Immunoelectron Microscopic Localization of Type 1 Plasminogen Activator Inhibitor on the Surface of Activated Endothelial Cells

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Abstract. Immunogold EM was employed to compare the distribution of type 1 plasminogen activator inhibitor (PAI-1) on the surface of agonist-activated human umbilical vein endothelial cells (HUVECs) with that of control, unactivated cells. As previously observed, (Schleef, R. R., T. J. Podor, E. Dunne, J. Mimuro, and D. J. Loskutoff. J. Cell Biol. 110:155-163), analysis of cross-sections of nonpermeabilized control HUVEC monolayers stained first with affinity-purified rabbit antibodies to PAI-1 and then with gold-conjugated goat anti-rabbit IgG, revealed the presence of relatively few gold particles (<1-2% of the total) on the apical cell surface. The majority of gold particles were detected primarily in the extracellular matrix between the culture substratum and the cell membrane. In contrast, treatment of HUVECs with tumor necrosis factor α (TNFα; 200 U/ml, 24 h) or with lipopolysaccharide (LPS; 10 μg/ml, 24 h) resulted in an increased staining of PAI-1 not only in the extracellular matrix, but also on the apical cell surface (10-fold increase). Immunoabsorption of the rabbit anti-PAI-1 with purified PAI-1, or treatment of HUVECs with tissue-type plasminogen activator (2.5 μg/ml, 2 h, 4°C) reduced the amount of staining both on the apical surface and in the extracellular matrix of agonist-activated HUVECs by 80-95%. The topographical location of PAI-1 on the cell surface was examined further by coupling immunogold staining with high resolution surface replication. Transmission EM of surface replicas from TNFα- or LPS-activated HUVECs revealed a general increase in PAI-1 staining both on planar regions and within indentations of the apical cell surface. Nonactivated HUVECs revealed PAI-1-specific immunogold particles only in areas of exposed extracellular matrix between the cells and occasionally at regions of cell-cell contacts. Analysis of activated bovine aortic endothelial cells by immunoelectron microscopy, immunologic assays, and flow cytometry revealed similar increases in surface PAI-1. These increases in surface PAI-1 could be detected by 3 h and continued over a 24-h period. The expression of PAI-1 on the luminal surface of endothelial cells during immune or inflammatory reactions could reduce endothelial fibrinolytic activity, thus, promoting the localized, pathologic formation of intravascular thrombi.

Increasing experimental evidence indicates that the surface of endothelial cells changes dramatically in response to inflammatory stimuli. For example, tumor necrosis factor α (TNFα), interleukin 1, and endotoxin (lipopolysaccharide [LPS]) cause the appearance of several adhesion molecules for leukocytes on the endothelial surface (Bevilacqua et al., 1989; Johnston et al., 1989). In addition, these compounds induce the appearance of tissue factor, a protein with procoagulant activity on endothelial surfaces (Bevilacqua et al., 1986a), and decrease the binding and surface activation of protein C (Nawroth et al., 1986; Nawroth and Stern, 1986). The latter effect results from a rapid internalization and degradation of cell surface–associated thrombomodulin (Moore et al., 1989).

1. Abbreviations used in this paper: BAE, bovine aortic endothelial cell; HUVEC, human umbilical vein endothelial cell; LPS, lipopolysaccharide; PA, plasminogen activator; PAI, PA inhibitor; TNFα, tumor necrosis factor α; t-PA, tissue type PA; u-PA, urokinase-like PA.

Agonist-induced alterations in the hemostatic balance at the level of vascular endothelial cells may also include changes in fibrinolytic components. For example, LPS and various cytokines stimulate the production and secretion of type 1 plasminogen activator inhibitor (PAI-1) (Bevilacqua et al., 1986b; Colucci et al., 1986; Dubor et al., 1986; Emes and Kooistra, 1986; Nachman et al., 1986; Schleef et al., 1988) and decrease tissue-type PA (t-PA) production (Schleef et al., 1988). These changes may promote the integrity and maintenance of the fibrin clot. Although four molecules with PAI activity have been detected, PAI-1 appears to be the physiologic inhibitor of tissue-type (t-PA) and urokinase-type PAs (u-PA) (for review see Sprengers and Kluft, 1987; Loskutoff et al., 1988). It has been shown that t-PA binds to cultured human umbilical vein endothelial cells (HUVECs) through a low- and a high-affinity binding site. The high-affinity binding site was identified as PAI-1 associated with the cells (Barnathan et al., 1988). We have recently demon-
Materials and Methods

