The effect of endothelial cell overexpression of plasminogen activator inhibitor-1 on smooth muscle cell migration

Richard R. Proia, MD, Peter R. Nelson, MD, Mary Jo Mulligan-Kehoe, PhD, Robert J. Wagner, BS, Arthur J. Kehas, BS, and **Richard J. Powell, MD,** *Lebanon, NH*

Introduction: **Plasminogen activator inhibitor-1 (PAI-1), a known inhibitor of plasminogen activators, may regulate smooth muscle cell migration (SMC) through alteration in matrix metalloproteinase (MMP) activity.**
Methods: **To study the effect of endothelial cell (EC) PAI-1 overexpression on SMC migration, RT-PCR was used to clone the full length PAI-1 gene, which was ligated into the pCMV/myc/ER expression vector. With electroporation, bovine aortic ECs were transfected with either the PAI-1 construct or the empty vector as control. EC PAI-1 overexpression was shown with a specific PAI-1 activity assay and enzyme-linked immunosorbent assay. The effect of EC PAI-1 overexpression on SMC migration was measured with a modified Boyden-chamber assay. SMC MMP expression was measured with zymography.**
Results: **Selected clones (EC9, EC21) had a three-fold to five-fold increase in PAI-1 activity compared with untransfected EC and empty vector EC (ECC). Similarly, enzyme-linked immunosorbent assay results showed a 3.5-fold to 5.5-fold increase in PAI-1 levels in EC9 and EC21 versus ECC. Untransfected EC and ECC had similar effects on SMC migratory patterns. Migration of SMC exposed to PAI-1 overexpressing EC was inhibited by 35% to 57% compared with ECC. This inhibitory effect was reversed with addition of exogenous urokinase-type plasminogen activator (uPA). Zymography showed downregulation of MMP-2 and MMP-9 in SMCs exposed to PAI-1 overexpressing EC.**
Conclusion: **PAI-1 overexpression with transfected EC inhibits SMC migration. This effect may be mediated through decreased SMC MMP activity. (J Vasc Surg 2002;36:164-71.)**

Intimal hyperplasia is the leading cause of restenosis and failure of peripheral angioplasty and bypass procedures. This intimal hyperplastic response seen in human arteries after vessel wall injury has been well described and is mediated predominantly with vascular smooth muscle cells (SMCs).[1] During the development of intimal hyperplasia, SMCs first migrate to the subintimal space where they proliferate and produce abundant extracellular matrix (ECM), which accounts for most of the mature lesion.[2,3]

For migration to the intima of the vessel wall, SMCs must degrade the surrounding matrix. Clowes and Schwartz[4] and other investigators[5,6] have shown that the urokinase-type plasminogen activator (uPA)-plasmin-matrix metalloproteinase (MMP) axis is involved in the degradation of this existing ECM. uPA is known to convert plasminogen to plasmin, which in turn activates MMPs.[7,8] These activated MMPs coordinate the degradation of existing ECM. Plasminogen activator inhibitor-1 (PAI-1), a known inhibitor of uPA, may play a significant role in regulating this pathway.[9] Evidence for this is provided by Carmeliet et al[10-13] with a PAI-1 knockout mouse model in which PAI-1 deficient mice had a more severe intimal hyperplastic response to arterial injury when compared with wild type controls. One explanation for these findings is that PAI-1 inhibits uPA-mediated activation of plasmin, which in turn limits MMP activity.[14] This may lead to less degradation of ECM and a potential decrease in SMC migration.[15]

Endothelial cells (ECs) are the major source of PAI-1 in the arterial wall. ECs have been shown to favorably affect arterial wall remodeling after arterial injury.[16] One possible mechanism with which this occurs is through inhibition of SMC migration as a result of PAI-1 secretion from the abluminal surface of the EC.[17,18] This has recently been shown by Redmond and coworkers.[19] These authors have shown that ECs inhibit SMC migration in a coculture system through a PAI-1 mechanism. In this study, we have developed EC clones that overexpress PAI-1. The purpose of this study was to determine whether EC overexpression of PAI-1 inhibited SMC migration. In addition, we examined the role of MMP-9 in this process. Lastly, we examined the spreading, attachment, and proliferation of the PAI-1 overexpressing EC to determine whether the transfection process altered the proliferation, attachment, or spreading of the experimental cell lines.

