Interleukin-1 Activation of Vascular Endothelium

Effects on Procoagulant Activity and Leukocyte Adhesion

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Interleukin-1 (IL-1), an inflammatory/immune mediator, acts directly and selectively on cultured human vascular endothelial cells to alter two important functional properties. First, IL-1 induces endothelial cell biosynthesis and surface expression of a tissue factor-like procoagulant activity. Second, IL-1 dramatically increases the adhesiveness of the endothelial cell surface for human peripheral blood polymorphonuclear leukocytes (6–42-fold increase) and monocytes (2–5-fold increase), as well as the related leukocyte cell lines HL-60 and U937. These IL-1 effects are concentration-dependent (maximum, 5–10 U/ml), time-dependent (peak 4–6 hours), and reversible. Cycloheximide and actinomycin D block these IL-1 actions on endothelium, which suggests the requirement for *de novo* protein synthesis. Human-monocyte–derived IL-1, cell-line–derived IL-1, and recombinant IL-1 exhibited comparable biologic activities in our assays, whereas two other mediators, IL-2 and immune interferon, were without effect. IL-1 stimulated procoagulant activity and leukocyte adhesion in human endothelial cells cultured from both umbilical veins and adult saphenous veins but not in other cultured cell types, including SV-40-transformed human endothelial cells and human dermal fibroblasts. Similar actions of IL-1 on vascular endothelium *in vivo* may contribute to the development of intravascular coagulation and enhanced leukocyte–vessel wall adhesion at sites of inflammation. (Am J Pathol 1985, 121:393–403)

RECENT STUDIES in our laboratory have focused on two specific aspects of the role of vascular endothelium in inflammation: the initiation of coagulation and the modulation of leukocyte–vessel wall adhesion.[1-3] In particular, we have tested the hypothesis that soluble mediators produced during inflammatory responses alter the functional surface properties of endothelial cells and thus promote their *active* participation in the initiation of coagulation and enhanced leukocyte adhesion. Interleukin-1 is a multifunctional inflammatory/immune mediator which is produced by stimulated mononuclear phagocytes.[4-6] Recent studies have established that IL-1 is composed of two related polypeptides encoded by separate genes.[7-11] Each of these peptides acts on lymphoid and nonlymphoid target cells to stimulate a variety of responses. These responses include elevation of body temperature, production of acute-phase reactants, activation and proliferation of lymphocytes, and secretory and metabolic alterations in hepatocytes, fibroblasts, and synoviocytes.[4-11] We have demonstrated

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that cultured human vascular endothelial cells are a target for IL-1.[1-3] Human monocyte-derived IL-1 was shown to stimulate the biosynthesis and cell-surface expression of a tissue factor-like procoagulant activity in endothelium[1,2] and to increase endothelial adhesiveness for blood leukocytes.[3] In this report, we review our studies of these effects of IL-1 on vascular endothelium and discuss certain implications for the pathophysiology of inflammation.

Materials and Methods

Cell Cultures

Human umbilical vein endothelial cells (HUVEs) were isolated from two- to five-cord segments, pooled, and grown in primary culture with the use of Medium 199 (M199, M. A. Bioproducts, Bethesda, Md) with 20% fetal calf serum (FCS, GIBCO, Grand Island, NY) and antibiotics, as previously described.[12] Three strains of human saphenous vein endothelium were kindly provided by Dr. P. Libby, Tufts University Medical School, Boston, Massachusetts. Cultures were serially passaged (1:3 split ratios) up to subculture level 20 with M199–20% FCS supplemented with endothelial cell growth factor (50–100 μg/ml, a gift of Dr. T. Maciag, Meloy Laboratories, Springfield, Va) and porcine intestinal heparin (50–100 μg/ml, Sigma Chemical Company, St. Louis, Mo)[13] in Costar tissue culture flasks (75 sq cm, Costar, Cambridge, Mass) coated with purified fibronectin (1 μg/sq cm, Meloy Laboratories) or 0.1% gelatin (Bactogelatin 0143-02, Difco Laboratories, Detroit, Mich). Most experiments were performed with cells at Passage Levels 2–4. A line of SV40-transformed HUVEs (SVHEC-F), previously established in this laboratory,[14] two strains of bovine aortic endothelial cells (BAECs), and two strains of human dermal fibroblasts (AIF21 and X1F30, kindly provided by Dr. J. Rheinwald, Dana Farber Cancer Institute, Boston) were maintained as previously described.[3,12] For experimental use, each cell type was plated ($2–4 \times 10^4$ cells/well) and grown to confluence (3–7 days) on the bottom of tissue culture wells 16 mm in diameter (Cluster 24, Costar) or on 15-mm Thermanox plastic coverslips (Miles Scientific, Naperville, Ill), which for the endothelial cells had been precoated with fibronectin (1–5 μg/sq cm).

