Hemodynamic Forces Modulate the Effects of Cytokines on Fibrinolytic Activity of Endothelial Cells

By Yohko Kawai, Yutaka Matsumoto, Kiyooki Watanabe, Hiroshi Yamamoto, Kumi Satoh, Mitsuru Murata, Makoto Handa, and Yasuo Ikeda

We investigated the effects of hemodynamic force on fibrinolytic activity of cultured human umbilical vein endothelial cells stimulated by cytokines, using a modified cone-plate viscometer in which well-controlled and defined shear forces were generated. Treatment of the cells with interleukin (IL)-β or tumor necrosis factor α (TNFα) under static conditions had no effect on tissue plasminogen activator (t-PA) secretion, while release of plasminogen activator inhibitor 1 (PAI-1) increased. When cells were exposed to increasing shear stress up to 24 dynes/cm², levels of t-PA and PAI-1/PAI-1 complex significantly increased relative to shear stress, while total PAI-1 and active PAI-1 secretion decreased gradually. In the presence of IL-1β or TNFα, the increase in production of t-PA and the t-PA/PAI-1 complex was further augmented. Dot blot hybridization analysis of cultured cells in similar experimental conditions using t-PA and PAI-1 cDNA probes revealed no t-PA mRNA in 3 μg total RNA from static endothelial cells under resting or cytokine-stimulated conditions, but abundant t-PA mRNA was detected in cells subjected to a shear force of 18 dynes/cm², and the increase was further augmented by addition of cytokines. In contrast, PAI-1 mRNA was detected in resting and cytokine-stimulated, nonsheared endothelial cells, but levels decreased after exposure to shear stress, even in the presence of cytokines. These results indicate a role for hemodynamic forces in regulating fibrinolytic activity with or without cytokine stimulation.

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Hemodynamic forces play a crucial role in regulating vascular function by modulating gene expression.12 Several recent lines of evidence have established that hemodynamic forces acting on the endothelial surface can induce a number of morphologic and functional changes. Shear stress induces endothelial cell elongation and formation of stress fibers and increases permeability, pinocytosis, and lipoprotein internalization.12 Endothelial cells have been proposed to act as local sensors of fluid shear stress and as effectors in the regulation of both vascular tone and structure; this occurs in conjunction with altered regulation of the gene for endothelium-derived relaxing factor (nitric oxide) prostacyclin, histamine, and endothelin-1. Shear stress also regulates adaptive vessel growth and angiogenesis1 and may be a local risk factor in the pathogenesis of atherosclerosis. Several candidates for the shear stress-responsive element (SSRE) were proposed, which suggests that the mechanotransduction mechanism of flow-induced vascular remodeling is regulated at the transcriptional level. Common promoter elements interacting with shear stress–induced transcription factors are reported including SSRE found in platelet-derived growth factor-B (PDGF-B) and transforming growth factor-β (TGF-β), and phorbol ester TPA-responsive elements (TRE) found in human monocyte chemotactic protein-1 gene.6

A variety of agents increase tissue plasminogen activator (t-PA) and/or plasminogen activator inhibitor (PAI-1) synthesis in cultured human endothelial cells.23 Thrombin and basic fibroblast growth factor induce both t-PA and PAI-1 release, whereas histamine and phorbol esters cause only t-PA secretion.24 Lipopolysaccharide (LPS), endotoxin, and cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) have been reported to induce PAI-1 release in endothelial cells, but have little effect on t-PA secretion.91112 Although other investigators found that IL-1 and TNF decrease endothelial cell t-PA secretion,10 shear stress can also influence the expression of t-PA mRNA, and t-PA secretion was observed with concomitant increases in t-PA mRNA levels after exposure to arterial levels of shear stress (>15 dynes/cm²), while PAI-1 secretion was unaffected by shear stress over the physiologic range.1314

To investigate the interplay between hemodynamic forces and cytokine stimulation in modulating the vascular fibrinolytic system, we analyzed t-PA and PAI-1 secretion and gene expression in cultured endothelial cells treated with IL-1β or TNFα and subjected to shear forces applied with a modified cone-plate viscometer. We found that t-PA expression is augmented at both secreted protein and mRNA levels by IL-1β or TNFα stimulation under shear stress, whereas the cytokines alone do not affect t-PA synthesis. Furthermore, PAI-1 secretion induced by IL-1β or TNFα is attenuated at both the secreted protein and mRNA levels by shear stress.