From the Department of Surgery, Dartmouth-Hitchcock Medical Center, Dartmouth Medical School.
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Reprint requests: Richard J. Powell, MD, Department of Surgery, Section of Vascular Surgery, Dartmouth-Hitchcock Medical Center, One Medical Center Dr, Lebanon, NH 03756 (e-mail: Richard.J.Powell@Hitchcock.org).
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MATERIALS AND METHODS

Construction of plasminogen activator inhibitor-1 plasmid. Total RNA was extracted from bovine EC with guanidium thiocyanate.[20] The isolated RNA was reverse transcribed into complementary DNA with the manufacturer's random primer protocol (Boehringer-Mannheim, Inc, Indianapolis, Ind). The 1.2-kilobase PAI-1 complementary DNA was made double stranded in a polymerase chain reaction containing primers specifically designed to amplify the 1.2-kilobase porcine PAI-1 DNA and incorporate 5′ Sal I and 3′ Not I restriction enzyme sites. The primer sequences are 5′ primer, 5′ GCGTCGACAAGCTTTTTGCCGAAGGC 3′, and 3′ primer 5′ ATATATGCGGCCGCGTCAAGGCTCCATCACTT 3′. PCR conditions were as follows: 95° C, 5 minutes; 58° C, 1 minute; add DNA Taq polymerase; 72° C, 1 minute 15 seconds for 1 cycle followed by 95° C, 1 minute; 58° C, 1 minute; add DNA Taq polymerase; 72° C, 1 minute 15 seconds for 34 cycles.

The restriction enzyme sites on the PCR product were activated. PAI-1 was ligated into the pCMV/myc/ER shuttle vector (Invitrogen, Inc, Carlsbad, Calif), enabling DNA amplification in prokaryotic cells and protein expression in eukaryotic cells. The vector contains both ampicillin and G-418 resistance genes for prokaryote and eukaryote cells, respectively. The ligated construct then was introduced into TOP 10 *Escherichia coli* cells (Invitrogen, Inc, Carlsbad, Calif) with electroporation with the BTX 600 Electro Cell Manipulator (Genetronics, Inc, San Diego, Calif). The transformed cells were selected on Luria broth agar plates containing 100 μg/mL of ampicillan. Individual colonies were isolated and grown at 37° C for 12 hours in Luria broth (Quality Biological, Inc, Gaithersburg, Md) containing 100 μg/mL ampicillan. DNA was isolated with a Qiagen plasmid purification kit (Qiagen, Inc, Valencia, Calif). Restriction enzyme digestion of plasmidal DNA was performed to confirm the presence of PAI-1. DNA sequencing was performed to verify the fidelity of the PAI-1/pCMV/myc/ER construct.

Cell culture and transfection. ECs were isolated from bovine thoracic aorta with collagenase technique and allowed to grow in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% calf bovine serum (HyClone Laboratories, Inc, Logan, Utah), penicillin (40 units/mL), streptomycin (40 mg/mL), and 4.8 mmol/L L-glutamine. EC identity was confirmed after each passage and after transfection with cobblestone morphology and also factor VIII-related antigen (cat# A616, DAKO Corp, Carpinteria, Calif) and lectin Bandeiraea simplicifolia BS-1 staining (cat# L-2140, Sigma, St Louis, Mo).[21] SMCs were isolated from bovine thoracic aorta with the explant technique. SMC identity was confirmed after each passage with staining for α-actin (Sigma).

Cells were transfected with the PAI-1 construct with the electroporation technique. Transfected cells were grown in DMEM containing the antibiotic G-418. Resistant clones were selected and tested for PAI-1 activity. The pCMV/myc/ER vector without the PAI-1 insert also was transfected into EC to function as a control (empty vector EC [ECC]).

Plasminogen activator inhibitor-1 assays. A modified spectrophotometric assay as described by Verheijen et al[22] was used to measure PAI-1 protein activity in the conditioned media from ECs. In this assay, plasmin cleaves Chromozym-PL, resulting in a p-nitroniline molecule that absorbs at 405 nm. Briefly, conditioned media were collected after 24 hours from confluent ECs (1×10^6 cells/25 mm^2-area circular well) and centrifuged to remove cellular debris. After this, 40 μL of fresh conditioned media were added to 0.2 units of uPA (American Diagnostica, Inc, Greenwich, Conn). This was incubated at 25° C for 1 hour, after which 15 μg/mL of Chromozym-PL and 1 unit of plasminogen (Boehringer Mannheim, Inc) were added. This was incubated at 37° C for 45 minutes, and PAI-1 activity was quantified with measuring absorbance at 405 nm on an EL-311 Microplate Autoreader (Bio-Tek Instruments, Burlington, Vt).

Quantification of PAI-1 protein also was performed with the IMUBIND tissue PAI-1 enzyme-linked immunosorbent assay (ELISA) kit as described by the manufacturer (American Diagnostica, Inc). Briefly, standards and samples were incubated in 96-well microtiter wells precoated with anti-human PAI-1 antibody. After washing, the secondary biotinylated anti-PAI-1 detection antibody was added along with the enzyme conjugate, streptavidin-horseradish peroxidase. The amount of streptavidin-horseradish peroxidase bound was determined with addition of perborate/3, 3′, 3, 5′-tetramethylbenzidine substrate. The reaction was stopped with sulfuric acid, and the assay was quantified with measurement of absorbance at 450 nm on an EL-311 Microplate Reader.