Cytokine Preparations and Cell Treatments

Human IL-1, isolated as a mixture of two ≅17,000-dalton polypeptides from the supernatant of *Staphylococcus albus*-stimulated human monocytes by immunoabsorption[15] and Sephadex chromatography, was obtained from Genzyme Inc., Boston, Mass. 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 costimulation activity, <1.0% T-cell growth factor, <1 U/ml interferon, and undetectable endotoxin activity. Thymocyte costimulation activity was independently confirmed (courtesy of Dr. C. Reiss, Dana Farber Cancer Institute, Boston, Mass) and the absence of endotoxin (<0.1 ng/ml) documented by Limulus assay (Sigma) in our laboratory. Human cell line-derived interleukin-1 (IL-1A, Genzyme) was purified by nonimmunologic methods and reportedly contains one of the two IL-1 polypeptides (pI-7). Lysates of an L-cell clone which expresses a human recombinant IL-1 polypeptide[9] and of a control L-cell clone were kindly provided by Dr. T. Livelli, Cistron Technology, Pine Brook, New Jersey. Human interleukin-2 (IL-2), isolated from Jurkat cell lines, was obtained from Genzyme Inc., and recombinant human IL-2 (*Escherichia coli*-derived) was provided by Biogen Inc., Cambridge, Massachusetts. Affinity-purified human gamma interferon (0.6×10^6 U/ml), isolated from lectin-stimulated leukocytes, was obtained from Interferon Sciences, Inc., New Brunswick, New Jersey; and recombinant gamma interferon (cloned Chinese hamster ovary cell line-derived) was kindly provided by Dr. W. Fiers (State University of Ghent, Belgium).

Cultured cell monolayers were washed three times with pretreatment media (see below), incubated for up to 60 minutes at 37 C, and washed again prior to the treatment with cytokines. IL-1, control media (IL-1 diluent, Genzyme, or 5% FCS–RPMI), or other cytokine preparations were added to each 16-mm well in a final volume of 0.5 ml of pretreatment media. Several pretreatment media, including RPMI–10% FCS (RPMI 1640, M. A. Bioproducts), RPMI–0.1% bovine serum albumin (BSA), and Tyrode's–0.1% BSA, were tested and produced comparable results. After incubation (37 C, 5% CO_2) for up to 28 hours, each well was prepared for evaluation of endothelial procoagulant activity or for the leukocyte–monolayer adhesion assay. Selective IL-1 pretreatment of leukocyte suspensions was performed in an analogous fashion prior to the adhesion assays.

In certain experiments, cycloheximide (10 μg/ml), actinomycin D (5 μg/ml) (Sigma), or acetylsalicylic acid (100 μM, Fisher Scientific Co., Medford, Mass) was added to the endothelial cultures 30 minutes prior to the addition of IL-1 and allowed to remain in the medium throughout the pretreatment phase. In pilot experiments, this concentration of cycloheximide blocked greater than 97% of ^{35}S-methionine incorporation into endothelial monolayers.

Evaluation of Procoagulant Activity (PCA)

At the end of the pretreatment phase, the endothelial monolayers were washed three times in 0.5 ml of RPMI-1640 or Dulbecco's phosphate buffered saline (DPBS, M. A. Bioproducts). For determination of total cellular PCA, a standard one-stage clotting (plasma recalcification) assay was performed, as described,[1] with the use of cell lysates (frozen-thawed three times, scrape harvested). In certain experiments, cell-surface-expressed PCA was assayed directly in the culture wells on intact, viable monolayers, with the use of a modified plasma recalcification technique.[1] Milliunits (mU) of PCA were defined by standard curves (log-log plot) developed with rabbit brain thromboplastin (Sigma) and citrated normal donor platelet-poor plasma; 10^3 mU PCA corresponded to a clotting time of 20 seconds.

Isolation and Radiolabeling of Leukocytes

Polymorphonuclear leukocytes (PMNs), monocytes, and erythrocytes were routinely isolated as described[3] from anticoagulated (sodium citrate dextrose 1:9, pH 6.5) whole blood collected by venipuncture from normal donors of both sexes. Suspensions of PMNs were greater than 95% pure by Wright-Giemsa staining and contained less than 1% platelet contamination by phase-contrast microscopy. Monocyte-enriched populations (78-88% pure) were prepared by modified density gradient centrifugation according to the method of Recalde.[16] For certain experiments, monocytes (>90% pure) were isolated from residual leukocytes in plateletpheresis preparations (obtained from Massachusetts Red Cross, Boston, Mass) by counterflow centrifugation elutriation.[3,17] Contaminating leukocytes were predominately T lymphocytes. The purity of the monocyte suspensions was determined using several criteria,[17] including 1) size, assessed with a Coulter Cell Counter, model ZF (Coulter Electronics, Hialeah, Fl); 2) morphology, evaluated by phase contrast microscopy; 3) histochemical staining for nonspecfic esterase activity; and 4) indirect immunoperoxidase staining (kindly performed by Dr. G. Pinkus, Department of Pathology, Brigham and Women's Hospital, Boston, Mass) using a monoclonal anti-monocyte antibody (MO-2, Bethesda Research Laboratories, Bethesda, Md) and antilymphocyte antibodies (T11 and B1, provided by Dr. L. Nadler, Dana Farber Cancer Institute, Boston, Mass).[18] Washed erythrocyte suspensions were prepared from anticoagulated blood after centrifugation and removal of the buffy coat layer. The monocytelike cell line U937 (kindly provided by Dr. C. Bianco, New York Blood Center, New York, NY) and the promyelocytic cell line HL-60 (kindly provided by Dr. V. Kelley, Brigham and Women's Hospital, Boston, Mass) were cultured in RPMI-1640 medium with 25 mM HEPES (RPMI, M. A. Bioproducts) and 10% FCS.

