

Lymphocyte Function-associated Antigen-1 (LFA-1) Interaction with Intercellular Adhesion Molecule-1 (ICAM-1) is One of At Least Three Mechanisms for Lymphocyte Adhesion to Cultured Endothelial Cells

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Abstract. Intercellular adhesion molecule-1 (ICAM-1) on the surface of cultured umbilical vein and saphenous vein endothelial cells was upregulated between 2.5- and 40-fold by rIL-1, rTNF, LPS and rIFN γ corresponding to up to 5×10^6 sites/cell. Endothelial cell ICAM-1 was a single band of 90 kD in SDS-PAGE. Purified endothelial cell ICAM-1 reconstituted into liposomes and bound to plastic was an excellent substrate for both JY B lymphoblastoid cell and T lymphoblast adhesion. Adhesion to endothelial cell ICAM-1 in planar membranes was blocked completely by monoclonal antibodies to lymphocyte function associated antigen-1 (LFA-1) or ICAM-1. Adhesion to artificial membranes was most sensitive to ICAM-1 density within the physiological range found on resting and stimulated endothelial cells. Adhesion of JY B

lymphoblastoid cells, normal and genetically LFA-1 deficient T lymphoblasts and resting peripheral blood lymphocytes to endothelial cell monolayers was also assayed. In summary, LFA-1 dependent (60–90% of total adhesion) and LFA-1-independent basal adhesion was observed and the use of both adhesion pathways by different interacting cell pairs was increased by monokine or lipopolysaccharide stimulation of endothelial cells. The LFA-1-dependent adhesion could be further subdivided into an LFA-1/ICAM-1-dependent component which was increased by cytokines and a basal LFA-1-dependent, ICAM-1-independent component which did not appear to be affected by cytokines. We conclude that ICAM-1 is a regulated ligand for lymphocyte-endothelial cell adhesion, but at least two other major adhesion pathways exist.

INTERCELLULAR and cell-matrix adhesion are critical during morphogenesis and in the adult organism. Adhesive interactions are mediated by a diverse array of cell surface and matrix molecules which show specialization for interactions involving particular cell types. Interaction of lymphocytes with endothelial cells is important for entry of lymphocytes into lymphoid tissues (15, 17, 45) and extravasation at sites of immune and inflammatory reactions (45) and is an interesting model for studying dynamic cell-cell interactions.

Adhesion of lymphocytes to many cell types is inhibited by monoclonal antibodies (mAbs) to lymphocyte function associated antigen-1 (LFA-1)¹ (CD11, CD18) (38, 46). LFA-1 is a member of a family of three leukocyte heterodimers which have distinct α subunits of 150–180 kD and a common β subunit of 95 kD (42). Extracellular matrix receptors are also heterodimers; recently their α and β subunits have been

found to be 25 to 48% homologous to those of LFA-1 (22, 23, 46, Larson, R. and T. A. Springer, manuscript in preparation). This relationship suggests that LFA-1 is a member of a super-family of heterodimeric, divalent cation-dependent adhesion molecules, designated the integrins (22). However, the LFA-1, Mac-1 and p150,95 antigens are the only members of this super-family which are known to be involved in cell-cell rather than cell-matrix adhesion.

We have identified a putative ligand for LFA-1 called intercellular adhesion molecule-1 (ICAM-1) (39). Monoclonal antibodies to ICAM-1 inhibit several LFA-1 dependent cell-cell interactions including the phorbol ester-stimulated homotypic adhesion of B-lymphoblastoid cell lines (39) and the binding of T lymphoblasts to fibroblasts (11). ICAM-1 expression on dermal fibroblasts and endothelial cells in vitro is increased by exposure to interferon γ (IFN γ), interleukin-1 (IL-1) and tumor necrosis factor (TNF) (11, 35). Increasing ICAM-1 on dermal fibroblasts correlates with a significant increase in T lymphoblast adhesion which requires the LFA-1/ICAM-1-dependent pathway.

To directly test the potential role of ICAM-1 in lymphocyte adhesion to endothelial cells we have examined the adhesion of resting or activated T and B lymphocytes to purified en-

1. *Abbreviations used in this paper:* FBS, fetal bovine serum; HDF, human dermal fibroblast; HSVEC, human saphenous vein endothelial cell; HUVEC, human umbilical vein endothelial cell; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; LAD, leukocyte adhesion deficiency; LFA, lymphocyte function associated; LPS, lipopolysaccharide; PBL, peripheral blood lymphocyte.

endothelial cell ICAM-1 reconstituted into planar membranes. We have also assayed the adhesion of lymphocytes to resting or activated cultured endothelial cells with attention to the correlation between ICAM-1 expression and adhesion, and the role of LFA-1 and ICAM-1. Our results show that lymphocyte-cultured endothelial cell adhesion is a complex process using at least three different adhesion pathways, one of which is regulated by the cell surface density of ICAM-1 and is mediated by interaction of LFA-1 on lymphocytes and ICAM-1 on endothelial cells.

Materials and Methods

Materials

Recombinant human interleukin (IL)-1 β expressed in *Escherichia coli* (2×10^7 U/mg), rabbit antisera to natural IL-1 (pI 7.0), and recombinant tumor necrosis factor (rTNF) were a gift of Dr. Charles A. Dinarello (Tufts University School of Medicine, Boston, MA). Recombinant human TNF (1×10^8 U/mg) was from Genentech (San Francisco, CA). Recombinant human IL-1 α (5×10^7 U/mg) was from Dr. Peter Lomedico (Hoffmann La Roche, Nutley, NJ). Recombinant human interferon (IFN) γ expressed in CHO cells (1×10^6 U/mg) was a gift of Dr. D. Novick, (Weizmann Institute, Rehovot, Israel). Endothelial cell growth supplement was obtained from Meloy Labs (Springfield, VA). Fetal bovine serum was obtained from Gibco (Grand Island, NY) or Hyclone (Logan, UT). Clinical grade human plasma fibronectin was obtained from the New York Blood Research Center (New York, NY). Clinical grade Polymyxin B sulfate was purchased from Pfizer (New York, NY). Sepharose CL 4B was purchased from Pharmacia (Uppsala, Sweden). Iodogen was obtained from Pierce (Rockford, IL). Lipopolysaccharide (LPS) (*E. Coli*, 0111:B4), heparin, and other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Carrier-free Na¹²⁵I and Na⁵¹CrO₄ were purchased from New England Nuclear (Boston, MA).

mAbs

TS1/22 (LFA-1 mAb, IgG1), TS2/18 (CD2 mAb, IgG1), TS2/9 (LFA-3 mAb, IgG1) (41), W6/32 (HLA-A,B mAb, IgG2a) (3) and RRI/1 (ICAM-1 mAb, IgG1) (39) were used as dilutions of hybridoma culture supernatants or ascites, or as purified IgG. R6.5 (ICAM-1 mAb, IgG 2a) IgG and Fab were a gift of Dr. Robert Rothlein (Boehringer Ingelheim, Ridgefield, CT). mAbs were purified from culture supernatants by protein A affinity chromatography. F(ab')₂ fragments of RRI/1 and TS2/9 were prepared by papain cleavage and their purity determined by SDS-PAGE (34). Purified mAb were covalently coupled to Sepharose CL-4B after CNBr activation (29).

Cell Culture. Human umbilical vein endothelial cells were a gift of Dr. Michael A. Gimbrone, Jr. (Brigham and Women's Hospital, Boston, MA) and human saphenous vein endothelial cells were a gift of Dr. Peter Libby (Tufts University School of Medicine, Boston, MA). These cells were maintained up to 25 doublings in RPMI 1640 with 20% FBS (Hyclone, [LPS] = 0.025 ng/ml), 5 μ g/ml bovine endothelial cell growth supplement, 10 μ g/ml heparin (porcine), 25 mM HEPES, 5 mM glutamine and 50 μ g/ml gentamicin (16, 50). Tissue culture surfaces were treated with 1 μ g/cm² human plasma fibronectin in Hanks' balanced salt solution (HBSS) for 20 min at 37°C to promote endothelial cell attachment. Endothelial cells (2,500) were seeded into 96-well plate wells and grew to confluence in 3 d. Endothelial cells were treated with cytokines or LPS in the presence of endothelial cell growth supplement and heparin and were washed three times with complete media before assay. Dermal fibroblasts were a gift of Dr. James Rheinwald (Dana Farber Cancer Institute, Boston, MA) and were maintained in RPMI-1640 with 10% FBS.