Smooth muscle cell migration studies. A modified Boyden-chamber assay was used to study the effect of EC overexpressing PAI-1 on SMC migration.[23,24] The assay was run with both coated and uncoated polycarbonate 8-μm porous membranes (Corning Costar Corp, Cambridge, Mass). Coated filters were covered with growth factor reduced basement membrane gel matrix (Matrigel, Becton Dickinson, Inc, Franklin Lakes, NJ) at a concentration of 100 μg/mL. The membrane was exposed to the Matrigel for 1 hour, then washed with Hank's Balanced Salt Solution and allowed to dry overnight. ECs were allowed to grow to confluence in the bottom of a 12-well plate. After the ECs had conditioned the media for 24 hours, the inserts containing porous membranes were placed into each well. Bovine aortic SMCs were grown to confluence in T-75 flasks and made quiescent in serum-free DMEM, harvested in 0.05% trypsin-ethylenediamine tetraacetic acid, and resuspended in serum-free DMEM. SMCs were seeded on the membrane supports at a concentration of 100,000 cells per well. Migration of the SMC toward the EC was assayed for 4 hours at 37° C. The membranes were removed, fixed in 70% ethyl alcohol for 20 minutes, and stained in hematoxylin overnight. The membranes were rinsed in water and then mounted on micro-

scope slides. Migration was quantified with counting cells in five high-power fields for each membrane with microscopy. All assays were run in triplicate.

Zymography. Gelatin zymography was performed as previously described with lysate from SMC grown in coculture with transfected and untransfected EC.[25] SMCs were seeded on a 0.4-μm porous polycarbonate membrane and cocultured with EC for 4 hours. SMCs were removed from the plate with homogenization buffer (50 mmol/L Tris-HCL, 10 mmol/L $CaCl_2$, 0.2% tritin X-100, pH 7.5). The cell membranes were fractured in three freeze/thaw cycles, after which protein levels were determined with a standard Bradford assay. Equivalent amounts of total soluble protein were electrophoresced on a 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis gel containing 0.1% gelatin (Bio-Rad, Inc, Hercules, Calif). After electophoresis, the gel was washed in 2.5% Triton X-100 to remove the sodium dodecylsulfate. The gel was developed for 18 hours at 37° C in a solution containing 50 mmol/L Tris-HCL (pH 7.9) and 5 mmol/L $CaCl_2$. The solution was removed, and the gel was fixed in 10% acetic acid/40% methanol and then stained with coomassie blue.

Control zymograms were performed in a similar fashion with samples known to express only MMP-2 (chondrosarcoma cell line) or both MMP-2 and MMP-9 (melanoma cell line).[26] Control cell lysates were obtained as a generous gift from Ulrike Benbow, PhD, Dartmouth Medical School, Hanover, NH. Gels were photographed, and densitometric analysis of MMP bands was performed with NIH image 1.62.

Western blot analysis. The SMC lysates isolated and analyzed with zymography also were used to analyze the latent form of MMP-9. Samples containing an equivalent number of SMCs grown in coculture with untransfected ECs or ECs transfected with pCMV/ER/EC-9 were isolated as described (zymography analysis). An equal amount of protein from the supernatant of pelleted, lysed cells was electrophoresed on a 4% to 20% gradient, sodium dodecylsulfate, nonreducing polyacrylamide gel. The proteins were visualized on a nitrocellulose membrane probed with 1 μg/mL of mouse anti-HT1080 latent MMP-9 (Oncogene Research Products, Boston, Mass). A rabbit antimouse heavy and light chain antibody (1 μg/mL) (Pierce, Rockford, IL) amplified the binding reaction. A 1h incubation with a horse radish peroxidase-conjugated antibody (donkey antirabbit immunoglobulin G, Amersham, Arlington Heights, Ill) diluted 1:10,000 further amplified the binding reaction. Detection was performed with addition of a chemiluminescent substrate (Amersham).

