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

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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 Peyer's 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 M_r heterogeneity in different cell types with a M_r 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 M_r ~73,000 intracellular precursor. The non-N-glycosylated form resulting from tunicamycin treatment has a M_r of 55,000. ICAM-1 isolated from phorbol myristic acetate (PMA) stimulated U937 and from fibroblasts yields an identical major product of M_r = 60,000 after chemical deglycosylation. ICAM-1 MAb interferes with the adhesion of phytohemaglutinin 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 requires 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 adherence 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[2] (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)[3] (14, 15, 18). In further support of the importance of LFA-1 in this adherence 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

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[2] **Rothlein, R., M. L. Dustin, S. D. Marlin, and T. A. Springer.** 1986. An intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *Submitted for publication.*

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

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.[2] 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-γ (IFN-γ) in a process that is dependent on de novo mRNA and protein synthesis, and ICAM-1 regulates the natural adherence of lymphocytes to dermal fibroblasts. Furthermore, the M_r 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 (6×10^6 U/mg) was a gift of Dr. P. Lomedico, Hoffman LaRoche Inc., Nutley, NJ. Recombinant human IFN-β (10^9 U/mg) and IFN-γ (10^8 U/mg) was a gift of Dr. D. Novick, Virology Department, Weizmann Institute, Rehovot, Israel. Tunicamycin (A:B: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.

Monoclonal antibodies (MAb). Mouse hybridomas were grown in RPMI 1640 or DME plus 20 mM L-glutamine, 50 μM 2-mercaptoethanol, 50 μg/ml gentamicin, 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,[2] 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, a γ1-producing myeloma cell line.

Purification of human monocyte (HM) IL 1. Human platelet-pheresis by-products were used as a source of mononuclear cells. The adherent cell population was stimulated with opsonized heat-killed *Staphylococcus albus* in the presence of methionine-free medium containing 50 μCi/ml of [^{35}S]methionine and was incubated for 36 hr. The supernatant was purified by sequential immunoabsorption, gel-filtration, and chromatofocusing. Details of the purification procedures and the antibody used to make the immunoabsorbent have been published (21, 22). IL 1 with isoelectric point (pI) 6.8 to 7.2 eluted from the chromatofocusing step as a homogeneous M_r = 17,500 molecule determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) fluorography (21); active fractions were pooled. IL 1 biological activity was determined by using the co-stimulator assay (23). The specific activity of HM IL 1 was approximately 1 U per ng as estimated from the gel. One IL 1 unit is defined as doubling the mitogen response.

Human recombinant (HR) IL 1. The IL 1 cDNA was expressed in *E. coli* by isolating the 1112 bp *NcoI-XmnI* fragment (bp 295-1407) from the IL 1 cDNA plasmid pcD 12-1-8 (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. The HR-IL 1 was extracted from the insoluble cellular fraction with 8 M urea. HR-IL 1 was purified by sequential ion exchange and either gel-filtration chromatography or FPLC. The 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 99%. The endotoxin concentration of the homogeneous HR-IL 1 was approximately 20 pg/μg of IL 1 protein.

Preparation of IL 1-containing U937 supernatant. U937 cells were incubated in Teflon beakers at 4×10^5 cells/ml with 2 ng/ml PMA for 3 days. The conditioned media was dialyzed against Hanks' balanced salt solution (HBSS) and 1% bovine serum albumin for 3 days with three dialysate changes to remove free phorbol myristic 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 endothelial cell system (25). However, the crude U937 supernatant probably contains other materials, such as tumor necrosis factor, which may also have IL 1 like activities. An anti-IL 1 antisera inhibited U937 IL 1 activity by 81%.

Cells and cell culture. In general cells were maintained in RPMI 1640, 20 mM L-glutamine, 50 μM 2-mercaptoethanol, 50 μg/ml gentamicin, and 10% FBS at 37°C in a 5% CO_2, 95% air humidified atmosphere. Human dermal fibroblasts from neonatal foreskins 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. The T lymphoma cell line SKW3 was obtained from Dr. P. Cresswell, Duke University, Durham, NC. An anti-JY CTL clone was obtained from Dr. S. Mentzer, Dana-Farber Cancer Institute, Boston, MA. The SV-40-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). Phytohemagglutinin (PHA) blasts 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 20% FBS and 1:1000 PHA-b (GIBCO). The cells were washed and were resuspended in CM plus IL 2 (28) and were always maintained between 0.5 and 5×10^6 cells/ml. The blasts were used between days 7 and 10.