To facilitate the quantitation of their adherence, we labeled blood cells or the leukocyte cell lines with ^{111}Indium-oxine (^{111}In, Amersham Corp., Arlington Heights, Ill) as described.[3]

Leukocyte-Monolayer Adhesion Assay

The adhesion of washed human leukocytes, leukocyte cell lines, and erythrocytes to cultured cells or noncellular surfaces was measured using quantitative monolayer adhesion assays, as previously described by our laboratory.[3,19] Test wells (16 mm in diameter) containing confluent endothelial monolayers on Thermanox coverslips were washed three times and then incubated with 0.5 ml of unlabeled or radiolabeled blood cell suspensions, for defined periods varying from 10 to 60 minutes at 37 C, under static conditions, in their respective assay media (Tyrode's-0.1% BSA for PMNs and erythrocytes; RPMI-0.1% BSA for monocytes; RPMI-1% FCS for HL-60 cells and U937 cells). Typi-

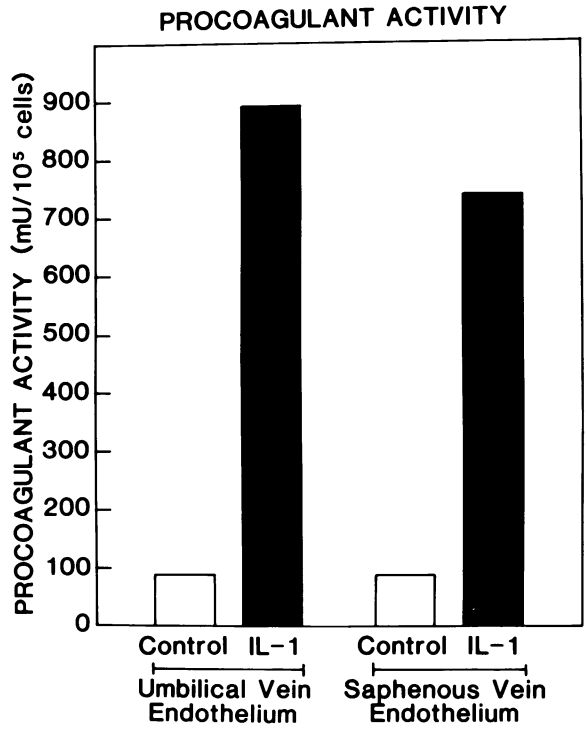

Figure 1—IL-1 induction of total cellular procoagulant activity in serially passaged (Level 2) human umbilical vein endothelium and saphenous vein endothelium. PCA was assayed in freeze-thaw endothelial cell lysates after a 4-hour pretreatment with control (*open bar*) or IL-1-containing (5 U/ml; *solid bar*) medium. Data represented are mean values from triplicate cultures in a single experiment. Similar results were obtained with more than 10 additional strains of umbilical vein endothelium and with 2 additional strains of adult saphenous vein endothelium.

cally, the blood cell concentrations were 5×10^6/ml for PMNs and erythrocytes, 4×10^5/ml for monocytes, and 2×10^6/ml for the cell lines. At the end of the assay incubation, the endothelial monolayers were subjected to a standardized wash procedure to remove unbound blood cells. Typically, monolayers on Thermanox coverslips were washed by repeated passage (three times) through the air–fluid interphase of a 250-ml beaker containing assay media or Hanks' balanced salt solution (HBSS) with calcium and magnesium (M. A. Bioproducts). The number of leukocytes bound per square millimeter was determined directly by microscopic counting or calculated from the monolayer-bound radioactivity and the known specific activity (counts per minute/cell) of the ^{111}In-labeled blood cell preparations.[3] Pilot experiments indicated that visual and radiometric methods gave comparable results, and that the ^{111}In-labeling procedure did not significantly alter the adhesive characteristics of the blood cells. Experiments using ^{111}In-labeled leukocytes routinely included morphologic monitoring of at least one well from each experimental group in order to assess the intactness of the endothelial monolayer and the nature of the endothelial cell–leukocyte interaction.

Results

Endothelial Procoagulant Activity

IL-1 Induces the Synthesis and Cell Surface Expression of PCA in Human Vascular Endothelial Cells

We have previously demonstrated that treatment of serially passaged HUVE monolayers with human monocyte-derived IL-1 results in a concentration- and time-dependent rise in PCA.[1,2] Induction of endothelial PCA was observed with as little as 0.5 U/ml of IL-1 and was maximal with 5–10 U/ml. Continuous exposure of the endothelial monolayers to IL-1 resulted in a rapid rise in PCA which peaked between 3 and 6 hours and subsequently declined toward basal levels by 24 hours. IL-1 induction of endothelial PCA was blocked by cycloheximide (10 μg/ml) or actinomycin D (5 μg/ml), which suggested the need for *de novo* protein synthesis. In contrast, this IL-1 action was not significantly affected by treatment with acetylsalicylic acid (100 μM), which interferes with arachidonate metabolism primarily by blocking the cyclooxygenase pathway. As seen in Figure 1, in addition to human umbilical vein endothelial cells, treatment of adult human saphenous vein endothelial cells for 4 hours with 5 U/ml of monocyte-derived IL-1 (immunoaffinity-purified) resulted in a dramatic rise in total cellular PCA as measured with a one-stage clotting assay. Similar results were obtained with adult human iliac artery endothelial cells (a gift of Dr. Robert Weinstein, Boston, Mass; data not shown).

Table 1—Interleukin-1 and Endotoxin Stimulate Endothelial Procoagulant Activity*

	Endothelial PCA (mU/10^5 cells)		
	Untreated	Heat-treated†	Polymyxin B-treated†
Control buffer	30	(N.D.)	29
Monocyte-derived IL-1	400	22	426
Cell-line–derived IL-1	515	50	585
Endotoxin	167	253	38

* HUVE cultures were incubated for 4 hours at 37 C with RPMI-10% FCS alone (control buffer) or with RPMI–10% FCS containing monocyte-derived IL-1 (5 U/ml), cell-line–derived IL-1 (5U/ml IL-1), or endotoxin (1 μg/ml LPS *E coli* 0111.B4, Difco Laboratories, Detroit, Mich) and assayed for total cellular PCA.