Peripheral blood mononuclear cells were obtained by dextran sedimentation and Ficoll-Hypaque (1.077) centrifugation. Lymphocytes were enriched by incubating mononuclear cells in RPMI-1640 10% FBS in tissue culture-treated plastic petri dishes for 1 h two times and saving the nonadherent cells. T lymphoblasts were prepared by incubating mononuclear cells at 10^6 cells/ml with 2 μ g/ml concanavalin A (Con A) in RPMI-1640 20% FBS for 3 d, washing out the Con A and then culturing for 4–10 d in RPMI-1640 20% FBS containing 2.5 ng/ml rIL-2. T lymphoblasts were prepared from mononuclear cells of severely affected LAD patients (BBN and ZJO). It was particularly important to maintain these cells at high density ($1-2 \times 10^6$ cells/ml) and rIL-2 was added during the first 3 d also. The JY B lym-

phoblastoid cell line was obtained from Dr. Jack Strominger (Dana Farber Cancer Institute, Boston, MA). It was maintained in complete media (RPMI-1640, 10% FBS, 2.5 mM glutamine, 50 μ g/ml gentamicin).

Immunofluorescence Flow Cytometry. Monodispersed suspensions of endothelial cells were prepared by brief trypsinization which was found to give the same levels for ICAM-1, HLA-A,B and LFA-3 as EDTA and mechanical dispersal (11). Cells were stained as previously described (11) except that phycoerythrin conjugated goat anti-mouse IgG (Biomed, Foster City, CA) was used instead of the fluorescein conjugate. Samples were analyzed on a Coulter Epics V flow cytometer (Coulter, Hialeah, FL).

Analytical Immunoaffinity Isolation and SDS-PAGE. Endothelial cell monolayers (4×10^6 cells) were treated with media, IL-1 β , TNF or IFN γ for 24 h. Monolayers were washed three times with 0.15 M NaCl, 0.025% Na₂S₂O₃, 25 mM Tris, pH 8 (TSA), and then lysed in 2 ml TSA + 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM iodoacetamide and 200 mU/ml aprotinin (lysis buffer) per flask for 30 min at 4°C. The lysates were centrifuged at 14,000 \times g for 15 min, were incubated overnight with 20 μ l mouse IgG Sepharose CL 4B, and the supernatant reserved. One-third of the lysate was incubated with 20 μ l of mAb Sepharose for 6 h. After washing (25) the mAb-Sepharose, protein was eluted by boiling in sample buffer with 50 mM iodoacetamide and electrophoresed (26).

Purification of ICAM-1 from LPS-Activated HUVEC. The procedure was modified from Marlin and Springer (30). Endothelial cell monolayers (8×10^7 cells) were treated with 10 μ g/ml LPS for 12–16 h. The cells were lysed in 20 ml of lysis buffer for 30 min at 4°C. The lysate was centrifuged 1,000 g for 10 min and this supernatant was centrifuged at 100,000 g for 1 h. The supernatant was passed over a 1 ml RRI/1-Sepharose CL-4B column (1.5 mg/ml IgG) at a flow rate of 0.1 ml/min. The column was washed with 10 vol of 0.1% Triton X-100 in PBS, then 5 vol of 50 mM triethylamine, pH 11, 0.1% Triton X-100, 0.15 M NaCl and finally 5 vol of 1% octyl- β -D-glucopyranoside in PBS. ICAM-1 was eluted with 50 mM triethylamine pH 12.4, 1% octyl- β -D-glucopyranoside, 0.15 M NaCl at a flow rate of 0.1 ml/min and fractions were collected in tubes containing 0.1 vol of 1 M Tris, pH 7.5, 1% octyl- β -D-glucopyranoside. ICAM-1 eluted in 1–2 column vol.

Reconstitution of ICAM-1 into Phospholipid Vesicles and Binding to Plastic. Egg phosphatidylcholine and cholesterol were combined at a 7:2 molar ratio in chloroform/methanol (9:1), dried under a stream of nitrogen and held under a vacuum for 1 h. The lipids were solubilized with PBS, 2% octyl- β -D-glucopyranoside at a lipid concentration of 0.4 mM. The solubilized lipids were combined with 1 vol of neutralized ICAM-1 eluate or as a control, glycophorin in the same buffer at molar lipid to protein ratios between 1,000:1 and 80,000:1. Vesicles were formed by dialysis against four changes of PBS (32). The ICAM-1 or glycophorin vesicle suspension (30 μ l/well) was added to 96-well tissue culture treated plates and incubated for 1 h at 37°C with a moist paper towel draped over the plate to reduce evaporation (30). The wells were washed five times with 200 μ l of complete media without exposing the bottom of the well to air. ICAM-1 vesicles bound to plastic at a similar efficiency as to glass (5) (not shown) as has also been found by Quill and Schwartz (37).

Radioimmunoassay for ICAM-1. Purified RRI/1 was iodinated using iodogen (14) to a specific activity of 10 μ Ci/ μ g. Iodinated antibody binding to cell monolayers was determined as previously described (11). The specific activity of the [¹²⁵I]RRI/1 was adjusted using unlabeled RRI/1 to obtain a linear signal over the range of antigen densities encountered in this study. Nonspecific binding was determined in the presence of a hundred fold excess of unlabeled RRI/1 and was subtracted from total binding to yield the specific binding. All points were done in quadruplicate.

Site-Number Determinations. RRI/1 was iodinated to a specific activity of around 0.5 μ Ci/ μ g as above. Specific activity was determined using a molecular weight of 75,000 assuming bivalent binding. Binding experiments were done as above except the [¹²⁵I]RRI/1 was added at increasing concentrations until saturation was evident. The number of cells was determined after trypsinization and counting of cells from four wells for each pretreatment condition.

Adhesion Assay. Lymphocytes, T lymphoblasts or lymphoid tumor cells were labeled with ⁵¹Cr by incubation of cells at $5-50 \times 10^6$ cells/ml with 200–400 μ Ci/ml Na₂ ⁵¹CrO₄ for 1–1.5 h at 37°C in complete media. The cells were washed four times with complete media (with 25 mM HEPES). T lymphoblasts were washed with complete media + 5 mM methyl- α -mannoside during the first two washes. Plastic-bound vesicles or confluent endothelial cell monolayers in 96-well plates were treated as described for each experiment. mAb and labeled cells were added to a final volume of 100 μ l. In some experiments cells, plastic-bound vesicles, or monolayers were treated with mAb and then unbound mAb removed by washing before the binding assay. The labeled cells were allowed to settle and adhere for 1 h

Table I. Upregulation of ICAM-1 Expression on HUVEC and HSVEC

Condition (16 h)	[¹²⁵ I]ICAM-1 mAb bound (cpm)			
	HUVEC	Δ	HSVEC	Δ
Experiment 1				
Control	497 ± 14	—	1,132 ± 31	—
100 ng/ml rIL-1 β	5,680 ± 633	11 ×	8,320 ± 766	7.3 ×
50 ng/ml rIL-1 α	9,910 ± 538	20 ×	—	—
50 ng/ml rTNF α	14,905 ± 1,324	30 ×	12,690 ± 657	11.2 ×
10 μg/ml LPS	9,530 ± 512	19 ×	10,459 ± 388	9.2 ×
10 ng/ml rIFNγ	1,306 ± 308	2.6 ×	4,002 ± 664	3.5 ×
rIL-1β + rTNF	17,943 ± 1,410	36 ×	—	—
rIL-1β + LPS	11,636 ± 761	23 ×	—	—
rIL-1β + rIFNγ	7,849 ± 601	16 ×	—	—
rTNF + LPS	21,004 ± 1,241	42 ×	—	—
rTNF + rIFNγ	20,232 ± 1,189	41 ×	—	—
LPS + IFNγ	14,509 ± 320	29 ×	—	—
Experiment 2				
Control	603 ± 11	—	—	—
Polymyxin B (10 μg/ml)	480 ± 23	0.79 ×	—	—
Polymyxin B + rIL-1	5,390 ± 97	8.9 ×	—	—
Polymyxin B + rTNF	14,801 ± 389	25 ×	—	—
1 μg/ml LPS	7,598 ± 432	13 ×	—	—
Polymyxin B + LPS	510 ± 44	0.85 ×	—	—

HUVEC or HSVEC were seeded into 96 well plates at 1:3 from a confluent monolayer and allowed to grow to confluence. Cells were then treated with the indicated materials or media for 16 h and the RIA done as in Materials and Methods.

at 37°C. Nonadherent cells were removed by washing with warm media (18). Wells were examined microscopically before and after washing to determine the evenness of cell settling and damage to the endothelial cell monolayer during washing. Damage to the monolayer was never significant (<5% area). Radioactivity was quantified after solubilization of the monolayer in 0.1 N NaOH. Percent adhesion was calculated as the (Experimental counts - Counter background)/(Input counts - spontaneous release).