Materials and Methods

Cell culture. Human umbilical vein endothelial cells were isolated with collagenase type I (Nitta Gelatin Inc, Osaka, Japan) as described previously.15 Cells from two or three umbilical cords were pooled and then grown in dishes coated with gelatin (Eastman Kodak Co, Rochester, NY) in complete medium 199 (GIBCO Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (Seromex, Vithofen, Germany), 30 μg/mL endothelial cell growth supplement (ECGS) Collaborative Research Inc, Bedford, MA), 6 U/mL sodium heparin (Shimizu Pharmaceutical Inc, Shizuoka, Japan), and penicillin/streptomycin (GIBCO) at 37°C in a 5% CO₂ atmosphere. Subcultures were obtained by trypsin/EDTA.

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Shear stress augments t-PA secretion and suppresses PAI-1 release. Confluent endothelial cells were exposed to constant shear stress at 0, 6, 12, 18, and 24 dynes/cm². Concentrations of t-PA secreted into the culture medium increased significantly as shear stress increased (0.46 ± 0.22 ng/10⁶ cells/h for resting cells vs. 1.36 ± 0.33 ng/10⁶ cells/h for sheared cells at 24 dynes/cm²). PAI-1 secretion gradually diminished (34.43 ± 10.78 ng/10⁶ cells/h for resting cells vs. 23.33 ± 7.63 ng/10⁶ cells/h for cells at 24 dynes/cm²), as shown in Fig. 1. Furthermore, active PAI-1 release was significantly decreased with increased intensity of shear stress (0.91 ± 0.44 ng/mL for resting cells vs. 0.09 ± 0.14 ng/mL at 24 dynes/cm²), as shown in Fig 2. All the effects of shear stress were significant: t-PA (P < .0001), t-PA-PAI complex (P < .0001), total PAI-1 (P < .05), and active PAI-1 (P < .0001).

Shear stress augments t-PA secretion and suppresses PAI-1 secretion from endothelial cells incubated with cytokines. An assay of t-PA and PAI-1 release from cytokine-treated cells under various shear stresses indicated that the gradual increase in t-PA secretion with increasing shear forces was markedly augmented in the presence of IL-1β or TNFα. At 24 dynes/cm², t-PA release was 2.9-fold higher than in resting cells in the absence of cytokines, 10.6-fold higher in the presence of IL-1β, and 7.9-fold higher with TNFα (Fig 1A). By contrast, PAI-1 secretion induced by cytokines at rest was gradually attenuated with increasing shear stress (Fig 1B). Similarly, the increment in active PAI-1 after IL-1β treatment at a split ratio of 1:2. Cells at the second passage were seeded at 4 x 10⁶ cells/cm² onto 35-mm petri dishes (Iwaki Inc., Chiba, Japan) precoated with 200 μg/mL collagen type IV (Iwaki) in 1 mL complete media. After 72 hours, replicate secondary confluent monolayers of 8 x 10⁶ cells on a 35-mm petri dish were exposed to shear stress. In some experiments, cells were cultured in the presence or absence of IL-1β or TNFα (Genzyme Inc, Cambridge, MD) and with or without ECGS and heparin.

Exposure of endothelial cells to shear stress. A modified cone-plate viscometer was used in a previous study for measurement of shear-induced platelet aggregation several to generate well-defined, constant shear conditions without producing turbulent flow. The cone-plate chamber made of polymethylmethacrylate is composed of a rotating cone and a base plate in which a 35-mm petri dish can be fitted. The distance from the cone apex to the bottom of the petri dish is adjusted to 0.004 cm by a micrometer screw in conjunction with the cone rotation unit. The cone rotation unit is provided by a rotor motor with an optical encoder. Rotation rates of 0 to 50 revolutions per second can be produced. Shear rate (γ) was calculated according to the formula γ = 6 N/π, where N is the rotational speed of the cone and θ is the cone angle. In the device used for these studies, the cone angle was 1°. Shear stress was calculated by multiplying shear rate by viscosity of the fluid, assumed to be 0.01 poise for culture medium tested here. Second-passage endothelial cells were grown to confluency in 35-mm petri dishes and then applied to the base plate for shear stress experiments. Cells were exposed to steady shear stress at 0, 6, 12, 18, and 24 dynes/cm² for 24 hours in a 5% CO₂ atmosphere in the presence or absence of 100 U/mL IL-1β or TNFα. Shear stress experiments were repeated at least three times using different pooled endothelial cells.