Endothelial cell proliferation, attachment and spreading studies. Transfected and untransfected ECs were examined to determine whether PAI-1 overexpression affected their attachment, spreading, or proliferation characteristics with similar methods to those previously described.[27] For attachment, cells were seeded on glass cover slips at 5×10^4 cells per well in 12-well plates. A portion of the cover slips were left uncoated, and others were precoated with either type I collagen or fibronectin (Sigma) at a concentration of 10 μg/mL in DMEM. Cells were allowed to attach for 20 minutes, and then each well was washed twice with Hank's Balanced Salt Solution. Cells were fixed by adding 4% paraformaldehyde to each well for 1 hour. Cover slips were stained in toluidine blue for 15 minutes, after which time they were rinsed in 70% ethyl alcohol, allowed to air dry, and mounted on slides. Attachment was quantified with counting total number of cells per five high-power fields. For spreading, cells were plated at 5×10^4 on cover slips in 12-well plates and fixed after 1 hour. Cover slips were stained as previously described, and cell area in μm^2 was calculated with NIH image 1.62. Proliferation assays were performed with plating 2.5×10^5 EC in triplicate into six-well plates. Cells were grown in DMEM with 10% calf serum. At 24, 48, and 72 hours, cells were trypsinized and counted with a hemocytometer.

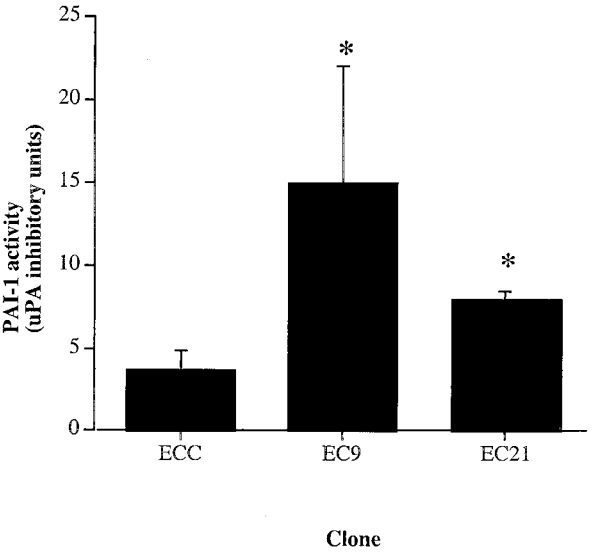

Fig 1. Graph shows results of pooled PAI-1 activity assays. PAI-1 activity was determined with spectrophotometric analysis and is expressed in uPA inhibitory units. Assays run in triplicate, n = 5 experiments with cell passages 4 to 8. $^*P < .05$ versus ECC.

Statistics. All cell culture experiments were performed in triplicate with EC passages 5 to 10 and SMC passages 1 to 5. Data were tabulated with Microsoft Excel, and statistical analysis was performed with Statview (Abacus Concepts, Inc, Berkeley, Calif) for the Macintosh. Comparisons were performed with analysis of variance or Student *t* test or both, with *P* less than .05 considered a significant difference. Error bars represent standard error of the mean.

RESULTS

Plasminogen activator inhibitor-1 construct. We successfully created a gene construct that overexpressed the PAI-1 gene. Positive clones were identified on the basis of size when examined with DNA gel electrophoresis. Cleavage of the construct produced two appropriately sized bands, a 5000-base pair band corresponding to the

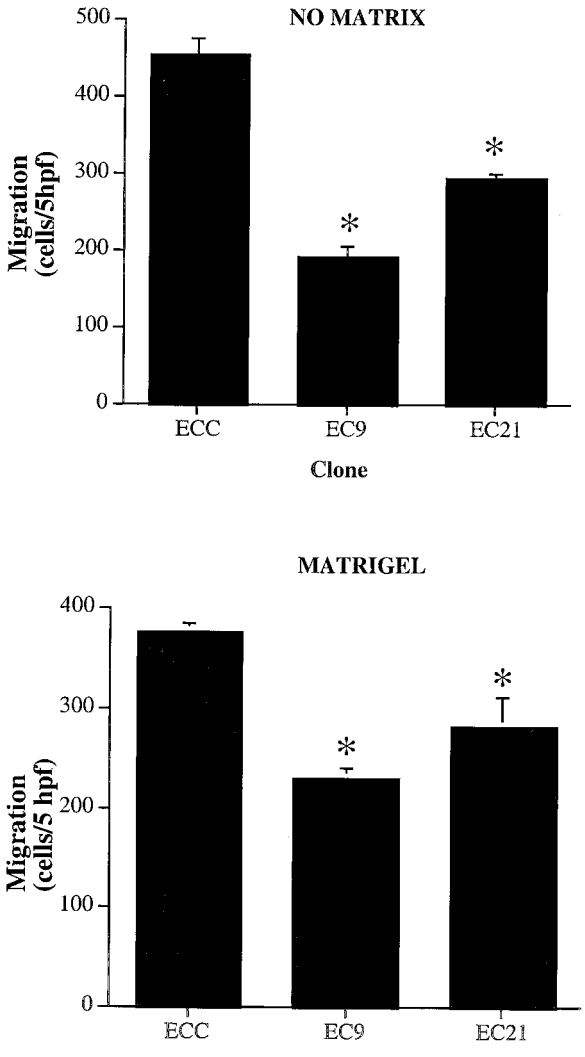

Fig 2. SMC migration results from modified Boyden-chamber assays. Assays were run with uncoated membranes and with Matrigel-coated membranes. Migration expressed as number of SMCs per five high-power fields per membrane. Assays run in triplicate, n = 6 experiments with cell passages 4 to 8. $^{*}P < .05$ versus ECC.

pCMV/myc/ER expression vector and a 1200-base pair band corresponding to the PAI-1 insert. These results were confirmed with DNA sequencing of the PAI-1 insert, which produced an exact match to the known genetic sequence.[28] Our control cell line, ECC, contained only the empty vector (data not shown).

