INDUCTION BY IL 1 AND INTERFERON-γ: TISSUE DISTRIBUTION, BIOCHEMISTRY, AND FUNCTION OF A NATURAL ADHESION MOLECULE (ICAM-1)

MICHAEL L. DUSTIN,* ROBERT ROTHLEIN,• ATUL K. BHAN,* CHARLES A. DINARELLO,* AND TIMOTHY A. SPRINGER*•

From the *Laboratory of Membrane Immunology, Dana-Farber Cancer Institute, Boston, MA; the †Committee on Cell and Developmental Biology, and the ‡Department of Pathology, Harvard Medical School, Boston, MA; the §Department of Pathology, Massachusetts General Hospital, Boston, MA; and the ‡Division of Experimental Medicine, Tufts University School of Medicine, Boston, MA

ICAM-1 is a cell surface glycoprotein originally defined by a monoclonal antibody (MAb) that inhibits phorbol ester-stimulated leukocyte aggregation. Staining of frozen sections and immunofluorescence flow cytometry showed intercellular adhesion molecule-1 (ICAM-1) is expressed on non-hematopoietic cells such as vascular endothelial cells, thymic epithelial cells, certain other epithelial cells, and fibroblasts, and on hematopoietic cells such as tissue macrophages, mitogen-stimulated T lymphocyte blasts, and germinal center dendritic cells in tonsils, lymph nodes, and Peyers patches. ICAM-1 staining on vascular endothelial cells is most intense in T cell areas in lymph nodes and tonsils showing reactive hyperplasia. ICAM-1 is expressed in low amounts on peripheral blood leukocytes. Phorbol ester-stimulated differentiation of myelomonocytic cell lines greatly increases ICAM-1 expression. ICAM-1 expression on dermal fibroblasts is increased threefold to fivefold by either interleukin 1 (IL 1) or interferon-γ at 10 U/ml over a period of 4 or 10 hr, respectively. The induction is dependent on protein and mRNA synthesis and is reversible. ICAM-1 displays M₅ heterogeneity in different cell types with a M₅ of 97,000 on fibroblasts, 114,000 on the myelomonocytic cell line U937, and 90,000 on the B lymphoblastoid cell JY. ICAM-1 biosynthesis involves a M₅ ∼73,000 intracellular precursor. The non-N-glycosylated form resulting from tunicamycin treatment has a M₅ of 55,000. ICAM-1 isolated from phorbol myristic acetate (PMA) stimulated U937 and from fibroblasts yields an identical major product of M₅ = 60,000 after chemical deglycosylation. ICAM-1 MAb interferes with the adhesion of phytohemagglutinin blasts, and the adhesion of the cell line SKW3 to human dermal fibroblast cell layers. Pretreatment of fibroblasts but not lymphocytes with ICAM-1 MAb, and of lymphocytes but not fibroblasts with lymphocyte function-associated antigen 1 MAb inhibits adhesion.

Intercellular adhesion is increased by prior exposure of fibroblasts to IL 1, and correlates with induction of ICAM-1.

Immune responsiveness of T lymphocytes to antigens and several effector activities of leukocytes require cell-cell contact and adhesion (1–3). Adhesion to both hematopoietic and non-hematopoietic cells is an obligate step in antigen presentation (4–7) and effector cell functions, such as cytolytic T lymphocyte-mediated killing (1). Molecules have been characterized that are involved in both antigen-specific and nonspecific contributions to these adhesion processes (8–10). One antigen nonspecific adhesion molecule, the lymphocyte function-associated-1 (LFA-1) antigen, is thought to strengthen adhesion to cells bearing specific antigens, perhaps by binding to unidentified molecules on the antigen-presenting cell or target cell (11, 12), thereby increasing the range of avidities over which antigen-specific interactions can be effective (13). Alternatively, LFA-1 may regulate adhesion without itself engaging in ligand-receptor interactions (13). Understanding the molecular nature and regulation of these antigen nonspecific interactions is important for an understanding of adhesion interactions in the immune response, inflammation, and other aspects of leukocyte biology.

As a model for leukocyte adhesion we recently studied lymphocyte self-aggregation (14, 15). Lymphocytes stimulated with specific antigen or with phorbol esters become adherent and form large cell clusters (16, 17). This aggregation is completely inhibited by anti-LFA-1 monoclonal antibody (MAb) (14, 15, 18). In further support of the importance of LFA-1 in this adhesion reaction, lymphocytes from LFA-1-deficient patients fail to self-aggregate. LFA-1-deficient lymphocytes, however, can form mixed aggregates with normal (LFA-1⁺) lymphocytes, suggesting the involvement of additional surface molecules (15).

To identify additional intercellular adhesion molecules (ICAM), MAb were prepared against LFA-1-deficient lymphocytes and were screened for their ability to inhibit

Received for publication January 30, 1986.
Accepted for publication April 8, 1986.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked
advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

* This work was supported by National Institutes of Health Grants AI15614, HL18646, CA31798 and Council for Tobacco Research grant I307. Michael L. Dustin is the recipient of a National Science Foundation predoctoral fellowship.


‡ List of abbreviations: ICAM-1, intercellular adhesion molecule-1; HM, human monocyte; HR, human recombinant MAb, monoclonal antibody; TFMS, trifluoromethane sulfonic acid.

245
aggregation of LFA-1 * lymphocytes. A MAb, RR 1/1, was obtained to an antigen distinct from LFA-1 that inhibited the phorbol ester-stimulated self-aggregation of a B lymphoblastoid and a myelomonocytic cell line. This antigen has been designated ICAM-1. Here we report that ICAM-1 is present on non-hematopoietic and hematopoietic cells. ICAM-1 surface expression on dermal fibroblasts is rapidly up-regulated by interleukin 1 (IL 1) and interferon gamma (> in a process that is dependent on de novo mRNA and protein synthesis. ICAM-1 regulates the natural adhesion of lymphocytes to dermal fibroblasts. Furthermore, the M, of mature ICAM-1 glycoprotein, its intracellular precursor, and the polypeptide backbone have been characterized in hematopoietic and non-hematopoietic cells.

MATERIALS AND METHODS

Reagents. Recombinant mouse IL 1 (6 x 10^5 U/mg) was a gift of Dr. P. Lomedico, Hoffman LaRoche Inc., Nutley, NJ. Recombinant human IFN-γ (10^8 U/mg) and IFN-α (10^8 U/mg) was a gift of Dr. D. Novick, Virology Department, Weizmann Institute, Rehovot, Israel. Turnip mosaic virus (C.D. 3.2.6.4.1.1.1) was a gift of Dr. R. Hamill, Eli Lilly, Indianapolis, IN. Affinity-purified goat anti-mouse IgG and fluorescein isothiocyanate (FITC)-goat anti-mouse IgG was purchased from Zymed, South San Francisco, CA. Actinomycin D was purchased from Calbiochem, San Diego, CA. Tissue culture reagents were purchased from Gibco, Grand Island, NY. Radiochemicals were obtained from New England Nuclear, Boston, MA. Protein A Sepharose and Sepharose CL-4B were purchased from Pharmacia Fine Chemicals, Piscataway, NJ. All other reagents were of the highest grade available and were obtained from Fisher, Fair Lawn, NJ, or Sigma Chemical Co., St. Louis, MO.