† Replicate cultures were incubated with heat-treated (80 C, 15 minutes) or polymyxin B sulfate-treated (50 μg/ml, Sigma) IL-1 or endotoxin. Data represent mean values from triplicate cell cultures.

As seen in Table 1, immunoaffinity-purified human monocyte-derived IL-1 and biochemically purified human-cell-line–derived IL-1 yielded comparable stimulations of endothelial PCA. In addition, lysates of murine L cells which expressed a recombinant human IL-1 polypeptide induced PCA in HUVE monolayers, whereas control L-cell lysates were inactive (data not shown). We[1,2] and others[20–22] have reported that endotoxin (bacterial LPS) induces endothelial PCA. However, the actions of IL-1 and LPS were readily distinguished by their relative sensitivities to heat treatment and polymyxin B (Table 1).

We also examined the effect of IL-1 on the cell surface expression of PCA in intact HUVE monolayers, using a modified one-stage clotting assay (see Materials and Methods). As seen in Table 2, untreated HUVE monolayers displayed negligible surface PCA. However, intact HUVE monolayers which were treated with 5 U/ml of IL-1 for 4 hours became highly procoagulant. In a series of four experiments, the IL-1-induced cell-surface PCA in HUVE represented between 50% and 85% of the total cellular PCA. Little or no PCA was released into the culture supernatant by the 4-hour time point. The effect of IL-1 on PCA appeared to be selective for human endothelial cells, since IL-1 did not stimulate PCA in several other cultured cell types. Human dermal fibroblasts (Table 2) and SV-40-transformed HUVE constitutively expressed large quantities of PCA. Treatment of these two cultured cell types with IL-1 did not alter their total cellular or cell-surface–expressed PCA. In addition, a strain of bovine aortic endothelium and the human monocyte-like cell

Table 2—Cell Surface Expression of Procoagulant Activity in Human Umbilical Vein and Human Dermal Fibroblasts

	Procoagulant activity (mU/10^5 cells)		
		Interleukin-1	
	Control	2.5 U/ml	10 U/ml
Endothelium			
Intact monolayer	0.5	17	120
Cell lysate	19	119	230
% Surface expression	3%	14%	52%
Dermal fibroblasts			
Intact monolayer	240	217	256
Cell lysate	≅500	≅500	≅500
% Surface expression	48%	42%	50%

* Replicate cultures of HUVE or human dermal fibroblasts were treated for 4 hours with control or IL-1–containing media and assayed as intact monolayers (surface-available PCA) or as cell lysates (total cellular PCA). Data represent mean values from duplicate cell cultures. Similar results were obtained in two additional experiments.

line U937 demonstrated very low levels of PCA and were not significantly stimulated by IL-1 (data not shown).

Characterization of IL-1-Induced Endothelial PCA

We have previously reported[1,2] that IL-1-induced endothelial PCA was expressed in plasma deficient in coagulation Factor IX but not in plasma deficient in Factor VII or Factor X. PCA activity was blocked by an antiserum to human apoprotein III (88–100% inhibition, three experiments) and abolished by treatment of the cell lysates with phospholipase. In addition, using a two-stage chromogenic assay with purified coagulation factors (kindly provided by Dr. D. Stern of Columbia University and Dr. W. Kisiel of the University of Washington), we have demonstrated that IL-1-induced PCA requires the presence of Factor VIIa in order to activate Factor X to Xa (data not shown). Taken together, these observations strongly suggest that most, if not all, of the IL-1-induced PCA in our cultured human endothelial cells is tissue factor-like.

Endothelial Cell–Leukocyte Adhesion

IL-1 acts on Human Vascular Endothelium to Increase Leukocyte Adhesion

Under basal conditions (ie, in the absence of added stimuli), 149 ± 13 (1.2% of total added) human PMNs bound per square millimeter of HUVE monolayers (mean ± SEM, 16 experiments) in a standardized 10-minute adhesion assay.[3] Pretreatment of the endothelial cultures with 5 U/ml of IL-1 for 4 hours resulted in a

