INTERLEUKIN 1 (IL-1) INDUCES BIOSYNTHESIS AND CELL SURFACE EXPRESSION OF PROCOAGULANT ACTIVITY IN HUMAN VASCULAR ENDOTHELIAL CELLS

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Vascular endothelium in situ normally acts to inhibit coagulation and thrombosis (reviewed in reference 1). However, recent observations suggest that under certain circumstances endothelial cells may actively promote coagulation by various mechanisms (2–7). For example, human endothelial cells in vitro (2–4), like monocytes and macrophages (8, 9), can be induced to express a tissue factor–like procoagulant activity (PCA), thereby activating coagulation via the extrinsic pathway. Since inflammatory and immunological processes in vivo are often associated with localized or disseminated intravascular coagulation, we hypothesized that soluble mediators produced during these processes might induce endothelial cell expression of PCA, thus modulating the thrombogenicity of the blood-vascular wall interface.

IL-1 is a potent intercellular mediator involved in inflammatory and immunological responses. It is elaborated by monocytes and macrophages during antigen presentation and in response to a variety of other stimuli including endotoxin. Although originally defined as a co-stimulator of thymocyte proliferation and an activator of T lymphocytes, IL-1 also acts upon nonlymphoid target cells to produce multiple biological effects, including fever, elaboration of acute phase reactants, and fibroblast proliferation (reviewed in references 10–12). In this report, we demonstrate that purified human monocyte–derived IL-1 is a potent inducer of a tissue factor–like PCA in cultured human endothelial cells.

Materials and Methods

Cell Cultures. Primary cultures of human umbilical vein endothelial cells (HEC) were established in Medium 199 (M.A. Bioproducts, Walkersville, MD) with 20% fetal calf serum (FCS, Hyclone, Logan, UT or Gibco, Grand Island, NY) as previously described (13), and several strains were serially passaged (up to subculture 30 at 1:3 split ratios) using Endothelial Cell Growth Supplement (50–100 μg/ml; a gift of Dr. T. Maciag, Meloy Laboratories, Springfield, VA) and porcine intestinal heparin (50–100 μg/ml, Sigma Chemical Co., St. Louis, MO). For experimental use, freshly isolated or subcultured HEC were plated (4.0×10^4 cells/well) and grown to confluence (3–7 d) in 16-mm diameter

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wells (Cluster 24, Costar, Cambridge, MA) coated with plasma fibronectin (1 $\mu g/cm^2$, Meloy Laboratories).

IL-1 Preparations. Human IL-1, isolated as a ~15,000-dalton polypeptide from *Staphylococcus albus*–stimulated human monocyte supernatants by immunoabsorption (14) and Sephadex chromatography, was obtained from Genzyme Inc., Boston, MA. This material was provided in sterile 0.15 M NaCl with 5% FCS (IL-1 diluent) and was reported to contain 100 U/ml thymocyte co-stimulation activity, <1.0% T cell growth factor, <1 U/ml interferon, and undetectable endotoxin activity. Thymocyte co-stimulation activity was independently confirmed (courtesy of Dr. C. Reiss, Dana-Farber Cancer Institute, Boston, MA) and absence of endotoxin (<0.1 ng/ml) documented by Limulus assay (Sigma) in our laboratory.

Experimental Procedure. HEC monolayers were washed three times with 10% FCS in RPMI-1640 (RPMI, M.A. Bioproducts), incubated for up to 60 min at 37°C, and washed again to remove growth media. IL-1, endotoxin in the form of lipopolysaccharide (LPS, *E. coli* 0111.B4, Difco Laboratories, Detroit, MI), or control media (IL-1 diluent, Genzyme, or 10% FCS-RPMI) were added to each 16-mm well in a final volume of 0.5 ml 10% FCS-RPMI. In certain experiments, cycloheximide and actinomycin D (Sigma) also were included. After incubation (37°C, 5% CO_2) for up to 28 h, each well was washed twice with 0.5 ml Dulbecco's phosphate buffered saline (DPBS).