Plasminogen activator inhibitor-1 activity assay and enzyme-linked immunosorbent assay. More than 30 clones of transfected EC were tested for PAI-1 activity. The range of activity varied from no increase over control EC (ECC) to a five-fold increase in activity. Clone EC9 was found to have an average three-fold increase over ECC in PAI-1 activity with activity assay ($P = .03$) and an average five-fold increase with ELISA. EC21 had an average two-fold increase over ECC with activity assay ($P = .005$; Fig 1) and an average 3.5-fold increase with ELISA (data not shown). Clones EC9 and EC21 were chosen for further cell culture experiments.

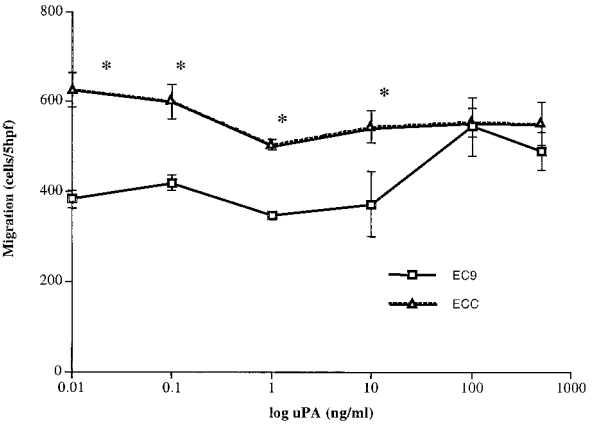

Fig 3. Effect on SMC migration after addition of exogenous uPA to modified Boyden-chamber assays. Assays run in triplicate, n = 6 experiments with cell passages 4 to 8. $^{*}P < .05$, EC9 versus ECC.

Smooth muscle cell migration. Bovine aortic SMCs were exposed to ECs in a modified Boyden-chamber assay to examine the effect of EC overexpression of PAI-1 on SMC migration. When nontransfected ECs were compared with ECCs, no difference was found in migration of SMC cultured opposite untransfected EC or EC transfected with the empty vector (ECC; data not shown). Compared with ECC, SMC migration of cells cultured opposite clone EC9 was decreased by 57% ± 4% ($P = .01$) and migration of SMC grown opposite clone EC21 was decreased by 35% ± 2% ($P = .008$; Fig 2, *A*). A similar trend was seen when Matrigel-coated membranes were used for the migration assay (Fig 2 , *B*). The inhibitory effect on SMC migration seen with exposure to EC9 could be reversed in a dose-dependent manner with the addition of increasing amounts of exogenous uPA to the modified Boyden-chamber media (Fig 3). At concentrations of uPA from 0.01 to 10 ng/mL, migration of SMC exposed to clone EC9 was significantly less than that of SMC exposed to control cells (ECCs). At higher concentrations of uPA, no difference was seen in SMC migration between EC9 and ECC.

Metalloproteinase expression. Gelatin zymography was performed with the cell lysate from SMC exposed to ECC, EC9, or EC21. A 56% ± 2% ($P = .02$) and 60% ± 3% ($P = .03$) decrease in MMP-2 expression of SMC exposed to EC9 and EC21 was found when compared with SMC exposed to ECC. Similarly, a 40% ± 4% ($P = .04$) and 44% ± 2% ($P = .03$) decrease in MMP-9 expression in SMC exposed to EC9 and EC21 was found when compared with control (Fig 4).