Light Microscopy. ICAM-1 and LFA-3 were incorporated into liposomes as above at a lipid:protein ratio of 1,000:1. LFA-3 was purified as previously described (12). Membranes were formed on 12-mm glass coverslips (Bellco Glass, Inc, Vineland, NJ) in 24 well plate wells (5, 12). T lymphoblasts (10⁶) were added and incubated for 1 h at 37°C and then washed five times with warm media. Coverslips with planar membranes and bound cells were attached to glass slides using two pieces of two-sided tape spaced 8-mm apart without allowing the membrane surface to come into contact with air. This could be done by turning the coverslip membrane side-down with forceps under media and rapidly transferring the inverted slide with a hanging drop of media. Pictures were taken using a Zeiss standard microscope with phase contrast optics.

Results

ICAM-1 Expression on Cultured Endothelial Cells

ICAM-1 expression was assayed on human umbilical vein endothelial cells (HUVEC) and human saphenous vein endothelial cell (HSVEC) using radioimmunoassay and immunofluorescence flow cytometry. The basal expression of ICAM-1 is 2-4-fold higher on HSVEC than on HUVEC, as determined both by [¹²⁵I]ICAM-1 mAb binding (Table I) and immunofluorescence flow cytometry (not shown). In our hands HSVEC grew more slowly than HUVEC and this may be the basis of the higher ICAM-1 expression on these cells. Quiescent fibroblasts express higher levels of ICAM-1 compared with rapidly dividing cells (11) and HUVEC maintained at confluence for several days gave immunofluorescence flow cytometry profiles similar to HSVEC. As previously reported

(35) exposure of HUVEC to recombinant rIL-1α, rIL-1β, and rTNFα increases ICAM-1 expression 10-30-fold (Table I). Used at optimal concentrations for this response, rIL-1α, rTNF and LPS were the most potent inducers and rIL-1β was less potent. These results should be interpreted conservatively since only single recombinant preparations were tested. The absolute number of ICAM-1 mAb-binding sites was quantified by saturation binding with [¹²⁵I]ICAM-1 mAb (Table II). Basal expression of ICAM-1 varied between 5-10 × 10⁴ sites/cell, but after rTNF stimulation it is a major HUVEC surface component at 3.5 × 10⁶ sites/cell. Combinations of rTNF and rIFNγ, and LPS and rIFNγ at optimal concentrations resulted in greater than additive effects on ICAM-1 expression (Table I). rIL-1β and rTNF increased ICAM-1 expression half-maximally at 15 ng/ml and 0.5 ng/ml, respectively (not shown). The results on ICAM-1 induction were confirmed at the single cell level by immunofluorescence flow cytometry (Fig. 1). Furthermore, flow cytometry showed that ICAM-1 staining on HUVEC and HSVEC (not shown) was heterogeneous showing a normal distribution with a half-height width spanning one log. While

Table II. Site-number Determination for ICAM-1

HUVEC treatment	Sites/cell ± SD
	× 10 ⁻⁵
Media	1.0 ± 0.1
LPS	31.8 ± 1.4
TNF	35.0 ± 2.0
LPS + TNF	54.2 ± 2.5

HUVEC at about 1 × 10⁴ cells/well were treated as indicated for 16 h at 37°C. The concentrations of LPS was 10 μg/ml and TNF was 10 ng/ml. Results are from two experiments.

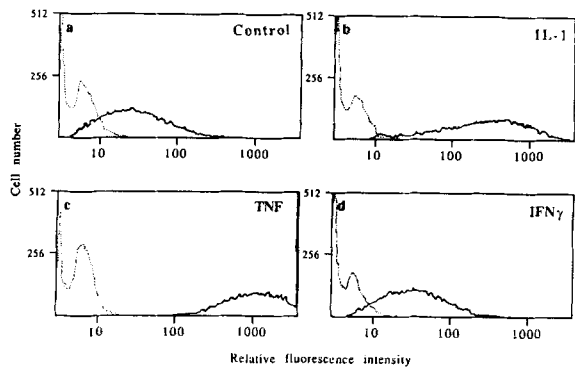


Figure 1. Immunofluorescence flow cytometry of HUVEC. HUVEC were treated with media (a), 10 ng/ml rIL-1 β (b), 10 ng/ml rTNF (c), or 1,000 U/ml rIFN γ (d) for 24 h. Monodispersed suspensions were prepared by brief trypsinization which does not affect ICAM-1 expression. (dotted line) Nonbinding control. (solid line) ICAM-1.

some of this heterogeneity in staining may be due to variation in cell size, staining with ICAM-1 mAb was more heterogeneous than with LFA-3 mAb or HLA-A,B mAb (not shown).

Low endotoxin serum (0.025 ng/ml) was used in all of these studies and experiments with the LPS neutralizing an-

tibiotic polymyxin B showed that the endotoxin levels in the growth media had little effect on expression of ICAM-1 on HUVEC (Table I). These experiments further showed that effects of recombinant IL-1 and TNF were not attributable to endotoxin contamination.

IL-1 and TNF caused ICAM-1 expression to be increased rapidly between 1 and 8 h and then more slowly over a period of days, as previously described (35). ICAM-1 expression remained elevated for 7 d in the presence of IL-1 or TNF, but returned to basal levels when the mediators were washed out (not shown). The time-course of LPS contrasted with that of IL-1 or TNF since maximum expression was seen at 12 h (25-fold basal) after which ICAM-1 expression decreased to an intermediate level by 24 h (15-fold basal).

Endothelial Cell ICAM-1 Biochemistry. ICAM-1 shows significant relative molecular mass (M_r) heterogeneity when immunoaffinity isolated from different cell types (11). ICAM-1 isolated from HUVEC had an M_r of $\sim 90,000$ under nonreducing conditions (Fig. 2 A). IL-1 and TNF treatment for 24 h resulted in an increase in the amount of isolated ICAM-1 corresponding to the increase in ICAM-1 surface expression. However there was no change in ICAM-1 M_r (Fig. 2 A). IFN γ treatment resulted in a small increase in immunoprecipitated ICAM-1, but a stronger increase in HLA-

