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Research Article

We examined the effects of human interleukin 1 (IL-1) on the production of fibrinolytic components by cultured human vascular endothelium. Conditioned media collected from IL-1-treated (5 U/ml, 24 h) monolayers exhibited decreased tissue-type plasminogen activator (tPA) activity and increased plasminogen activator inhibitor (PAI) activity, as assessed by fibrin and reverse fibrin-autography. Quantitative immunological assays revealed a 35% decrease in tPA antigen and a 360% increase in active PAI antigen, after incubation for 24 h with 0.6 U/ml IL-1. Maximal effects (approximately 50% decrease in tPA antigen; 400-800% increase in active PAI antigen) were observed with 2.5-5 U/ml IL-1. Changes in tPA and PAI reached a maximum at approximately 24 h and persisted for greater than 48 h. IL-1 induction of endothelial procoagulant activity was more rapid and transient, peaking by 6 h and subsiding by 24 h. Natural monocyte-derived IL-1 and two species of recombinant IL-1 had comparable effects. Heat and polymyxin-B treatments differentiated IL-1 actions from those of endotoxin, which promoted similar endothelial alterations. IL-1 effects on endothelial procoagulant and fibrinolytic activities may contribute to the generation and maintenance of fibrin in pathophysiological settings in vivo.

Regulation of the Fibrinolytic System of Cultured Human Vascular Endothelium by Interleukin 1

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Abstract

We examined the effects of human interleukin 1 (IL-1) on the production of fibrinolytic components by cultured human vascular endothelium. Conditioned media collected from IL-1-treated (5 U/ml, 24 h) monolayers exhibited decreased tissue-type plasminogen activator (tPA) activity and increased plasminogen activator inhibitor (PAI) activity, as assessed by fibrin and reverse fibrin-autography. Quantitative immunological assays revealed a 35% decrease in tPA antigen and a 360% increase in active PAI antigen, after incubation for 24 h with 0.6 U/ml IL-1. Maximal effects (~50% decrease in tPA antigen; 400–800% increase in active PAI antigen) were observed with 2.5–5 U/ml IL-1. Changes in tPA and PAI reached a maximum at ~24 h and persisted for >48 h. IL-1 induction of endothelial procoagulant activity was more rapid and transient, peaking by 6 h and subsiding by 24 h. Natural monocyte-derived IL-1 and two species of recombinant IL-1 had comparable effects. Heat and polymyxin-B treatments differentiated IL-1 actions from those of endotoxin, which promoted similar endothelial alterations. IL-1 effects on endothelial procoagulant and fibrinolytic activities may contribute to the generation and maintenance of fibrin in pathophysiological settings in vivo.

Introduction

Increasing experimental evidence supports the concept of a "hemostatic balance" between prothrombotic and antithrombotic activities at the level of the vascular endothelial lining (1). Inflammatory mediators that can act on endothelial cells may play a central role in the regulation of these activities. We have previously reported that interleukin 1 (IL-1),[1] a product of stimulated mononuclear phagocytes, can induce a relatively rapid and transient expression of tissue factor procoagulant activity in cultured human endothelial monolayers (2–4). This IL-1–stimulated alteration in endothelial cell surface thrombogenicity could potentially contribute to localized or disseminated activation of coagulation at the blood-vascular wall interface in vivo.

In the current studies, we have examined the effects of IL-1 on endothelial components of the fibrinolytic system. Vascular endothelial cells produce both a tissue-type plasminogen activator (tPA) and a β-migrating 50,000-mol wt plasminogen activator inhibitor (PAI) that can form a complex with tPA and neutralize its activity (5). We now report that treatment of cultured human endothelial cells with IL-1 results in a concomitant decrease in the production of tPA and increase in PAI, thereby suppressing net fibrinolytic activity. The effects of IL-1 on these fibrinolytic components were found to be delayed and prolonged relative to its effects on procoagulant activity. The complementary actions of IL-1 on procoagulant and fibrinolytic activities thus could shift the "endothelial hemostatic balance" toward the generation and maintenance of fibrin, and thereby influence inflammatory and immune reactions in vivo.