Evaluation of PCA. A standard one-stage clotting (plasma recalcification) assay was performed, essentially as described (9), at 37°C using glass tubes containing 100 μl citrated normal donor platelet-poor plasma, or coagulation Factor VII-, IX-, or X-deficient plasma (George King Bio-Medical, Overland Park, KS), to which 100 μl cell lysate (frozen-thawed three times, scrape harvested) and 100 μl $CaCl_2$ (30 mM) were added. In certain experiments, PCA was assayed directly in the culture wells on intact, viable or frozen-thawed monolayers, using a modified plasma recalcification technique (4, 15). To each well containing an intact or disrupted monolayer in 200 μl DPBS, 200 μl normal citrated plasma and 200 μl 30 mM $CaCl_2$ were added, and the clotting time determined. Milliunits (mU) of PCA were defined by reference to standard curves (log-log plot) developed with rabbit brain thromboplastin (Sigma); 10^3 mU PCA corresponded to a clotting time of 20 seconds in the standard assay with normal plasma.

Results

Purified IL-1 Induces Procoagulant Activity in Cultured HEC. Unstimulated passaged HEC (subcultures 3–25; 5 strains) demonstrated low levels of total cellular PCA (27 ± 3 mU/10^5 cells, mean ± SE, 15 experiments) in our standard clotting assay. After treatment with human monocyte-derived IL-1 (10 U/ml) for 4–6 h, cellular PCA was dramatically increased (271 ± 38 mU/10^5 cells, 6 experiments). Negligible PCA (<2 mU/10^5 cells) was liberated into the media of control or IL-1–treated HEC cultures. IL-1 preparations did not contain detectable PCA. Fig. 1 *a* illustrates the concentration dependence of IL-1 induction of HEC-PCA. Stimulation was observed with 0.5–1.0 U/ml IL-1 and was maximum (4–15 times control, 4 experiments) at ~10 U/ml. The majority of our experiments were carried out with immunoaffinity purified, monocyte-derived IL-1 (Genzyme, Inc.); however, monocyte-derived IL-1 prepared by alternate biochemical methods (kindly provided by K. Matsushima and J. J. Oppenheim, National Cancer Institute, Frederick, MD) had comparable HEC-PCA-inducing activity (126–135% of that of immunoaffinity-purified preparations at 5–10 U/ml, 3 experiments).

Significant increases in HEC-PCA (total cellular) were detectable following 1 h of continuous exposure to 5 U/ml IL-1 (Fig. 1 *b*). Activity peaked between 3 and 6 h and declined to near basal levels by 24 h. Addition of fresh IL-1 at 24 h gave negligible restimulation by 28 h, suggesting that the HEC had become

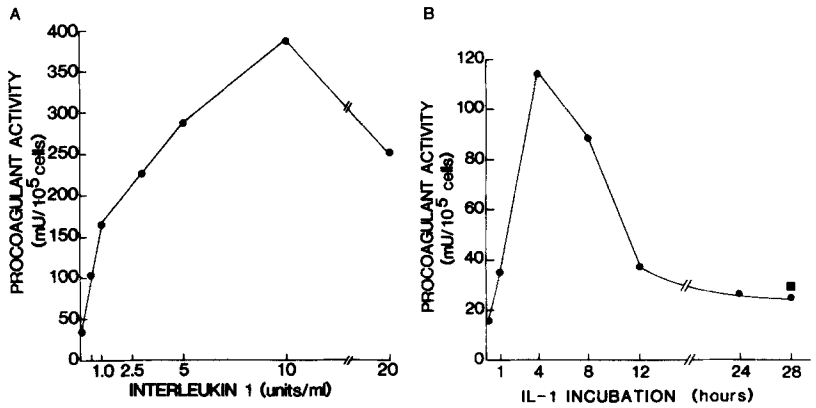

FIGURE 1. (*a*) Concentration dependence of IL-1 induction of total cellular procoagulant activity in passaged human endothelial cells. PCA was assayed in freeze-thaw HEC lysates after 4 h incubation with the indicated concentrations of IL-1. Each point represents mean values from duplicate cultures. Similar results were obtained in three additional experiments. (*b*) Time course of IL-1 induction of total cellular procoagulant activity in passaged human endothelial cells. Replicate cultures were incubated in 10% FCS-RPMI with 5 U/ml IL-1 for up to 28 h (●). At 24 h control (▲) and IL-1 treated (■) wells were stimulated with fresh IL-1 (5 U/ml) for 4 additional h. Each point represents the mean of duplicate cultures. Similar results were found in two additional experiments.

refractory. Treatment with cycloheximide or actinomycin D (5 μg/ml) essentially blocked (>95% inhibition) IL-1 induction (4 h, 5 U/ml) of HEC-PCA, without causing microscopically detectable cell toxicity.

