SPARC Induces the Expression of Type 1 Plasminogen Activator Inhibitor in Cultured Bovine Aortic Endothelial Cells

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SPARC, a Ca²⁺-binding glycoprotein that is expressed during tissue morphogenesis and functions as an inhibitor of cell spreading in vitro, was found to induce the secretion of an M₉, 45,000 protein in bovine aortic endothelial (BAE) cells. This protein was identified as type 1 plasminogen activator inhibitor (PAI-1) on Western blots with anti-PAI-1 antiserum. SPARC stimulated the secretion of PAI-1 protein into the medium of subconfluent BAE cells, but not confluent BAE cells, in a dose- and time-dependent manner. Secretion of PAI-1 into the culture medium was progressive and exhibited an increase of 3- to 7-fold over control values within 24 h after the addition of SPARC. Levels of PAI-1 mRNA were elevated 2-fold within 4 to 24 h after the addition of SPARC and did not increase with higher concentrations of SPARC. Since the induction of PAI-1 mRNA by SPARC was not blocked by cycloheximide, de novo protein synthesis was apparently not required for this stimulation. Control experiments showed that the induction of PAI-1 was not due to contamination of the SPARC preparations with endotoxin. These data demonstrate that SPARC induces the biosynthesis of PAI-1 in BAE cells and suggest a role for SPARC in the regulation of fibrinolysis and in the control of proteolytic events in remodeling tissues.

Endothelial cells form a nonthrombogenic lining of the vascular system. Thromboresistance is achieved not only by the formation of a continuous sheet of cells, but also by the production of substances that function to maintain the hemostatic balance. In addition, several proteins are secreted that are associated with the cell surface and/or the underlying endothelial extracellular matrix (ECM). Vascular endothelial cells interact with components of the ECM to ensure thromboresistance, promote angiogenesis, and transduce molecular signals (1-3), which in turn could result in the induction of gene products that modulate cellular behaviour.

One of these products has been identified as SPARC (secreted protein, acidic and rich in cysteine), a secreted Ca²⁺-binding glycoprotein that is associated with morphogenesis, remodeling, proliferation, and cellular migration (4-7). Previous studies have shown that addition of purified SPARC to bovine endothelial cells, fibroblasts, and smooth muscle cells causes a rounded cell morphology and inhibits the spreading of plated cells. The binding of SPARC to several ECM components, including type III and type V collagens, has led to the hypothesis that the rounding effect of SPARC on endothelial cells is mediated through these interactions, which create in part an environment permissive for cellular migration and remodeling.

Activation of plasminogen also plays a key role in these processes, since it provides an important source of proteolytic activity during ovulation, embryonic implantation, cell migration, neovascularization, tumor invasion, epithelial differentiation, and fibrinolysis (8-11). Regulation of plasminogen activator activity is therefore a crucial event in many biological processes. In this regard, specific inhibition of plasminogen activators by the action of plasminogen activator inhibitors (PAIs) is an important and fundamental mechanism of biological control (12-14). Although at least four distinct molecules with PAI activity have been identified, type 1 plasminogen activator inhibitor (PAI-1) appears to be the primary physiologic inhibitor of both tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator.

The biosynthesis and secretion of PAI-1 in cultured endothelial cells is increased by various physiologic or pathologic mediators, such as thrombin, endotoxin or lipopolysaccharide (LPS), lipoprotein (a), transforming growth factor-β (TGF-β), basic fibroblast growth factor, interleukin-1, lymphokinin, and tumor necrosis factor-α (15-26). These agents can alter systemic or local concentrations of PAI-1. Localized deposition of PAI-1 in the ECM might effectively limit the generation of extracellular proteolytic activity.

In this study, we demonstrate that SPARC induces PAI-1 expression in subconfluent bovine aortic endothelial (BAE) cells in a dose- and time-dependent manner, and that this induction occurs at both the protein and mRNA levels. These data suggest a role for SPARC in the control of proteolytic events in tissues that undergo remodeling, as well as in the regulation of fibrinolysis.