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 for 10 min and were stained with the MAb RR 1/1 by an immunoperoxidase technique by using 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-amino-9-ethyl-carbazole (Aldrich Chemical Co., Inc., Milwaukee, WI) to develop color reaction. They were fixed in 4% 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 10% heat-inactivated FBS (EDTA buffer), and were resuspended in the same to 10^7 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. Cells were suspended, and clumps were dispersed by using a rotating Teflon pestle homogenizer at 50 rpm for 30 sec at 4°C. The suspension was underlayed with Ficoll-Hypaque (d = 1.08) and was centrifuged at 1000 × G for 25 min. The cells at the interface were collected and were washed three times with the EDTA buffer at 4°C. In this way 10 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 10^5 to 10^6 cells/well. For indirect immunofluorescence staining, the cells were pelleted by centrifugation at 200 × 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 × G for 10 min to remove aggregated IgG. Cells were incubated for 30 min at 4°C, and then were washed three times, and were resuspended in 100 μl of EDTA buffer. 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 5 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 to 8×10^4 cells/well (0.32 cm^2). The cells were washed twice with CM and once with HBSS, 10 mM HEPES, 0.05% NaN_3, and 10% heat-inactivated FBS (binding 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, ^{125}I-goat anti-mouse IgG, was added at 50 nCi in 100 μl. The ^{125}I-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 and a 100-μl wash were 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 with MAb) − (cpm with X63). All steps, including induction with specific reagents such as IL 1 were carried out in quadruplicate so the SD reported include error from both induction and assay procedures.

Preparation of RR 1/1 (anti-ICAM-1) Sepharose (32). RR 1/1 IgG1 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 was dialyzed against three 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 $NaHCO_3$ 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 $NaHCO_3$, 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 preclearing 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 phenyl methyl sulfonyl fluoride, 5 mM iodoacetamide, and 0.2 trypsin inhibitor U/ml Aprotinin at 4°C for 30 min at the indicated cell density. Insoluble material was sedimented by centrifugation at 12,000 × G for 10 min at 4°C. Lysates were precleared by incubation with 50 μl activated-quenched Sepharose (Sepharose) for 1 hr at 4°C. The precleared lysate was incubated for 16 hr with 25 μl/ml of a 50% suspension of RR 1/1-Sepharose or 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 cm^2 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 twice with methionine-free CM supplemented with 9% dialyzed FBS and 1% normal FBS and then were incubated for 1 hr at 37°C in 2 ml of the same with 10% IL 1-containing U937 supernatant. At this time 200 μCi of [^{35}S]methionine was added (20 μl) to each flask and was incubated for 10 min at 37°C (pulse). 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 0.5 mM methionine, and then lysing the cells with1 ml lysis buffer and 1% bovine hemoglobin for 30 min at 4°C. The lysates were centrifuged 12,000 × G for 10 min at 4°C. Immunoprecipitations were done from lysates of 10^5 cells containing from 10^5 to 10^6 cpm. Preclearing was done as above and immunoprecipitation was 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.

Trifluoromethane sulfonic acid (TFMS) treatment (32). [^{35}S]-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 a 2:1 solution of TFMS:anisole, the tube was flushed with nitrogen, and 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).

SDS-PAGE (33). Protein was eluted from beads after immunoprecipitation by boiling in non-reducing SDS-sample buffer. For reducing gels 2-mercaptoethanol was added to 5% (w/v) to the supernatant of the bead eluate. Silver staining was done by the procedure of Morrissey et al. (34). Fluorography was carried out by using the procedure of Bonner and Laskey (35).

Cell attachment assays. PHA-blasts and SKW3 cells were labeled with ^{51}Cr for quantitation of attachment. Cells were pelleted and were resuspended in 0.5 ml of complete medium to which 200 μl of saline containing 200 μCi of $^{51}CrO_4^{-2}$ was added. The suspension was incubated for 2 hr at 37°C and then was washed three times with complete media. Spontaneous release of ^{51}Cr during subsequent incubations was evaluated by running parallel 37°C incubations and counting cell-free supernatants. None of the MAb used enhanced spontaneous release under these conditions and the spontaneous release did not exceed 5%. Human dermal fibroblasts were grown to confluency in 2.01 cm^2 wells. The cell layers were washed three times with CM and 10 mM HEPES. ^{51}Cr-labeled cells (2×10^5) were added in a total vol of 1 ml, which also contained a 1/10 dilution of the appropriate hybridoma supernatant. After a 1-hr incubation at 37°C, the wells were washed four times with 1 ml of phosphate-buffered saline by running the buffer down one side of the well (~1 ml/sec) and aspirating from the opposite side with each successive wash. Attached ^{51}Cr was released by addition of 200 μl of 0.1 N NaOH. This and a 200-μl wash were counted in the gamma counter. All points were determined from quadruplicate wells, and cells bound were calculated as (cpm released by NaOH − counter bkg)/(total cpm of cells − spontaneous release).