In addition to passaged umbilical vein endothelial cells, IL-1 also enhanced total cellular PCA in primary umbilical vein endothelial cultures (8.7 ± 1.1 times control, mean ± SE, 5 experiments). However, IL-1 (10 U/ml, 4–6 h) did not stimulate total cellular PCA in a human dermal fibroblast strain (A1F21, basal PCA 511 mU/10^5 cells, kindly provided by Dr. J. Rheinwald, Dana-Farber Cancer Institute) and in a human lymphoblastoid B cell line (basal PCA 2 mU/10^5 cells, kindly provided by Dr. J. L. Strominger, Harvard University).

Endotoxin has been reported to induce PCA in primary HEC cultures (3, 4). We confirmed this observation and found a similar result in serially passaged HEC. In comparison, IL-1 consistently induced more HEC-PCA than did endotoxin (data not shown). Furthermore, IL-1 induction of HEC-PCA appeared to be *independent* of endotoxin in that: (*a*) our IL-1 preparations were free of endotoxin by Limulus assay; and (*b*) heat treatment (80°C, 15 min) completely abolished both the PCA-inducing activity (96 ± 4% inhibition, mean ± SE, four experiments) and the thymocyte co-stimulating activity (100% inhibition, one experiment) of IL-1, but did not affect the PCA-inducing activity of endotoxin in our system (−4 ± 3% inhibition, three experiments).

Cell Surface Expression of HEC-PCA. Unstimulated intact HEC monolayers expressed little or no surface-available PCA (0–2 mU PCA/10^5 cells, four experiments), consistent with previous observations (3, 4, 15). However, incubation of HEC monolayers with IL-1 (10 U/ml, 4 h, 37°C) resulted in the development of a highly clot-promoting surface (94 mU PCA/10^5 cells, Table I). This surface-available PCA represented >70% of the total HEC-PCA, as determined by freeze-thaw lysis of replicate cultures. In contrast, IL-1 did not substantially affect the constitutively high surface PCA associated with human

TABLE I
Cell Surface Expression of Endothelial Procoagulant Activity

Treatment	Endothelial procoagulant activity (mU/10^5 cells)		
	Intact monolayer	Cell lysates	% Surface expression
Control	1	21	5
Interleukin 1			
2.5 U/ml	29	63	46
10 U/ml	94	131	71
Endotoxin			
10 μg/ml	14	42	33

Replicate HEC cultures in 16-mm wells were incubated for 4 h in 10% FCS-RPMI with IL-1 or endotoxin (LPS, *E. coli* 0111.B4) and assayed, either as intact monolayers (surface-available PCA), or as cell lysates (total cellular PCA). Data represent means from duplicate wells in two separate experiments.

dermal fibroblast cultures (240 mU/10^5 cells). IL-1 treatment (up to 20 U/ml, 4 h) did not result in loss of cellular integrity in HEC monolayers as judged by ^{111}In release (16) (−7 to 4% specific release, two experiments) and trypan blue exclusion (96–103% of control, 10 experiments).

Characterization of IL-1–induced Endothelial Procoagulant Activity. IL-1–induced total cellular and cell surface HEC-PCA were not demonstrable in coagulation Factor X-deficient plasma (clotting times >450 and >600 seconds, respectively), and were reduced to control HEC-PCA levels when measured in Factor VII-deficient plasma (Table II). Total cellular PCA was also tested in a Factor IX-deficient plasma in which IL-1–treated HEC caused a marked reduction of clotting time when compared with control HEC. Treatment of HEC lysates and intact monolayers with a goat anti-human apoprotein III antiserum (3) (kindly provided by Prof. H. Prydz, Research Institute for Internal Medicine, University of Oslo, Oslo, Norway) substantially diminished IL-1–induced PCA (90% and 100% inhibition, respectively, Table II), while treatment with a control goat antiserum had no effect. In addition, IL-1–induced PCA was completely abolished by treatment of HEC lysates with phospholipase C (*Bacillus cereus*, Sigma; 6 U/ml, 10 min at 37°C). These data suggest that most, if not all, of the IL-1–induced HEC-PCA acts via the extrinsic pathway (Factor VII dependent) and is immunochemically related to or identical with tissue factor.