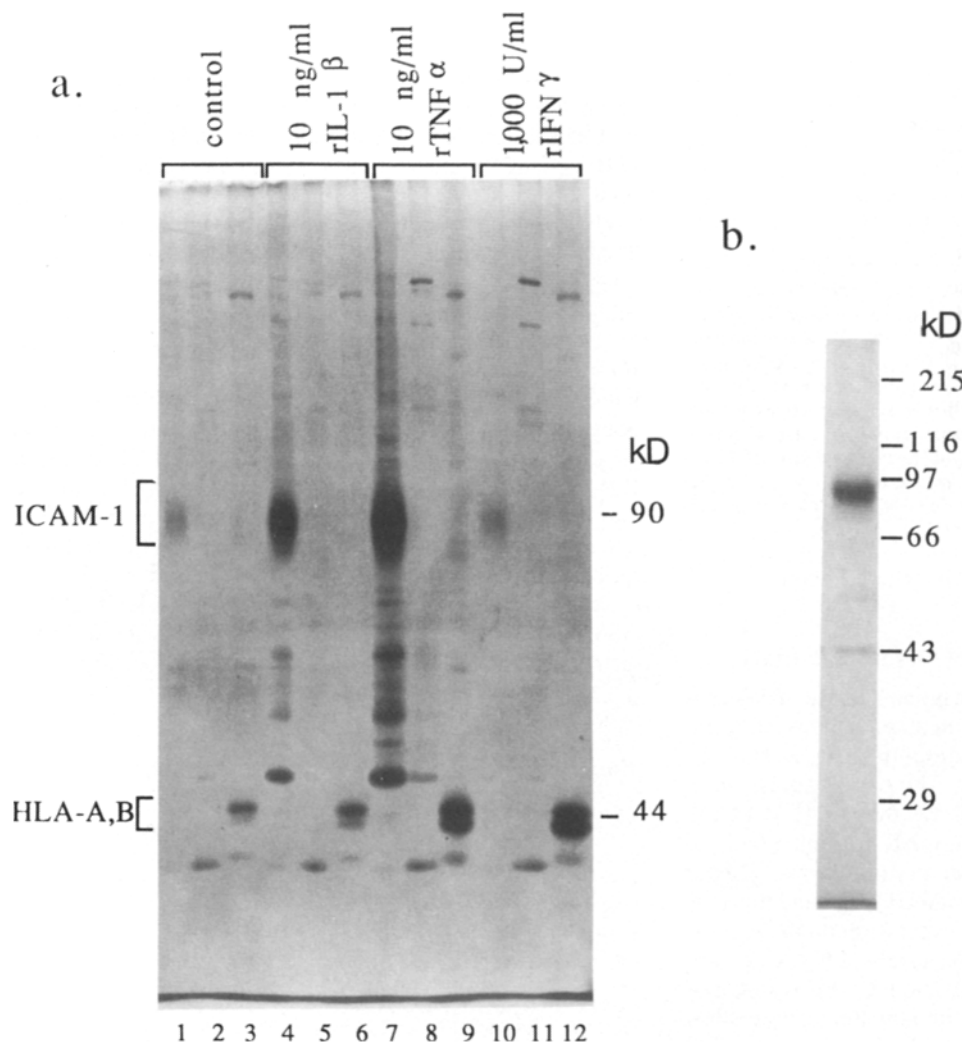


Figure 2. SDS-PAGE of ICAM-1. (a) HUVEC were treated with media (lanes 1-3), 10 ng/ml rIL-1 β (lanes 4-6), 10 ng/ml TNF (lanes 7-9), and 1,000 U/ml rIFN γ (lanes 10-12). Lysates from 4×10^6 cells were prepared and immunoaffinity isolation was performed with RR1/1 ICAM-1 mAb Sepharose (lanes 1, 4, 7, and 10), TS2/9 LFA-3 mAb Sepharose (lanes 2, 5, 8, and 11) or W6/32 HLA-A,B mAb Sepharose (lanes 3, 6, 9, and 12). Protein was eluted at 100°C with SDS and then run on nonreducing 8% SDS-PAGE and the gel silver stained. (b) 500 μ g of purified ICAM-1 from HUVEC stimulated with LPS, run on a reducing 10% SDS-PAGE and silver stained (33).

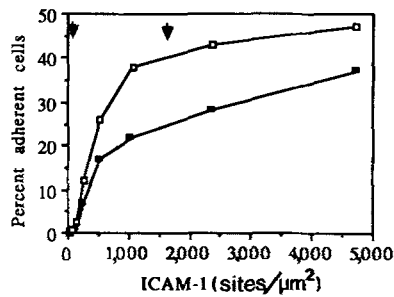


Figure 3. Adhesion of T lymphoblasts (open squares) and JY cells (solid squares) to HUVEC ICAM-1 at different densities. Cell-binding percentages are means of quadruplicate determinations. Standard deviations did not exceed 10%. Resting (hatched arrow) and TNF (solid arrow)-HUVEC equivalents are based on 3.5×10^6 sites/cell over 0.28 cm^2 . Data shown are representative of 5 experiments with T lymphoblasts (d7-13) and two with JY.

A,B (Fig. 2 A). Other bands appeared to be contaminants or weakly associated with ICAM-1, since only the 90,000-M_r ICAM-1 band was isolated when HUVEC plasma membranes were initially solubilized in 1% N-lauroylsarcosine detergent followed by addition of 2% Triton X-100 (not shown).

Reconstitution of Purified Endothelial Cell ICAM-1

The ability of endothelial cell ICAM-1 to act as a ligand for lymphocyte adhesion was directly tested using purified HUVEC ICAM-1 reconstituted into phospholipid vesicles and bound to plastic. ICAM-1 was purified from LPS activated HUVEC by immunoaffinity chromatography using conditions established to recover functional ICAM-1 from JY cells (30). SDS-PAGE and silver staining confirmed that ICAM-1 was the major species (Fig. 2 B). ICAM-1 protein (90-kD band) isolated under these conditions can be completely reabsorbed to anti-ICAM-1-Sepharose demonstrating retention of the epitope recognized by RRI/1 after purification (not shown). Immunofluorescence light microscopy of ICAM-1 in plastic bound liposomes suggests that the bound liposomes remained discrete vesicles (not shown). Average expression of ICAM-1 by plastic bound liposomes was quantified using saturating ^{125}I -anti-ICAM-1 and sites per square micron were calculated assuming ICAM-1 distribution over a flat surface. Reconstitution of ICAM-1 at different lipid:protein ratios resulted in different densities of

ICAM-1 when bound to plastic (Fig. 3). The range of densities covered included the density on confluent unstimulated HUVEC ($40 \text{ sites}/\mu\text{m}^2$) and TNF-stimulated HUVEC monolayers ($1,600 \text{ sites}/\mu\text{m}^2$) (arrows in Fig. 3) again assuming ICAM-1 distribution on a flat surface.

Adhesion to ICAM-1 in planar membranes was tested using the B lymphoblastoid cell line JY and T lymphoblasts. JY has been used previously in studies of LFA-1 dependent, phorbol ester stimulated, homotypic adhesion (39, 40). Low numbers of JY cells per well ($<5 \times 10^4$) were used such that contact between JY cells was minimal and no phorbol esters were used such that homotypic aggregation was not observed in this study. T lymphoblasts have been used in a previous study as a model for activated T lymphocytes (11). Both JY and T lymphoblasts showed efficient adhesion to purified reconstituted ICAM-1 which was dependent on ICAM-1 density (Fig. 3). Adhesion was most sensitive to ICAM-1 density in the physiologic range defined by expression on resting and TNF-treated endothelial cells (arrows in Fig. 3). Adhesion was completely blocked by ICAM-1 mAb and LFA-1 mAb and adhesion was not seen to membranes containing glycophorin (Table III). LFA-1 mAb inhibited by binding to the T lymphoblasts, while ICAM-1 mAb inhibited by binding to the reconstituted ICAM-1 (Table III). ICAM-1 purified from HUVEC or a B lymphoblastoid cell line, JY, were similar in activity at the one density of the JY material tested. In several experiments no adhesion of either cell type was seen below a threshold value of $100 \text{ sites}/\mu\text{m}^2$ (Fig. 3 and not shown).

T and B lymphocytes bound to ICAM-1 in plastic-bound vesicles or planar membranes had a distinctive morphology. This was particularly striking relative to T lymphoblast adhesion to LFA-3 in planar membranes (12). T lymphoblasts adhering to ICAM-1 were spread and irregular in shape (Fig. 4 a), while T lymphoblasts adhering to LFA-3 were mostly rounded (Fig. 4 b). The morphology of T lymphoblasts adhering to ICAM-1 is most similar to that of T lymphoblasts adhering to HUVEC (not shown).