Splenocytes (MAH) Mouse thymic lymphomas were grown in RPMI 1640 or DMEM plus 20 mM l-glutamine, 50 μg/ml glutamine, and 5% or 10% fetal bovine serum (FBS). The supernatants from post-log cultures were collected and were complement inactivated by heating to 56°C for 30 min. Preparation of the hybridomas used was described. RR1/1/1.1, TS 1/22.1.1 (19), TS 2/9/1.1 (19), TS 2/18.1 (19), and W6/32 (20). Control supernatants were from P3X63Ag8.6.5 producing myeloma cell supernatants.

Purification of human monocyte (HM) IL 1. Human platelet-lymphoblastoid cell products were used as a source of mononuclear cells. The adherent cell population was stimulated with opsonized heat-killed Staphylococcus aureus in the presence of methionine-free medium containing 50 μg/ml of 125I-methionine and was incubated for 36 hr. The supernatant was purified by sequential immunoadsorption, gel-filtration, and chromatofocusing. Details of the purification of the antibody used to make the immune complexes have been published (21, 22). IL 1 was isolated by the following procedures: (a) the antibody was isolated by protein A-Sepharose chromatography (23). The specific activity of HM IL 1 was approximately 1 U per mg as estimated from the gel. One IL 1 unit is defined as doubling the mitogenic response.

Human recombinant (HR) IL 1. The IL 1 cDNA was expressed in E. coli by isolating the 1112 bp Ncol-XmnII fragment (bp 295-1407) from the IL 1 cDNA plasmid pc 12.1-16 (24). The HR-IL 1 included 46 amino acids of the IL 1 precursor peptide that are present before the alanine at position 117. This alanine is the N-terminus of the processed IL 1 found in the supernatants of stimulated human blood monocytes (2). The cDNA was transfected into 100 μl of COS-1 insolution/106 cells/well. HR-IL 1 was stored at −70°C in a 0.15 M phosphate-buffered saline, pH 6.8. The identity of the purified HR-IL 1 was confirmed by amino acid composition and the sequence of the amino terminus. Purity (assessed by SDS-PAGE) was greater than 95%. The endotoxin concentration of the homogenous HR-IL 1 was approximately 20 ng/ml of IL 1 protein.

Preparation of IL 1-containing U937 supernatant. U937 cells were incubated in Teflon beakers at 4 x 10^5 cells/ml with 2 ng/ml PMA. The suspension was dialyzed against 200 mM Hanks' balanced salt solution (HBSS) and 1% bovine serum albumin for 3 days with three dialysis changes to remove free phorbol myristate acetate (PMA). This source of IL 1 activity was used for biochemical characterization and other procedures requiring large volumes of cells. It has been demonstrated that IL 1 from U937 has identical effects to HM IL 1 in an erythroleukemia cell line (25). Therefore, crude U937 supernatant probably contains other materials, such as tumor necrosis factor, which may also have IL 1 like activities. An anti-IL 1 antiserum inhibited 95% IL 1 activity in crude U937 supernatant.

Cells and cell culture. For general studies, cells were maintained in RPMI 1640, 20 mM l-glutamine, 50 μg/ml penicillin, 50 μg/ml streptomycin, and 10% FBS at 37°C in 5% CO2, 95% air humidified 100% humidified atmosphere. Human dermal fibroblasts derived from foreskin were obtained from Dr. J. Rheinwald, Dana-Farber Cancer Institute, Boston, MA. The premyelocytic leukemia-derived cell line HL-60 and the erythroleukemia cell line K562 were obtained from Dr. J. Strominger, Dana-Farber Cancer Institute, Boston, MA. The SKW3 was obtained from Dr. P. Cresswell, Duke University, Durham, NC. An anti-JY CTL clone was obtained from Dr. S. Metzger, Dana-Farber Cancer Institute, Boston, MA. The SV40-transformed fibroblast cell line M1 (26) was provided by Dr. C. Terhorst, Dana-Farber Cancer Institute. Epstein-Barr virus (EBV)-transformed human B lymphocytes from whole blood were prepared as described (27). Phorbolmyristate acetate (PMA) blastins were prepared from isolated peripheral blood mononuclear cells (28). Briefly, mononuclear cells isolated from whole blood by dextran sedimentation and Ficoll-Hypaque (d = 1.08) centrifugation were incubated for 4 days in complete medium (CM) plus 5% FBS and 1,000 PHA (GIBCO). The cells were washed and were resuspended in CM plus IL 2 (28) and were maintained between 0.5 and 5 x 10^6 cells/ml. The blastins were washed once.

Immunohistochemical staining. Frozen tissue sections (4 μm thick) of normal human tissues (thymus, lymph nodes, tonsil, kidney, liver, small and large intestine, and skin) were fixed in acetone. Tissue sections with the MAB culture supernatant and the MAB culture supernatant with the MAB culture supernatant were incubated with avidin-biotin complex method (Vector Laboratories, Burlingame, CA) as described (29). After incubation with the RR 1/1 antibody, the sections were sequentially incubated with biotinylated horse anti-mouse IgG and avidin-biotinylated peroxidase complexes. The sections were finally dipped in a solution containing 3-amin-9-ethyl-carbazole (Aldrich Chemical Co., Inc., Milwaukee, WI) and hydrogen peroxide solution (1:1). The sections were counterstained with formaldehyde for 5 min and were counterstained with hematoxylin. Controls included sections incubated with unrelated MAB instead of RR 1/1 antibody.