Preparation of Fab' (36). Protein A affinity-purified RR 1/1 (5 mg) was incubated with 400 μg cysteine-activated papain for 24 hr at 37°C in 1 ml 0.05 M citrate buffer (pH 5.5). The reaction was stopped by addition of iodoacetamide to 5 mM and was dialyzed against Tris pH 8.6. The Fc fragment was removed by protein A affinity chromatography. The $F(ab')_2$ were reduced and were alkylated by using 10 mM cysteine for 1 hr at 37°C followed by 30 mM iodoacetamide for another 1 hr at 37°C. Analysis on non-reducing SDS-PAGE showed >90% in Fab' form, with <1% $F(ab')_2$ and 5 to 10% free heavy and light chain fragments.

Light microscopy. To allow unambiguous identification of lymphoid and fibroblast cells, PHA blasts were incubated with 40 μg/ml carboxyfluorescein diacetate (CFD) in complete media 1% FBS for 15 min at 37°C and then were washed (37). This made the PHA blasts fluorescent and therefore easily differentiated from dividing fibroblasts, which are also round. CFD-PHA blasts were added to chamber slides (Titertek) with sparsely plated fibroblasts so as to form a monolayer of PHA-blasts. After 1 hr at 37°C the unattached cells were washed out, and the slide was examined by using a Zeiss Microscope 14 (Carl Zeiss, Inc., New York, NY) equipped for epifluorescence.

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 endothelium staining was more intense in the interfollicular (paracortical) areas in lymph nodes, tonsils (Fig. 1), and Peyer's 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

Distribution of ICAM-1 in normal human tissues[a]

Vascular endothelium
Germinal center cells (dendritic reticulum cells, B cells), interdigitating reticulum cells, and macrophages in lymphoid tissue (tonsil, lymph node, Peyer's patches)
Fibroblast-like cells and dendritic cells in all organs including skin, intestine, kidney, liver, and thymus
Epithelial cells (thymic epithelial cells, mucosal epithelium in tonsil and sometimes tubular epithelial cells in kidney)

[a] Organs studied: skin, kidney, liver, thymus, tonsil, lymph node, and intestine.

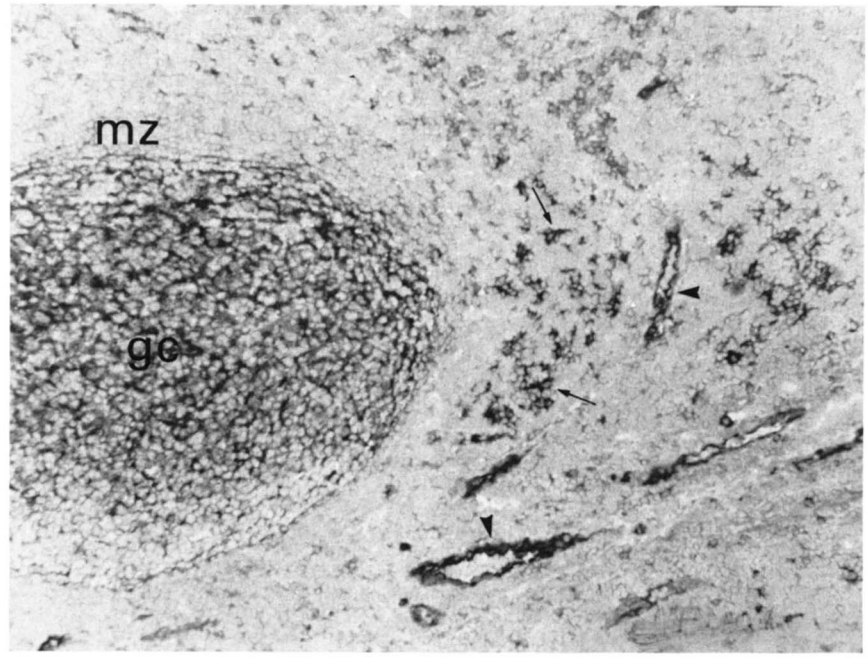

Figure 1. Immunoperoxidase staining of a tonsil with ICAM-1 MAb. Intense staining of germinal center (*gc*) area of the secondary follicle is present; the staining most likely represents reactivity with dendritic reticulum cells, as well as B cells. Faint staining of many cells in the mantle zone (mz) of the follicle is also seen. There is intense staining of vascular endothelial cells (*arrow heads*), as well as interdigitating reticulum cells in the interfollicular (T cell) area (*arrows*). (original ×125).