Discussion

This study defines monocyte-derived IL-1 as a potent inducer of procoagulant activity in HEC. Cultured HEC contained low levels of PCA, and expressed little or no activity at their surface. Incubation of HEC with IL-1 led to a marked increase in total cellular PCA which was dependent upon de novo protein synthesis. Continuous exposure to IL-1 resulted in a transient PCA elevation, followed by hyporesponsiveness to fresh IL-1. In vivo, this pattern of response might serve to limit the extent of endothelial-mediated intravascular coagulation. IL-1 also induced a dramatic increase in PCA expression on the surface of intact monolayers, suggesting that this activity would be accessible to the plasma clotting

TABLE II
Characterization of IL-1–Stimulated Endothelial Procoagulant Activity

Endothelial procoagulant activity	Net IL-1–induced procoagulant activity (mU/10^5 cells)*					
	Normal plasma	Factor-deficient plasmas			Antibody treatment	
		VII	X	IX	Control	Anti-apoprotein III
Total cellular‡	140	−6	0	119	133	14
Cell surface‡	66	0	0	ND	57	0

* Net IL-1–induced PCA = IL-1–induced PCA − Control PCA.
‡ Determined in separate experiments, using normal and coagulation factor-deficient plasmas. Milliunits (mU) of activity were established from standard curves developed with a single source of rabbit tissue thromboplastin. PCA of cell lysates and intact monolayers were also evaluated in normal human plasma after treatment with a goat anti-human apoprotein III antiserum and a control (anti-human gamma globulin) goat antiserum, essentially as described (3), using final antisera dilutions of 1:200 for 20 min at 37°C followed by washing for intact monolayers, and for 15 min at 37°C and 1 h at 4°C for cell lysates.

system in vivo. Data obtained with coagulation factor–deficient plasma and an anti-apoprotein III antiserum suggested that most, if not all, of the IL-1–induced HEC-PCA measured in intact cell monolayers and cell lysates was tissue factor–like. Preliminary experiments with purified human factors X and VIIa (generous gifts of Dr. David Stern, Columbia University and Dr. Walter Kisiel, University of Washington) further support this conclusion (unpublished observations). IL-1–induced HEC total cellular and surface-available procoagulant activity may reflect increases in the amount and/or activity of tissue factor–like molecules. However, given the multiple potential roles of endothelial cells in coagulation (1–7), IL-1 effects on other procoagulant mechanisms cannot be ruled out.

Recently, it has been reported that primary cultures of HEC develop tissue factor–like procoagulant activity during coculture with peripheral blood lymphocytes and certain leukocyte cell lines (3). Furthermore, it has been well established that monocytes and macrophages produce procoagulant activity in response to a variety of stimuli, some of which require lymphocyte cooperation (8, 9). These observations suggest that interactions among lymphocytes, monocytes, and endothelial cells may be involved in the development of intravascular coagulation in certain pathological settings. Our data indicate that the elaboration of IL-1 and its action on vascular endothelium may be central to this process.

Summary

Human monocyte-derived interleukin 1 (IL-1) was found to be a potent inducer of procoagulant activity in cultured human vascular endothelium. IL-1–induced human umbilical vein endothelial cell procoagulant activity (HEC-PCA) was transiently expressed, manifest in intact cell monolayers, and required protein synthesis. Data obtained with coagulation factor–deficient plasma and a goat anti-human apoprotein III antiserum suggested that most, if not all, of IL-1–induced endothelial cell procoagulant activity is tissue factor–like. IL-1 induction

of HEC-PCA may be important in the pathogenesis of intravascular coagulation in a variety of immunological and inflammatory conditions.

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