B Lymphoblastoid Cell Adhesion to HUVEC

Since it was clear that both JY cells and T lymphoblasts could adhere to endothelial cell ICAM-1 at physiological densities, the ability of ICAM-1 mAb to block adhesion of cells to resting and activated HUVEC was tested. Binding of JY cells to HUVEC and human dermal fibroblast (HDF) monolayers was assayed (Table IV). Adhesion of JY to un-

Table III. Adhesion of T Lymphoblasts to ICAM-1 in Plastic-Bound Vesicles

Membrane composition	mAb pretreatment					
	None	ICAM-1		LFA-1		CD2 Both
		Cells	Vesicles	Cells	Vesicles	
HUVEC ICAM-1 ($1,900/\mu\text{m}^2$)	44 ± 1.2	47 ± 1.9	0.5 ± 0.1	0.2 ± 0.1	42 ± 2.6	40 ± 4.2
JY ICAM-1 ($1,300/\mu\text{m}^2$)	39 ± 2.3	40 ± 3.8	0.8 ± 0.3	0.4 ± 0.1	36 ± 2.4	37 ± 2.0
Glycophorin	0.3 ± 0.2	—	—	—	—	—

ICAM-1 was purified from LPS activated HUVEC or JY in an identical manner. Binding of ^{51}Cr -labeled T lymphoblasts was determined as in Materials and Methods and is expressed as percentage bound \pm SD. These results are representative of two experiments.

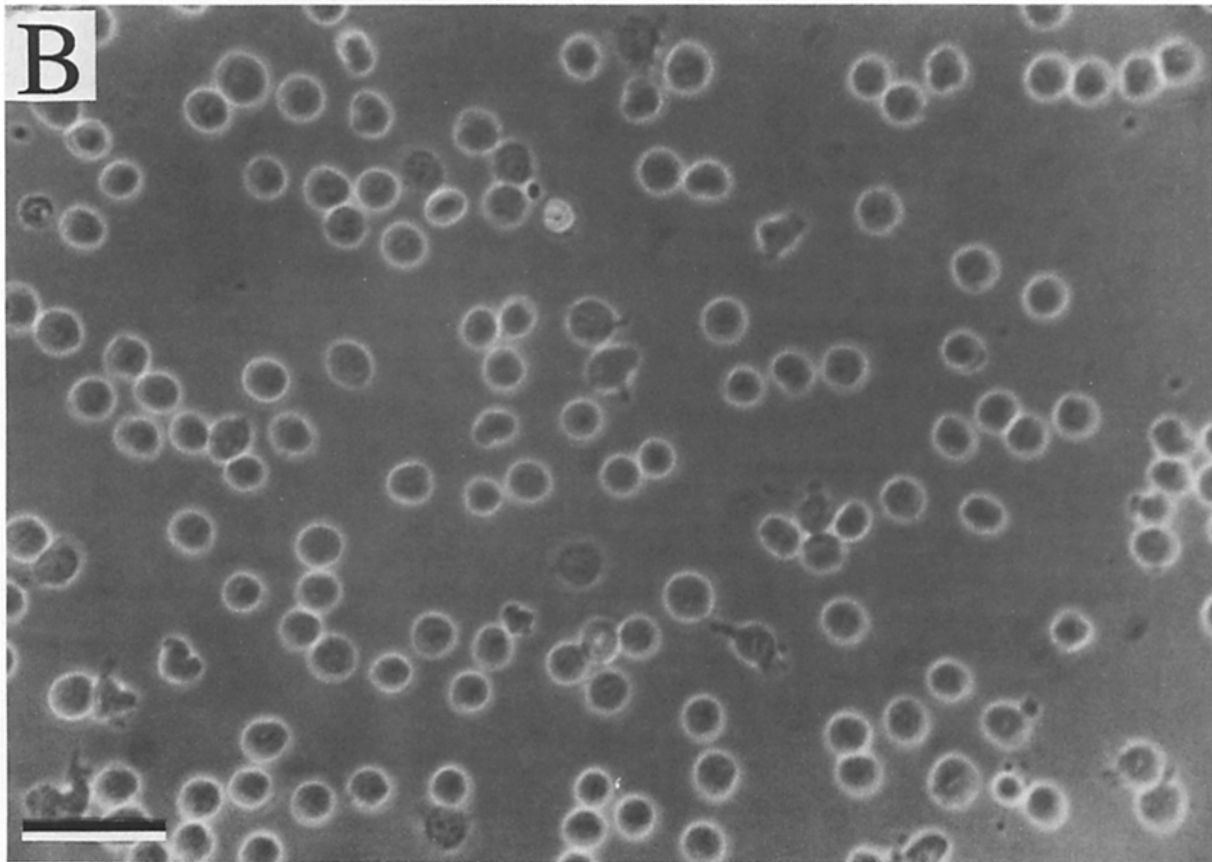
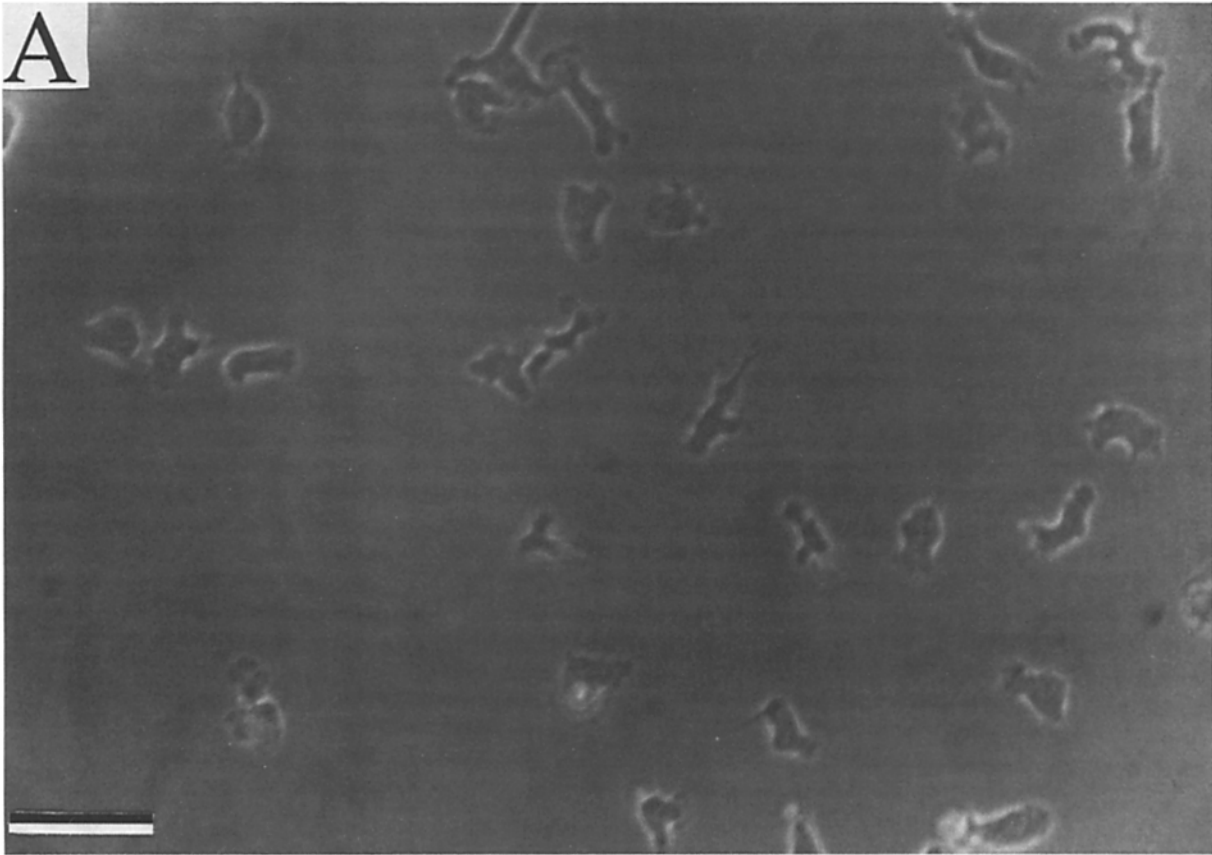


Figure 4. Photomicrographs of T lymphoblasts adhering to glass supported planar membranes with ICAM-1 (a) or LFA-3 (b). Bars, 20 μ m.

