Urokinase and Tissue-type Plasminogen Activator Are Required for the Mitogenic and Chemotactic Effects of Bovine Fibroblast Growth Factor and Platelet-derived Growth Factor-BB for Vascular Smooth Muscle Cells*

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The present study was undertaken to evaluate *in vitro* the relative importance of tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) in the mitogenic and chemotactic potential of bovine fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF-BB) for smooth muscle cells (SMC). Aortic SMC were isolated from transgenic mice showing single inactivations of the t-PA, u-PA, plasminogen activator inhibitor-1, or urokinase-type plasminogen activator receptor (u-PAR) genes. With regard to serum-induced proliferation, all cell types showed similar responses. However, SMC isolated from t-PA-deficient mice did not proliferate or migrate in response to PDGF, whereas SMC isolated from u-PA-deficient animals appeared to be much less sensitive to bFGF than the cells isolated from the other animals. Supplementation of cells deficient with factors with regard to both migration and proliferation. The mitogenic and chemotactic responses of bFGF were specifically inhibited in u-PAR-deficient cells and in wild-type SMC, cultured in the presence of antibodies to u-PAR. The role of u-PA and t-PA in bFGF and PDGF-induced growth and migration of SMC was not dependent on plasmin generation and activity as demonstrated by the inactivity of e-amino caproic acid and aprotinin. A 4–5-fold increase in the steady-state levels of u-PA and t-PA mRNA and proteins were observed after 24 h of incubation of the cell cultures with bFGF and PDGF-BB, respectively. These results therefore indicate that, at least *in vitro*, t-PA is an important element of the activity of PDGF-BB with regard to the proliferation and migration of SMC whereas u-PA is a key factor in the effect of bFGF on SMC.

The accumulation of neointimal SMC resulting from media smooth muscle proliferation and migration in response to vascular injury is believed to be one of the main events involved in the initiation of atherosclerosis or during restenosis following angioplasty (1, 2). Although the general contribution of thrombosis to the development of atherosclerosis has been acknowledged for a long time, recent investigations suggested that the fibrinolytic system may also play an important role in the process of cell proliferation or migration (3, 4). Indeed, several authors described both a mitogenic and a chemotactic effect of two types of plasminogen activators: t-PA and u-PA for several cell types including vascular smooth muscle cells (5–8) and a recent study (10) showed that both u-PA and t-PA produced by endothelial cells were sequestered in an active form by the subendothelial extracellular matrix suggesting that they may participate in sequential matrix degradation during cell invasion but also function in the release of extracellular matrix-bound growth factor-like bFGF, that will stimulate SMC growth, a crucial step in atherosclerosis or post-percutaneous transluminal coronary angioplasty restenosis.

Most interesting were the works of Clowes et al. (7) showing that vascular SMCs express u-PA during mitogenesis and t-PA during migration in balloon-injured rat carotid artery. These data which suggested that both u-PA and t-PA might have specific functions related to SMC proliferation and migration during atherosclerosis were recently confirmed by the same authors (11, 12) who provided further evidence for the involvement of both u-PA and t-PA in the myointimal hyperplasia following endothelial injury. Moreover, the recent gene transfer and gene targeting studies performed on mice for several fibrinolytic components such as t-PA, u-PA, PAI-1, u-PAR, and plasminogen provided further evidence for the major role played by these factors in the process leading to neointima formation and atherosclerosis (3). These studies provided direct genetic evidence for a significant role of fibrinolytic enzymes in vascular wound healing and arterial neointima formation after injury, most likely by affecting cellular migration and proliferation (3, 13). Since growth factors and components of the fibrinolytic system seem to be closely related, the main aim of this *in vitro* study was to investigate the effect of bFGF and PDGF-BB on the growth and migration of aortic SMC isolated from mice deficient in t-PA, u-PA, PAI-1, and u-PAR.

**MATERIALS AND METHODS**

*Animals—* Homozygous t-PA−/−, u-PA−/−, PAI-1−/−, and u-PAR−/− mice were obtained and characterized as described previously (3, 14, 15). Isolation and Culture of SMC—SMC were isolated as described previously (16). Briefly, media fragments of the aorta were incubated for 16 h at 37 °C in DMEM containing 0.15% collagenase, 5% FCS, penicillin–streptomycin, and aprotinin. A 4–5-fold increase in the steady-state levels of u-PA and t-PA mRNA and proteins were observed after 24 h of incubation of the cell cultures with bFGF and PDGF-BB, respectively. These results therefore indicate that, at least *in vitro*, t-PA is an important element of the activity of PDGF-BB with regard to the proliferation and migration of SMC whereas u-PA is a key factor in the effect of bFGF on SMC.