**EXPERIMENTAL PROCEDURES**

Materials—Reagents were obtained as follows: Dulbecco’s modified Eagle’s medium (DMEM), amphotericin, penicillin, streptomycin sulfate, cycloheximide, polymyxin B, LPS, rabbit brain cephalin, N-ethylmaleimide, phenylmethanesulfonyl fluoride, TWEEN 20, and COOMASSIE Brilliant Blue R-250 from Sigma; fetal calf serum (FCS) from Flow Laboratories; tissue culture plates from Costar; papastin A from Peninsula Laboratories; dithiothreitol (DTT) and acrylamide...
from Bio-Rad; molecular weight standards from Bethesda Research Laboratories; sodium dodecylsulfate (SDS) from British Drug House; nitrocellulose from Schleicher & Schuell; Multiprime DNA labeling system, 1,2,3,4,5-[^32]P-CTP from Amersham; 125I-Protein A from Du Pont-New England Nuclear; GeneClean® from Bio 101; X-Omat AR x-ray film from Kodak; normal pooled human plasma from George King Biomedical; kaolin from Fischer Scientific; and Limulus amebocyte lysate (Pyrotell) from Associates of Cape Cod. Purified annexin V (placental anticoagulant protein-I) was a kind gift from Dr. K. Fujikawa (University of Washington, Seattle, WA). Antiserum to purified bovine PAI-1 was obtained as previously described (27). TGF-β1 was a gift from Elaine Raines (University of Washington, Seattle, WA). Partially purified thromboplastin was a gift from Dr. W. Kisiel (University of New Mexico, Albuquerque, NM).

**Purification of SPARC—**SPARC protein was purified from parietal yolk sac (PYS)-2 cells as previously described (7). The SPARC preparations employed in these studies had less than 0.02 ng of LPS/μg of SPARC as determined with the Limulus amebocyte lysate assay (28).

**Cell Culture—**BAE cells were cultured as previously described (7). The cells were grown in DMEM containing 10% FCS, 250 μg/ml amphotericin B, 100 units/ml penicillin, and 100 units/ml streptomycin. Experiments were performed in a highly controlled culture system with several separate cultures of BAE cells between passages 4 and 8. For the experiments with subconfluent and confluent cultures, the cells were plated in 6-well tissue culture plates (Costar) at a density of 2.5 x 10⁶ cells/cm². Twelve hours after plating (subconfluence)(subconfluences) or 1 day after reaching confluence (confluence)(confluences), the cells were washed twice with serum-free DMEM and incubated for 16 h in serum-free DMEM to remove all serum components. After an additional wash, 1 ml of serum-free DMEM or DMEM/1% FCS was added to each well. Additions of purified SPARC, TGF-β1, LPS, cycloheximide, and polymyxin B were done as indicated. To study the biosynthetic profile of SPARC-treated and control BAE cells, the cells were metabolically labeled for 24 h with 50 μCi/ml[^35]SPro. All experiments in this study were performed at least three times with different strains of BAE cells and different preparations of SPARC.

**Isolation and Analysis of Proteins in Conditioned Medium—**Conditioned medium of BAE cells was collected at indicated times, clarified by centrifugation (10 min at 1,200 × g), and dialyzed against 0.1 M acetic acid in the presence of the protease inhibitors N-ethylmaleimide (5 mM), phenylmethylsulfonyl fluoride (0.2 mM), and pepstatin A (10 μg/ml). The dialyzed samples were lyophilized and subsequently used for SDS-PAGE (29). Proteins from conditioned medium were analyzed by SDS-PAGE in the presence or absence of 50 mM DTT, followed by fluorescence autoradiography or Western blotting. For autoradiography, gels were stained with 0.2% Coomassie Blue in 45% methanol, 9% acetic acid, and dried. After autoradiography, proteins were transferred to nitrocellulose and (pre)hybridizations of blots were performed to a final stringency of 0.6 X SSC, 0.5% SDS at 65 °C for the PAI-1 probe and 0.1 X SSC, 0.1% SDS at 65 °C for the 28 S RNA cDNA probe. Blots were exposed to preflashed x-ray film with intensifying screens at −70 °C.

**RNA Extraction, Northern Blotting, and Hybridization—**Total cytoplasmic RNA was isolated from BAE cells by the single-step method described by Chomczynski and Sacchi (32). Electrophoresis of RNA, transfer to nitrocellulose, and (pre)hybridizations of blots were performed as previously described (33). Hybridization washes were performed in 0.6 X SSC, 0.5% SDS at 65 °C for the PAI-1 cDNA probe and 0.1 X SSC, 0.1% SDS at 65 °C for the 28 S RNA cDNA probe. Blots were exposed to preflashed x-ray film with intensifying screens at −70 °C. The density of autoradiographic signals was quantitated with a laser scanning densitometer equipped with an Integrator (110 Ultramark III SpectroKodak) and a normal pocket histogrammer. The amounts of PAI-1 mRNA and 28 S ribosomal subunit RNA were expressed as the number of integrator units corresponding to individual bands. Values of 28 S RNA were used to correct for differences in loading of RNA. Within one experiment, the amounts of PAI-1 mRNA were normalized and expressed as relative values. A relative value of 1 refers to the normalized level of PAI-1 mRNA 8 h after the addition of PBS to the cells.