Reagents

Reagents were obtained as follows: FCS, trypsin, penicillin, and streptomycin from Gibco Laboratories (Grand Island, NY); medium 199 (M199) from M.A. Bioproducts (Bethesda, MD); tissue culture plasticware from Corning Glass Works (Corning, NY); endothelial cell growth factor, Trypsin, porcine intestinal heparin, purified goat immunoglobulin (IgG), fibroblast growth factor, paraformaldehyde, LPS, trypsin, penicillin, and streptomycin from Sigma Chemical Co. (St. Louis, MO); cyanogen bromide (CNBr)-activated Sepharose 4B from Pharmacia Fine Chemicals (Piscataway, NJ); 10-nM gold conjugated-goat anti-rabbit (Rb) IgG from Janssen Life Sciences Products (Piscataway, NJ); recombinant TNFα (2 x 10^7 Units/mg) from Genzyme Corp. (Boston, MA).

Fibrinolytic Proteins

Human PAI-1 was purified from the conditioned media conditioned by a transformed human lung fibroblast cell line (SV40 WI38 VA13 2RA) according to procedures described previously (Hekman and Loskutoff, 1988). Antiserum to purified PAI-1 was raised in New Zealand Rb according to standard procedures. Antibodies to PAI-1 were affinity purified using human PAI-1 bound to CNBr-activated Sepharose 4B. Briefly, PAI-1 (500 µg) was coupled to 3 ml of CNBr-activated Sepharose 4B according to the manufacturer's instructions. The IgG fraction of Rb antiserum to PAI-1 was isolated (Schleef et al., 1985) and 100 μg of IgG was circulated (16 h, 4°C) through the PAI-1-Sepharose column at 10 ml/h. The column was washed with PBS, and then eluted with 0.2 M glycine-HCl, pH 2.5. The eluted antibodies were immediately neutralized with 1 M Tris-HCl, pH 8.1. Protein concentration was determined by the method of Bradford (1976).

Growth and Treatment of HUVECs

HUVECs were isolated from two to five freshly collected umbilical cords, pooled, and grown in culture dishes coated with purified fibronectin (1 µg/cm²). The growth medium consisted of M199 with 20% FCS, and was supplemented with endothelial cell growth factor (75 µg/ml), porcine intestinal heparin (50 µg/ml), and antibiotics (Schleef et al., 1988). For experimental use, HUVECs were grown to confluence on either fibronectin-coated glass coverslips (12 mm diameter, Fisher Scientific Co., Pittsburgh, PA) or fibronectin-coated 24-well Costar culture plates, and maintained at confluency for 3 d before use. To activate HUVECs, the monolayers were washed 3x with M199 and incubated for 24 h with growth media supplemented with either PBS, 200 U/ml TNFα, or LPS (10 µg/ml). Experiments were performed with HUVECs cultured between the first and third passage.

Immunologic Assays and Flow Cytometric Analysis for BAE PAI-1

BAEs were isolated and grown to confluence as described previously (Mimuro et al., 1987). BAEs were utilized between the tenth and twentieth passage. For experimental use, the cells were washed and incubated with 0.1% serum-containing media supplemented with or without 10 ng/ml LPS (Sawadey et al., 1989). The conditioned media (CM) was harvested at the indicated times and the washed cells were lysed with PBS containing 0.5% Triton X-100 by incubation for 10 min at 22°C. Both the CM and the Triton X-100-soluble cell lysates were analyzed using a two-site enzyme linked immunonasometric assay as described previously (Mimuro et al., 1987; Seifert and Podor, 1991). PAI-1 associated with the insoluble ECM was analyzed by incubating the plates with an mAb (mAb 12C2) specific for bovine PAI-1 (10 µg/ml, 2 h, 37°C) followed by incubation with 125I-goat anti-mouse IgG (2 x 10^5 cpm/ml, 1 h, 37°C). A standard curve of PAI-1 was prepared by drying overnight known concentrations of purified PAI-1 onto 24-well Costar plates and then processing the plates as described above.