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 > erythroleukemia 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 monoblasts 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 II

Relative surface antigen expression determined by immunofluorescence flow cytometry[a]

Cell Line/Type	Specific Fluorescence Intensity			
	αICAM-1 (RR1/1)	αLFA-1 (TS1/22)	αHLA-A,B,C (W6/32)	αLFA-3 (TS2/9)
JY B lymphoblastoid	28	51	460	29
SLA LFA-1⁻ B lymphoblastoid	50	0	300	55
HFO LFA-1⁺ B lymphoblastoid	11	46		
SKW3 T-lymphoma	1	130	55	
PHA-blasts	3.2	65		
α JY CTL line	9.1	53		
K562 erythroid/myeloid	9.7	<1	0	14
K562 + IFN-γ	32		5.8	14
U937 monoblastoid	7.4	22		
U937 + PMA	114	50		
HL60 myeloblastoid	0	8.1		
HL60 + PMA	49	45		
Dermal fibroblasts (5–10th passage)	29	0	140	19
M1 fibroblast	0.3	0	99	14
Granulocytes	1.0			
Monocytes	2.1			
Lymphocytes	2.0		59	

[a] K562 cells were treated with 10 U/ml recombinant IFN-γ for 24 hr. U937 and HL-60 cell lines 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 ^{125}I-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. 2*A*) 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. 2*B*) 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 E_2 (PGE_2) (0.28 to 28 μM) had no effect on fibroblast ICAM-1 expression at 24 hr (not shown). The concentrations of IFN-β that were 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 HM IL 1 and IFN-γ effects. The kinetics of HM IL 1 and IFN-γ effects on ICAM-1 expression on dermal fibroblasts were determined using a ^{125}I goat anti-mouse IgG binding assay. The effect of IL 1 with a $t_{½}$ for ICAM-1 induction of 2 hr was more rapid than that of IFN-γ with a $t_{½}$ of 3.75 hr (Fig. 3). No significant change in HLA-A,B,C expression was seen with HM IL 1, whereas

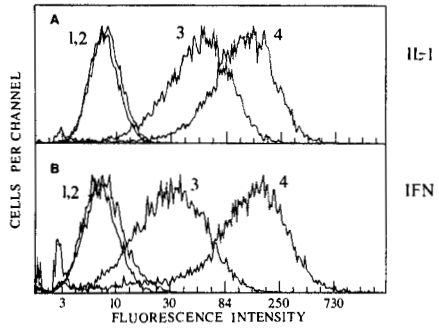

Figure 2. Increased ICAM-1 expression on IL 1 and IFN-γ-treated fibroblasts measured by immunofluorescence flow cytometry. In *Panel A*, human dermal fibroblasts were grown to 2×10^7 cells/75 cm^2 flask, and one flask received a 1/10 dilution of IL 1-containing U937 conditioned media. In *Panel B*, fibroblasts were grown to 2×10^6 cells/75 cm^2 flask, and one flask received 100 U/ml recombinant human IFN-γ. Cells with IL 1 were incubated for 4 hr, whereas cells with IFN-γ were incubated 18 hr before harvesting and processing for flow cytometry. *Panel A, Peak 1*, untreated cells with X63 supernatant; *Peak 2*, IL 1-treated cells with X63; *Peak 3*, untreated cells with anti-ICAM-1 supernatant; and *Peak 4*, IL 1-treated cells with anti-ICAM-1. *Panel B, Peak 1*, untreated cells with X63; *Peak 2*, IFN-γ-treated cells with X63; *Peak 3*, untreated cells with anti-ICAM-1; *Peak 4*, IFN-γ-treated cells with anti-ICAM-1.