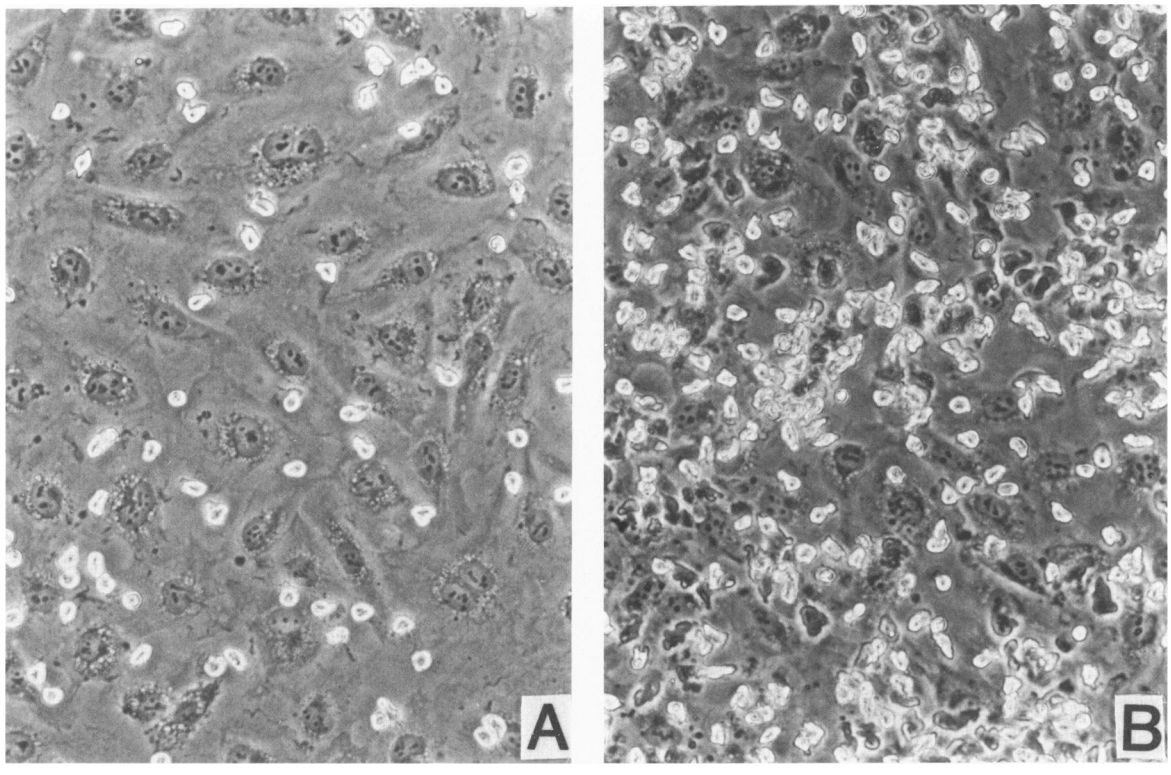

Figure 2—Phase-contrast photomicrographs (× 200 magnification) of the adhesion of human polymorphonuclear leukocytes to control (**A**) and IL-1-treated (5 U/ml, 4 hours) HUVE monolayers (**B**) at the end of a standard 10-minute monolayer adhesion assay.

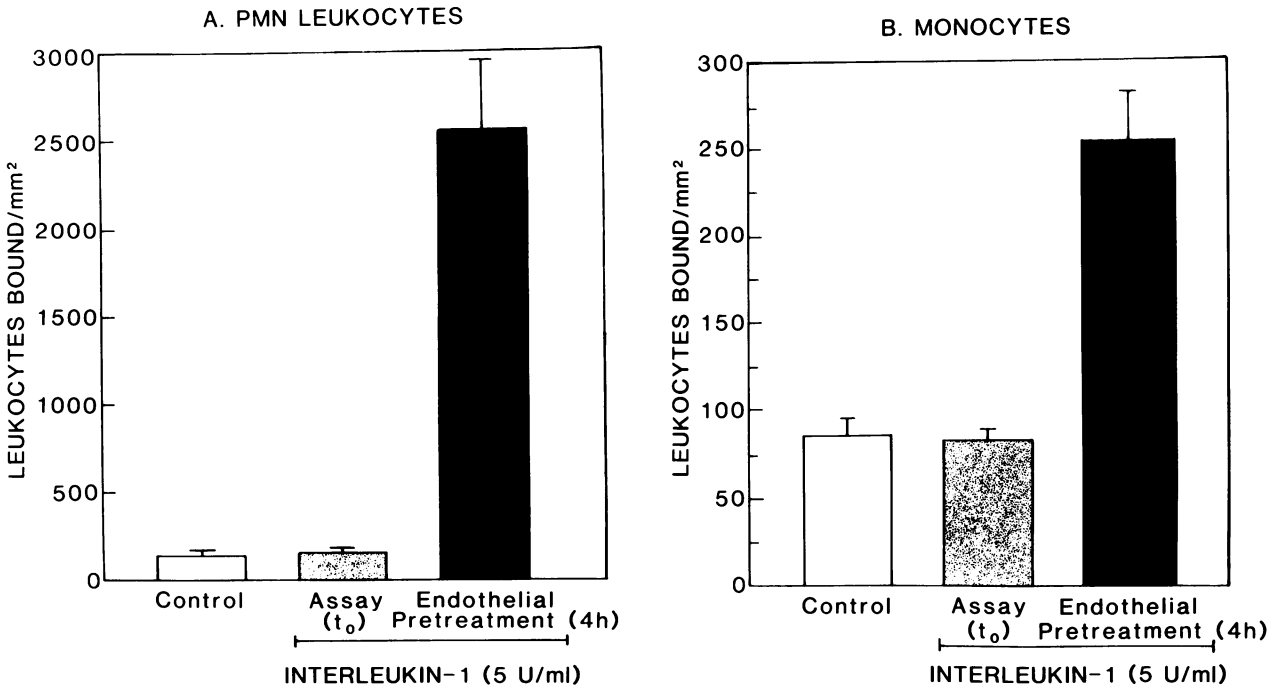

Figure 3—IL-1 stimulation of human PMN and monocyte adhesion to cultured HUVE monolayers. Confluent HUVE monolayers (Passage Levels 2–4) were incubated for 4 hours at 37 with control or IL-1- containing (5 U/ml) pretreatment media (see Materials and Methods) and then washed prior to start of the leukocyte–monolayer adhesion assays. ^{111}In-labeled PMNs (5.0×10^6/ml) (A) or monocytes (4.0×10^5/ml) (B) were added to the wells in 0.5 ml of assay media (see Materials and Methods) and incubated for 10 minutes at 37 C. IL-1 (5 U/ml) was added at the start of the monolayer adhesion assay to one set of replicate wells (assay, t_0). Each *bar* represents the mean (± SEM) of leukocyte adhesion in a given experimental group calculated from 6–16 separate experiments.