Control zymography was performed with cell lysate obtained from a chondrosarcoma cell line expressing only MMP-2 and cell lysate from a melanoma cell line known to express both MMP-2 and MMP-9. The MMP-9 band is at

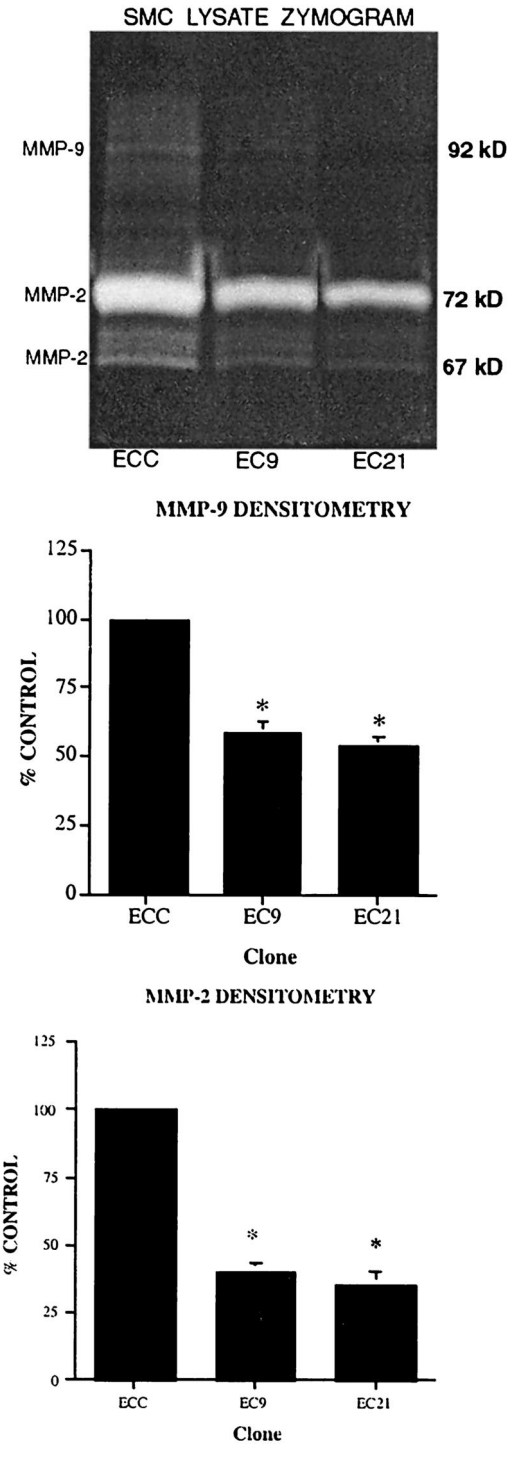

Fig 4. Zymogram and densitometric analysis of lysate from SMC exposed to various EC clones. *Clear bands* represent MMP degradation of gelatin contained within zymogram substrate. Gelatinolytic band at 92 kD corresponds with MMP-9, and 72 kD and 67 kD bands correspond with MMP-2. Densitometric analysis shows diminished activity of MMP-2 and MMP-9 in SMC cultured opposite clones EC9 and EC21 versus ECC (n = 3 experiments with cell passages 4 to 8). $^{*}P < .05$, EC9 versus ECC.

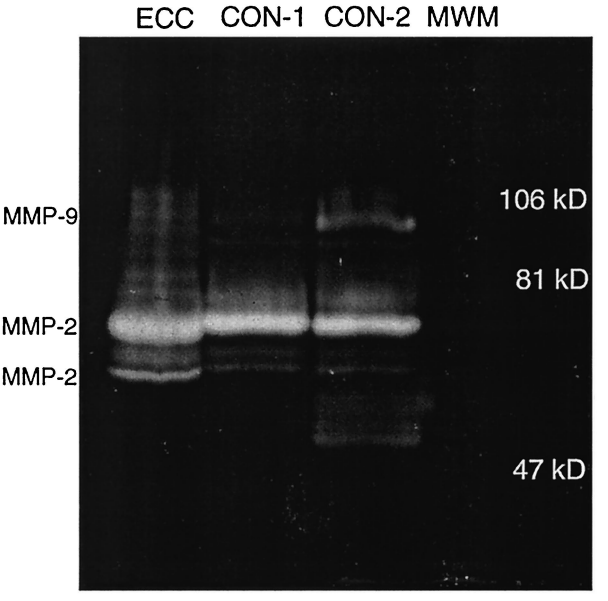

Fig 5. Control zymography shows correlation of MMP-9 and MMP-2 bands with cell lines known to express specific MMPs. *Lane 1*, Lysate from SMC exposed to ECC with bands present for both MMP-9 and MMP-2; *lane 2*, lysate from chondrosarcoma cell line *(CON-1)* known to express MMP-2 only; *lane 3*, lysate from melanoma cell line *(CON-2)* known to express MMP-9 and MMP-2; *lane 4*, low range molecular weight marker *(MWM)*.

92 kD, and the bands at 72 and 67 kD correlate with MMP-2 (Fig 5).

Western analysis also showed decreased latent MMP-9 in SMCs cultured opposite control ECs compared with SMCs cultured opposite EC9. On the other hand, SMCs cocultured with EC9 had lower amounts of active MMP-9 compared with controls (Fig 6).