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1. The abbreviations used are: SMC, smooth muscle cells; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator; bFGF, basic fibroblast growth factor; PAI-1, plasminogen activator inhibitor-1; u-PAR, urokinase-type plasminogen activator receptor; PDGF-BB, platelet-derived growth factor (BB dimer); DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; LRP, low density lipoprotein receptor-related protein.

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cillin (100 IU/ml), streptomycin (100 μg/ml), and glutamine (4 mM). After incubation, SMCs were sedimented by gentle centrifugation (400 g for 10 min), resuspended in DMEM + 10% FCS, and grown at 37 °C in a humidified atmosphere of 5% CO₂ in air. Culture medium (DMEM + 10% FCS) was changed every 3 days and a confluent SMC monolayer was obtained after about 7 days. Cells were routinely used from the third to the sixth passage.

Proliferation Assays—Cells were plated sparsely (10⁴ cells/well) in 24-well cluster plates (Nunc, Denmark) in DMEM + 0.5% FCS. After 3 days, growth-arrested cells were exposed to increasing concentrations of FCS (A), bFGF (B), or PDGF-BB (C). After 3 days, cells were detached from triplicate wells by trypsin treatment and counted in a Coulter counter. Results are expressed as mean cell numbers ± S.D. (n = 6). p < 0.05 versus wild-type by analysis of variance.

Migration Assays—Wound assays were performed as described previously (17). Briefly, confluent monolayers of SMC in 35-mm dishes were wounded with a razor blade. After wounding, the cells were washed with PBS and further incubated for 20 h at 37 °C in DMEM containing 0.1% gelatin and increasing concentrations of FCS, bFGF, or PDGF. The cells were then fixed with absolute methanol and stained with Giemsa. Cells that had migrated from the edge of the wound were counted in seven successive 125-μm increments at 100 × magnification using a light microscope with an ocular grid. The cell numbers represent the mean determined from six different fields.

Expression of t-PA and u-PA by SMC—Levels of t-PA and u-PA mRNA in SMC, total cellular RNA was isolated from 5 × 10⁶ cells according to Chomczynski and Sacchi (19) and u-PA and t-PA mRNA expression was analyzed by Northern blotting. Samples were size-fractionated by electrophoresis on 1% agarose gels containing 7.5% formaldehyde. Capillary transfer to Nylon N1 (Amersham, United Kingdom) was performed overnight in standard saline citrate. Hybridizations were carried out at 42 °C for 18 h in hybridization buffer (50% formamide, 1 M NaCl, 10 × Denhardt’s solution, 0.5% SDS, and 100 μg/ml salmon sperm DNA). Nick-translated labeled cDNA were used as hybridization probes for t-PA and u-PA as already described (11). Washes were performed at room temperature in 2 × SSC, 0.1% SDS and finally at 60 °C in 0.1 × SSC, 0.1% SDS. Filters were rehybridized with a probe for human glyceraldehyde-3-phosphate dehydrogenase as a control for the amount of RNA in each lane and washed in the same conditions as above. Autoradiograms were obtained by exposure of filters to Amersham MP films at −80 °C using two intensifying screens.

Statistical Analysis—Values shown are means ± S.D. Statistical significant differences between groups were calculated by analysis of variance followed by Bonferroni correction. Statistical values were considered significant with a p < 0.05 value.

RESULTS

Mitogenic and Chemotactic Responses of SMC Isolated from Transgenic Mice—When added to growth-arrested aortic SMCs of control mice, FCS exhibited a dose-dependent mitogenic
effect (Fig. 1A). A similar mitogenic effect was observed with regard to the SMC isolated from mice deficient in t-PA, u-PA, PAI-1, or u-PAR. With regard to bFGF-induced SMC proliferation, all cell types responded normally compared with controls except SMC isolated from u-PA- or u-PAR-deficient mice which did not proliferate in response to bFGF (Fig. 1B). Similarly, all SMC types showed similar proliferative responses in the presence of increasing concentrations of PDGF-BB except SMC isolated from t-PA-deficient mice which did not proliferate in response to PDGF-BB.