Determination of antigen levels in cultured medium. After exposure of cells to shear stress and/or cytokines, culture supernatants were collected with 5 mmol/L EDTA and 6 mmol/L N-ethylenemide (Sigma Chemical Co, St Louis, MO), filtered with a 0.45-μm filter to avoid floating debris, and then brought to a volume of 1 mL with complete medium. Concentrations of t-PA and PAI-1 antigens in these culture supernatants were measured in duplicate by enzyme-linked immunosorbent assay (ELISA) using commercially available kits: total t-PA antigen, including uncomplexed and inhibitor-bound t-PA, was assayed by the Imulyse t-PA kit (Biopool, Umeå, Sweden), and total PAI-1, including active, latent, and t-PA–complexed PAI-1, was assayed by the TintElize PAI-1 kit (Biopool). Concentrations of t-PA-PAI complex and active PAI-1 in conditioned medium were measured by the TDC-88 kit (Teijin Co, Tokyo, Japan) using a sandwich ELISA technique. In this assay, active PAI-1 is calculated by measuring baseline t-PA-PAI complex and complex generated after adding fresh t-PA to bind free, active PAI-1. No t-PA-PAI complex or active PAI-1 was detected in complete medium before addition of endothelial cells.

Data were obtained from at least three different culture dishes on different days and are presented as nanograms per 10⁶ cells per hour. Numbers of separate experiments are shown in parentheses in figure legends.

RNA isolation and hybridization. Total cellular RNA was extracted from human umbilical vein endothelial cells as previously described, with some minor modifications. Briefly, cells were washed with Hanks balanced salt solution (GIBCO), followed by addition of lysis buffer (GIB solution: 4 mol/L ultra PURE guanidine isothiocyanate [Life Technologies Inc, Gaithersburg, MD], 0.5% N-laurylsarcosine sodium salt, 2-mercaptoethanol [Nacalai Tesque Inc, Kyoto, Japan], 5 mmol/L trisodium citrate dihydrate [Wako Pure Chemical Inc, Osaka, Japan], pH 7.0) directly to the cells in the culture dish. Lysates were collected from three dishes, layered over a 5.7-mol/L CsCl cushion, and ultracentrifuged at 120,000 g for 18 hours. The RNA pellets were dissolved in diethylpyrocarbonate-treated sterile water and ethanol-precipitated, and RNA concentrations were measured by spectrophotometry. Equal amounts of total RNA from different sources were analyzed for t-PA and PAI-1 mRNA content by dot blot hybridization. Serial dilutions of total RNA were blotted to nitrocellulose membranes using a MINIFOLD S apparatus (Schleicher & Schuell, Dassel, Germany). Prehybridization and hybridization were performed at 65°C as previously described. Hybridization was performed with probes labeled by the random-primer method. Probes used in this study were as follows: 608-bp t-PA cDNA was kindly provided by Dr Desire Collen, and 1.3-kb PAI-1 cDNA was a generous gift from Dr Atsushi Mimuro and Dr Youichi Sakata. A CDNA probe for γ-actin was used for comparison.

Statistics. Data are presented as the mean ± SD and were analyzed with a two-factor analysis of variance (ANOVA) followed by Scheffe’s test. Differences were considered significant at P < .05.

RESULTS

IL-β and TNFα increase secretion of PAI-1 but not t-PA from endothelial cells. Endothelial cells were grown to confluency in 35-mm petri dishes and exposed to complete medium containing IL-1β or TNFα for 24 hours. Culture supernatants were then assayed for t-PA and PAI-1. Levels of t-PA in cultured medium from resting, IL-1β–treated, and TNFα–treated cells were 0.46 ± 0.22, 0.27 ± 0.12, and 0.46 ± 0.23 ng/10⁶ cells/h, respectively, and respective PAI-1 levels were 34.43 ± 10.78, 98.8 ± 19.34, and 145.33 ± 23.72 ng/10⁶ cells/h. Neither IL-1β nor TNFα affected t-PA secretion, but each cytokine significantly increased PAI-1 secretion as compared with control media (Fig 1, at 0 dyne/cm²), IL-3 used as a control cytokine had no effect (data not shown).