Methods

Reagents. Human monocyte-derived IL-1 (hmIL-1), isolated as a mixture of two (or more) polypeptides (~17 kD) from the supernatant of *Staphylococcus albus*-stimulated monocytes by immunoabsorption and Sephadex chromatography (6), was obtained from Genzyme, Inc., Boston, MA. hmIL-1 was provided in sterile 0.15 M NaCl with 5% fetal calf serum (FCS) and reportedly contained 100 U/ml thymocyte costimulation activity, <1.0% T cell growth factor, <1 U/ml interferon, and undetectable endotoxin. Lysates of control L cells and L cells that express human recombinant IL-1 (rIL-1), derived from the complementary DNA (cDNA) cloned by Auron et al. (7), and corresponding to the predominant species (pI 7) in hmIL-1 (6), were provided by Dr. T. Livelli, Cistron Technology, Pine Brook, NJ. Purified rIL-1β (pI 7) produced by expression of this same cDNA in *Escherichia coli* was purchased from Cistron; a distinct species, rIL-1α (pI 5) (8), was obtained from Genzyme, Inc. Bacterial endotoxin (lipopolysaccharide, *E. coli* 0111.B4), was purchased from Difco Laboratories, Detroit, MI; polymyxin-B sulfate was obtained from Sigma Chemical Co., St. Louis, MO. Human tPA was isolated from melanoma cells as described (9). PAI was purified from bovine aortic endothelial cells by fractionation on concanavalin A-Sepharose and preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10). Rabbit antisera to tPA and PAI were prepared by standard methods (10); goat antiserum to tPA was obtained from Bio-pool, Hornefors, Sweden.

Endothelial cell cultures. Human umbilical vein endothelial cells (HUVEC) were isolated from 2 to 5 cords, pooled, and grown in primary culture using medium 199 (M199, M.A. Bioproducts, Bethesda, MD) with 20% FCS (Gibco, Grand Island, NY) and antibiotics (11). HUVEC and human adult saphenous vein endothelial cells (HSaVEC; provided

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1. *Abbreviations used in this paper:* CM, conditioned medium; ECGF, endothelial cell growth factor; FCS, fetal calf serum; hmIL-1, human monocyte-derived IL-1; HSaVEC, human saphenous vein endothelial cells; HUVEC, human umbilical vein endothelial cells; IL-1, interleukin 1; IRMA, immunoradiometric assay; M199, medium 199; PA, plasminogen activator; PAI, plasminogen activator inhibitor; rIL-1, human recombinant IL-1; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; tPA, tissue-type plasminogen activator.

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by Dr. P. Libby, Tufts University Medical School, Boston, MA) were passaged (1:3 split ratios) using M199-20% FCS (HUVEC) or M199-10% FCS (HSaVEC) with endothelial cell growth factor (ECGF, 50–150 μg/ml; provided by Dr. T. Maciag, Meloy Laboratories, Springfield, VA) and porcine intestinal heparin (50–100 μg/ml, Sigma). For experimental use, cells were grown to confluence (3–7 d) in culture dishes (Costar, Cambridge, MA) coated with purified fibronectin (1–5 $\mu g/cm^2$; Meloy).

Cell treatments. Natural hmIL-1, rIL-1, or endotoxin were added to washed monolayers in various pretreatment media, including M199-20% FCS with ECGF and heparin, RPMI 1640 (RPMI, M.A. Bioproducts) with 10% FCS or with 0.1% bovine serum albumin (BSA, Sigma), all of which produced comparable results. In certain cases, the mediators were heat treated (80°C, 15 min) or mixed with polymyxin-B sulfate (50 μg/ml, 10 min) prior to their addition to monolayers. After incubation for up to 72 h, the conditioned media (CM) and cells were assayed for fibrinolytic components and procoagulant activity, respectively.

Evaluation of endothelial procoagulant activity. Monolayers (16-mm wells) were washed three times in 0.5 ml of RPMI. Total cellular procoagulant activity was determined in a one-stage clotting assay (2), using glass tubes containing 100 μl pooled, normal donor, platelet-poor, citrated plasma, to which 100 μl cell lysate (frozen-thawed three times, scrape harvested) and 100 μl $CaCl_2$ (30 mM) were added. Milliunits of procoagulant activity were defined by standard curves (log-log plot) developed with rabbit brain thromboplastin (Sigma); 10^3 mU corresponded to a clotting time of 20 s.