Immunofluorescence flow cytometry. Nonadherent cells were isolated by centrifugation, were washed twice at 4°C with HBSS plus 10 mM HEPES, no Ca++ or Mg++, 2 g/l EDTA, 0.05% sodium azide, and 1% heat-inactivated FBS (EDTA buffer), and were resuspended in the same to 10^6 cells/ml. Fibroblast cell layers were washed once with HBSS, no Ca++ or Mg++, 10 mM HEPES, and 2 g/l EDTA and were incubated for 15 min at 37°C in the same cell layers. Cells were suspended with 7.5% FBS and clumps were dispersed by using a rotating Teflon pestle homogenizer at 50 rpm for 30 sec at 4°C. The suspension was centrifuged with Ficoll-Hypaque (d = 1.08) and was centrifuged at 1000 x g for 25 min. The cells at the interface were collected and resuspended in the EDTA buffer. Fifteen to 20% of the initial cells were recovered as a single cell dispersion with >95% viability. Trypsin was avoided because the binding of RR 1/1 (anti-ICAM-1) is reduced by trypsin. Once collected and washed, the cells were aliquoted into wells of 96-well V-bottomed microtiter plates at 10^4 cells/well. For indirect immunofluorescence staining, the cells were pelleted by centrifugation at 200 x g for 2 min at 4°C, resuspended in 30 μl of EDTA buffer and 50 μl of the appropriate hybridoma supernatant including one well with P3X63Ag8 (X63) supernatant as a negative control. Cells were incubated for 30 min at 4°C with vigorous agitation. The cells were pelleted, were washed twice, and were resuspended in 80 μl of EDTA buffer containing 5 μg of FITC-goat anti-mouse IgG that had been centrifuged at 12,000 x g for 10 min to remove aggregated IgG. Cells were incubated for 30 min at 4°C, and then were washed twice with PBS. The samples were either analyzed immediately or were fixed with 1% paraformaldehyde and were analyzed within 5 days. Samples were analyzed on a Coulter Epics 8 fluorescence flow cytometer, Coulter Epics Div., Hialeah, FL.

Binding assay. Human dermal fibroblasts were grown in 96-well microtiter plates (Costar) to a density of 2 x 10^4 cells/well (0.32 cm²). The cells were washed twice with CM and once with HBSS, 10 mM HEPES, 0.05% NaCl, and then resuspended in HBSS (10% FBS) buffer at 4°C. To each well was added 50 μl binding buffer and 50 μl of the appropriate hybridoma supernatant with X63 and W6/32 as the negative and positive controls, respectively. After incubation for 30 min at 4°C with gentle agitation, the wells were washed twice with binding buffer, and the second antibody, 125I-goat anti-mouse IgG, was added at 50 nCi in 100 μl. The 125I-goat anti-mouse IgG was prepared by using iodogen (Pierce) (30). After 30 min at 4°C, the
wells were washed twice with 200 μl of binding buffer, and the cell layer was solubilized by addition of 100 μl of 0.1 N NaOH. This 0.1 N NaOH was counted in a Beckman Gamma 5500 gamma counter. Beckman Instruments Inc., Irvine, CA. All points were determined in quadruplicate. Specific cpm bound was calculated as (cpm bound in binding assay - cpm bound in non-binding assay) with specific reagents such as IgG. 1 were carried out in quadruplicate so that the SD reported error from both induction and assay procedures.

Preparation of RR 1/1 (anti-ICAM-1) Sepharose (32). RR 1/1 IgG was purified by protein A Sepharose chromatography by loading at pH 8.6 and eluting with citrate buffer at pH 5.5. The IgG was immediately neutralized and then dialyzed against 3 changes of 50 mM NaCl and was concentrated by ultrafiltration to 2.4 mg/ml. The coupling to Sepharose CL-4B was carried out in 0.1 M NaHCO3 after activating the CL-4B with 13 mg/ml CNBr for 10 min at 4°C. The coupling was allowed to proceed for 20 hr at 4°C, and remaining reactive sites were quenched by incubation with 50 mM glycine. 0.1 M NaHCO3, pH 8.5 for 4 hr at 4°C. The IgG was coupled at 1.3 mg/ml packed volume. Activated quenched CL-4B was used for preincubation and in controls and was prepared by omitting the incubation with MAb.

Immunoprecipitation. Cells were washed twice with borate-buffered saline (BBS) pH 8.0 at 4°C. The cells were lysed in BBS, 0.5% Triton X-100, 5 mM phenylmethylsulfonyl fluoride, 5 mM iodoacetamide, and 0.2 mg/ml protein inhibitor U/A protinin at 4°C for 30 min at the indicated cell density. Insoluble material was sedimented by centrifugation at 12,000 x g for 10 min at 4°C. The supernatants, which are not used here, were precipitated by incubation with 50 μl activated-quenched Sepharose (Sepharose) for 1 hr at 4°C. The preincubated lysate was incubated for 16 hr with 25 μl/m of a 50% suspension of RR 1/1-Sepharose. After this incubation, the beads were washed once with lysis buffer, once with BBS and 0.1% Triton X-100, once with BBS, and once with 50 mM Tris HCl, pH 6.8 (at 24°C) all at 4°C.

Metabolic labeling. Dermal fibroblasts grown to near confluency in 25 cm² flasks (Falcon) were incubated for 2 hr at 37°C with 1/10 dilution of IL 1-containing U937 supernatant. The cell layers were washed with methionine-free serum supplemented with 150 mM NaCl and 1% FBS for 3 h at 37°C and then incubated for 1 hr at 37°C in 2 ml of the same with 10% IL 1-containing U937 supernatant. At this time 20 μCi of [35S]methionine was added (20 μl) to each flask. The samples were incubated for 10 min at 37°C (pulsed). Methionine was then added to 0.5 mM, and the cells were incubated for the indicated time (chase). The chase was terminated by washing the cell layer twice with ice cold BBS and 5 mM methionine, and then lysing the cells with 1 ml lysis buffer and 1% bovine hemoglobin for 30 min at 4°C. The lysates were centrifuged 12,000 x g for 10 min at 4°C. Immunoprecipitations were done from lysates of 105 cells containing from 105 to 106 cpm. Precipitation was done as above and immunoprecipitation was performed for 2.5 hr at 4°C by using 10 μl of 1:1 RR 1/1 Sepharose-CL-4B or control Sepharose CL-4B in a final vol of 200 μl. Washing was as described above.

Sodium sulfonic acid (NaSM) treatment (32). [35S]-ICAM-1 was eluted from MAb-Sepharose by heating to 56°C in the presence of 1% SDS. A carrier protein (20 μg of myoglobin) was added, and the protein was precipitated by 10% trichloroacetic acid (TCA). The precipitate was washed three times with cold acetone, was resuspended in water, and was lyophilized. The lyophilized protein was resuspended in 200 μl of 2:1 solution of TFFS/amido, the tube was flushed with nitrogen, and it was sealed. The sample was then incubated 2 hr at 0°C and 2 hr at -20°C. The reaction was stopped with 10 mM triethanolamine. 0.2% Nonidet P-40 (500 μl) followed by addition of 100 μl 1 M triethanolamine. The sample was brought to 10% TCA, and the precipitate was washed twice with cold acetone and was resuspended in sample buffer for electrophoresis (see below).