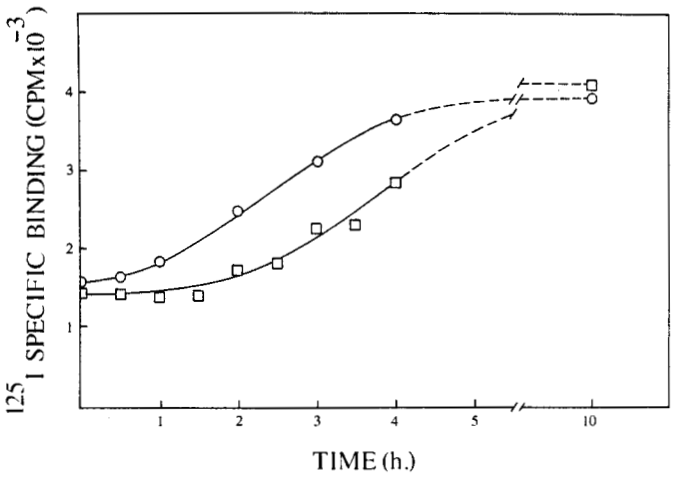

Figure 3. Kinetics of IL 1 and IFN-γ effects on ICAM-1 expression on human dermal fibroblasts. Human dermal fibroblasts were grown to a density of 8×10^4 cells/0.32 cm^2 well. HM IL 1 (10 U/ml) (○) or rIFN-γ (10 U/ml) (□) was added, and at the appropriate time, the plate was cooled to 4°C, and an indirect ^{125}I-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.

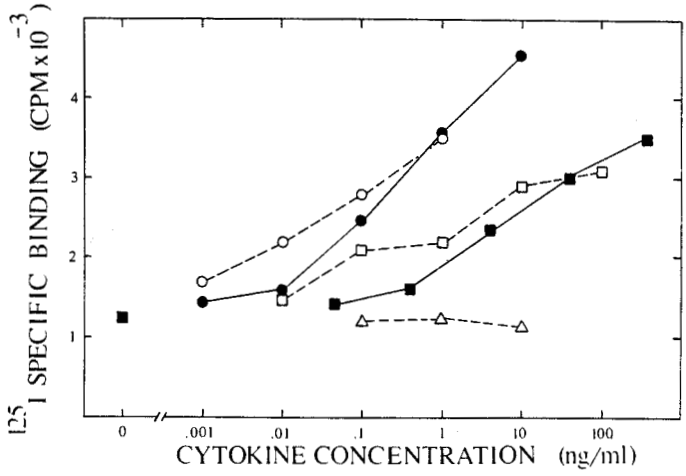

Figure 4. Concentration dependence of IL 1 and IFN-γ effects on ICAM-1. Human dermal fibroblasts were grown to 8×10^4 cells/0.32 cm^2 well. HM IL 1 (○), recombinant human IL 1 (□), recombinant mouse IL 1 (■), recombinant human IFN-γ (●), and recombinant human IFN-β (△) were added at the indicated dilution and were incubated for 4 hr (IL 1) or 16 hr (IFN-γ and -β). The results are from ^{125}I-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.

IFN-γ increased expression of those antigens by approximately twofold at 10 hr (not shown). The time-course of return to resting levels of ICAM-1 appears to depend on the cell cycle or rate of growth. In quiescent cells, the HM IL 1 and IFN-γ effects are stable for 2 to 3 days, whereas in log phase cultures, ICAM-1 expression is near baseline 2 days after removal of IL 1 or IFN-γ (not shown).

Concentration dependence of IL 1 and IFN-γ effect. The dose response curves for induction of ICAM-1 by recombinant mouse and human IL 1, for purified HM IL 1 and for recombinant human IFN-γ were compared (Fig. 4). IFN-γ and HM IL 1 have similar concentration dependencies with nearly identical effects at 1 ng/ml. The human and mouse recombinant IL 1 also have similar curves but are much less effective than the HM IL 1. Both recombinant IL 1 were purified under denaturing conditions, and contain sequences that are removed by processing in HM IL 1. The recombinant human IL 1 is presently 100-fold less effective than the HM IL 1 in the endogenous pyrogen assay (Dinarello, unpublished ob-

servation). IFN-β 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-γ 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-γ-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 100,000 M_r (Fig. 5, lane 2), ICAM-1 from PMA-stimulated U937 cells is 114,000 M_r (Fig. 5, lane 7), and ICAM-1 from JY cells is 90,000 M_r (Fig. 5, lane 9). This heterogeneity in M_r appears to reflect differences in glycosylation (see below). M_r estimates from reducing and nonreducing gels were similar[2]. ICAM-1 immunoprecipitated from hairy cell leukemia spleen lysates was similar in size to the M_r = 90,000 species isolated from JY (not shown).