Evaluation of endothelial fibrinolytic components. CM from cultures (35-mm dishes; 2 ml) were collected, centrifuged (10,000 *g*, 3 min), transferred to fresh tubes, and frozen at −80°C after the addition of 0.01% Tween 80. Aliquots were fractionated by SDS-PAGE (12) (10 cm gels, 7.5% acrylamide) and analyzed by fibrin-autography for PA activity(ies) and by reverse fibrin-autography for PAI activity(ies) (13). In fibrin-autography, the SDS gel was incubated with 2.5% Triton X-100 (two changes; 250 ml each) and placed on a fibrin-agar indicator film containing 1% Seaplaque agarose (FMC Corp., Rockland, ME), 25 μg/ml plasminogen, 2.4 mg/ml fibrinogen and 0.5 U/ml thrombin. Development of clear lytic zones in the opaque indicator film (2–4 h, 37°C) reveals PA activity. In reverse fibrin-autography, indicator films also contained 0.05 U/ml urokinase, which converts the plasminogen into plasmin, thus hydrolyzing the fibrin. Development of opaque, lysis-resistant zones in the otherwise clear indicator film reveals PAI activity.

CM were also analyzed by quantitative immunoradiometric assays (IRMAs) (14). To assess tPA antigen, samples were diluted in IRMA buffer (phosphate-buffered saline, PBS, 3% BSA, 5 mM EDTA, 0.1% Tween 80, and 0.02% NaN_3) and incubated (1.5 h, 37°C) in microtiter wells precoated with goat anti-tPA (50 μl/well, 10 μg/ml). Plates were blocked with 3% BSA and washed with PBS containing 0.1% BSA, 0.05% NaN_3, and 0.05% Tween 80. Bound tPA was quantitated radiometrically (gamma counter) after washing and incubating with rabbit anti-tPA (1:75 dilution), followed by ^{125}I-labeled goat anti-rabbit IgG (10^5 cpm/well). Free PAI antigen (not complexed with tPA) was evaluated by the tPA binding assay (14). CM and purified PAI standards in IRMA buffer were incubated (1 h, 37°C) in microtiter wells precoated with tPA (50 μl/well, 1 μg/ml; overnight, 4°C) and blocked as described above. Endothelial PAI exists in CM both as active and latent forms. Active PAI was directly measured in diluted CM. Latent PAI was measured after converting it into the active form by treatment with 0.1% SDS (1 h, 37°C) (15), followed by dialysis (PBS-0.01% Tween 80). PAI bound to tPA-coated wells was detected radiometrically after incubation (1.5 h, 37°C) with rabbit antiserum to the PAI (1:75 dilution, 50 μl/well), followed by ^{125}I-labeled goat anti-rabbit IgG ($2.5–5 \times 10^4$ cpm/well; 1.5 h, 37°C) (14).

Results

IL-1 alters endothelial cell production of tPA and PAI. CM were collected from HUVEC after incubation for 24 h in the presence or absence of 5 U/ml hmIL-1, fractionated by SDS-PAGE, and analyzed by fibrin-autography and reverse fibrin-autography. IL-1–treated HUVEC elaborated less high molecular weight tPA activity (Fig. 1 *A*) and more PAI (50,000 relative molecular weight) activity (Fig. 1 *B*) than did untreated cultures. Neither control nor IL-1–treated HUVEC produced detectable urokinase-like PA activity.

Immunologic assays were used to quantitate the effects of IL-1 on endothelial tPA and PAI. As seen in Fig. 2, incubation of serially passaged HUVEC and HSaVEC with hmIL-1 for 24 h resulted in a concentration-dependent decrease in secreted tPA antigen and increase in PAI. Changes were observed with as little as 0.6 U/ml hmIL-1, and were maximal with 2.5–5 U/ml. In six experiments, 24 h incubation with 5 U/ml hmIL-1 promoted a 49±12% decrease in tPA antigen, a 550±140% increase in active PAI antigen, and a 234±60% increase in latent PAI antigen (mean±SD).