RESULTS

Tissue distribution of ICAM-1. Immunohistochemical studies were performed on frozen tissue sections of normal human organs to determine distribution of ICAM-1 in thymus, lymph nodes, intestine, skin, kidney, and liver. ICAM-1 was found to have a distribution most similar to that of major histocompatibility complex (MHC) class II antigens [Table I]. Most of the blood vessels (both small and large) in all tissues showed staining of endothelial cells with ICAM-1 antibody. The vascular endothelial staining was more intense in the interfollicular (paracortical) areas in lymph node, tonsils [Fig. 1], and Peyers patches as compared with vessels in kidney, liver, and normal skin. In the liver, the staining was mostly restricted to sinusoidal lining cells; the hepatocytes and the endothelial cells lining most of the portal veins and arteries were not stained.

In the thymic medulla, diffuse staining of large cells and a dendritic staining pattern was observed. In the cortex, the staining pattern was focal and predominantly dendritic. Thymocytes were not stained. The staining pattern most likely represented reactivity with thymic epithelial cells. In the peripheral lymphoid tissue, the germinal center cells of the secondary lymphoid follicles were intensely stained [Fig. 1]. The staining pattern most likely reflected reactivity with dendritic reticulum cells.

### Table I

<table>
<thead>
<tr>
<th>Distribution of ICAM-1 in normal human tissues*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vascular endothelium</strong></td>
</tr>
<tr>
<td>Germinal center cells (dendritic reticulum cells, B cells)</td>
</tr>
<tr>
<td>Macrophages and T cells and macrophages in lymphoid tissue (tonsil, lymph node, Peyers patches)</td>
</tr>
<tr>
<td>Epithelial cells (thyroid epithelial cells, mucosal epithelium in tonsil and sometimes tubular epithelial cells in kidney)</td>
</tr>
</tbody>
</table>

*Organs studied: skin, kidney, liver, thymus, tonsil, lymph node, and intestine.
INTERCELLULAR ADHESION MOLECULE-1 CHARACTERIZATION

Figure 1. Immunoperoxidase staining of a tonsil with ICAM-1 MAb. Intense staining of germinal center (gc) area of the secondary follicle is present; the staining most likely represents reactivity with dendritic reticulum cells, as well as B cells. Faint staining of many cells in the mantle zone (mz) of the follicle is also seen. There is intense staining of vascular endothelial cells (arrowheads), as well as interdigitating reticulum cells in the interfollicular (T cell) area (arrows). (original x125).

as well as B cells. In some lymphoid follicles the staining pattern was mostly dendritic with no recognizable staining of lymphocytes. Faint staining of cells in the mantle zone was also observed. In addition, dendritic cells with cytoplasmic extensions (interdigitating reticulum cells) and a small number of lymphocytes in the interfollicular or paracortical areas stained with ICAM-1 antibody.

Cells resembling macrophages were stained in the lymph nodes and lamina propria of small intestine. Fibroblast-like cells (spindle shaped cells) and dendritic cells scattered in the stroma of most of the organs studied stained with ICAM-1 antibody. However, there was no recognizable staining of Langerhans/indeterminate cells in the epidermis. Smooth muscle did not stain.

The staining of epithelial cells was consistently seen in the mucosa of the tonsils. Although hepatocytes, bile duct epithelium, intestinal epithelial cells, and tubular epithelial cells in kidney did not stain in most instances, sections of normal kidney tissue obtained from a nephrectomy specimen with renal cell carcinoma showed staining of many proximal tubular cells for ICAM-1. Interestingly, the tubular epithelial cells in this case also stained with an anti-HLA-DR antibody.

Immunofluorescence flow cytometry. Flow cytometry analysis of tumor cell lines and peripheral blood leukocytes supported the results obtained in frozen tissue sections (Table II). Peripheral blood leukocytes had low but significant expression of ICAM-1. ICAM-1 expression on cell lines followed the trend: EBV-transformed B lymphoblastoid lines > erythroid/myeloid cell line K562 > anti-JY CTL line > PHA blasts. The T cell lymphoma cell line SKW3 expressed ICAM-1 at approximately the same level as peripheral blood mononuclear cells. The myelomonocytic cell lines U937 and HL-60 can be induced to express monocyte/macrophage-like characteristics by incubation with PMA over a period of 3 days. This induction of more differentiated properties that may be analogous to the normal differentiation of mononoblasts to monocyte/macrophages (38) was accompanied by a dramatic increase in ICAM-1 expression. HL-60 expression of ICAM-1 went from negative to strongly positive, and U937 expression was increased 15-fold.

A human dermal fibroblast explant from a normal donor and a SV-40-transformed fibroblast cell line M1 were also analyzed by flow cytometry (Table II). Expression on the dermal fibroblast varied from 28 to 49 fluorescence units depending on cell density, with cells in log phase growth expressing less ICAM-1 than quiescent cells (see below Fig. 2, profile A3 and B3). In at least five experiments, there was a positive correlation between cell density and ICAM-1 expression (not shown). ICAM-1

<table>
<thead>
<tr>
<th>Cell Line/Type</th>
<th>Specific Fluorescence Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aICAM-1 (RR1/1)</td>
</tr>
<tr>
<td>JY B lymphoblastoid</td>
<td>28</td>
</tr>
<tr>
<td>SLA LFA-1- B lymphoblastoid</td>
<td>50</td>
</tr>
<tr>
<td>HFO LFA-1- B lymphoblastoid</td>
<td>11</td>
</tr>
<tr>
<td>SKW3 T-lymphoma</td>
<td>1</td>
</tr>
<tr>
<td>PHA blasts</td>
<td>3.2</td>
</tr>
<tr>
<td>a JY CTL line</td>
<td>9.1</td>
</tr>
<tr>
<td>K562 erythroid/myeloid</td>
<td>9.7</td>
</tr>
<tr>
<td>K562 + IFN-γ</td>
<td>32</td>
</tr>
<tr>
<td>U937 monoblastoid</td>
<td>7.4</td>
</tr>
<tr>
<td>U937 + PMA</td>
<td>114</td>
</tr>
<tr>
<td>HL60 myeloblastoid</td>
<td>0</td>
</tr>
<tr>
<td>HL60 + PMA</td>
<td>49</td>
</tr>
<tr>
<td>Dermal fibroblasts (5-19th passage)</td>
<td>29</td>
</tr>
<tr>
<td>M1 fibroblast</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* K562 cells were treated with 10 U/ml recombinant IFN-γ for 24 hr. U937 and HL-60 cells were treated with 2 and 10 ng/ml PMA, respectively, for 3 days. Monodisperse suspensions were obtained by trituration. Peripheral blood leukocytes were separated into granulocyte and mononuclear cell fractions by Ficoll-Hypaque centrifugation. Monocytes and lymphocytes were resolved during the analysis by forward angle and 90° light scattering. Selection of the correct populations was confirmed by using monocyte markers. Fluorescent beads were used to calibrate the cytometer such that one unit was equal to 1/20th the fluorescence of the 1/32 (2%) bright fluorosphere (Coulter).
expression on the cell-line M1 was very low but was reproducibly detectable by both immunofluorescence and binding of 125I-labeled ICAM-1 MAb (not shown).