For flow cytometric analysis, BAEs were grown in 60-mm culture dishes, and the washed cells were treated for 3, 8, and 24 h in the presence or absence of 10 ng/ml LPS. The cells were washed with ice-cold PBS and detached by treatment with PBS containing 10 mM EDTA and 0.2 M urea. The tryptase were then suspended in a wash buffer at 4°C and the cells were incubated (30 min, 4°C) with 10 µg/ml mAb 12C2 anti-PAI-1 followed by fluoroscein-labeled goat anti-mouse IgG. The washed cells were analyzed using a FACScan (Becton-Dickinson & Co., Mountain View, CA) flow cytometer. Fluorochrome was excited with 250 mW of 488 nm light using a 2 W argon laser. Light emission was detected on a photomultiplier tube using a band pass filter (530/30 nm) with 10^6 cells analyzed over a log scale of fluorescent intensity using the Consort 30 Software data package.

Cells were gated using forward scatter and side scattered light to include only single endothelial cells in order to exclude both dead or damaged cells and aggregated cells.

Immunogold Staining Procedure

HUVEC and BAE monolayers were washed and fixated in 1.5% paraformaldehyde in M199 for 20 min at room temperature. The fixed monolayers were washed, incubated for 20 min with 0.2 M glycine in M199 to neutralize residual paraformaldehyde, and then incubated for 30 min with 5 µg/ml goat IgG in M199 (M199-IgG) to block nonspecific IgG binding sites. Washed HUVEC monolayers were stained for 1 h at room temperature with a primary antibody (1 µg/ml) of either affinity-purified Rb anti-human PAI-1 (or normal Rb IgG diluted in M199-IgG), washed and then stained again with 10 nM gold-conjugated goat anti-Rb IgG diluted (1/25) in M199-IgG. BAE monolayers were stained with either mAb 12C2 anti-bovine PAI-1 or normal mouse IgG followed by 5 nM gold-conjugated goat anti-normal IgG. After washing the endothelial monolayers with M199, the cells were stored in cold Karnovsky's fixaton buffer (1.5% gluteraldehyde, 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4) until processed.

Thin-section EM

After immunogold staining, monolayers in 24-well costar plates were briefly rinsed with PBS, postfixed with 1.5% OsO4 in 0.1 M cacodylate buffer, pH 7.2, for 1 h at room temperature, and dehydrated in agraded ethanol series. The wells were flat embedded in Epon 8122 and after polymerization, stained en bloc with uranyl acetate. To quantify the amount of anti-PAI-1 specific gold particles bound to the cell surface or culture substratum, four strips of 3 x 8 mm from each group were cut out from the Epon sheet containing cell monolayers. Two such strips from the same group were sandwiched together, cells apposed, with fresh Epon. Sections from these sandwiched blocks bearing two layers of cells were cut perpendicular to the culture substratum, double stained with uranyl acetate and lead citrate, and mounted on 100-mesh parallel lined grids (Ted Pella, Inc., Tustin, CA) so that both cell layers ran longitudinally between two grid bars. Quantification of gold particles on nucleated cells was carried out at 15,000 x magnification on a Hitachi 12-12-UA electron microscope as previously described (Schleef et al., 1990). Briefly, one section from each block was used for quantitative analysis and the gold particles associated with the apical cell surface or the ECM were counted in each section over a total of 25 nucleated cells, representing 800-1,000 µM of linear culture substratum surface. A total of four blocks, each derived from a separate tissue culture well, were examined in a group. The density of gold markers was expressed as the number of particles (mean ± SD) per nucleated cell from the four representative sections. Data were statistically analyzed with a paired r test.

Surface Replication

Platinum-carbon replicas of the surface of HUVEC cultures were prepared according to the procedure described by Robenek et al. (1984). Fixed and immunostained monolayers on glass coverslips were dehydrated and critical-point dried. The coverslips were mounted on the stage of a Balzers 360 M freeze-fracture apparatus (Balzers Union, Hudson, NH) and the bell jar was
Figure 1. Immunoelectron microscopic localization of PAI-1 on the surface of control and TNFα-activated HUVECs. HUVEC monolayers in 24-well Costar plates were incubated for 24 h in growth media either in the absence (A and B) or presence (C-F) of 200 U/ml TNFα. The washed cultures were blocked with M199-IgG, stained with affinity-purified rabbit antibodies to PAI-1 followed by gold-conjugated goat anti-rabbit IgG, and then processed for thin-section EM as described in Materials and Methods. (arrowheads) Immunogold particles. (A-C, inset) Low-power magnification of HUVEC with arrow indicating approximate area enlarged in each respective panel. (D) Low-magnification of HUVEC with left arrow and right arrow indicating area enlarged in E and F, respectively. Bars: (A–C, E, and F) 250 nm; (D) 2 μm.
Particles Associated with Control and Activated HUVECs