Table IV. Adhesion of JY to HUVEC

mAb in assay	Adherent cells (percentage ± SD)				
	HUVEC treatment			HDF treatment	
	None	IL-1	TNF	None	TNF
Control	4.6 ± 1.7	13 ± 2.2	20 ± 2.8	3.8 ± 1.0	13 ± 2.8
ICAM-1 (RR1/1)	3.3 ± 0.4	3.7 ± 0.5	4.9 ± 1.2	1.3 ± 0.3	1.1 ± 0.4
LFA-1α	0.7 ± 0.2	0.8 ± 0.3	1.0 ± 0.1	1.7 ± 0.2	2.1 ± 0.3
LFA-3	5.9 ± 1.1	10 ± 1.3	18 ± 2.3	3.8 ± 0.5	11 ± 2.9

Confluent monolayers of HUVEC or HDF in 96 well plates were treated for 24 h with 10 ng/ml recombinant IL-1α or TNF as indicated. Binding of ⁵¹Cr-labeled JY cells was determined as described in Materials and Methods. These results are representative of four experiments.

stimulated HUVEC was inhibited 85% by LFA-1 mAb and 28% by ICAM-1 mAb. In contrast, adhesion of JY cells to HDF was inhibited 65% by ICAM-1 mAb and 55% by LFA-1 mAb. ICAM-1 mAb F(ab)₂ fragments did not increase the amount of inhibition for either HUVEC or HDF. These experiments were repeated with the R6.5 ICAM-1 mAb and R6.5 Fab fragments which binds to a different epitope on ICAM-1 (Marlin, S. M., personal communication), but yielded identical results to RR1/1 (not shown). Treatment of HUVEC and HDF with IL-1, TNF or LPS resulted in a significant increase in JY adhesion (Table IV and not shown). This adhesion was inhibited 95% by LFA-1 mAb and 75% by ICAM-1 mAb. The adhesion in the presence of ICAM-1 mAb to resting and activated cells was similar for both endothelial cells and fibroblasts, suggesting that ICAM-1 mAb inhibited the cytokine and LPS stimulated component. Inclusion in the adhesion assay of neutralizing antisera to TNF or IL-1, or of polymyxin B at 10 μg/ml had no effect on the efficiency of JY adhesion to HUVEC stimulated with TNF, IL-1 and LPS respectively (not shown). Similarly, inclusion of TNF, IL-1 or LPS in the adhesion assay only (1 h) did not affect the efficiency of JY binding to cells which received no prior stimulation (not shown); ICAM-1 expression does not increase until after 1 h (11, 35). Furthermore, the time course of the mediator effects on endothelial adhesiveness paralleled the kinetics of the increase in ICAM-1 expression (not shown).

ICAM-1 is expressed on JY cells as well as HUVEC. To determine on which cell ICAM-1 is functionally important,

cells were pretreated with mAb, washed, and tested in the adhesion assay (Table V). ICAM-1 mAb pretreatment of TNF activated HUVEC resulted in inhibition equivalent to that obtained when ICAM-1 mAb was included in the assay, while pretreatment of JY cells with ICAM-1 mAb had no effect. LFA-1 mAb inhibited only when JY cells were pretreated which is consistent with the expression of LFA-1 on JY only in this system. This result demonstrates that ICAM-1 mAb inhibits this interaction by binding to HUVEC ICAM-1.

JY adhesion and ¹²⁵I-anti-ICAM-1 binding to HUVEC treated with increasing amounts of TNF was studied to determine the correlation between adhesion and ICAM-1 expression. JY adhesion increased with increasing TNF concentration (Fig. 5 A). At all TNF concentrations LFA-1 mAb inhibited almost completely, while ICAM-1 mAb inhibited partially with the amount of ICAM-1 mAb resistant adhesion remaining constant (Fig. 5 A). The relative amount of ICAM-1 expression was determined at each experimental point with [¹²⁵I]anti-ICAM-1, and was plotted against the adhesion dependent on ICAM-1 or on LFA-1, which was calculated by subtracting the adhesion in the presence of ICAM-1 mAb or LFA-1 mAb from the adhesion in the presence of the non-binding control mAb (Fig. 5 B). When the percentage of adhering JY cells was plotted against ICAM-1 expression a very strong positive correlation was obtained both with LFA-1-dependent and ICAM-1-dependent adhesion. While LFA-1 dependent adhesion extrapolates to 5% at zero

Table V. Cellular Site of mAb Blocking

mAb Pretreatment	Adherent cells	
JY	(% ± SE)	
—	HUVEC (TNF 24 h)	
—	—	22.9 ± 4.5
ICAM-1	—	24.2 ± 3.1
LFA-1α	—	0.5 ± 0.7
LFA-3	—	21.0 ± 2.7
—	ICAM-1	3.7 ± 1.4
—	LFA-1α	22.2 ± 1.9
—	LFA-3	22.4 ± 3.1

HUVEC were grown to confluence in 96 well plates. HUVEC or ⁵¹Cr-labeled JY cells were pretreated with 50 μg/ml mAb for 30 min at 24°C and then washed four times with media. The cell adhesion assay was then done as in Materials and Methods in the absence of unbound mAb. ICAM-1 mAb was RR1/1. These results are representative of two experiments.

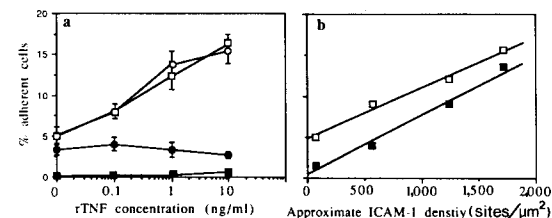


Figure 5. Effect of TNF treatment on JY adhesion and ICAM-1 expression. HUVEC were grown to confluence in 96 well plates and were incubated with the indicated concentration of rTNF for 16 h. Adhesion of ⁵¹Cr JY cells was measured as described in methods. Binding of [¹²⁵I]ICAM-1 mAb was determined in parallel. (a) (Open squares) control mAb; (open circles) anti-HLA A,B; (solid squares) LFA-1 mAb; (solid circles) ICAM-1 mAb. (b) Adhesion data same as in a. (Solid squares) binding inhibitable by LFA-1 mAb; (solid circles) binding inhibitable by ICAM-1 mAb. Density is approximated assuming all ICAM-1 is expressed on an area with the dimension of the well (0.28 cm²). Similar results were obtained in two other experiments.

Table VI. Inhibition of Adhesion of Peripheral Blood Lymphocytes and Con A Lymphoblasts to HUVEC and HSVEC

mAb in assay	Adherent cells (percentage \pm SD)			
	HUVEC		HSVEC	
	PBL	Blasts	PBL	Blasts
Control IgG 1	22 \pm 1.2	81 \pm 6.2	30 \pm 3.0	91 \pm 7.7
ICAM-1	20 \pm 1.5	77 \pm 5.6	28 \pm 4.5	81 \pm 9.2
LFA-1 α	6 \pm 0.8	24 \pm 1.4	16 \pm 3.9	49 \pm 7.8
LFA-1 β	4 \pm 0.2	—	—	—
LFA-3	25 \pm 1.3	83 \pm 7.0	31 \pm 2.0	92 \pm 4.9
CD2	21 \pm 2.0	80 \pm 4.1	—	—
ICAM-1 + LFA-1 α	6 \pm 1.6	22 \pm 1.9	15 \pm 2.8	45 \pm 6.3
ICAM-1 + LFA-3	23 \pm 1.4	79 \pm 3.1	—	—

HEC were seeded into 96 well plates at a 1:3 dilution and allowed to grow to a confluent monolayer. 5×10^4 ^{51}Cr -labeled PBL were added with the indicated mAb (10 $\mu\text{g}/\text{ml}$ IgG) and incubated at 37°C for 1 h. The plates were washed as described in Materials and Methods. ICAM-1 mAb was RR1/1. These results are representative of four experiments with HUVEC and two experiments with HSVEC.

ICAM-1, consistent with the presence of the ICAM-1 independent adhesion, the ICAM-1 dependent adhesion extrapolates to near zero adhesion at zero ICAM-1 expression (Fig. 5). Therefore, the relationship between ICAM-1 expression and the efficiency of adhesion of JY cells was linear for the range of ICAM-1 expression observed on endothelial cells.

Resting and Activated Lymphocyte Adhesion to HUVEC

Adhesion of PBL and T lymphoblasts to resting endothelial cells was much more efficient than the basal adhesion of JY (Table VI). In both cases adhesion was inhibited by 50–80% by LFA-1 α or β subunit mAb, as previously reported (19), but only 10% or less by ICAM-1 mAb. RR1/1 F(ab')₂ fragments were no more effective than IgG. The R6.5 ICAM-1 mAb and Fab fragments of R6.5 gave identical results to RR1/1 (not shown). The combination of LFA-1 mAb and ICAM-1 mAb was no more effective than LFA-1 mAb alone.