Modulation of ICAM-1 expression by IL 1 and IFN-γ. The effects of inflammatory and immune cytokines on ICAM-1 expression were investigated, because several surface molecules have been shown to be modulated by these factors (39–41) and staining of frozen tissue sections showed increased ICAM-1 expression in reactive lymphoid tissue and in delayed type hypersensitivity inflammatory sites [data not shown]. Human dermal fibroblasts were used as targets, because these cells were readily available, had been used in these kinds of studies previously (40), and may play a significant role in inflammatory and immune responses (40, 42). Incubation of human dermal fibroblasts with IL 1-containing U937 supernatant for 5 hr [Fig. 2A] increased ICAM-1 expression 4.3 ± 1.1-fold (four experiments with cells both in log and stationary growth). Incubation with 100 U/ml recombinant human IFN-γ for 18 hr [Fig. 2B] resulted in a fivefold increase in ICAM-1 expression. Incubation with IL 1-containing U937 supernatant resulted in a small increase in HLA-A,B,C expression, whereas IFN-γ increased HLA-A,B,C expression by 2.5-fold and resulted in detectable expression of HLA-DR (not shown). Forward angle light scattering was not affected by incubation with IL 1 or IFN-γ, suggesting that no significant change in cell size or cytoplasmic contents occurred. Although basal ICAM-1 expression was density dependent, cell density did not affect the level to which ICAM-1 could be induced by a given activity of IL 1 or IFN-γ. IFN- β (10 to 1000 U/ml) and prostaglandin E2 (PGE2) (0.28 to 28 μM) had no effect on fibroblast ICAM-1 expression at 24 hr (not shown). The concentration of IFN-γ that was used were sufficient to increase HLA-A,B,C expression at 24 hr and had no effect on HLA-DR expression, which remained negative (not shown). IFN-γ induced a threefold increase in ICAM-1 on the K562 erythroleukemia cell line that accompanied HLA class I antigen induction (Table II).

Time course of HML 1 and IFN-γ effects. The kinetics of HML 1 and IFN-γ effects on ICAM-1 expression on dermal fibroblasts were determined using a 125I-goat anti-mouse IgG binding assay. The effect of IL 1 with a t½ for ICAM-1 induction of 2 hr was more rapid than that of IFN-γ with a t½ of 3.75 hr (Fig. 3). No significant change in HLA-A,B,C expression was seen with HML 1, whereas

![Figure 3. Kinetics of IL 1 and IFN-γ effects on ICAM-1 expression on human dermal fibroblasts. Human dermal fibroblasts were grown to a density of 10^4 cells/cm² in 24-well plates. SMEC (3.3×10^4 cells/ml) or IFN-γ (10 U/ml) was added, and at the appropriate time, the plate was cooled to 4°C, and an indirect 125I-goat anti-mouse IgG binding assay was performed with X63 and anti-ICAM-1 as primary MAb for each time point. SD did not exceed 10% and are not shown.](https://example.com/figure3)

![Figure 4. Concentration dependence of IL 1 and IFN-γ effects on ICAM-1. Human dermal fibroblasts were grown to 8×10^4 cells/cm² in 24-well plates. SMEC (3.3×10^4 cells/ml) or recombinant human IL 1 (3), recombinant mouse IL 1 (3), recombinant human IFN-γ (3), or recombinant human IFN-β (3) were added at the indicated dilution and were incubated for 4 hr (IL 1) or 16 hr (IFN-γ and -β). The results are from 125I-goat anti-mouse IgG binding assay and represent means of quadruplicate determinations with anti-ICAM-1 as primary MAb. SD did not exceed 10% and are not shown.](https://example.com/figure4)
INTERCELLULAR ADHESION MOLECULE-1 CHARACTERIZATION

IFN-\(\beta\) has no effect at concentrations up to 10 ng/ml.

Requirement for protein and mRNA synthesis. Cycloheximide, an inhibitor of protein synthesis, and actinomycin D, an inhibitor of mRNA synthesis, abolished the effects of both IL 1 and IFN-\(\gamma\) on ICAM-1 expression on fibroblasts (Table III). Furthermore, tunicamycin, an inhibitor of N-linked glycosylation, only inhibited the IL 1 effect by 43\%. These results suggest that protein and mRNA synthesis, but not N-linked glycosylation, are required for IL 1 and IFN-\(\gamma\)-stimulated increases in ICAM-1 expression.

Molecular characterization of ICAM-1. ICAM-1 was isolated from different cell types by using MAb coupled to Sepharose, subjected to SDS-PAGE, and visualized by silver-staining (Fig. 5). ICAM-1 from fibroblasts is

<table>
<thead>
<tr>
<th>Treatment</th>
<th>125I Goat Anti-Mouse IgG-Specifically Bound (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (4 hr)</td>
<td>1524 ± 140 11928 ± 600</td>
</tr>
<tr>
<td>+ cycloheximide</td>
<td>1513 ± 210 10676 ± 471</td>
</tr>
<tr>
<td>+ actinomycin D</td>
<td>1590 ± 46 12276 ± 608</td>
</tr>
<tr>
<td>+ tunicamycin</td>
<td>1461 ± 176 12340 ± 940</td>
</tr>
<tr>
<td>IL 1 (10 U/ml) (4 hr)</td>
<td>4264 ± 249 12155 ± 510</td>
</tr>
<tr>
<td>+ cycloheximide</td>
<td>1610 ± 381 12676 ± 446</td>
</tr>
<tr>
<td>+ actinomycin D</td>
<td>1613 ± 88 12294 ± 123</td>
</tr>
<tr>
<td>+ tunicamycin</td>
<td>3094 ± 133 13434 ± 691</td>
</tr>
<tr>
<td>IFN-(\gamma) (10 U/ml) (18 hr)</td>
<td>4659 ± 109 23675 ± 500</td>
</tr>
<tr>
<td>+ cycloheximide</td>
<td>1461 ± 59 10675 ± 800</td>
</tr>
<tr>
<td>+ actinomycin D</td>
<td>1326 ± 186 12089 ± 550</td>
</tr>
</tbody>
</table>

*Human fibroblasts were grown to a density of 8 x 10^6 cells/0.32 cm² well. Treatments were carried out in a final vol of 50 μl containing the indicated reagents. Cycloheximide, actinomycin D, and tunicamycin were added at 20 μg/ml, 10 μM, and 2 μg/ml, respectively, at the same time as the cytokines. All points are means of quadruplicate wells ± SD.