TABLE III

Effects of cycloheximide, actinomycin D, and tunicamycin on ICAM-1 induction by IL 1 and IFN-γ on human dermal fibroblasts[a]

Treatment	^{125}I Goat Anti-Mouse IgG Specifically Bound (cpm)	
	αICAM-1	αHLA-A,B,C
Control (4 hr)	1524 ± 140	11928 ± 600
+ cycloheximide	1513 ± 210	10678 ± 471
+ actinomycin D	1590 ± 46	12276 ± 608
+ tunicamycin	1461 ± 176	12340 ± 940
IL 1 (10 U/ml) (4 hr)	4264 ± 249	12155 ± 510
+ cycloheximide	1619 ± 381	12676 ± 446
+ actinomycin D	1613 ± 88	12294 ± 123
+ tunicamycin	3084 ± 113	13434 ± 661
IFN-γ (10 U/ml) (18 hr)	4659 ± 109	23675 ± 500
+ cycloheximide	1461 ± 59	10675 ± 800
+ actinomycin D	1326 ± 186	12089 ± 550

[a] Human fibroblasts were grown to a density of 8×10^4 cells/0.32 cm^2 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.

Pulse-chase metabolic labeling of ICAM-1 and metabolic labeling in the presence of tunicamycin were used to study the biosynthesis and the unglycosylated m.w. of ICAM-1. Dermal fibroblasts treated with IL 1-containing U937 supernatant were pulsed with [^{35}S]methionine for 10 min followed by a variable chase period after which the cells were lysed and ICAM-1 was isolated by using MAb-Sepharose. ICAM-1 has a precursor of M_r = 73,000 (Fig. 6, lane 1), which is converted to the mature M_r = 97,000 form between 20 and 50 min (Fig. 6, lanes 3 through 5). Labeling in the presence of tunicamycin followed by isolation of ICAM-1 results in a sharp band at

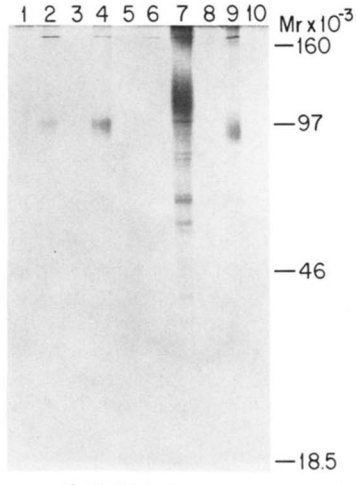

Figure 5. Isolation of ICAM-1 by immunoprecipitation from fibroblasts, U937 cells, and JY cells. *Lane 1*, untreated fibroblasts immunoprecipitated with control Sepharose CL-4B; *Lane 2*, untreated fibroblasts with anti-ICAM-1 Sepharose; *Lane 3*, IL 1-treated fibroblasts with control Sepharose; *Lane 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 5×10^6 fibroblasts, from 10^7 U937 cells, and from 5×10^7 JY cells were subjected to nonreducing SDS 9% PAGE and silver staining. The faint, sharp band at ~160,000 M_r 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).

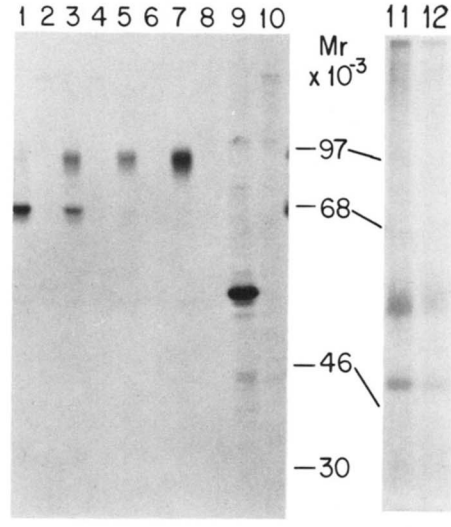

Figure 6. Pulse-chase metabolic labeling of dermal fibroblast ICAM-1 and TFMS treatment of ICAM-1 from dermal fibroblasts and U937. Fibroblasts were grown to 2×10^6 cells/25 cm^2 flask. Fibroblasts were treated with U937 conditioned media for 3 hr before labeling and were in methionine free media for 1 hr before labeling. Labeling conditions: *lanes 1* and *2*, 10 min pulse, 10 min chase; *lanes 3* and *4*, 10 min pulse, 20 min chase; *lanes 5* and *6*, 10 min pulse, 40 min chase; *lanes 7* and *8*, 10 min pulse, 80 min chase; *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 *odd 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 M_r = 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 M_r = 60,000 and a minor band at an M_r = 47,000 (Fig. 6, lanes *11* and *12*). This result shows that dermal fibroblast and U937 ICAM-1 have polypeptides with identical M_r, suggesting the polypeptides are identical and carbohydrate processing leads to the different M_r 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 ^{51}Cr-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.[2] 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 α and β 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 inhibitable by anti-ICAM-1 showed a similar increase. Binding not inhibitable 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