dramatic increase in PMN adhesion (Figure 2A and B). In a series of 16 experiments using 10-minute adhesion assays, IL-1-treated HUVE monolayers bound 18.3 ± 2.6-fold more PMNs than control monolayers ($P < 0.0005$, Student *t* test, two-tailed), whereas addition of IL-1 to the adhesion assay itself (9 experiments) was without effect (Figure 3A). Selective pretreatment of PMNs for 10 or 30 minutes did not appear to significantly increase endothelial–PMN adhesion (data not shown).

Human peripheral blood monocytes, isolated by density gradient centrifugation or by countercurrent flow elutriation, demonstrated higher levels of adhesion to untreated HUVE monolayers (11.2 ± 0.8% of total added during a 10-minute adhesion assay, 11 experiments) than did PMNs (1.2% of total added, 16 experiments). Pretreatment of the endothelial monolayers with IL-1 (5 U/ml, 4 hours) resulted in a 2–5-fold increase in monocyte adhesion (11 experiments, $P < 0.005$), whereas the addition of IL-1 during the adhesion assay did not significantly alter adhesion. (Figure 3B). Endothelial-adherent monocytes were esterase-positive and were recognized by the anti-monocyte monoclonal antibody MO-2. Lymphocytes (esterase-negative, T11-antigen-positive), which contaminated the monocyte preparations, adhered to both control and IL-1-treated endothelial monolayers but appeared to be more reactive with the latter.

Adhesion of the Promyelocytic Cell Line HL-60 and the Monocyte-like Cell Line U937 to Human Vascular Endothelium

In order to further examine certain aspects of endothelial–leukocyte adhesion, we have employed the promyelocytic cell line HL-60 and the human monocyte-like cell line U937. Unlike PMNs and monocytes, HL-60 cells and U937 cells demonstrated little or no attachment to plastic or glass surfaces (<5 cells/sq mm), thus allowing us to focus directly on the cell–cell interactions which occur in endothelial–leukocyte adhesion assays.[2,3] IL-1 pretreatment (5 U/ml, 4 hours) of passaged HUVE monolayers resulted in a 33.0 ± 6-fold increase in HL-60 cell adhesion (mean ± SEM, 6 experiments) and a 4.9 ± 0.5-fold increase in U937 cell adhesion (15 experiments) over basal levels.[3] As demonstrated for HL-60 cells in Figure 4, the addition of IL-1 to the adhesion assay itself or pretreatment of the cell lines with IL-1 for up to 4 hours did not significantly increase leukocyte adhesion.

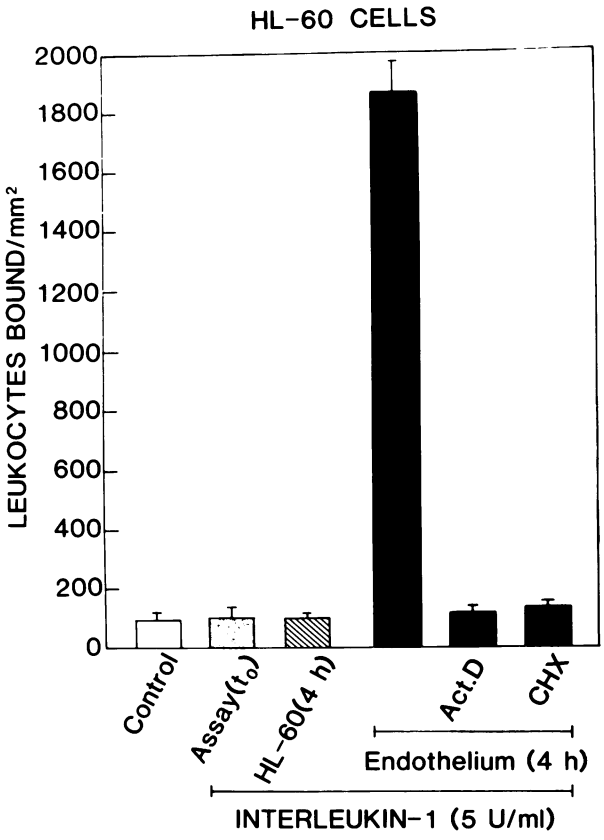

Figure 4—Effects of IL-1 on the adhesion of HL-60 cells to cultured human endothelial monolayers (Passage Level 3). Confluent HUVE monolayers (*solid bars*) or the leukocyte cell line HL-60 (*hatched bar*) was selectively pretreated with IL-1 (5 U/ml, 4 h, 37) in RPMI–10% FCS. In certain wells actinomycin D (*Act.D*, 5 μg/ml) or cycloheximide (*CHX*, 10 μg/ml) was added 30 minutes prior to the IL-1 and allowed to remain throughout the pretreatment phase. At the start of the adhesion assay (30 minutes; 2.0 × 10^6 ^{111}In-labeled leukocytes/ml), IL-1 (5 U/ml) was added to one set of wells (assay, t_0, *stippled bar*). Basal adhesion is shown by the *open bar* (*control*). Each *bar* represents the mean (± SD) of triplicate wells in a single experiment. Similar results were obtained in three additional experiments.