The effect of monokine activation of HUVEC on adhesion of T lymphoblasts was determined. IL-1 and TNF did not increase adhesion of normal T lymphoblasts over the high basal level of 80%, which may represent the maximal adhesion in our assay (Fig. 6). However, inclusion of LFA-1 mAb showed that these cytokines stimulated an increase in the LFA-1-independent component of adhesion as indicated by a dramatic decrease in the ability of LFA-1 mAb to inhibit adhesion. To confirm our findings on LFA-1 dependent and independent components of adhesion, LFA-1 deficient T lymphoblasts were used. Lymphocytes from patients with an inherited disease, leukocyte adhesion deficiency (LAD), are genetically deficient in LFA-1 due to a mutation in its β subunit (1, 23). LFA-1⁻ T lymphoblasts (line ZJO with less than 1% normal expression) from an LAD patient showed greatly decreased adhesion compared to LFA-1⁺ T lymphoblasts (Fig. 6). Adhesion of LFA-1⁻ cells was 10% basally and was increased to 30% after HUVEC treatment with IL-1 for 6 h, or to 58% after HUVEC treatment with TNF. While adhesion of LFA-1⁻ T lymphoblasts was optimal at 6 h after initiation of lymphokine treatment, it had not returned to basal levels at 48 h. Similar results were seen with short term cultured LFA-1⁻ EBV transformed B lymphoblastoid cell lines from LAD patients (not shown), however JY, a long term EBV transformed B cell line, has apparently lost this LFA-1-independent adhesion pathway. ICAM-1 mAb had no effect on adhesion by LFA-1⁺ or LFA-1⁻ T lymphoblasts to endothelial cells. Hence, a completely LFA-1 independent component of adhesion is present which is stimulated by monokines and is not blocked by ICAM-1 mAb.

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An ICAM-1 dependent component was detected in PBL adhesion to stimulated endothelial cells (Table VII). As noted above, resting PBL have a level of adhesion to unstimulated endothelial cells which is higher than for JY B lymphoblastoid cells and lower than for T lymphoblasts. Basal adherence of 22% was increased to 36 to 41% by LPS and TNF and to a lesser extent by IL-1 β , correlating with the effects of these agents on ICAM-1 expression. This increase in total adhesion was accompanied both by an increase in the ICAM-1 dependent adhesion, similarly to JY B lymphoblastoid cell binding, and in LFA-1 independent adhesion, similarly to T lymphoblast adhesion. The composition of adherent PBL was not determined with respect to T and B lymphocytes which make up 70–75% and 15–20% of PBL respectively, with \sim 10% large granular lymphocytes. Similarly, it was not determined whether the blocking effect of ICAM-1 mAb in binding of PBL to activated endothelial cells was directed against T cells, B cells or both.

Discussion

We have found that purified endothelial cell ICAM-1 is a ligand for lymphocyte adhesion and have tested the ability of

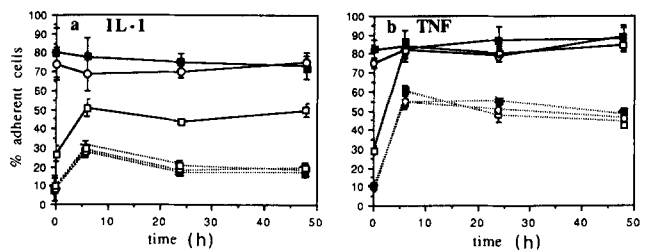


Figure 6. Adhesion of LFA-1⁺ and LFA-1⁻ T lymphoblasts to IL-1 and TNF treated HUVEC-HUVEC were grown to confluent monolayers in 96 well plates and were treated with 10 ng/ml rIL-1 β (a) or rTNF (b) for the indicated amount of time. Adhesion of ^{51}Cr -labeled LFA-1⁺ (solid lines) or LFA-1⁻ (dashed lines) T lymphoblasts was determined in the presence of control mAb (solid squares), ICAM-1 mAb (open circles) or LFA-1 mAb (open squares). This experiment was with ZJO T lymphoblasts; similar results were obtained with BBN T lymphoblasts.

Table VII. PBL Adhesion to Activated HUVEC

HUVEC pretreatment	Adherent PBL (percentage ± SD)			
	Control	ICAM-1	mAb LFA-1α	LFA-3
None	22.7 ± 1.2	19.2 ± 2.0	7.8 ± 1.1	22.8 ± 1.9
10 ng/ml rIL-1β	30.1 ± 1.3	26.9 ± 1.2	14.9 ± 0.2	32.7 ± 1.0
10 ng/ml rTNF	41.2 ± 0.8	33.1 ± 1.1	27.7 ± 1.5	40.3 ± 1.8
10 μg/ml LPS	36.0 ± 1.6	28.4 ± 0.6	21.2 ± 1.3	37.9 ± 3.4
1 ng/ml rIFNγ	26.3 ± 3.4	24.8 ± 1.1	10.0 ± 1.3	29.3 ± 0.8

Confluent HUVEC in 96-well plates were treated as indicated for 30 h. Cell binding assay with ⁵¹Cr-labeled PBL was done as in Materials and Methods. ICAM-1 mAb was RR1/1. This experiment is representative of three experiments with PBL from different donors.

ICAM-1 mAb to inhibit the adhesion of lymphocytes to HUVEC. At least three lymphocyte adhesion pathways were found to be used in adhesion of three types of lymphocytes to endothelial cells (summarized in Fig. 7): (a) an LFA-1 dependent, ICAM-1 independent basal adhesion which was not significantly changed by cytokines or LPS; (b) an LFA-1 and ICAM-1 dependent pathway which was weakly expressed basally, but strongly up regulated by monokines and LPS; and (c) an LFA-1 independent pathway which also may have a low basal expression on cultured cells and was also up-regulated by monokines and LPS. Pathways 1 and 3 above may actually consist of more than one pathway at the molecular level. Our findings extend previous studies using LFA-1 mAb which had defined only LFA-1 dependent basal adhesion and a monokine stimulated LFA-1-independent pathway (6, 18).

Adhesion of T lymphoblasts and JY cells to endothelial cell ICAM-1 in planar membranes shows that LFA-1-dependent adhesion of both of these cell types is completely inhibited by ICAM-1 mAb when ICAM-1 is the only LFA-1 ligand available for adhesion. The complex pattern of mAb inhibition or lack of inhibition seen for T lymphoblast, PBL and JY cell binding to endothelial cells (Fig. 7) may be the result of additional adhesion ligands expressed by endothelial cells. In general, if several adhesion pathways available to mediate interaction of two cells are capable of allowing stable cell adhesion under a given set of binding and washing conditions, then mAb to any one pathway may be

ineffective in reducing the number of cells bound, although the pathway blocked by that mAb may be used. This may be the case in ICAM-1 mAb ineffectiveness against PBL adhesion to resting endothelial cells and T lymphoblast adhesion to resting or activated endothelial cells. Detection of ICAM-1's relative contribution to these interactions and accurate assessment of ICAM-1's contribution to systems such as PBL adhesion to activated endothelial cells, in which partial inhibition was detected with ICAM-1 mAb, will require development of mAb to the other adhesion pathways (both LFA-1 dependent and independent) or use of different types of assays. Adhesion assays which measure the force required for separation of cells (49) and the use of flow devices which simulate conditions in blood vessels (27) should be useful in further evaluating the role of the different adhesion pathways in lymphocyte-endothelial cell adhesion.

ICAM-1 is distinct from other endothelial cell antigens induced or upregulated by cytokines both in its pattern of expression (35) and in size. HUVEC ICAM-1 at ~90 kD under nonreducing conditions and ~100 kD under reducing conditions (48) is of about average size; ICAM-1 on different cells ranges from 80 to 114 kD nonreduced, the heterogeneity arising from N-glycosylation (11, and our unpublished results). ICAM-1 is clearly distinct from the endothelial cell antigen E-LAM 1, which is involved in HL-60 myelomonocytic cell and granulocyte adhesion, by expression pattern and size in SDS-PAGE (4). ICAM-1 structure based on cDNA sequencing (44, 48) is in excellent agreement with the biochemical data on ICAM-1 (11) and further show that ICAM-1 from endothelial cells and the HL-60 myelomonocytic cell line are identical. ICAM-1 is a member of the immunoglobulin super-family based on sequence homology (44, 48).