Transfected endothelial cell proliferation and attachment. When ECs were compared with ECCs, no difference in spreading, attachment, or proliferation was seen (data not shown). The same morphologic characteristics for EC9 and EC21 were compared with ECC (Fig 7). We found no differences in 20-minute attachment between ECC, EC9, and EC21, even when different attachment matrices were used. All cell lines had similar spreading dimensions. No significant differences were seen in proliferation of clones at any time point in the 72-hour assay.

DISCUSSION

Neointimal plaque formation occurs frequently in human arteries after injury or manipulation during interventional or surgical procedures. This phenomenon has been studied by other investigators, and various key factors have been identified that contribute to this process.[29] Specifically, uPA and its known antagonist PAI-1 are thought to play a role in the development of intimal hyperplasia.[30-32] Carmeliet et al[12] have shown that PAI-1-deficient mice displayed accelerated neointimal formation after vascular

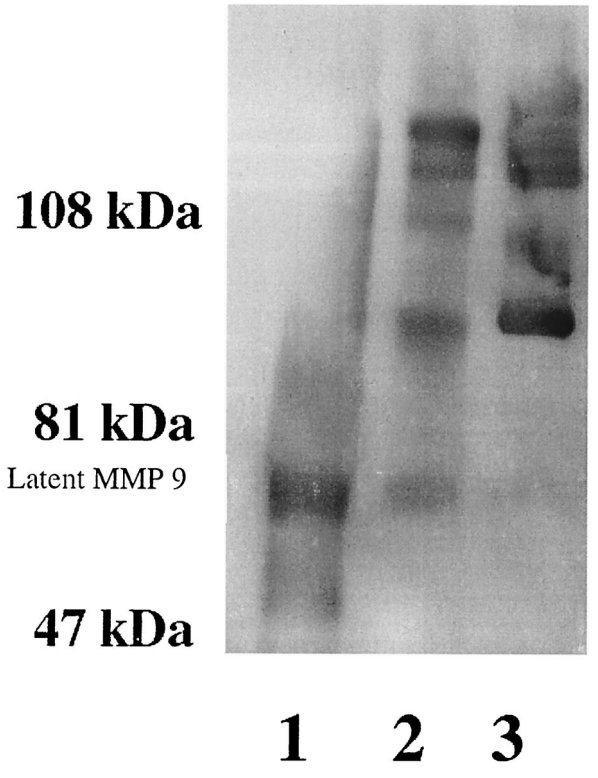

Fig 6. Representative Western blot for latent MMP-9. *Lane 1*, SMC lysate from EC coculture; *lane 2,* SMC lysate from pCMV/ER/PAI-1 isolate EC9 coculture; *lane 3,* purified latent MMP-9.