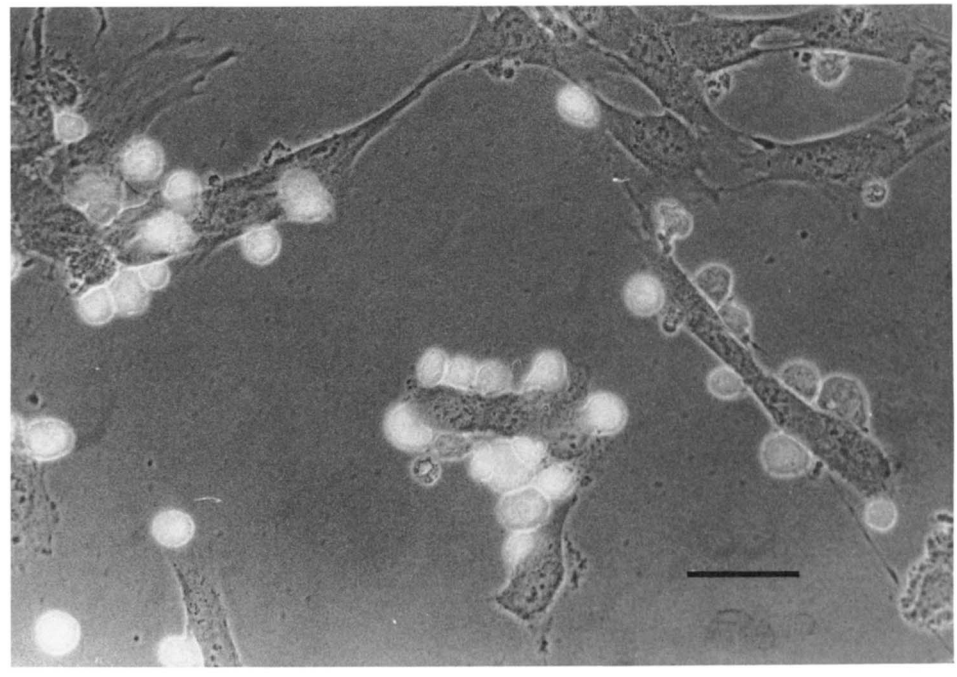

Figure 7. PHA-blasts binding to human dermal fibroblasts. PHA blasts (10 days) were labeled with carboxyfluorescein diacetate (40 μg/ml) by incubation in CM with 1% serum. The PHA blasts were washed and were added to plastic slides (Titertek) with sparsely plated fibroblasts at >10 PHA blasts: 1 fibroblast. After 1 hr at 37°C, the unattached cells were washed out, and the slide was observed immediately by using a combination of visible wavelength phase illumination and fluorescence without fixation. Bar = 20 μ.

TABLE IV

Binding of lymphoid cells to human dermal fibroblasts[a]

Antibody	Cells Bound × 10^{-4}	
	PHA blasts	SKW3
X63	2.4 ± 0.2	2.1 ± 0.04
αICAM-1	0.7 ± 0.1	0.47 ± 0.03
αLFA-1, α	0.8 ± 0.1	0.50 ± 0.08
αLFA-1, β	0.65 ± 0.1	ND[b]
αHLA-A,B,C	2.6 ± 0.1	2.01 ± 0.07
αLFA-3	2.6 ± 0.2	2.30 ± 0.04
αLFA-2	2.4 ± 0.1	2.21 ± 0.05

[a] Human dermal fibroblasts were grown to 5 × 10^5 cells/2.01 cm^2 well. PHA blasts or SKW3 (2 × 10^5 cells labeled with ^{51}Cr) were added for 1 hr at 37°C. PHA blasts had an activity of 58,000 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.

[b] ND, not done.