Comparison of the effects of IL-1 on endothelial fibrinolytic and procoagulant activities. The kinetics of IL-1–mediated changes in tPA and PAI were compared with those of IL-1 induction of procoagulant activity (Fig. 3). As previously reported (2), the stimulation of total cellular procoagulant activity in cultured HUVEC was rapid and transient. Stimulated procoagulant activity reached a peak by 6 h after the addition of 5 U/ml hmIL-1, and declined toward untreated control levels by 24 h.

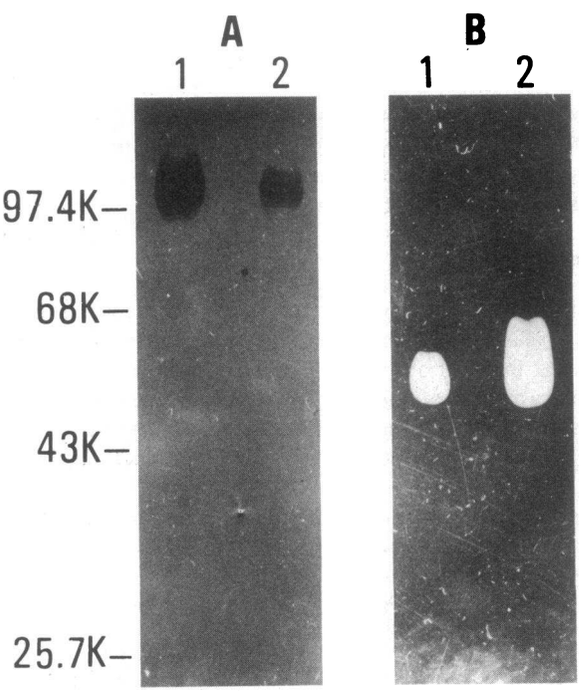

Figure 1. Effect of IL-1 on the PA and PAI profiles of HUVEC. Confluent HUVEC (first passage) were incubated in RPMI-0.1% BSA with or without 5 U/ml hmIL-1. After 24 h, the CM were harvested and 50-μl aliquots were subjected to SDS-PAGE. Electrophoresed gels were analyzed for PA activity(ies) by fibrin-autography (panel *A*) and for PAI activity(ies) by reverse fibrin-autography (panel *B*). Lane *1*, CM from control HUVEC; lane *2*, CM from IL-1-treated-HUVEC. Molecular weight standards included phosphorylase B (97,400 relative mol wt), human serum albumin (68,000 relative mol wt), ovalbumin (43,000 relative mol wt), and α-chymotrypsinogen (25,700 relative mol wt) (Sigma).

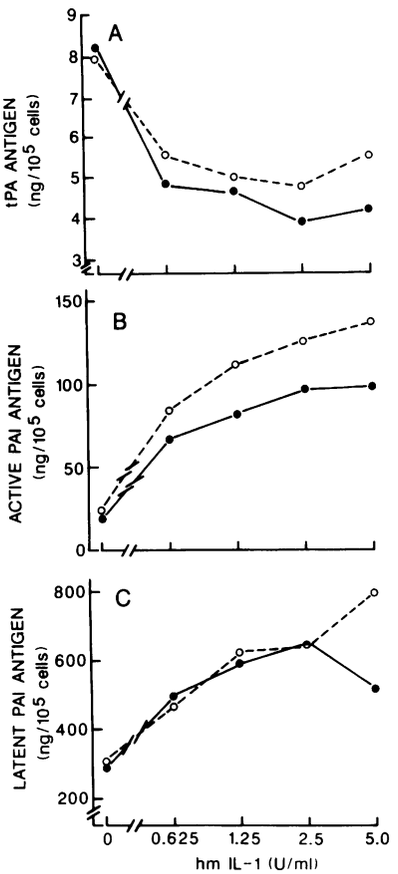

Figure 2. Effect of IL-1 concentration on the secretion of tPA and PAI by HUVEC and HSaVEC. Confluent HUVEC (second passage; solid line) and HSaVEC (fifth passage; broken line) were washed and incubated in complete growth media (M199-20% FCS plus ECGF and heparin) supplemented with the indicated concentration of hmIL-1. After 24 h, CM were harvested and assayed by IRMAs for tPA antigen (*A*), and for active (*B*) and latent (*C*) PAI antigen. Data points represent the mean values from triplicate cultures.