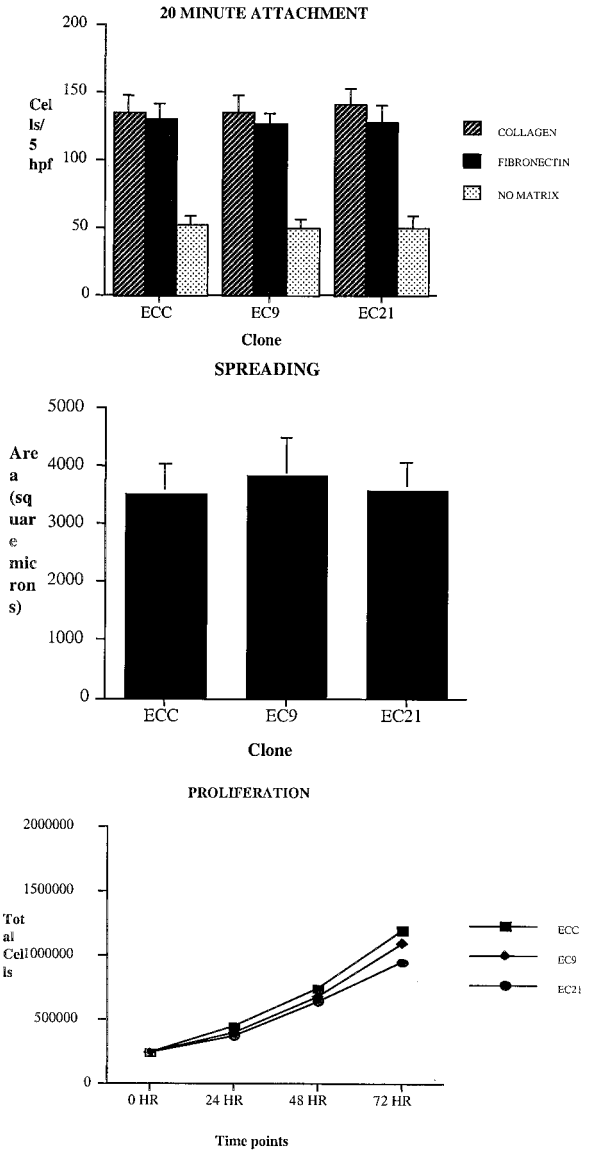

Fig 7. Effect of PAI-1 transfection on EC proliferation, attachment, and spreading. *Top graph* shows EC attachment for various clones after 20 minutes. Attachment assays were done with different types of matrix (type I collagen and fibronectin) and on uncoated glass cover slips. *Middle graph* shows cell spreading after 40 minutes. *Bottom graph* shows cell proliferation at various time points up to 72 hours. Assays run in triplicate, n = 6 experiments with cell passages 4 to 6. *P* = not significant between clones for all morphologic characteristics.

injury compared with mice with normal levels of PAI-1. Recently, Redmond and coworkers[19] have shown that EC release of PAI-1 inhibits flow induced SMC migration in coculture. Zempo et al[33] found that SMC migration into the subintima in injured rat carotid arteries is regulated by MMP-2 and MMP-9 activity. These data were further validated by the work of Forough et al[34,35] and Lijnen et al[36] who found that inhibition of MMPs caused a significant decrease in vascular SMC migration both in vitro and in vivo. In light of these prior findings suggesting that PAI-1 may play a role in regulation of vascular wall remodeling, we examined whether EC overexpression of PAI-1 inhibited SMC migration in vitro. We also sought to determine whether the mechanism by which this occurs is through PAI-1 inhibition of SMC MMP activation. In addition, we examined the morphologic characteristics of the PAI-1 overexpressing clones to determine whether the transfection process altered the cell function.

Our control cells, ECCs, which contained the pCMV/myc/ER vector without the PAI-1 insert, exhibited PAI-1 protein levels similar to untransfected EC. Stimulation of SMC migration with EC or ECC was equivalent. The EC cell population that overexpressed PAI-1 inhibited SMC migration. With the addition of increasing amounts of uPA to the media of the modified Boyden-chamber assay, the decrease in SMC migration caused by PAI-1 overexpressing clone EC9 was reversed. These data support the hypothesis that PAI-1 is responsible for the decrease in migration that we observed.

Zymographic analysis of SMC lysate from cells exposed to various EC clones revealed significant differences in MMP activity. Gelatin-based zymography showed diminished MMP-2 and MMP-9 levels in the SMC exposed to

PAI-1 overexpressing EC. Mason et al[37] have shown that MMP-9 overexpression increased SMC migration and intimal hyperplasia in a rat model. These findings are similar to ours in which EC overexpression of PAI-1 diminished SMC MMP activity and decreased SMC migration. The decrease in MMP-9 activity of the SMC found both on zymography and Western blot likely inhibits the ability of these cells to digest their surrounding matrix and migrate. Our data suggest that EC overexpression of PAI-1 may favorably affect arterial intimal hyperplasia by downregulating MMP activity and the subsequent migration of SMC.

A second explanation for the increase in MMP levels observed in cell lysates of SMCs cocultured opposite control ECs compared with the PAI-1 overexpressing ECs could be explained with increased secretion of MMP-2 and MMP-9 by the ECs themselves. The MMPs secreted by control ECs possibly could simply cross the semipermeable membrane and become bound to the SMC. This is unlikely to have occurred because no differences were observed between PAI-1 overexpressing and control ECs in the cell attachment and spreading studies. That a difference would be found in EC MMP-2 and MMP-9 secretion yet no difference found in EC spreading or attachment, especially in view of the multiple types of matrix the studies were conducted on, is unlikely. Nevertheless, because of the nature of the coculture system used in these experiments, this possibility cannot be excluded.

The studies performed to assess the effect of PAI-1 transfection on EC proliferation, attachment, and spreading revealed no differences between overexpressing clones and control cells. The transfection process did not seem to alter the attachment, spreading, or proliferation characteristics of the PAI-1 overexpressing clones. Our attachment results, as expected, are different from those of Dunn et al,[38] whose studies showed that EC transfected with a tissue-type plasminogen activator encoding vector had significantly diminished attachment abilities compared with control. This difference is most likely related to the opposing proteolytic properties of tissue-type plasminogen activator and PAI-1. However, even though PAI-1 inhibits proteolysis and one might expect to find enhanced attachment, our transfected cells exhibited normal attachment properties with the conditions studied.

In summary, our studies revealed that EC overexpression of PAI-1 inhibits SMC migration and MMP expression. The mechanism for the decrease in SMC migration is likely related to PAI-1 inhibition of plasmin generation, which in turn may diminish SMC MMP-9 activity. EC overexpression of PAI-1 may ultimately have a favorable effect on arterial wall repair and remodeling.

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