Confluent HUVEC monolayers were incubated (24 h, 37°C) in growth media supplemented with either PBS, TNFα (200 U/ml) or LPS (10 μg/ml). The cells were washed, fixed, and incubated with the indicated primary antibody (1 μg/ml) followed by gold-conjugated goat anti-Rb IgG. In experiment II, anti-PAI-1 (1 μg/ml) were preabsorbed by incubation with a 40-fold excess of purified PAI-1. Immunostained cells were then processed for transmission EM as described in Materials and Methods. Data are expressed as the number of immunogold particles (mean ± SD per cell) detected on either the apical cell surface or associated with the ECM and were derived by analyzing 25 nucleated cells/section (four sections/group).

| HUVEC treatment | Primary antibody | Apical surface | ECM |
|-----------------|-----------------|----------------|-----|
| I. Control | Rb anti-PAI-1 | 0.44 ± 0.50 | 30.12 ± 11.66 |
| TNFα | Rb anti-PAI-1 | 6.36 ± 1.82* | 44.06 ± 16.06 |
| LPS | Rb anti-PAI-1 | 5.52 ± 1.44* | 47.28 ± 12.11 |
| Control | Nonimmune Rb IgG | 0.24 ± 0.43 | 0.79 ± 0.59 |
| TNFα | Nonimmune Rb IgG | 0.36 ± 0.43 | 0.72 ± 0.61 |
| LPS | Nonimmune Rb IgG | 0.28 ± 0.45 | 0.85 ± 0.56 |
| II. LPS PBS + Rb anti-PAI-1 | 6.08 ± 2.17 | 47.28 ± 15.31 |
| LPS PAI-1 + Rb anti-PAI-1 | 0.40 ± 0.51 | 0.89 ± 0.61 |

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Gold particles could be detected not only in the ECM but also on the apical surface of all cells (Fig. 1, C-F). Gold particles on the apical surface of TNFα-activated HUVECs were present either as widely dispersed single gold particles (E), or in isolated clusters adhering to fibrous material (C and F). Heat treatment (100°C, 10 min) of the cytokine preparations were found to prevent not only the cytokine-mediated increase in PAI-1 production (Bevilacqua et al., 1986b; Schleef et al., 1988), but also the increase in PAI-1 associated with apical cell surfaces (data not shown), suggesting that these responses were not mediated by LPS in the recombinant preparations. However, LPS treatment of HUVECs also increased the amount of gold particles present on the apical surface of cells stained with antibodies to PAI-1 (Fig. 2). As observed with TNFα, gold particles were present either in clusters (A), as doublets (B and C), or as singletons (D). Quantitation of the total number of particles distributed over 25 nucleated cell lengths (~800-1,000 μM linear length) demonstrated a ~10-fold increase in the amount of gold particles detected on the dorsal surfaces of the TNF- and LPS-activated cells compared to the untreated control cells (Table I). Pre-absorption of the antibodies with an excess of purified PAI-1 reduced the number of gold particles on the cell surface and ECM by over 90% (Table I).

Although PAI-1 is synthesized as an active molecule, it is relatively unstable in solution and rapidly decays into a latent, inactive form at 37°C (Levin, 1986; Kooistra et al., 1986). Localization of PAI-1 on the surface of endothelial cells
would have physiological relevance for hemostasis if this inhibitor was in an active conformation and capable of interacting with PAs. We assessed the activity of surface-bound PAI-1 by determining whether it reacted with exogenously added t-PA. Solution-phase t-PA reduced the concentration of PAI-1 in the ECM of TNFα-activated HUVECs (Table II), consistent with previous results for nonactivated HUVECs (Schleef et al., 1990). The addition of exogenous t-PA also reduced the amount of PAI-1-specific gold particles detected on the apical cell surface of TNFα-activated HUVECs by over 80% (Table II). Again, HUVECs stained with nonimmune IgG as the primary antibody exhibited low gold staining on both the apical and the ECM (Table II).

