furthermore, active PAI-1 might be converted into its latent (inactive) form during tissue extraction procedures, as has been experienced by other investigators.11,33 To prevent potential artifacts, we refrained from tissue extraction procedures and performed in situ reverse zymography on intact tissues. Consequently, we demonstrated that active PAI-1 is abundantly present in the arterial vessel wall. As expected, most of the PAI-1 did not colocalize with thrombin, since PAI-1 is synthesized and secreted by endothelial and smooth muscle cells and, subsequently, is bound to heparin-containing proteoglycans and Vn.45 In contrast, active thrombin will be generated in the vicinity of infiltrating cells,32 thereby explaining that the majority of these reactants will be present in different intravascular compartments. In aggregate, the observed colocalization of thrombin, PAI-1, and Vn in the neointima of atherosclerotic arteries by immunofluorescence analysis and the demonstration of the active forms of PAI-1 and thrombin are consistent with a regulatory mechanism of thrombin activity by PAI-1/Vn complexes.

Our previous in vitro studies on the interaction between thrombin and an excess of PAI-1/Vn complexes have taught us that thrombin inhibition, accompanied by the formation of ternary complexes, is actually a minor reaction pathway.9 The major reaction constitutes the substrate pathway, resulting in cleavage of PAI-1 by thrombin and regeneration of the active protease. We appreciate that this branched suicide substrate mechanism may have profound effects on the progression of atherosclerosis, as exemplified by migration and proliferation of smooth muscle cells. First, direct inhibition of thrombin by PAI-1/Vn complexes would eliminate thrombin as an important trigger for proliferation of smooth muscle cells. The resulting ternary thrombin/PAI-1/Vn complexes are bound by the LRP16 that is exposed on the surfaces of these cells15 and will be subsequently internalized and degraded. Hence, it is expected that this pathway efficiently attenuates proliferation of smooth muscle cells. Second, solid evidence has been reported that PAI-1 is an essential inhibitor of u-PA–mediated migration of murine smooth muscle cells in both an electric injury model of carotid arteries and a wound healing model.94 Accordingly, PAI-1–deficient mice display more pronounced neointima formation than do wild-type mice. In addition, neointima formation in PAI-1–deficient mice can be restored on infection with an adenovirus preparation that harbors and expresses intact PAI-1 cDNA. Taking together these observations and our previous findings on the predominance of PAI-1 cleavage by thrombin, it is concluded that thrombin generation would largely result in PAI-1 degradation, ultimately favoring u-PA–mediated smooth muscle cell migration. Thus, paradoxically, we predict that the generation of thrombin in the vessel wall and the subsequent regulation of its activity by PAI-1/Vn according to the branched suicide substrate mechanism promote migration but reduce proliferation of smooth muscle cells. Evidence for this prediction may come from studies with mice that are deficient in 1 or more of the components described in this study and are thus challenged for the development of a neointima. As mentioned before, PAI-1–deficient mice have been generated and display increased neointima formation.96 At present, this phenotype has not been analyzed in Vn-deficient mice in comparison with wild-type mice.35 Unfortunately, direct studies on the effect of thrombin generation on migration and proliferation of smooth muscle cells are not feasible, since homozygous thrombin-deficient mice are not viable.36 In that case, valuable information might be obtained by studying mice that are deficient in PAR-1,37 which is present on smooth muscle cells and is responsible for thrombin-induced proliferation. We expect that future studies with the indicated genetically modified experimental animals may provide further insight into the mechanisms and consequences of the regulation of thrombin activity by PAI-1/Vn complexes.

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References


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