Shear stress augments t-PA secretion and attenuates PAI-1 release. Endothelial cells were grown to confluency at 0, 6, 12, 18, and 24 dynes/cm². Concentrations of t-PA secreted into the culture medium increased significantly as shear stress increased (0.46 ± 0.22 ng/10⁶ cells/h for resting cells vs. 1.36 ± 0.33 ng/10⁶ cells/h for sheared cells at 24 dynes/cm²). PAI-1 secretion gradually diminished (34.43 ± 10.78 ng/10⁶ cells/h for resting cells vs. 23.33 ± 7.63 ng/10⁶ cells/h for cells at 24 dynes/cm²), as shown in Fig 1. Furthermore, active PAI-1 release was significantly decreased with increased intensity of shear stress (0.91 ± 0.44 ng/mL for resting cells vs. 0.09 ± 0.14 ng/mL at 24 dynes/cm²), as shown in Fig 2. All the effects of shear stress were significant: t-PA (P < .0001), t-PA-PAI complex (P < .0001), total PAI-1 (P < .05), and active PAI-1 (P < .0001).

Shear stress augments t-PA secretion and attenuates PAI-1 secretion from endothelial cells incubated with cytokines. An assay of t-PA and PAI-1 release from cytokine-treated cells under various shear stresses indicated that the gradual increase in t-PA secretion with increasing shear forces was markedly augmented in the presence of IL-1β or TNFα. At 24 dynes/cm², t-PA release was 2.9-fold higher than in resting cells in the absence of cytokines, 10.6-fold higher in the presence of IL-1β, and 7.9-fold higher with TNFα (Fig 1A). By contrast, PAI-1 secretion induced by cytokines at rest was gradually attenuated with increasing shear stress (Fig 1B). Similarly, the increment in active PAI-1 after IL-1β
treatment gradually decreased with increasing shear stress (Fig 2A). At rest, t-PA-PA1 complex formation was unchanged by treatment with cytokines, but increased with addition of shear forces, and IL-1β enhanced t-PA-PA1 complex formation at each shear value (Fig 2B).

Levels of t-PA, active PAI-1, and t-PA-PA1 complex were significantly affected by the interaction of cytokines and shear stress ($P < .0001$ for t-PA and t-PA-PA1 complex, and $P < .005$ for active PAI-1), whereas total PAI-1 levels showed no significant changes. Thus, cytokines and shear stress have synergistic effects on t-PA, t-PA-PA1 complex, and active PAI-1 levels, but not on total PAI-1.

Synergistic effect of shear stress and cytokines on t-PA secretion and t-PA-PA1 complex formation is significant in endothelial cells cultured without ECGS and heparin. To investigate the effects of ECGS and heparin on the synergistic interaction of shear stress and cytokines, culture media were discarded and replaced with ECGS- and heparin-free media 2 hours before stimulation with IL-1β or TNFα and the loading of shear stress with 18 dynes/cm².