Characterization of IL-1-Stimulated Endothelial-Leukocyte Adhesion

IL-1 induction of endothelial–leukocyte adhesion was found to be concentration-dependent (maximum, 10 U/ml) and time-dependent (peak activity 4–6 hours).[3] The presence of cycloheximide (10 μg/ml) or actinomycin D (5 μg/ml) throughout the pretreatment phase blocked IL-1 stimulation of endothelial leukocyte adhesion (Figure 4), which suggests the requirement for *de novo* protein synthesis.

As noted above for the induction of procoagulant activity, monocyte-derived IL-1, human-cell-line–derived IL-1, and lysates of an L-cell clone expressing a recombinant human IL-1 polypeptide all stimulated endothelial–leukocyte adhesion in a comparable fashion (Table 3) Endotoxin also promoted an increase in endothelial adhesivity for leukocytes as measured with HL60 and U937 cell lines. The effect of endotoxin was easily distinguished from that of IL-1 by their relative sensitivities to heat and polymyxin B (Figure 5). Two other inflammatory/immune mediators, IL-2 and gamma interferon, did not promote endothelial–leukocyte adhesion (data not shown).

Human umbilical vein endothelium and human saphenous vein endothelium responded to IL-1 treatment with comparable increases in adhesivity for leukocytes (data not shown). However, several other cultured cell types, including SV40-transformed HUVE, two strains of bovine aortic endothelium, and two strains of human dermal fibroblasts, did not demonstrate a significant increase in leukocyte adhesion after IL-1 treatment.[3]

Discussion

Localized intravascular coagulation and increased leukocyte–vessel wall adhesion at sites of inflammation or vascular injury have long been noted as part of the inflammatory process.[23–28] The vascular endothelium generally has been considered as either a passive participant or a target of injury in these events (reviewed by Harlan[29]). More recently, it has been appreciated that endothelial cells can display a variety of functional states and thus potentially may play more active roles in the inflammatory process. For example, endothelial cells have been shown to express several procoagulant activities.[20–22,30–32] We have hypothesized that inflammatory mediators act on vascular endothelium to alter its functional surface properties and thus promote its active participation in inflammation. In this report, we have reviewed our studies on the actions of interleukin-1, a pleiotropic multifunctional mediator, on cultured human vascular endothelial cells. First, IL-1 induces the

Table 3—Stimulation of Endothelial Cell–Leukocyte Adhesion by Monocyte-Derived IL-1, Cell-Line–Derived IL-1, and Recombinant IL-1

	Leukocytes bound/mm²	
Treatment	PMNs	HL-60
Control	58 ± 21	37 ± 11
Monocyte-derived IL-1	1498 ± 156	768 ± 136
Cell-line–derived IL-1	1887 ± 187	1105 ± 220
Recombinant IL-1	1046 ± 114	1015 ± 166

Replicate HUVE cultures were treated with RPMI–10% FCS (control) with RPMI–10% FCS containing 5 U/ml of monocyte-derived IL-1 or cell-line–derived IL-1 or a 1:2000 dilution of crude lysate of L cells which express a recombinant IL-1 polypeptide for 4 hours, washed, and assayed for leukocyte adhesion with PMNs and HL-60 cells. A lysate of control L cells did not significantly stimulate endothelial–leukocyte adhesion (data not shown). Data represent the mean (± SD) of triplicate determinations. Similar results were obtained in two additional experiments.

biosynthesis and cell-surface expression of a tissue factor-like procoagulant activity in endothelial cells, thus potentially making them actively thrombogenic. Second, IL-1 dramatically and selectively induces endothelial adhesiveness for blood leukocytes. We propose that endothelium, which has been "activated" by IL-1 *in vivo*, could mediate the development of intravascular coagulation and/or promote localized adhesion of blood leukocytes at sites of inflammation.

In addition to mononuclear phagocytes, several cell types, including epidermal cells[33] and endothelial cells themselves,[34,35] have been shown to produce IL-1-like activities after appropriate stimulation (eg, bacterial products). Thus, at a site of infection or in a wound, it is likely that one or more cell types would secrete IL-1. Locally produced IL-1 could then act on target cells, including the vascular endothelium, in order to promote the development of an inflammatory response. Under certain circumstances, it is possible that excessive production of IL-1 could lead to widespread changes in the vascular endothelium contributing to the development of pathologic states such as disseminated intravascular coagulation.

The actions of IL-1 on endothelial procoagulant activity and endothelial-leukocyte adhesion are concentration-dependent (maximum, 5-10 U/ml) and time-dependent (peak, 4-6 hours). In addition, these effects are reversible, since upon removal of the IL-1 both the PCA and leukocyte adhesivity fall to near basal levels by 24 hours and the endothelium can be restimulated by fresh IL-1. IL-1 treatment of the endothelium does not appear to cause cell damage.[1-2] Thus, *in vivo*, it is possible that the vascular endothelium could be activated by IL-1 to transiently express increased PCA and adhesiveness for leukocytes, and then return to a basal functional state, susceptible to subsequent restimulation.