**Preparation of DNA Probes—**The PAI-1 probe was a fragment from the human cDNA (24, 34). A 280-base pair bovine 28 S ribosomal subunit cDNA probe was used for normalization (35). DNA fragments were isolated from gel slices with the adsorbent GeneClean and were nick-translated with a Multiprime DNA labeling system in the presence of [α-32]P-CTP to yield specific activities of 15 and 10 X 10⁶ cpm/μg for the PAI-1 and 28 S probes, respectively. Probes were separated from the reaction mixture with GeneClean.

**In Vitro Coagulation Assays—**Activated partial thromboplastin time (APTT) and prothrombin time (PTT) were performed by the tilttube method in siliconized glass tubes as previously described (36). A cephalin stock solution was prepared by resuspending one vial of rabbit brain cephalin in 100 ml of saline. For the APTT, 20 μl of cephalin was preincubated for 30 min at 37 °C with an equal volume of SPARC (0.6–20 μg), PBS, or annexin V (2 μg). Twenty microliters of pooled normal plasma and 20 μl of kaolin (5 mg/ml) were added to the mixture. After 3 min at 37 °C, coagulation was started by the addition of 20 μl of 50 mM CaCl₂, and the clotting time was measured in triplicate. For the PTT, 20 μl of both human brain thromboplastin and SPARC, PBS, or annexin V were preincubated for 30 min at 37 °C, after which 20 μl of pooled normal plasma was added. After an incubation of 1 min at 37 °C, 20 μl of 50 mM CaCl₂ was added, and the clotting time was measured in triplicate.

**RESULTS**

Initially, we assessed the effect of exogenous SPARC on the biosynthetic profile of BAE cells. Subconfluent BAE cells were incubated for 24 h in the presence of SPARC (20 μg/ml) and [^35]SPro. Subsequently, radiolabeled proteins secreted into the conditioned medium were analyzed by SDS-PAGE and fluorescence autoradiography. In comparison to untreated BAE cells, the addition of SPARC resulted in a significant increase in the secretion of a nonreducible Mr = 45,000 protein into the culture medium (Fig. 1). Immunoreactivity with an antisemir to bovine PAI-1 in Western blots indicated that this protein was PAI-1.

These data led us to investigate the induction of PAI-1 by the addition of SPARC.
SPARC. To rule out the possibility that the effect of SPARC on the induction of PAI-1 was caused by a contamination of the SPARC preparations with LPS, we performed several control experiments. First, the antibiotic polymyxin B was used to neutralize the effect of LPS. Addition of polymyxin B (10 μg/ml) to BAE cell cultures diminished the induction of PAI-1 mRNA by LPS (10 ng/ml), but also significantly reduced the induction of PAI-1 mRNA in control cultures and in cultures that were treated with TGF-β1 (2.5 ng/ml) or SPARC (20 μg/ml) (Fig. 2). Previous studies showed that BAE cells are extremely sensitive to LPS and significantly up-regulate PAI-1 with concentrations of LPS >20 ng/ml. However, in the absence of FCS, significant induction of PAI-1 mRNA was found only with concentrations of LPS ≥10 ng/ml (24, 37). Since the LPS content in the added preparations was less than 0.006 ng/2.5 ng of TGF-β1 and less than 0.4 ng/20 μg of SPARC, as measured by Limulus assay, the inhibitory effect of polymyxin B was probably caused by a toxic effect of polymyxin B on BAE cells rather than by specific neutralization of LPS. To assess directly the sensitivity of BAE cells to LPS, confluent monolayers of BAE cells were incubated for 9 h with increasing concentrations of LPS (0.1–100 ng/ml) in the absence or presence of 1% FCS. In the presence of FCS, induction of PAI-1 mRNA was seen with concentrations of LPS ≥0.3 ng/ml; the maximum level of induction was found with 1 ng of LPS/ml (Fig. 5A). However, in the absence of FCS, significant induction of PAI-1 mRNA was found only with concentrations of LPS ≥30 ng/ml. A similar result was obtained with respect to secreted PAI-1 protein (Fig. 3B). Whereas significant secretion of PAI-1 into the medium was seen with all concentrations of LPS tested, the same concentrations did not induce secretion of PAI-1 in the absence of FCS. Since BAE cells are apparently much less sensitive to LPS in the absence of FCS, all experiments were performed under serum-free conditions. In comparison to confluent cultures, LPS showed a somewhat different effect on subconfluent BAE cell cultures in the absence of FCS (Fig. 4). All concentrations of LPS (0.1–30 ng/ml) caused secretion of PAI-1 into the medium (from 1.3-fold with 0.1 ng of LPS/ml to 2-fold with 30 ng/ml). However, addition of 25 μg/ml SPARC (containing <0.5 ng of LPS/ml) elicited a 5-fold increase in the secretion of PAI-1. This observation supports the conclusion that the effect of SPARC on PAI-1 was not caused by LPS as a contaminant.