Figure 5. Isolation of ICAM-1 by immunoprecipitation from fibroblasts, U937 cells, and JY cells. Lane 1, untreated fibroblasts immunoprecipitated with control Sepharose CL-4B; Lane 2, untreated fibroblasts with anti-ICAM-1 Sepharose; Lane 3, IL 1-treated fibroblasts with control Sepharose; Lane 4, IL 1-treated fibroblasts with anti-ICAM-1 Sepharose; Lane 5, untreated U937 with control Sepharose; Lane 6, untreated U937 with anti-ICAM-1 Sepharose; Lane 7, PMA-treated (3 days) U937 with anti-ICAM-1 Sepharose; Lane 8, PMA-treated (3 days) U937 with control Sepharose; Lane 9, JY with anti-ICAM-1 Sepharose; Lane 10, JY with control Sepharose. Immunoprecipitates from 5 x 10^6 fibroblasts, from 10^7 U937 cells, and from 5 x 10^7 JY cells were subjected to nonreducing SDS 9% PAGE and silver staining. The faint, sharp band at ~160,000 M, in lanes 2, 4, 6, 7, and 9 is ICAM-1 MAb that dissociated from the Sepharose. The high m.w. material at the top of Lane 7 is probably aggregated monomer, because this material is not seen on reducing gels (not shown).

Figure 6. Pulse-chase metabolic labeling of dermal fibroblast ICAM-1 and TFMS treatment of ICAM-1 from dermal fibroblasts and U937. Fibroblasts were grown to 2 x 10^6 cells/25 cm² flask. Fibroblasts were treated with U937 conditioned media for 3 hr before labeling and were in methionine-free media for 1 hr before labeling. Labeling conditions: lanes 1 and 2, 10 min pulse; lanes 3 and 4, 10 min pulse; lanes 5 and 6, 10 min chase; lanes 7 and 8, 10 min pulse; lanes 9 and 10, 60 min pulse with 2 μg/ml tunicamycin present and added 1 hr before labeling. Lysates were immunoprecipitated with ICAM-1 MAb-Sepharose in cold lanes and control Sepharose in even lanes. For TFMS treatment, dermal fibroblasts (lane 11) or PMA-stimulated U937 cells (lane 12) were labeled by pulsing 1 hr and chasing 1 hr. ICAM-1 was immunoprecipitated with ICAM-1 MAb-Sepharose, was eluted, and was treated with TFMS. Precipitates were subjected to reducing SDS 9% PAGE and fluorography.
an Mₐ = 55,000 (Fig. 6, lane 9). To evaluate the contribution of glycosylation to the heterogeneity of ICAM-1 from different cell types, we made use of trifluoromethane sulfonic acid, which removes both N- and O-linked oligosaccharides [32]. This treatment results in essentially identical banding patterns for fibroblast and PMA-stimulated U937 ICAM-1, with a major band at an Mₐ = 60,000 and a minor band at an Mₐ = 47,000 (Fig. 6, lanes 11 and 12). This result shows that dermal fibroblast and U937 ICAM-1 have polypeptides with identical Mₐ, suggesting the polypeptides are identical and carbohydrate processing leads to the different Mₐ of the mature proteins in these two cell types. The presence of two species after TFMS modification makes it unclear whether or not ICAM-1 contains O-linked oligosaccharides. Incomplete removal of O-linked oligosaccharides could account for the two bands. Alternatively, a site-specific cleavage of the polypeptide chain occurring to a similar extent in both samples could also yield two bands.

Natural attachment assay. The adhesion of in vitro activated T lymphocytes to dermal fibroblasts in a non-antigen-specific manner has been described and is referred to as natural attachment [43–45]. This system has been used here to study lymphocyte adhesion to cells on which ICAM-1 expression can be modulated and for which adhesion can be readily assayed by measuring attachment of radiolabeled lymphoid cells to fibroblast cell layers. To obtain activated T lymphocytes, peripheral blood lymphocytes were stimulated with PHA, were washed, and then were cultured in the absence of PHA for 3 to 6 days. Under these conditions, surface-bound PHA is internalized and would not contribute to the adhesion reaction. Figure 7 shows the adhesion of fluorescently labeled PHA blasts to sparsely plated fibroblasts. This demonstrates the nature and specificity of the interaction. Very few PHA blasts were bound to the plastic slide, whereas those bound to fibroblasts were often seen to spread out on the fibroblast apparently increasing the area of contact between cell surfaces.

MAb to ICAM-1 and other cell surface molecules were evaluated for their effect on the adhesion of ⁵¹Cr-labeled PHA-blasts to continuous layers of dermal fibroblasts (Table IV). Natural attachment of PHA blasts was inhibited 70% by anti-ICAM-1 MAb. The T lymphoma cell line SKW3 was also studied because homotypic adhesion by this cell line was inhibited by anti-LFA-1 but not anti-ICAM-1 MAb. It was therefore an important question to determine whether heterotypic adhesion between SKW3 and ICAM-1 fibroblasts would be inhibited by anti-ICAM-1 MAb. Natural attachment of SKW3 cells to fibroblasts was inhibited 78% by anti-ICAM-1 MAb. Attachment of PHA blasts and SKW3 cells was inhibited 66 to 76% by MAb to LFA-1 a and b subunits. In contrast, MAb to HLA-A, B, C, LFA-2, and LFA-3 had no effect. Anti-HLA-A, B, C binds to fibroblasts at greater density than any of the effective antibodies (Table II), which suggests that the inhibition seen with anti-ICAM-1 and anti-LFA-1 MAb is not due to nonspecific effects of surface-bound IgG. Purified anti-ICAM-1 IgG and Fab' half-maximally inhibited attachment of SKW3 at 0.2 μg/ml and 0.5 μg/ml, suggesting that anti-ICAM-1 is very efficient at inhibiting attachment and inhibition is not dependent on bivalency of the MAb (Fig. 8).

Preincubation of one cell population with MAb followed by natural attachment assay was used to identify the cell on which ICAM-1 and LFA-1 are required for adhesion in this heterotypic system (Table V), When fibroblasts were preincubated with anti-ICAM-1 MAb, adhesion was inhibited by 66 and 73%. Despite the presence of ICAM-1 on the lymphoid cells, preincubation of these cells with anti-ICAM-1 MAb had no effect on attachment. Preincubation of the lymphoid cells with anti-LFA-1 MAb inhibited attachment, but preincubation of fibroblasts with anti-LFA-1 MAb had no effect.