**Surface Replicas**

Drying of cultured fibroblasts (Robenek et al., 1983), macrophages (Robenek et al., 1984) and platelets (Isenberg et al., 1987) using the critical point method with the subsequent preparation of surface replicas has permitted the visualization of a number of cell surface proteins over extended areas of plasma membrane surfaces using the transmission electron microscope. To determine whether activation of endothelial cells might cause changes in the surface distribution of PAI-1 not appreciated in conventional thin sections, we studied surface replicas of immunostained unstimulated and TNFα-stimulated endothelial cells. Examination of the apical cell surface of control HUVECs by this technique revealed the presence of numerous invaginations in an otherwise smooth cell surface (Fig. 3). Control HUVECs immunostained for PAI-1 contained low quantities of gold particles on the apical cell surface (Fig. 3, B and D). However, in areas between two cells where the ECM was exposed, as well as on the edges of the cell in the vicinity of the ECM, PAI-1-specific immunogold particles were readily detected bound to the ECM (Fig. 3 C).

Stolpen et al. (1986) reported that TNFα treatment disrupts the normal cobblestone morphology of HUVECs, resulting in a more fibroblastic-like appearance. TNFα-activated HUVECs possessed more cytoplasmic extensions and an increase in the intercellular spaces (Fig. 4, A and D), thus confirming this observation. Again, PAI-1-specific gold particles were readily detected in the ECM exposed between adjacent HUVECs (Fig. 4 B). However, the amount of PAI-1-specific gold particles on the cell's apical surfaces was also dramatically increased (Fig. 4, B and C). PAI-1 was present both on planar regions of the cell surface (Fig. 4 B) and in invaginations of the cell's membrane (Fig. 4 C), and both singletons and clusters of two to three gold particles were routinely detected. Gold particles were rarely detected either on the ECM or the cell surface of TNFα-activated HUVECs stained with nonimmune IgG (Fig. 4 D).

**Association of PAI-1 with the Surfaces of LPS-activated BAEs**

To demonstrate that the association of PAI-1 with the surface of activated-endothelial cells is not unique to HUVECs, we also have investigated changes in the distribution of PAI-1 in BAEs after their treatment with LPS. In comparison to HUVECs, a 1,000-fold lower concentration of LPS was utilized in experiments with BAEs, as higher concentrations were cytotoxic as shown by trypan blue exclusion and by flow cytometry studies. Thin-section electron microscopic examination of these cells revealed a pattern of PAI-1 on the surfaces of LPS-activated BAEs (data not shown) that was similar to that described above for activated HUVECs. Immunologic assays indicated that the increases in PAI-1 associated with BAEs could be detected in the Triton X-100-soluble cell extract and in the ECM within 4 h after exposure to LPS and remained elevated for at least 24 h (Fig. 5, B and C). To investigate the kinetics of the LPS-induced increases in the surface expression of PAI-1 in more detail, we immunofluorescence control and activated BAEs for cell surface PAI-1 after 3, 8, and 24 h exposure to LPS, and then analyzed the cells by flow cytometry. A slight shift to the right in the fluorescent labeling intensity could be detected within 3 h using this procedure (Fig. 6 A). The fluorescent labeling intensity continued to shift to the right over the 24-h treatment period (B and C), indicating further increases in PAI-1 associated with the cell surface. All cells seemed to have PAI-1 on the cell surface since only a single, rather homogeneous population of cells were detected after 24 h, and these were well displaced from the untreated control cells.

**Discussion**

Research over the past few years has revealed that key surface-related functions of the vascular endothelium appear to be dynamically modulated (for review see Gimbrone, 1986). For example, the adhesivity of leukocytes and tumor cells to endothelial cells is dependent on endothelial surface molecules which are inducible by a number of physiological stimuli (e.g., LPS, TNFα, etc.; Bevilacqua et al., 1989). Alteration in surface thrombogenic properties include the rapid induction of tissue factor on the cell surface and the concomitant depression of cell surface antithrombotic activities (Nawroth et al., 1986; Nawroth and Stern, 1986). In this report, we provide data which indicate that the dynamic changes in the cell surface properties of cultured endothelial cells extend to the expression of PAI-1.

Thin-section transmission EM was employed to demonstrate that the concentration of PAI-1 was increased ~10-fold on the apical surface of agonist-activated HUVECs and BAEs (Figs. 1 and 2; Table I). Flow cytometric analysis of
LPS-activated BAEs confirmed these results and indicated that the surface expression of PAI-1 increased over a 24-h period (Fig. 6). In contrast to the LPS-treated BAEs, control BAEs exhibited a slight decrease in cell surface PAI-1 staining over the 24-h period (Fig. 6). This decrease may have resulted from the relative decline in the rate of PAI-1 production caused by reducing the serum concentration in this system (Sawdey et al., 1986, 1989). Evidence that the antibodies were actually detecting changes in the surface distribution of PAI-1 was provided by the >95% reduction in the concentration of gold particles associated with the cell surfaces after immunoabsorption of the antibody preparations with purified PAI-1 (Table I), or upon treatment of the cells with t-PA (Table II). Moreover, we could detect similar changes in PAI-1 on surfaces of activated BAEs using mAbs specific for bovine PAI-1 (Fig. 6).