Subconfluent and confluent cultures of BAE cells were subsequently incubated for 24 h in the absence or presence of SPARC (25 μg/ml), and the levels of PAI-1 secreted into the medium were analyzed by Western blotting (Fig. 5A). Whereas SPARC, added to subconfluent BAE cells, induced a 3-fold increase in the secretion of PAI-1, there was no apparent effect of SPARC on confluent BAE cells. Therefore, all subsequent experiments were performed on subconfluent cells.

Subconfluent BAE cell cultures were incubated for various times under serum-free conditions in the absence or presence of 30 μg/ml SPARC, and the secreted levels of PAI-1 were quantitated by scanning densitometry of Western blots (Fig. 5B). PAI-1 was first observed in the conditioned medium 12 h after the addition of SPARC, and increased levels of 3-fold over control were apparent within 24 h.

As part of the same experiments, total RNA was extracted and analyzed. Fig. 6 shows the Northern blot and the values obtained from scanning densitometry of PAI-1 mRNA, nor-
SPARC induces the secretion of PAI-1 in subconfluent BAE cells: time course. A, subconfluent and confluent BAE cell cultures were incubated for 24 h in the absence (closed bars) or presence (hatched bars) of 25 µg/ml SPARC. Proteins secreted into the medium were analyzed by SDS-PAGE and Western blotting. Shown is the densitometry scan of the autoradiographic signals. B, densitometry scan of PAI-1 that is secreted by subconfluent BAE cells over time when incubated in the absence (0) or presence (0) of 30 µg/ml SPARC. The amounts of secreted PAI-1 protein are expressed as relative values (mean ± S.D. of three experiments), a value of 1 corresponding to the amount of PAI-1 secreted by subconfluent BAE cells after an incubation of 24 h in the absence of SPARC.

Fig. 5.

Time course of the induction of PAI-1 mRNA by SPARC. Subconfluent cultures of BAE cells were incubated in the absence (●) or presence (○) of 30 µg/ml SPARC (SP). Total cytoplasmic RNA was extracted at the indicated times and analyzed by Northern blotting (5 µg of RNA/lane). A, the blot was hybridized with a PAI-1 cDNA probe and subsequently with a 28 S RNA cDNA probe. B, PAI-1 mRNA levels were quantitated by densitometry and expressed as relative values (mean ± S.D. of three experiments).

SPARC induces a dose-dependent expression of PAI-1 protein. BAE cells were incubated for 16 h with the indicated concentrations of SPARC (0–60 µg/ml). A, proteins that were secreted into the medium were resolved by SDS-PAGE (−DTT), transferred to nitrocellulose, and analyzed by immunoblotting with rabbit anti-PAI-1 serum followed by 125I-Protein A. Positions of molecular weight standards are indicated (M, ×10^6). B, total cytoplasmic RNA was extracted and analyzed by Northern blotting with cDNA probes for PAI-1 and 28 S rRNA. Levels of PAI-1 mRNA were quantitated by densitometry and expressed as relative values (mean ± S.D. of three experiments).

Table I. Effect of cycloheximide on the expression of PAI-1 mRNA induced by SPARC

| Addition  | PAI-1 mRNA level (relative value)* |
|-----------|-----------------------------------|
| PBS       | 1.0                               |
| SPARC     | 1.6 ± 0.3                         |

Values are relative mean ± S.D. of three experiments.

7-fold increase in the amount of secreted PAI-1 protein was found when the cells were incubated with 60 µg/ml SPARC. The dose-dependent induction of PAI-1 mRNA is shown in Fig. 7B. PAI-1 mRNA levels were similar to control values in the presence of 1–15 µg/ml SPARC. In contrast to the levels of induced protein, a 2-fold induction of PAI-1 mRNA was found upon incubation of the cells with 30 or 60 µg/ml SPARC, as quantitated by densitometric scanning of the autoradiogram and normalization for cell number with the 28 S RNA signal.