IL-1 induction of endothelial-procoagulant activity and endothelial-leukocyte adhesion were blocked by the presence of cycloheximide and actinomycin D, suggesting the need for *de novo* protein synthesis. In contrast, inhibition of endothelial arachidonate metabolism via the cyclooxygenase pathway did not affect these IL-1 actions. Neither the generation of soluble mediators nor alterations of endothelial cell matrix appear to be essential for IL-1 stimulation of endothelial procoagulant activity or leukocyte adhesion.[1-3] Rather, both effects appear to reflect alterations in the surface properties of intact endothelial monolayers, involving the biosynthesis and expression of new protein-associated structures. Though these two IL-1-inducible alterations in functional surface properties of endothelial cells may be related, at least two lines of evidence suggest that they are distinct. First, extensive adhesion of U937 cells and normal monocytes is observed with unstimulated primary cultures of human umbilical vein endothelium,[2,36] which express little or no procoagulant activity[1,2]; conversely, there is relatively little adhesion of U937 cells to SV-40-transformed human endothelial cells, which constitutively express large amounts of procoagulant activity (M. P. Bevilacqua, unpublished observations). Second, our preliminary experiments indicate that IL-1-induced endothelial procoagulant activity, but not endothelial-U937 cell adhesion, can be blocked by a polyclonal antiserum to human apoprotein III (kindly provided by H. Prydz, University of Oslo, Oslo, Norway) (M. P. Bevilacqua, unpublished observations). In addition, hirudin did not significantly alter IL-1 stimulation of endothelial-leukocyte adhesion, thus suggesting that the generation of thrombin was not essential for this effect.[3] Further understanding of the mechanisms of endothelial-leukocyte adhesion and definition of IL-1-induced endothelial cell structures may allow clarification of the relation-

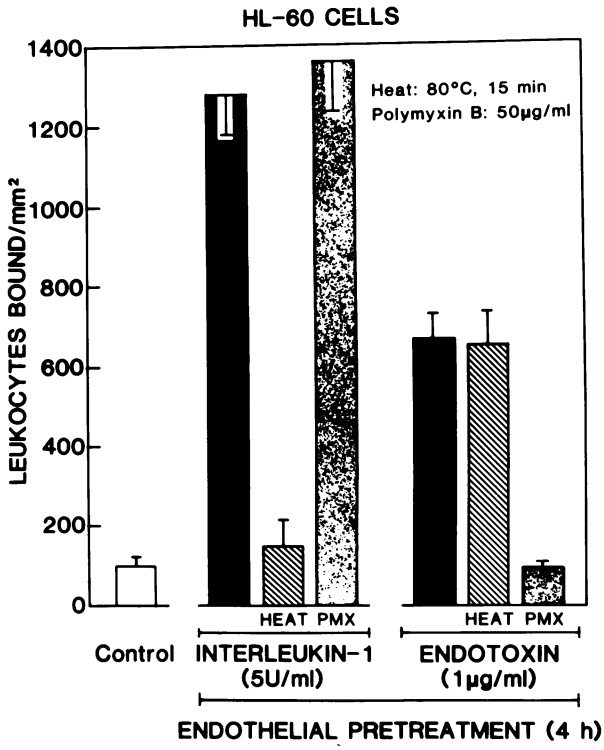

Figure 5—Stimulation of endothelial cell-HL-60 cell adhesion by IL-1 or endotoxin. HUVE monolayers were pretreated for 4 hours with maximal doses of IL-1 (5 U/ml) or endotoxin (1 µg/ml) (*solid bars*) prior to the performance of a 30-minute monolayer adhesion assay (2.0×10^6 ^{111}In-labeled HL-60 cells/ml). Replicate cultures were treated with IL-1 or LPS preparations which had been heated (80 C, 15 minutes) or which contained 50 µg/ml of polymyxin B (*PMX*). The bars represent the means (± SD) of triplicate determinations. Similar results were obtained in an additional experiment.

ship between IL-1-induced endothelial procoagulant activity and leukocyte adhesion.

We hypothesize that these IL-1 responses are part of the activation of a broader genetic program. Recently, Rossi et al[37] demonstrated that IL-1 increases endothelial production of prostacyclin. To explore further the endothelial response to IL-1, we have developed murine monoclonal antibodies to IL-1-treated endothelial cells in collaboration with Dr. D. Mendrick of our department. In four separate hybridoma fusions, we have identified approximately 15 monoclonal antibodies which show enhanced binding to IL-1-treated endothelium. One of these antibodies, H4/18, has been characterized more fully.[38] H4/18 recognizes a cell-surface molecule inducible by IL-1 in primary or serially passaged HUVE and demonstrates little or no binding to control monolayers. The H4/18 binding site is not basally expressed or inducible in SV-40-transformed HUVEs, human dermal fibroblasts, or blood leukocytes. Similar to endothelial PCA and leukocyte adhesion, induction of H4/18 binding by IL-1 is concentration-dependent and time-dependent and requires *de novo* protein synthesis. In preliminary experiments, H4/18 did not appear to alter IL-1-induced endothelial PCA (M. P. Bevilacqua, unpublished observations) but did partially inhibit IL-1-induced endothelial–leukocyte adhesion, as demonstrated with HL-60 cells.[39] We propose that the IL-1 effect on leukocyte adhesion involves the biosynthesis and endothelial cell-surface expression of endothelial–leukocyte adhesion molecules (E-LAMs). Our future studies will focus on defining the molecular nature of these putative E-LAMs.

In summary, we have demonstrated that the soluble mediator IL-1 acts selectively upon cultured human endothelial cells to induce a tissue factor-like procoagulant activity and to alter their adhesivity for blood leukocytes. Our data strongly support the hypothesis that the vascular lining can play an active role in the localized intravascular coagulation and leukocyte–vessel wall adhesion *in vivo*, and implicate the inflammatory mediator interleukin-1 as a potent modulator of these endothelial functions.

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