t-PA release after cytokine stimulation (0.052 ± 0.01 ng/10⁶ cells/h by IL-1β stimulation and 0.021 ± 0.01 by TNFα stimulation, respectively) was similar to t-PA basal levels (0.029 ± 0.01 ng/10⁶ cells/h). Under shear stress with 18 dynes/cm², t-PA release was significantly ($P < .0001$) increased (0.084 ± 0.016 ng/10⁶ cells/h), and in the presence of cytokines, it was further augmented (0.469 ± 0.078 ng/10⁶ cells/h by IL-1β and 0.385 ± 0.151 by TNFα stimulation, Fig 3A). By contrast, PAI-1 release after cytokine stimulation was increased (to 185.24 ± 13.56 ng/10⁶ cells/h by IL-1β and 168.72 ± 10.18 by TNFα, respectively) as compared with basal levels (66 ± 5.59 ng/10⁶/h). Under shear forces with 18 dynes/cm², PAI-1 release was significantly ($P < .05$) decreased (to 45.83 ± 4.26 ng/10⁶ cells/h). Shear forces of 18 dynes/cm² also attenuated PAI-1 release induced by cytokines to 162.35 ± 7.2 ng/10⁶ cells/h by IL-1β and 140.42 ± 6.49 ng/10⁶ cells/h by TNFα, respectively (Fig 3B) and attenuated active PAI-1 release from cytokine-stimulated endothelial cells (Fig 3C), although in neither case was the effect statistically significant. However, shear stress significantly augmented t-PA-PA1 complex
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Fig 3. t-PA (A), PAI-1 (B), active PAI-1 (C), and t-PA/PAI complex (D) secretion from human umbilical vein endothelial cells cultured for 24 hours in the absence of ECGS and heparin. t-PA, PAI-1, active PAI-1, and t-PA/PAI complex were assayed by sandwich ELISA (see Figs 1 and 3). Lane 1 (control), unstimulated and unsheared cells (n = 6); lane 2 (cytokine), cells treated with IL-1β or TNFα (n = 3 each); lane 3 (shear stress 18 dynes/cm²), unstimulated cells under shear stress with 18 dynes/cm² (n = 6); lane 4 (cytokine-stimulated sheared cells), sheared cells with 18 dynes/cm² treated with IL-1β or TNFα (n = 3 each). **Significant (P < .0001) increase in specific protein secretion from stimulated and/or sheared cultured cells versus unstimulated and unsheared cells. *Significant (P < .05) decrease in PAI-1 or active PAI-1 secretion from stimulated and/or sheared cultured cells versus unstimulated and unsheared cells. NS, Synergistic effect was not significant by two-way ANOVA.

A

B

C

D

formation from cytokine-stimulated endothelial cells (Fig 3D).

Thus, without ECGS and heparin, levels of t-PA and t-PA/PAI complex were significantly increased by the interaction of cytokines and shear stress (P < .0001 in t-PA and t-PA/PAI complex) although basal levels of t-PA were decreased, whereas total PAI-1 and active PAI-1 levels showed no significant change although basal secretion of PAI-1 was slightly increased. Thus, the synergistic interaction of shear stress and cytokines was observed on t-PA and t-PA/PAI complex but not on PAI-1 in the presence or absence of ECGS and heparin.

Effects of shear stress on mRNA levels of t-PA and PAI-1 in cytokine-stimulated endothelial cells. Dot hybridization analysis was performed to compare changes in mRNA levels for t-PA and PAI-1. Experiments were performed three times, and the representative data are shown in Fig 4. When 3 μg total RNA was applied, t-PA mRNA was not detectable at 0 dyne/cm² with or without cytokine stimulation; however, t-PA mRNA was clearly detectable when shear forces were applied and was further enhanced in the presence of IL-1β (Fig 4, upper panel, lanes 5 to 8). Scanning densitometry indicated 2.1-fold higher t-PA mRNA levels from cytokine-treated sheared cells as compared with sheared cells without cytokine. This increase in t-PA mRNA was also observed in experiments in which ECGS and heparin were omitted in the culture medium (Fig 4, upper panel, lanes 1 to 4). By contrast, PAI-1 mRNA levels were detectable in stationary cultured cells and were enhanced 6.8-fold after cytokine (IL-1β) stimulation. However, PAI-1 mRNA levels decreased to one fifth of control levels under shear stress in the absence of IL-1β and to one half of control levels with IL-1β–treated cells exposed to shear stress (Fig 4, middle panel). γ-Actin mRNA used as a control was present at similar levels in all lanes. Changes similar to those described with IL-1β were also induced with TNFα (data not shown).

Effects of shear stress and cytokines on endothelial cell shape. Cell shapes of resting, sheared, cytokine-treated, and cytokine-treated sheared cells were compared under the phase-contrast microscope. Resting cells assumed a cobble-
stone shape, and shear forces at 18 dynes/cm² caused cells to elongate to a teardrop shape. IL-1β enhanced the elongation of sheared cells at each shear stress applied, although IL-1β in the absence of shear had only a slight effect on cell shape (Fig 5). Similar changes were observed in TNFα-treated cells (data not shown).