To determine whether de novo protein synthesis was required for the induction of PAI-1 mRNA by SPARC, total RNA was isolated from subconfluent BAE cells that were incubated for 6 h with 25 µg/ml SPARC in the absence or presence of cycloheximide (4 µg/ml), an inhibitor of protein synthesis. As shown in Table I, cycloheximide did not block the effect of SPARC. Incubation of BAE cells with cyclohex-
imide alone increased the level of PAI-1 mRNA 2.5-fold.

The NH2-terminal domain of SPARC (domain I) is a glutamic acid-rich domain that is responsible for most of the Ca2+-binding properties of the protein (38, 39). Although previous studies have demonstrated that Gla is not present in the molecule (7), a potential effect of SPARC in in vitro coagulation has never been investigated. Since SPARC induces the antifibrinolytic protein PAI-1, we questioned whether SPARC also exerts its effect on intrinsic and/or extrinsic coagulation pathways and thereby contributes to the regulation of the hemostatic balance. To test this possibility, SPARC was included in incubation mixtures of APTT and PTT assays, and the resulting clotting times were measured. In contrast to the anticoagulant protein annexin V (placental anticoagulant protein I), the presence of which resulted in significant prolongation of both the APTT and PTT, SPARC (0.6–20 μg) did not alter the normal (control) coagulation time (Table II).

**DISCUSSION**

SPARC is a secreted, Ca2+-binding glycoprotein that is induced in conjunction with cellular differentiation, proliferation, stress, and certain developmental signals (4–7, 40). Molecular cloning and protein sequence analysis have demonstrated that SPARC is identical with osteonectin, a protein that was originally described as a major noncollagenous protein of bone that bound hydroxyapatite and type I collagen (5, 38, 41, 42). SPARC is also identical with BM-40, a product of a basement membrane-secreting tumor cell line (43, 44), and to an Mr = 43,000 "culture shock" protein that was increased after LPS-mediated injury of endothelial cells (40). It has been shown previously that addition of SPARC to cells in culture elicited a rounded morphology that was correlated with an inhibition of cell spreading, and that SPARC bound specifically to several components of the ECM (7). This interaction is thought to be permissive for cellular migration and morphogenesis.

Proteolytic degradation or modification of the ECM has a significant influence on many biological processes, such as cellular proliferation, differentiation, adhesion, migration, and tissue morphogenesis. An important source of proteolytic activity during these processes is the serine protease plasminogen activator, which converts the inactive zymogen plasminogen into the broad spectrum protease plasmin (9–11). It has become clear that PAI-1, formerly called the endothelial cell inhibitor (45–47) plays an important role in the regulation of plasminogen activation. PAI-1 is secreted by various cell types ranging from 0.6-20 pg, produced similar results. Annexin V (2 pg) inhibits gene transcription by inhibiting the synthesis of a repressor for the PAI-1 gene. Alternatively, cycloheximide might inhibit the synthesis of a labile RNA-degrading protein and thereby increase the stability of PAI-1 mRNA. Whether the observed increases in steady state PAI-1 mRNA by SPARC in BAE cells result from enhanced transcription of the PAI-1 gene or from mRNA stabilization is presently unknown. Most likely, SPARC primarily affects the rate of PAI-1 gene transcription, as has been demonstrated with BAE cells that were stimulated with LPS, TGF-β, and tumor necrosis factor, that elicited maximal induction of PAI-1 mRNA within 6–18 h of incubation (24, 25, 37), both lipoprotein (a) and SPARC induced a 2-fold elevation of PAI-1 mRNA that lasted at least 24 h.

Experiments with cycloheximide indicated that de novo protein synthesis is not required for the induction of PAI-1 mRNA by SPARC. In fact, cycloheximide itself increased the accumulation of PAI-1 mRNA. This result confirms previously reported data (24) and implies that cycloheximide activates gene transcription by inhibiting the synthesis of a repressor for the PAI-1 gene. Alternatively, cycloheximide might inhibit the synthesis of a labile RNA-degrading protein and thereby increase the stability of PAI-1 mRNA. Whether the observed increases in steady state PAI-1 mRNA by SPARC in BAE cells result from enhanced transcription of the PAI-1 gene or from mRNA stabilization is presently unknown. Most likely, SPARC primarily affects the rate of PAI-1 gene transcription, as has been demonstrated with BAE cells that were stimulated with LPS, TGF-β, and tumor necrosis factor (24).