The effect of IL-1 pretreatment of dermal fibroblasts on natural attachment was determined to additionally evaluate the role of ICAM-1 in this adhesion process. There was a significant correlation between ICAM-1 surface expression and natural attachment when the dose responses for HM IL-1 were compared (Fig. 9). Furthermore, the amount of natural attachment that was inhabitable by anti-ICAM-1 showed a similar increase. Binding not inhabitable by anti-ICAM-1 changed little over the range of IL-1 concentrations used. A significant increase in SKW3 binding of twofold to threefold is also
TABLE IV

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Cells Bound x 10^-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA Blasts</td>
<td>SKW3</td>
</tr>
<tr>
<td><strong>XG3</strong></td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td><strong>aICAM-1</strong></td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td><strong>aLFA-1α</strong></td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td><strong>aLFA-1β</strong></td>
<td>0.65 ± 0.1</td>
</tr>
<tr>
<td><strong>aHLA-A.B.C</strong></td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td><strong>aLFA-2</strong></td>
<td>2.6 ± 0.1</td>
</tr>
</tbody>
</table>

*Human dermal fibroblasts were grown to 5 x 10^6 cells/2.01 cm^2 well. PHA blasts or SKW3 (2 x 10^6 cells) labeled with ^51Cr were added for 1 hr at 37°C. PHA blasts had an activity of 56,000 cpm/10^5 cells and SKW3 of 120,000 cpm/10^5 cells. Hybridoma supernatants were added at a dilution of 1/10 just before addition of the labeled cells. The final vol was 1 ml. The results are means of quadruplicate determinations ± SD.

**ND**: Not done.

![Graph](image)

Figure 8. Effect of anti-ICAM-1 IgG and Fab' on PHA blast binding to human dermal fibroblasts. Fibroblasts were grown to 2.5 x 10^6 cells/2.01 cm^2 well, PHA blasts (2 x 10^6) and the MAB (M) or MAB fragment (M) were added and were incubated 1 hr at 37°C. Final volume was 1 ml.

TABLE V

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Lymphoid cell</th>
<th>Fibroblast pretreatment with MAB</th>
<th>Cells Bound x 10^-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA Blasts</td>
<td>SKW3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>2.57 ± 0.00</td>
<td>2.04 ± 0.06</td>
</tr>
<tr>
<td>aICAM-1</td>
<td>None</td>
<td>0.88 ± 0.08</td>
<td>0.56 ± 0.03</td>
</tr>
<tr>
<td>None</td>
<td>aLFA-1α</td>
<td>2.52 ± 0.05</td>
<td>1.98 ± 0.08</td>
</tr>
<tr>
<td>None</td>
<td>aHLA-A.B.C</td>
<td>2.40 ± 0.21</td>
<td>1.85 ± 0.18</td>
</tr>
<tr>
<td>aICAM-1</td>
<td>None</td>
<td>2.84 ± 0.18</td>
<td>1.97 ± 0.06</td>
</tr>
<tr>
<td>None</td>
<td>aLFA-1β</td>
<td>0.73 ± 0.16</td>
<td>0.87 ± 0.07</td>
</tr>
<tr>
<td>None</td>
<td>aHLA-A.B.C</td>
<td>2.40 ± 0.31</td>
<td>2.10 ± 0.08</td>
</tr>
</tbody>
</table>

*Human dermal fibroblasts were grown to 2 x 10^6 cells/2.01 cm^2 well. Fibroblasts or T lymphoid cells were incubated with a 1/10 dilution of hybridoma supernatant for 30 min at 24°C and then were washed three times with CM. PHA blasts or SKW3 (2 x 10^6) were added to wells in a final vol of 1 ml of CM with no more addition of hybridoma supernatant. The results are the mean of quadruplicate determinations ± SD.

![Graph](image)

Figure 9. Correlation between IL-1 up-regulation of ICAM-1 and increase in PHA blast binding to dermal fibroblasts. Fibroblasts were grown to 5 x 10^6 cells/2.01 cm^2 well. IL-1 was added at the appropriate concentration 4 hr before addition of ^51Cr-PHA blasts. PHA blasts were added at 2 x 10^6 cells/well just after addition of a 1/10 dilution of the appropriate hybridoma supernatant to a final vol of 1 ml. The plates were incubated for 1 hr at 37°C, and unattached cells were washed out. Binding in the absence (O) and presence (O) of anti-ICAM-1 hybridoma supernatant is shown. The IL-1 dose-response curve for a parallel assay of ICAM-1 MAb binding by using ^125I-goat anti-mouse IgG is shown for comparison (——). Each point is a mean of four wells. The SD did not exceed 10% for cell binding or 20% for ^125I goat anti-mouse IgG binding and are not shown.

on the myelomonocytic cells lines U937 and HL-60 after treatment with PMA for 3 days. PMA treatment causes maturation of these cells to a monocyte/macrophage phenotype. These observations suggest that increased ICAM-1 expression is associated with activation of lymphocytes and with maturation of macrophage-like cells.

The overall distribution of ICAM-1 in thin tissue sections is similar to HLA-DR. ICAM-1 is present on non-lymphoid cells including vascular endothelial cells, thymic epithelial cells, mucosal epithelial cells, and dendritic cells in germinal centers and T cell areas in lymphoid tissue. These cells are recognized for their role in immune and inflammatory responses, particularly endothelial and dendritic cells, which may be antigen-presenting cells (3, 6, 7). Furthermore, mucosal epithelial cells and fibroblasts are important potential targets of invading viruses and may also be targets for lymphocyte functions involving adhesion.

These studies establish that IL-1 and IFN-γ increase ICAM-1 expression on fibroblasts and suggest that ICAM-1 may have a role in inflammatory and immune responses. IL-1 and IFN-γ have been reported to have other effects on dermal fibroblasts. For example, IL-1 stimulates proliferation (45), PGE₂ synthesis, and collagenase production (42, 47), whereas IFN-γ decreases collagen synthesis, causes a late inhibition of proliferation (48), increases PGE₂ elaboration (49), and decreases expression of class I and II MHC antigens (40). IFN-β, which does not increase ICAM-1 expression on dermal fibroblasts, also decreases collagen synthesis, causes growth inhibition (48), and increases expression of class I, but not class II MHC antigens (40). The regulation of ICAM-1 expression on dermal fibroblasts is novel in that it is the only antigen expression modulation system in which IL-1 and IFN-γ have the same effect. It is also notable that non-immune IFN-β, which shares several effects on dermal fibroblasts with IFN-γ, does not up-regulate the expression of ICAM-1.

IL-1 and IFN-γ also increase ICAM-1 expression on endothelial cells. A comparison of IL-1-induced surface expression of ICAM-1 and another antigen that is specific

for endothelial cells and is identified by the MAb H4/18 (41) has been made on cultured umbilical vein endothelial
cells. ICAM-1 expression was increased threefold to fourfold on endothelial cells by IL 1 and eightfold by
tumor necrosis factor. The expression of the antigen
recognized by H4/18 was induced by IL 1 on endothelial
cells but was not detected on resting endothelial cells.
The antigen recognized by H4/18 also shows different
kinetics of induction with a peak at 4.5 hr followed by
decay of expression down to almost background levels by
24 hr even in the presence of IL 1 or tumor necrosis
factor. In contrast, ICAM-1 expression continues to
increase for 24 hr and remains stable for 72 hr.