Thin-section immunoelectron microscopic analysis reveals only small amounts of the cell's membrane and does not readily permit the visualization of PAI-1 molecules and cell surface structures on either side of a particular section. Therefore, to investigate the topographical distribution of PAI-1 on the surface of control and agonist-activated HUVECs in more detail, we prepared platinum-carbon surface replicas of our immunogold-labeled HUVECs (Figs. 3 and 4). This technique is readily applicable to immunogold staining since the gold particles are resistant to digestion with sodium hypochlorite, which is used to digest the cell monolayers, and thus appear as circular black dots (i.e., 10 nM in diameter) in the surface replicas. Examination of replicas of immunostained control HUVECs in the transmission electron microscope (Fig. 3) confirmed our observation that PAI-1 was present only at low amounts on their apical cell surface. Surface replicas of agonist-activated HUVECs (Fig. 4) not only confirmed the observation that PAI-1 was increased, but also revealed that it was increased throughout the cell surface, both on planar areas of the cell membrane and within invaginations of the cell surface. Indentations in surface replicas of human skin fibroblasts (Robenek et al., 1983) and mouse peritoneal macrophages (Robenek et al., 1984) have been reported to localize gold-conjugated native and modified LDL, and are believed to represent endocytic structures (i.e., coated pits) in the cell membranes of these two types of cells. On the other hand, the ability of t-PA to reduce the concentration of PAI-1 associated with HUVECs suggests the possibility that PAI-1 is in the active conformation on the apical cell surface of HUVECs, a hypothesis that is consistent with the known biochemical properties of active PAI-1 when bound to a stabilizing protein (Loskutoff et al., 1988). One protein presently identified as capable of both binding and stabilizing this inhibitor in an active form is the adhesive glycoprotein, vitronectin. PAI-1 bound to immobilized (Mimuro et al., 1987; Mimuro and Loskutoff, 1989) or soluble (De-
c lerck et al., 1988; Salonen et al., 1989) vitronectin still rapidly interacts with t-PA, and the resulting t-PA/PAI-1 complex dissociate from the vitronectin surface. Since both LPS and TNFα cause morphological rearrangement of the monolayer, it is also possible that some of the cell surface PAI-1 located at the periphery may originate from the ECM and be relocated to apical cell surfaces during the rearrangement of the cells. Further experiments will be necessary to define the role of vitronectin and/or other proteins in the binding of PAI-1 to endothelial cells, and to establish the importance of these membrane invaginations in the expression of endothelial-associated PAI-1.

The interaction of t-PA with endothelial cells has previously been studied by several laboratories utilizing nonactivated HUVECs (Hajjar et al., 1987; Barnathan et al., 1988; Sakata et al., 1988). These reports have led to the concept that t-PA interacts with endothelial cells through high-affinity and low-affinity binding sites (Hajjar et al., 1987; Barnathan et al., 1988). Although the dissociation constants and number of binding sites for these endothelial t-PA “receptors” varies from laboratory to laboratory, the high-affinity binding site for t-PA appears to be PAI-1 (Barnathan et al., 1988).

The data reported here indicate that PAI-1 associated with the ECM and cell surfaces of both control and activated HUVECs is accessible to soluble t-PA. Since compounds present in different serum lots have been found to alter the production of PAI-1 in endothelial cells (Sawdey et al., 1986), one possibility that would account for the reported discrepancies in the t-PA binding data may be the altered surface expression of PAI-1 induced by these compounds which may be present at varying degrees in the different serum preparations. The accessibility of the ECM-associated PAI-1 to t-PA may be caused by the absence of tight junctions between the endothelial cells when they are grown on plastic surfaces (Schlee et al., 1990).

In summary, we have demonstrated that TNFα and LPS can act on cultured endothelial cells to increase the expression of PAI-1 on the apical cell surface. In vivo, these actions could lead to the development and maintenance of thrombotic and perivascular fibrin in a variety of pathophysiologic settings, thereby influencing the evolution of thrombotic and inflammatory processes. Additional experiments will be required to delineate the location, concentration, and role of PAI-1 associated with both unstimulated and agonist-activated endothelial cells in vivo.

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