As shown in Table I, similar results were observed concerning the migratory response of SMC to bFGF or PDGF-BB. Indeed, SMC isolated from u-PA-/- or u-PAR-/- animals did not migrate in response to bFGF whereas SMC isolated from t-PA-deficient animals appeared to be much less sensitive to PDGF-BB than the cells isolated from the other animals.

Effect of Antibodies on the Mitogenic and Chemotactic Response of SMC—As shown in Figs. 2, A and B, when a polyclonal antibody which neutralized the activity of t-PA was added in the presence of PDGF-BB, both the mitogenic and the chemotactic responses of cells isolated from wild-type animals were selectively inhibited. Similarly, a polyclonal antibody directed against u-PA reduced bFGF-induced proliferation and migration of control SMCs and did not affect the responses of these cells to PDGF-BB. Preliminary experiments had demonstrated that neutralizing antibodies selectively and completely abolished the PA activity evaluated in a chromogenic assay of both purified enzymes and conditioned media of FCS-treated wild-type SMC.

We then determined if supplementation of t-PA- or u-PA-deficient SMC by purified exogenous fibrinolytic enzymes would affect their mitogenic and chemotactic response of bFGF or PDGF-BB. For that purpose, proliferation and migration of SMC from u-PA- or t-PA-deficient mice was measured in the presence of bFGF or PDGF-BB and exogenous murine u-PA and t-PA prepared as described (15). As shown in Figs. 3, A and B, the addition of exogenous u-PA to u-PA-deficient cells and of t-PA to t-PA-deficient cells restored both the mitogenic and chemotactic response of bFGF or PDGF-BB, respectively. This effect was highly specific since the addition of exogenous t-PA did not restore the response of u-PA-deficient cells to bFGF and the addition of u-PA did not restore the mitogenic and chemotactic activity of PDGF-BB for t-PA-/- cells (Fig. 3, A and B).

To further investigate the ability of u-PAR to modulate bFGF-induced SMC growth and migration, we performed blocking experiments in the presence or absence of an antibody to u-PAR known to inhibit the binding of u-PA to u-PAR (20). This monoclonal antibody (number 3936 from American Diagnostica) which cross-reacted with murine u-PAR (not shown), inhibited bFGF-induced SMC growth and migration when tested at 25 μg/ml (87 and 79% inhibition, respectively; p < 0.001). The present data are consistent with a possible role of u-PAR in signal transduction and/or plasmin generation. It is noteworthy that this monoclonal antibody did not affect PDGF-induced SMC growth and migration (not shown).

Since cleavage of protease-inhibitor complexes from the cell surface has been postulated to be a critical cell function that may enable returning unoccupied urokinase receptors to the cell surface and since u-PA/PAI-1, t-PA/PAI-1, and scu-PA have been demonstrated to be internalized via the low density lipoprotein receptor-related protein (LRP) (21–23), we hypothesized that LRP function may be necessary for SMC migration and growth. When we incubated SMC with an anti-LRP antibody (25 μg/ml, American Red Cross) we found that specific...
inhibition of LRP significantly inhibited bFGF-induced migration and growth of wild-type SMC (35 and 44% inhibition, respectively, \( p < 0.05 \)) but totally abrogated the effect of PDGF-BB with regard to both the migration and growth of control SMC (98 and 89% inhibition, respectively, \( p < 0.001 \)). These observation shows that, 1) as suggested by others (24), the importance of LRP in SMC migration and growth may enable the return of functional unoccupied u-PARs to the cell surface, 2) that the effect of PDGF-BB on growth and migration of SMC occurs via the binding and internalization of t-PA by LRP on SMC, and 3) LRP plays a similar role for t-PA as for u-PA in binding and internalization of t-PA alone or complexed with PAI-1, and recycling of t-PA binding sites/receptors, but this latter mechanism needs to be further defined.