We observed that subconfluent BAE cells, but not quiescent, confluent cells, up-regulate PAI-1 after the addition of SPARC. This result implicates SPARC as a mediator during endothelial cell injury. In this regard, it is interesting that SPARC itself is secreted at higher levels by subconfluent cells and after LPS-mediated injury and is diminished in confluent monolayers (33, 40). Unfortunately, we were unable to study the secretion of PAI-1 into an underlying ECM, because subconfluent BAE cells make negligible ECM in the short period of time following plating. In previous experiments with confluent BAE cells, we found that LPS stimulated the secretion of PAI-1 into both the ECM and the culture medium (data not shown). These secretion patterns exhibited a differential time course and indicated that BAE cells secreted PAI-1 preferentially into the ECM, from which it was subsequently

### Table II

**Function of SPARC in coagulation assays in vitro**

Coagulation assays were performed as described under “Experimental Procedures.” Addition of different concentrations of SPARC, ranging from 0.6–20 μg, produced similar results. Annexin V (2 μg) was used as an anticoagulant control. Results are expressed as the mean clotting time ± S.D. of triplicate experiments.

|        | APTT (s) | PTT (s) |
|--------|----------|---------|
| PBS    | 62 ± 0   | 48 ± 1  |
| SPARC  | 61 ± 1   | 48 ± 2  |
| Annexin V | >300    | 94 ± 3  |
released into the culture medium. Most likely, this secretion is accompanied by the loss of PAI-1 activity because the molecule is unstable in solution (49–50). The temporal association of active PAI-1 with vitronectin in the ECM (53), however, could create an environment that controls localized plasminogen activation-mediated proteolytic degradation. In this regard, it is interesting to note that several ECM proteins, in particular type IV collagen and gelatin, are effective stimulators of plasminogen activation by t-PA (54). Plasminogen activation plays an important role in tissue morphogenesis and fibrinolysis. Although the association of SPARC with morphogenetic processes has also been noted (4–7), a potential role for SPARC in the regulation of thrombosis and hemostasis has, to our knowledge, not been investigated.

Stenner et al. (55) have reported that SPARC/osteonectin is present in blood platelets. Moreover, SPARC is secreted by platelets upon activation with the platelet agonists collagen and thrombin (56). Because one of the primary physiologic functions of platelets is the secretion of components involved in the regulation of hemostasis and thrombosis, it is conceivable that SPARC plays a role in these processes. Our study demonstrates that this role might be the induction of PAI-1, which is felt to be an important regulator of the fibrinolytic system in vivo. Diéval and co-workers (57) have recently described a patient with a severe deficiency of PAI-1 who suffered from a delayed type bleeding tendency. Elevated levels of PAI-1 activity in blood, on the other hand, correlate with thromboembolic diseases, although a direct causal relationship has not been demonstrated (14). In addition, in a recent study by Erickson et al. (58) with transgenic mice, strong evidence was presented that elevated levels of PAI-1 were closely associated with the development of venous occlusions. The precise mechanism of thrombosis in this model, however, has not been elucidated. Another function of SPARC in the regulation of hemostatic processes might be direct interference with intrinsic and/or extrinsic coagulation pathways. These reactions could include activation or inhibition of specific coagulation factors, as well as (Ca2+-dependent) binding to phospholipid surfaces, as demonstrated with annexin V (36, 59). Addition of SPARC to APPT and PTT incubation mixtures did not, however, affect normal clotting times in vitro. Several mediators that induce the expression of PAI-1 in endothelial cells, including interleukin-1, LPS, tumor necrosis factor, and thrombin, also induce tissue factor procoagulant activity on the surface of endothelial cells (60–64). Whether SPARC is able to induce endothelial tissue factor activity is currently under investigation. Although we did not find an effect of SPARC in coagulation assays in vitro, SPARC might still affect either of these pathways by altering the expression of pro- and/or anticoagulant factors.

We have shown that a high local concentration of SPARC, as might be released from activated blood platelets at sites of vascular injury or from endothelial cells under conditions of stress (40), will induce the expression of PAI-1 in endothelial cells. A temporal accumulation of the active form of PAI-1 in the endothelial cell ECM very likely plays a critical role in protecting both ECM components and ECM-associated fibrin from the degradation mediated by plasminogen activation. We hypothesize that SPARC, by inducing PAI-1 in endothelial cells, functions indirectly in the regulation of fibrinolysis and in the control of proteolytic events in tissues that undergo remodeling.

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