**Discussion**

We have shown that physiologic fluid shear stress increases t-PA release and decreases PAI-1 secretion. Furthermore, shear forces alter the effects of IL-1β and TNFα, enhancing t-PA secretion and attenuating the PAI-1 response induced by these cytokines. These interacting effects of shear forces and cytokines were statistically significant. These results clearly indicate that shear stress can enhance fibrinolytic activity induced by cytokines at the transcriptional level, and thus may play a crucial role in regulating the fibrinolytic system at sites of inflammation and cell-mediated immune response.

Several recent lines of evidence have indicated that physiologic levels of shear stress can upregulate the expression of several pathophysiologically relevant genes in cultured endothelial cells, including t-PA, TGF-β1, and PDGF-A and -B, based on measurement of steady-state levels of mRNA. Resnick et al. have identified a common promoter element that interacts with shear stress-induced transcription factors and the core binding sequence (GAGACC) of the putative SSRE in proteins for which mRNA levels are increased by flow-loading, such as PDGF, TGF-β, t-PA, and intercellular adhesion molecule (ICAM)-1. By contrast, Ohno et al. postulated the existence of novel SSRE cis and
trans elements based on deletion analysis that revealed no
effect of GAGACC on the induction of TGF-β1 gene expres-
sion in bovine aortic endothelial cells in response to shear
stress. It is now recognized that hemodynamic forces can
influence endothelial cell structure and function. In par-
cular, wall shear stress, the force that is imparted to the vascula-
ture by blood flow, can involve activation of constitutive
stimulus-response effector mechanisms and further cause de
novo protein synthesis and secretion. Diamond et al. have
shown that shear stress induces a large change in t-PA
mRNA levels but only a threefold enhancement of t-PA
secretion, suggesting that an additional level of posttran-
scriptional regulation of t-PA may be present. Thus, shear
forces could modulate t-PA synthesis through gene regula-
tion.

T-PA and PAI-1 are synthesized and secreted mainly by
endothelial cells to regulate fibrinolytic activity. A variety
of agents have been shown to perturb endothelium, resulting
in changes in t-PA and PAI-1 secretion that may alter fi-
brinolytic activity and possibly induce thrombosis. Cyto-
kines are one of the most important modulators of t-PA
and PAI-1 synthesis by vascular endothelium. Cytokines
are released from aggregating platelets, from activated leuko-
cyes, and from cells present in the vascular wall; both endo-
theelial cells and smooth muscle cells have receptors for many
of these agents. Among the various cytokines, IL-1 and
TNF have frequently been reported to alter t-PA and PAI-1
secretion. In agreement with most studies, we find that t-
PA secretion is unchanged and PAI-1 secretion increases
after stimulation with 100 U/mL IL-1β or TNFα in human
umbilical vein endothelial cells, although others have re-
ported a decrease in t-PA secretion. Since ECGS and hepa-
rin may alter t-PA and PAI-1 secretion, we also conducted
experiments without ECGS and heparin; again, t-PA secre-
tion was essentially unaffected by cytokines. However,
ECGS and heparin affected basal secretion of t-PA and PAI-
1; t-PA levels of cells cultured in the presence of ECGS and
heparin were 16-fold higher than those of cells in the absence
of these reagents, whereas PAI-1 levels of cells cultured in
the presence of ECGS and heparin were one half of those
cells cultured with ECGS and heparin. Konkle and Gins-
burg reported that addition of ECGS and heparin to cultured
cells for at least 24 hours up to 3 days resulted in a threefold
to 10-fold decrease in PAI-1 activity secreted into the condi-
tioned media and no significant change in t-PA antigen, using
primary to fifth-passage confluent human umbilical vein en-
dotheelial cells. Our finding of a significant decrease of t-PA
and slight increase of PAI-1 in the absence of ECGS and

Fig 5. Effect of shear stress, IL-1β, or both on the shape of cultured endothelial cells after 24 hours. Control, resting cells; 18 dynes/cm², shear-stressed cells at 18 dynes/cm²; IL-1β, IL-1β-treated cells; IL-1β + 18 dynes/cm², IL-1β-treated cells at 18 dynes/cm².
heparin may be due to differences in culture time in the absence of ECGS and heparin, endothelial cell conditions, or measurement of t-PA and PAI-1.