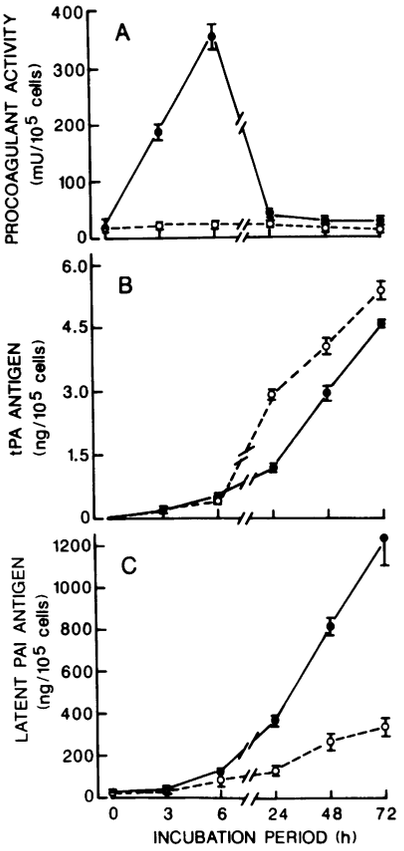

Figure 3. Kinetics of IL-1 effect on procoagulant and fibrinolytic activities of HUVEC. Confluent HUVEC (third passage) were washed and incubated in complete growth media either in the presence (solid line) or absence (broken line) of 5 U/ml hmIL-1. At the indicated times the CM and cells were harvested. Procoagulant activity (*A*) was determined in the cell lysates using a one-stage clotting assay, while tPA antigen (*B*) and latent PAI antigen (*C*) in the CM were quantitated by IRMAs. Data represent mean±SD from triplicate cultures.

The kinetics of the IL-1 mediated changes in tPA and PAI were relatively slow by comparison. Maximal change in tPA and PAI did not occur until after 24 h, at which time IL-1–stimulated procoagulant activity had nearly disappeared. Moreover, the cells continued to express decreased tPA and increased PAI for the next 48 h. The kinetics of the changes in endothelial tPA and PAI thus were quite different from those observed for procoagulant activity.

Two distinct species of rIL-1 act on endothelial cells. It has been established recently that hmIL-1 consists of several biochemically distinct species that appear to be encoded by at least two different genes (6–8). As seen in Table I, two purified rIL-1 species, derived from strains of *E. coli* expressing distinct cDNAs, altered endothelial production of tPA and PAI in a manner comparable to hmIL-1. In a separate experiment, mammalian cell-derived (L cell lysate) recombinant human IL-1 (pI 7) behaved similarly, inducing a 56% decrease in tPA antigen, and a 200% increase in latent PAI antigen, whereas a control L cell lysate had no significant effect.

In agreement with previous reports (2–4, 16–19), we found that endotoxin stimulated endothelial procoagulant and PAI activity. In addition, we observed that endotoxin decreased elaboration of tPA, and thus mimicked the biological activities of IL-1 in our studies (data not shown). To demonstrate that the results obtained with IL-1 were not due to the presence of endotoxin in the IL-1 preparations, we examined the effects of polymyxin-B and heat treatment on the biological activities of both endotoxin and IL-1 (3). Polymyxin-B treatment (50 μg/ml; premixed, 10 min) abolished the effect of 1 μg/ml endotoxin (90–100% inhibition) on endothelial procoagulant activity, and on tPA and PAI, but did not affect the activity of IL-1 preparations (<3% inhibition). Moreover, as we previously described (3), heat treatment (80°C, 15 min) did not significantly alter endotoxin activity on HUVEC, while it completely inactivated IL-1 preparations (data not shown). IL-1 and endotoxin thus can act in a similar fashion to alter endothelial procoagulant and fibrinolytic activities, and the actions of these mediators can be distinguished in vitro.