Effect of bFGF and PDGF-BB on the Expression of t-PA and u-PA by SMC—Since we have previously demonstrated that both the mitogenic and chemotactic responses of SMC to bFGF and PDGF-BB were related to the presence and/or activity of u-PA and t-PA, we determined if these growth factors affected the expression of these fibrinolytic enzymes in growth-arrested wild-type SMC. As shown in Fig. 4 and Table II, no significant modifications of the level of u-PA protein and mRNA were observed in PDGF-treated cultures of SMC. Similarly, the addition of bFGF did not affect the level of t-PA mRNA and protein but, on the contrary, when bFGF was added, a strong increase of steady state levels of u-PA mRNA and protein was observed in wild-type SMC (Fig. 4) but also in SMC from transgenic animals (Table II). These observations confirm previous results showing that the addition of exogenous bFGF to endothelial cells induced a rapid increase in steady-state levels of both u-PA mRNA and protein (8, 25, 26). Similarly the addition of PDGF to SMC resulted in a significant modification of the levels of both t-PA protein and transcript (Fig. 4 and Table II). It is noteworthy that, under basal conditions, in the absence of the specific gene, there was no compensatory changes in other closely related genes (not shown).

Effect of Inhibitors on the Mitogenic and Chemotactic Activity of bFGF and PDGF—The requisite role of bFGF and PDGF-induced u-PA and t-PA expression in SMC growth and migration was further defined by the results of the experiment illustrated in Fig. 5 which shows that the addition of \( \alpha \)-aminocaproic acid or aprotinin at doses which abrogated the activity of plasmin were unable to inhibit cell growth and movement induced by these growth factors. These results, in agreement with those published by us and others (5, 8, 27) show that the receptor binding but not the plasminogenolytic activity of t-PA and u-PA was necessary for their ability to stimulate growth and migration of vascular cells and demonstrate that this effect does not occur through the generation of plasmin by t-PA and u-PA.

To determine if the catalytic activity of t-PA and u-PA was necessary for the mitogenic and chemotactic activity of bFGF and PDGF, the effect of PAI-1 and \( \alpha \)-antitrypsin, both natural inhibitors of these fibrinolytic enzymes, was tested. As shown in Fig. 6, these two proteins reduced in a dose-dependent manner the mitogenic and chemotactic activity of PDGF but did not
While proliferation in the arterial wall are still poorly characterized. The involvement of fibrinolytic enzymes in such a process came from a number of studies (3, 5, 32–36). The first evidence for the involvement of fibrinolytic enzymes in the arterial wall was provided by Clowes et al. (7) showing that u-PA was expressed by vascular SMC during mitogenesis and t-PA during migration. These authors suggested that these plasminogen activators may act on SMC growth and migration through their ability to convert plasminogen into plasmin which degraded extracellular matrix glycoproteins and released active heparan sulfate-bFGF complexes that activated SMC growth (37). However, this hypothesis did not take into account a direct effect of plasminogen activators such as u-PA or t-PA on the SMC proliferation or migration processes. In that respect, we reported for the first time a direct and selective mitogenic activity of t-PA for SMC (5) raising the possibility of a direct active role of the fibrinolytic system in vascular SMC replication following injury. More recently, targeted gene manipulation and transfer of the plasminolytic system performed in mice for several fibrinolytic components such as u-PA, t-PA, PAI-1, u-PAR, and plasminogen confirmed the possibility of a direct active role of the fibrinolytic system in vascular SMC replication following injury. More recently, targeted gene manipulation and transfer of the plasminolytic system performed in mice for several fibrinolytic components such as u-PA, t-PA, PAI-1, u-PAR, and plasminogen confirmed the major role played by these factors during neointima formation and atherosclerosis (3, 13). In the present work, using SMC isolated from these animals, we provide evidence that the induction of u-PA and t-PA are fundamental mechanistic components of SMC migration and growth induced by bFGF and PDGF-BB, respectively.

Several works already reported that the induction of u-PA was observed in migrating endothelial cells after wounding was

$${a}$$ BDL, below detection limits.

| Cells          | bFGF (10 ng/ml) t-PA | PDGF (100 ng/ml) t-PA |
|---------------|---------------------|-----------------------|
| Wild-type     | 22 ± 3              | 4 ± 1                 |
| t-PA $^{+/−}$ | 25 ± 6              | 5 ± 2                 |
| u-PA $^{+/−}$ | 8 ± 1               | 23 ± 3               |
| PAI-1 $^{+/−}$| 21 ± 4              | 5 ± 1                 |
| u-PAR $^{+/−}$| 25 ± 3              | 9 ± 2                 |