Although both hemodynamic forces and cytokines are physiologically important in regulating endothelial functions, the interacting effects of shear stress and cytokines on vascular functions have not been well characterized. Our analysis of t-PA and PAI-1 synthesis at the mRNA level in sheared endothelial cells under cytokine stimulation produced results similar to those of Diamond et al., i.e., shear stress of 18 dynes/cm² increases t-PA secretion severalfold, coupled with a large increase in t-PA mRNA levels, regardless of the presence of ECGS and heparin. In addition, cytokines may further enhance t-PA synthesis at the transcriptional level under the influence of shear force, even though cytokines alone did not affect t-PA synthesis. In contrast, PAI-1 was decreased in proportion to the shear stress applied, regardless of cytokines, and mRNA levels were similarly decreased. Cultured human umbilical vein endothelial cells contain two PAI-1 mRNA species, both encoded by a single gene and differing by 1 kb in the 3' untranslated region.25 Dichek and Quertermous26 found that levels of PAI-1 mRNA were different in human umbilical vein endothelial cells of different lineage and culture time, and a shift in the proportion of PAI-1 mRNA from the 3.4- to 2.4-kb species was observed in the different-passage cells. Our dot hybridization analysis did not address whether only one or both PAI-1 mRNA species decreased. Diamond et al have also reported that PAI-1 levels were only slightly decreased under the stimulation of shear force, whereas we find a significant PAI-1 decrease, a discrepancy that may rest in the use of different systems (cone-plate vs parallel-plate flow chamber), endothelial cell conditions (second-passage vs primary cultured cell), and measurements of PAI-1 (total PAI-1 vs uncomplexed PAI-1).

Kooistra et al.27 have demonstrated that the protein kinase C activator, PMA, caused an increase in t-PA antigen levels with parallel increases in t-PA mRNA levels. Furthermore, an increase in intracellular cyclic adenosine monophosphate (cAMP) levels by forskolin only slightly diminished t-PA secretion, but further enhanced PMA-induced increases in t-PA synthesis and mRNA levels. The regulatory mechanisms involved in this phenomenon are still poorly understood. The action of cytokines, which enhance shear stress-induced t-PA secretion without an effect on static cells, resembles the effect of cAMP described by Kooistra et al.,27 who suggested that protein kinase C may modulate t-PA synthesis through intracellular signal transduction by cytokines. Recently, Sampath et al.28 reported that sequential application of shear stress (25 dynes/cm²) and LPS (25 ng/mL) induced greater expression of ICAM-1 than simultaneous application. Synergy between cytokines has been studied previously in cytokadhesion molecules such as ICAM-1 and VCAM-1.29,30 Shear stress has also been shown to increase the release of IL-1 and IL-6 in aortic endothelial cells.31 Our present findings clearly demonstrate synergy between shear stress and cytokines. The interacting effects of shear stress and cytokines were also observed on cell shape, although the mechanisms underlying this effect are unknown.

More recently, the proto-oncogenes c-fos, c-jun, and c-myc have been shown to respond to shear stress, and DNA-binding affinities of NFkB and AP-1 complex are changed in endothelial cells exposed to flow.32,34 Cytokines also affect these proto-oncogenes, which are located at promoter regions, through intracellular signal transduction.35,36 Shyy et al.4 found that genes known to be regulated by shear stress, including c-fos, c-jun, PDGF, t-PA, endothelin-1, ICAM-1, and TGF-β1, contain sequences with homology to TRE, TGA(C or G)/TCA, and the human MCP-1 gene, which is also modulated by shear forces and might be regulated through protein kinase C.5 Further study is needed to elucidate the common pathways by which biomechanical forces influence gene expression or posttranscriptional events. Whether the decrease in PAI-1 mRNA by shear forces is controlled at the transcriptional level or by stabilization of mRNA also remains to be clarified, since no SSRE element is located in the promoter region of PAI-1.

ACKNOWLEDGMENT

We thank Dr Desire Collen for providing the cDNA probe for t-PA, and Dr Youichi Sakata and Dr Atsushi Mimuro for providing the cDNA probe for PAI-1.

REFERENCES

34. Lan Q, Mercurius KO, Davies PF: Stimulation of transcription factors NFκB and API in endothelial cells subjected to shear stress. Biochem Biophys Res Commun 201:950, 1994
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