Discussion

These studies were based on the premise that vascular endothelium plays an active role in coagulation and inflammatory responses in vivo. We had previously demonstrated (2–4) that IL-1 can act directly on cultured human endothelial cells to induce

*Table I. hmIL-1 and Two Distinct Species of rIL-1 Affect Endothelial tPA and PAI**

Endothelial cell treatment	tPA Antigen	PAI antigen	
		Active	Latent
24 h	*ng/10⁵ cells*	*ng/10⁵ cells*	*ng/10⁵ cells*
Control	2.55±0.26‡	9±1	121±16
hmIL-1	1.76±0.05	46±4	398±20
rIL-1 α(pI 5)	1.76±0.09	49±5	409±27
rIL-1 β(pI 7)	1.81±0.03	40±8	404±35

* Confluent HUVEC (second passage) were incubated for 24 h with control media (RPMI-0.1% BSA) alone or supplemented with maximal doses of hmIL-1 (5 U/ml), rIL-1α (pI 5) (Genzyme) or rIL-1β (pI 7) (Cistron). The concentrations of the rIL-1 preparations used gave responses in a lymphocyte comitogenesis assay equivalent to 5 U/ml of hmIL-1 (courtesy of Dr. A. Abbas, Brigham and Women's Hospital, Boston, MA). Endothelial CM were collected and assayed for tPA and PAI antigen by IRMAs.

‡ Data represent mean±SD of determinations from triplicate cultures.

the biosynthesis and cell surface expression of tissue factor procoagulant activity. Induction of endothelial procoagulant activity could conceivably result in the intravascular activation of coagulation enzymes. In the present report, we examined the hypothesis that IL-1 also can regulate endothelial expression of key components of the fibrinolytic system. We found that exposure of cultured human endothelium to IL-1 resulted in a coordinated decrease in tPA and increase in the 50,000 relative mol wt PAI. The IL-1-mediated decrease in tPA appeared to be less dramatic and more variable than the stimulation of PAI. Elucidation of the cellular mechanisms involved in these alterations will require further investigation.

IL-1-induced changes in endothelial tPA and PAI were found to be concentration and time dependent. In comparison to the stimulation of procoagulant activity, the effects of IL-1 on tPA and PAI followed a similar dose-response pattern but a different kinetic pattern. Procoagulant activity peaked at ~6 h and then declined toward basal levels, whereas alterations in tPA and PAI reached a plateau at ≃24 h and persisted for at least 72 h. Induced tissue factor procoagulant activity appears to be predominantly cell associated while tPA and PAI are secreted. It is interesting that IL-1 stimulation of an endothelial surface activation antigen (20) follows a kinetic pattern similar to the induction of procoagulant activity. In addition, IL-1 stimulation of another endothelial secretory product, prostacyclin (21, 22), is more closely aligned with its effects on tPA and PAI.

The potential importance of plasma tPA and PAI in patients with thromboembolic diseases, including venous thrombosis and myocardial infarction, has been suggested by recent studies (5, 23, 24). However, regulation of plasma tPA and PAI is still poorly understood. Endothelial cells are a likely source of plasma fibrinolytic components (5). In our studies, cultured human endothelial cells isolated from umbilical and saphenous veins, were found to produce tPA and the rapidly acting PAI of 50,000 relative mol wt. Erickson et al. (25), demonstrated that this endothelial PAI species is immunologically related to the primary PAI found in platelets, serum, and plasma. It has been observed recently that endotoxin (18, 19) and thrombin (26) can stimulate endothelial cell production of PAI activity(ies). It is interesting that both endotoxin and thrombin can induce endothelial cell secretion of an IL-1-like activity, suggesting that these mediators may act, at least in part, through endogenous IL-1 (27).

In summary, IL-1 can act on vascular endothelium to rapidly and transiently increase procoagulant activity available at its surfaces and subsequently to suppress its secreted fibrinolytic activity through a concomitant decrease in tPA and increase in PAI. In vivo, these IL-1 actions could lead to the development and maintenance of intravascular and perivascular fibrin in a variety of pathophysiological settings, thereby influencing the evolution of thrombotic and inflammatory processes.

Note added in proof. Since submission of this manuscript, Emeis and Kooistra (28) and Nachman et al. (29) have also reported thet IL-1 stimulates PAI activity production by cultured human endothelial cells.

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