FIG. 5. Effect of plasmin inhibitors on the mitogenic and chemotactic effects of bFGF and PDGF-BB. A, growth: SMCs from wild-type mice (100 cells/well) were cultured in 24-well cluster plates in DMEM + 0.5% FCS. After 3 days, bFGF (10 ng/ml) or PDGF-BB (100 ng/ml) were added in the presence of 0.5% FCS and e-aminocaproic acid (full bars) or aprotinin (open bars) (100 μM). After 3 days, cells were detached from triplicate wells by trypsin treatment and counted in a Coulter counter. B, migration: confluent SMCs from wild-type mice cultured in DMEM + bFGF (100 ng/ml) or PDGF-BB (100 ng/ml) in the presence of e-aminocaproic acid (full bars) or aprotinin (open bars) (100 μM) as in A were wounded with a razor blade and incubated for 20 h at 37 °C. Cells that had migrated from the edge of the wound were counted using a light microscope with an ocular grid. Results are expressed as percent inhibition compared with the controls (n = 6).

FIG. 6. Effect of PAI-1 and α1-antitrypsin on the mitogenic and chemotactic effects of bFGF and PDGF-BB. A, growth: SMCs from wild-type mice (100 cells/well) were cultured in 24-well cluster plates in DMEM + 0.5% FCS. After 3 days, bFGF (10 ng/ml) (open symbols) or PDGF-BB (100 ng/ml) (full symbols) were added in the presence of 0.5% FCS and increasing concentrations of PAI-1 (circles) or α1-antitrypsin (squares). After 3 days, cells were detached from triplicate wells by trypsin treatment and counted in a Coulter counter. B, migration: confluent SMCs from wild-type mice cultured in DMEM + bFGF (100 ng/ml) (open symbols) or PDGF-BB (100 ng/ml) (full symbols) in the presence of increasing concentrations of PAI-1 (circles) or α1-antitrypsin (squares) as in A were wounded with a razor blade and incubated for 20 h at 37 °C. Cells that had migrated from the edge of the wound were counted using a light microscope with an ocular grid. Results are expressed as percent inhibition compared with the controls (n = 6).
dependent on endogenous bFGF (8) and that bFGF induced up-regulation of u-PA expression in endothelial cells by acting both at the transcriptional and post-transcriptional/translational levels (38). Our works confirm these observations, provide the first evidence for the selectivity of such an effect for u-PA, and extend these observations to another important growth factor: PDGF-BB which selectively induced an up-regulation of t-PA mRNA and protein in SMC. Basic FGF and PDGF-BB have been shown to affect the transcription of several genes in different cell types. A partial list includes nerve growth factor receptor in neuroblastoma cells (39), skeletal α-actin in cardiac muscle cells (40), type I collagen in osteoblastic cells (41), u-PAR (42), PDGFα receptor (43), and angiogenin converting enzyme (44) in SMC.

One important result of our present work is the high level of selectivity observed for bFGF and PDGF to induce the expression and release of u-PA and t-PA, respectively, but the underlying molecular mechanisms of this particular observation will have to be investigated thoroughly. Although the data shown in the present work correspond to the general idea of different functions of u-PA and t-PA during neointima formation or during the atherosclerosis process (7, 45–47), recent data showed that myointimal formation and neointimal cell accumulation was reduced in u-PA−/− and u-PA−/−; t-PA−/− mice, but not in t-PA−/− mice so therefore showing that u-PA plays a significant role in vascular wound healing and arterial neointima formation after injury, most likely by affecting cellular migration. Indeed, the role of u-PA in cell movement has been the object of several studies showing spacial and/or temporal changes in u-PA in migrating cells (8, 49–52). These studies suggested that u-PA expression was part of the program associated with onset of proliferation and migration and may at times be absolutely necessary for migration between tissue compartments. Our data demonstrate that, in response to a specific stimulus (bFGF), SMCs are capable of increased expression of u-PA and confirm the relation existing between proliferation, migration, and u-PA expression. A similar observation of u-PA as a mechanistic component of bFGF-induced migration has already been made for endothelial cells (8) and supports the conclusion drawn by several authors suggesting that the expression of u-PA is absolutely required for SMC proliferation and migration in vivo following vascular injury (6–8, 48).5

In addition to the stimulatory effects of u-PA and t-PA, these studies demonstrate an important modulatory role for u-PA and LRP in both SMC migration and growth. SMC isolated from u-PAR-deficient mice did not respond to bFGF and antibodies to u-PAR were able to inhibit both bFGF-induced migration and proliferation of SMC even when aprotinin and e-aminocaproic acid could not, suggesting that u-PA may modulate migration/invasion in a plasmin-independent fashion. Interestingly, a similar observation demonstrating a possible role of u-PA, independent of plasmin production, in cell adhesion and migration has been recently described (53).