The ICAM-1 glycoprotein displays M, heterogeneity in
different cell types. On the basis of the results of chemical
deglycosylation with TFMS, it appears that this hetero-
genuity is based on differential glycosylation of a common
polypeptide, although it remains to be rigorously
demonstrated that the polypeptides from dermal fibroblasts and
UB37 are identical. Pulse-chase metabolic labeling of
fibroblast ICAM-1 shows that a precursor of M, = 73,000
is converted to a mature form of M, = 97,000 in 20 to 30
min. If maturation in the Golgi complex is followed by
transport to the cell surface within a few minutes, then
this data would be consistent with the rapid mRNA and
protein synthesis-dependent up-regulation of ICAM-1 by
IL 1, which is apparent within 1 hr. The MAB RR 1/1
obeys to bind to a protein epitope on ICAM-1, because
RR 1/1 can immunoprecipitate ICAM-1 from cells labeled
in the presence of tunicamycin. This is notable because
the dermal fibroblast form of ICAM-1 is 45% carbohy-
drate as estimated by the mobilities of the glycosylated
and nonglycosylated forms in SDS-PAGE. The product
synthesized in the presence of tunicamycin was M, =
55,000. Estimating approximately 2,000 to 3,000 M, U
per high mannose oligosaccharide on the intracellular
precursor of M, = 73,000, ICAM-1 would contain approx-
imately 6 to 9 such oligosaccharide units per molecule.

Previous studies demonstrated the importance of
ICAM-1 and LFA-1 in PMA-stimulated self-adhesion of
several leukocyte cell types (15). PHA blasts and the T
lymphoma cell line SKW3 were used here to study the
binding of lymphocytes to non-hematopoietic ICAM-1
positive cells, which lack leukocyte markers such as LFA-
1. Natural attachment assay in which activated but not restoring T lymphocytes have been shown to
adhere to fibroblasts in a species-specific manner (45).

We found that both T lymphocyte blasts and SKW3 T
lymphoma cells bind to dermal fibroblasts, and this bind-
ing is inhibitable by either anti-LFA-1 or anti-ICAM-1
MAb. Furthermore, it has been established here that
ICAM-1 is required on the dermal fibroblast, whereas
LFA-1 is required on the lymphocyte. On incubating der-
mal fibroblasts with IL 1 natural attachment was signifi-
cantly increased in an IL 1 concentration-dependent
manner that was correlated with ICAM-1 surface expres-
sion.

The importance of ICAM-1 and LFA-1 and the regula-
tion by IL 1 suggest natural attachment is a functionally
significant assay. Natural attachment may represent part
of the non-antigen-specific component of leukocyte adhe-
sion in functions such as antigen presentation (50), and
cytokine T lymphocyte-mediated killing (13). ICAM-1
modulation by IL 1 and IFN-γ at sites of monocyte or T
lymphocyte activation might increase the tendency of T
lymphocytes to adhere to connective tissue cells such as
fibroblasts on which ICAM-1 expression is stimulated
and increase the probability ofafferent or efferent T
lymphocyte functions.

A possible role for ICAM-1 in lymphocyte diapedesis is
suggested by immunoperoxidase staining of sections of
tonsils and lymph nodes reacting to inflammation that
show very strong ICAM-1 expression on vascular endo-
thelial cells in T lymphocyte areas. Furthermore, areas of
delayed hypersensitivity reaction in skin show more
intense staining of ICAM-1 on vascular endothelial cells
as compared with vessels in normal skin (unpublished
observation). ICAM-1 induction on endothelial cells by
inflammatory mediators may facilitate margination and
evrasvatation of T and possibly B lymphocytes at sites of
inflammation or a localized immune response. It could be
speculated that because ICAM-1 upregulation on endo-
thelial cells by IL 1 is rapid, increased adhesion of
lymphocytes mediated by ICAM-1 might be an event mediat-
ing lymphocyte influx into inflammatory loci. Subse-
quent production of IFN-γ by activated lymphocytes at
the inflammatory site might mediate additional amplifi-
cation of the local inflammatory immune response.

ICAM-1 has some properties in common with the pa-
pain and trypsin sensitive molecule proposed to be LFA-
1 ligand (11, 12). ICAM-1 on JY cells is trypsin sensitive
under the same conditions as those used to define the
hypothetical ligand (unpublished observations). Furth-
more, ICAM-1 appears to be required in two LFA-1-de-
pendent adhesion systems, leukocyte self-aggregation
and natural attachment. These results suggest that
ICAM-1 could be a cell surface molecule that interacts
with LFA-1 on other cells.

The work reported here suggests that ICAM-1 is impor-
tant in leukocyte adhesion and is regulated in a manner
consistent with its being an important molecule in in-
fammatory and immune responses. Additional work is
required to define the receptor for ICAM-1 and the exact
molecular interactions involved in ICAM-1-dependent
cell-cell adhesion.

Acknowledgments. We are grateful to Linda Miller for
her valuable contribution of U937 and HL-60 differentiation
data and to Dr. Jordan Pober for sharing the result
that IL 1 increases ICAM-1 expression on dermal fibro-
blasts and endothelial cells. We also thank Dr. Steve
Marlin, Dr. Mariam Plunkett, Kei Kishimoto, and Linda
Miller for their advice and discussion of results, and
Janet Casaubon for secretarial assistance.

REFERENCES
1. Martz, E. 1977. Mechanism of specific tumor cell lysis by alloimmune
T-lymphocytes: resolution and characterization of discrete steps in
the cellular interaction. Cont. Top. Immunobiol. 7:301.
interaction. II. antigen-mediated physical interactions between immu-
ne guinea pig lymph node lymphocytes and syngeneic macro-
lymphocytes exhibit distinct stimulatory [antigen-presenting cell]
requirements for growth and lymphokine release. J. Exp. Med.
160:1717.
5. Chesnut, R. W., S. M. Colon, and H. M. Grey. 1982. Antigen pres-
entation by normal B cells. B cell tumours, and macrophages: func-
entation properties of human vascular endothelial cells. J. Exp. Med.
152:2495.
7. Hirschberg, H. 1981. Presentation of viral antigens by human vac-
Schlissman, and E. L. Reinherz. 1983. Clonotypic structures in-
volved in antigen-specific human T cell function. Relationship to the T3
blocking cytotoxic T-cell function. I. Relationship between the