ICAM-1 purified from endothelial cells and reconstituted into artificial vesicles mediated efficient adhesion of JY cells and T lymphoblasts which was LFA-1 dependent. The relationship between percent JY cell or T lymphoblast adhesion and ICAM-1 expression in the reconstituted system showed a threshold for adhesion at low ICAM-1 density, a linear phase over the range of densities found on cultured endothelial cells, and approach to a plateau at higher ICAM-1 densities. The activity of isolated ICAM-1 in plastic bound vesicles was very similar to the activity of ICAM-1 on the surface of HUVEC for JY binding. When the ICAM-1 independent adhesion pathway for JY adhesion to HUVEC was subtracted out there was a linear relationship between percent adhesion and ICAM-1 expression level which is very similar to the relationship seen for ICAM-1 in plastic bound vesicles. The finding that ICAM-1 in an inert substrate is

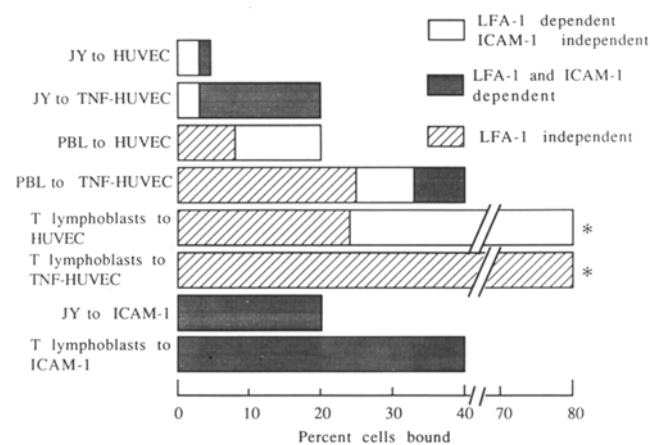


Figure 7. Summary of adhesion results. TNF HUVEC data is for endothelial cells treated with 10 ng/ml TNF for 24 h. (*) Binding of T lymphoblasts may be at saturation for one or two pathways, but the other pathways may be functioning, but not required.

as effective as ICAM-1 on endothelial cells suggests that HUVEC may not have an active role in LFA-1 dependent adhesion. This has been suggested previously since peripheral blood T lymphocytes adhere to glutaraldehyde fixed cultured HUVEC no less efficiently than to live HUVEC (19). Factors such as surface charge density and protein lateral mobility may differ significantly between the HUVEC and in plastic bound vesicles and these parameters will be useful in further interpreting these results when they are measured and their influence is systematically studied.

The proposal of other LFA-1 ligands besides ICAM-1 is consistent with prior observation that some cell lines show LFA-1-dependent phorbol ester stimulated aggregation which is not inhibited by ICAM-1 mAb (39) and that ICAM-1 mAb inhibits LFA-1 dependent CTL mediated lysis of only a subset of targets (28). Two ICAM-1 mAb which recognize different epitopes gave identical results, and we have ruled out the involvement of Fc receptors and antibody cross-bridging using appropriate fragments of IgG, so the failure to see inhibition of lymphocyte adhesion to HUVEC equivalent to that given by LFA-1 mAb is a strong negative result. The adhesion to endothelial cells which is blocked by LFA-1 mAb, but not by ICAM-1 mAb appears not to be due to differences in the efficiency of these mAb in blocking adhesion, since ICAM-1 mAb and LFA-1 mAb are equally effective in blocking adhesion to ICAM-1 in planar membranes, to dermal fibroblasts (11 and this report), and to epidermal keratinocytes (13).

Lymphocytes adhere efficiently to cultured endothelial cells, but not to the endothelial surface of sections of large veins and arteries (9, 10). The same level of lymphocyte adhesion, including the presence of a monokine inducible, LFA-1 independent pathway, is also seen for cultured microvascular endothelial cells (20), so the phenomenon described here and previously (18) is not peculiar to large vessel endothelial cells. Cultured endothelial cells appear to be in an intermediate state of activation in which endothelial cell adhesiveness for lymphocytes is significantly enhanced, but other indications of endothelial cell activation such as ELAM-1 are not expressed (4, 35). Endothelial cells in intact vessels may be less intrinsically adhesive for lymphocytes than cultured endothelial cells. ICAM-1 upregulation may be important in regulating lymphocyte interaction with endothelial cells at the site of inflammation. ICAM-1 upregulation has been observed in inflammation *in vivo* (8). Based on the planar membrane experiments it is clear that HUVEC ICAM-1 can be used as a ligand for T lymphoblast adhesion. The use of ICAM-1 as a ligand by other leukocytes which express significant levels of LFA-1 (24), such as monocytes and granulocytes remains to be assessed.

We used lymphocytes from genetically LFA-1 deficient individuals with leukocyte adhesion deficiency (LAD) (1) and showed their T lymphoblasts are totally deficient in the LFA-1-dependent adhesion pathways, but demonstrate the LFA-1-independent inducible adhesion. These defects correlated with the impaired primary responses to specific antigen *in vitro*, and with near normal secondary responses. *In vivo*, defects in lymphocyte function in LAD are less dramatic than those in granulocyte function (1). The ability of LFA-1⁻ lymphocytes to extravasate at sites of inflammation is consistent with the presence of the LFA-1-independent pathway, but does not rule out a role for LFA-1 since insufficient data is available to detect a relative deficiency. Use of animal models

for leukocyte-endothelial interactions using LFA-1, ICAM-1 and other antibodies *in vivo* is the only practical way to directly assess the physiological role of these molecules (2).

We and others (18, 31) have been unable to demonstrate adhesion of PBL (70–80% T cells), T lymphoblasts or cytolytic T cell lines to endothelial cells which can be blocked by CD2 mAb or LFA-3 mAb, which partially block the interactions of cytolytic T lymphocytes and some target cells (24, 43), and of thymocytes and thymic epithelial cells (51). The expression of LFA-3 on HUVEC is only 15% of that on the JY cell line which forms CD2/LFA-3 dependent conjugates with cytolytic T lymphocytes and this relatively low expression may partially explain the failure to detect the activity of this adhesion pathway (our unpublished observations). It is also possible that the LFA-3 on endothelial cells has a low affinity for CD2. Our results and those of others (18, 31) appear to be in conflict with earlier reports that killing of endothelial cells by cytolytic T lymphocyte/natural killer lines was inhibited partially by CD2 mAb and LFA-3 mAb (7). This difference could be resolved if the CD2/LFA-3 interaction in this system was not important for adhesion, but for some other process required for cytolysis. Heterogeneity in the ability of LFA-3 expressing cells to bind to thymocytes or T lymphocytes via the CD2/LFA-3 adhesion pathway has been observed in other systems (51). Our results are in agreement with the finding of Collins et al. (7) that LFA-1 mAb could partially block killing of endothelial cells.

This study reveals some interesting parallels between the function of LFA-1 and several extracellular matrix receptors, which are members of the integrin superfamily. First, the density of reconstituted ICAM-1 required to obtain efficient adhesion of 25–40% of input cells is very similar to the surface density of fibronectin required to allow spreading of fibroblastoid cells: on the order of 10^3 – 10^4 sites/ μm^2 (21). Second, cells adhering to ICAM-1 bearing substrates have a distinctive morphology which includes cell spreading. In contrast, T lymphoblasts adherent to LFA-3 bearing planar membranes were more refractile by phase-contrast microscopy indicating a lower degree of spreading. Third, binding of multiple ligands, as suggested for LFA-1 here and earlier, is a property of some of the integrin family receptors, such as platelet glycoprotein IIb/IIIa which binds to fibrinogen, fibronectin, vitronectin, von Willebrandt factor and probably thrombospondin (36). However, unlike receptor-ligand binding by many members of the integrin family, binding of T lymphoblasts to ICAM-1 in planar membranes is not inhibited by the hexapeptide GRGDSP (30) and ICAM-1 contains no RGD sequences (48).

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