In our study PAI-1 and α1-antitrypsin, both inhibitors of the catalytic activity of u-PA did not show any effect on bFGF-induced SMC migration/growth, therefore suggesting that single binding to u-PA may be adequate to initiate signal transduction. These studies also demonstrate a role for LRP in cell migration/growth induced by both bFGF (through u-PA) and PDGF-BB (through t-PA). This concept was supported by experiments that demonstrated inhibition of growth and migration by antibodies. Whether this effect of antibodies was related to diminished unoccupied u-PAR as suggested by Okada et al. (23) in another experimental system or any other reason remains, however, to be investigated. In that respect, altered expression of LRP and u-PA may also significantly affect the vascular response to injury as suggested by Watanabe et al. (54) who demonstrated up-regulation of LRP mRNA in aortas of rabbits that were fed a high-cholesterol diet and by Lupu et al. (55) who demonstrated both mRNA and protein in normal and atherosclerotic human arteries. Moreover, Noda-Heiny et al. (56) demonstrated increased vessel expression of u-PAR in cholesterol-fed rabbits and human atherosclerotic arteries. Our data therefore suggest that, since they may modulate the activity of u-PA and t-PA, both u-PA and LRP may also regulate bFGF- and PDGF-induced SMC migration and proliferation.

We have previously reported that t-PA exhibits a direct and selective mitogenic activity for SMCs (5). As observed in this work, this effect occurred at physiological concentrations of t-PA and was not mediated by t-PA-generated plasmin because plasmin inhibitors did not alter t-PA-induced SMC growth. Moreover, as already found for the mitogenic activity of t-PA for SMC, the catalytic activity of the enzyme was necessary for promotion of PDGF-induced t-PA-mediated migration and growth of SMC. Indeed, PDGF-induced SMC growth and migration was strongly affected by PAI-1, or α1-antitrypsin, both natural inhibitors of t-PA, but also by synthetic compounds such as diisofluoropropyl or Pefabloc-TPA (not shown), for all of which a good correlation between their inhibition of the catalytic activity of the enzyme and the mitogenic activity of PDGF was demonstrated. This observation suggests that SMC may possess a t-PA receptor analogous to the SMC thrombin or factor Xa receptors which exhibits a proteolytic mechanism of receptor activation (34–36, 57). The fact that PAI-1 has been shown to be expressed by SMC following PDGF, transforming growth factor β, bFGF, or thrombin stimulation (58, 59), together with the identification of a functionally active PAI-1 accessible to t-PA, identified in the extracellular matrix of cultured endothelial cells and SMC (9, 10, 48, 60) gives an added dimension to our observation showing that PAI-1 inhibited the mitogenic effect of t-PA. Indeed, it suggests that SMCs might thereby regulate their own plasminogen activators in an autocrine fashion. Such an hypothesis is reinforced by a recent paper (10) showing that, within the extracellular matrix of cultured SMCs, a large proportion of the endogenous resident t-PA is accounted for by binding to PAI-1, forming a stable complex in the extracellular matrix. Complex formation of t-PA with PAI-1 may serve to localize, concentrate, and protect t-PA activity on the cell surface and within the matrix, thereby providing a reservoir of PA activity. Moreover, although we did not observe differences in either the mitogenic or chemotactic responses of SMCs isolated from PAI-1-deficient mice to bFGF or PDGF (Fig. 1, Table I), neointima formation following vascular injury was accelerated in PAI-1-deficient mice suggesting that indeed, PAI-1 might act as a regulator of the activity of t-PA but also of u-PA which role in neointima formation has been demonstrated.3

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required to elucidate the precise mechanisms by which t-PA and u-PA act on SMC proliferation and migration in vivo. Nevertheless, it seems reasonable to propose that antagonism of t-PA and u-PA expression and/or activity within the vessel wall may be of therapeutical relevance in vascular occlusive disorders such as atherosclerosis and restenosis following balloon angioplasty or stent placement.

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