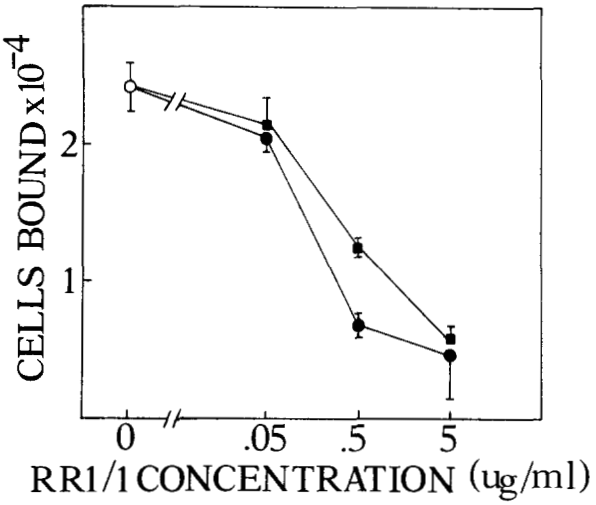

Figure 8. Effect of anti-ICAM-1 IgG and Fab' on PHA blast binding to human dermal fibroblasts. Fibroblasts were grown to 2.5 × 10^5 cells/2.01 cm^2 well. PHA blasts (2 × 10^5) and the MAb (●) or MAb fragment (■) were added and were incubated 1 hr at 37°C. Final volume was 1 ml.

TABLE V

Lymphoid cell or fibroblast pretreatment with MAb[a]

Pretreatment		Cells Bound × 10^{-4}	
Lymphoid cell	Fibroblast	PHA blasts	SKW3
None	None	2.57 ± 0.00	2.04 ± 0.06
None	αICAM-1	0.88 ± 0.08	0.56 ± 0.03
None	αLFA-1	2.52 ± 0.09	1.99 ± 0.08
None	αHLA-A,B,C	2.40 ± 0.21	1.85 ± 0.18
αICAM-1	None	2.84 ± 0.18	1.97 ± 0.06
αLFA-1	None	0.73 ± 0.16	0.87 ± 0.07
αHLA-A,B,C	None	2.40 ± 0.31	2.10 ± 0.08

[a] Human dermal fibroblasts were grown to 2 × 10^5 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 × 10^5) 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.

seen with fibroblasts incubated with 10 U/ml HM IL 1 for 4 hr (not shown).

DISCUSSION

ICAM-1 is a glycoprotein expressed on the surface of both hematopoietic and non-hematopoietic cells. Although ICAM-1 expression is uniformly low on peripheral blood leukocytes, it is expressed to higher levels on EBV-transformed B lymphocyte blasts, mitogen-stimulated T lymphocyte blasts, a cloned CTL line, macrophage-like cells in several organs, and dendritic cells and possibly B lymphocytes in germinal centers. ICAM-1 is also induced

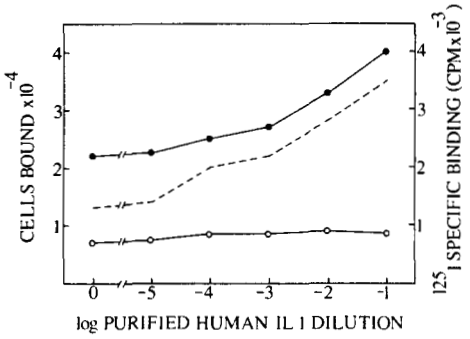

Figure 9. Correlation between IL 1 up-regulation of ICAM-1 and increase in PHA blast binding to dermal fibroblasts. Fibroblasts were grown to 5 × 10^5 cells/2.01 cm^2 well. HM IL 1 was added at the appropriate concentration 4 hr before addition of ^{51}Cr-PHA blasts. PHA blasts were added at 2 × 10^5 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 (●) and presence (○) 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 ^{125}I-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 ^{125}I 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 (46), PGE_2 synthesis, and collagenase production (42, 47), whereas IFN-γ decreases collagen synthesis, causes a late inhibition of proliferation (48), increases PGE_2 elaboration (49), and increases 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[4]. A comparison of IL 1-induced surface expression of ICAM-1 and another antigen that is specific

[4] **Pober, J. S., M. A. Gimbrone, Jr., L. A. Lapierre, D. L. Mendrick, W. Fiers, R. Rothlein, and T. A. Springer.** 1986. Activation of human endothelial cells by lymphokines: overlapping patterns of antigenic modulation by interleukin 1, tumor necrosis factor, and immune interferon. *Submitted for publication.*

for endothelial cells and is identified by the MAb H4/18 (41) has been made on cultured umbilical vein endothelial cells[4]. 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_r heterogeneity in different cell types. On the basis of the results of chemical deglycosylation with TFMS, it appears that this heterogeneity is based on differential glycosylation of a common polypeptide, although it remains to be rigorously demonstrated that the polypeptides from dermal fibroblasts and U937 are identical. Pulse-chase metabolic labeling of fibroblast ICAM-1 shows that a precursor of $M_r = 73,000$ is converted to a mature form of $M_r = 97,000$ 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 appears 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% carbohydrate as estimated by the mobilities of the glycosylated and nonglycosylated forms in SDS-PAGE. The product synthesized in the presence of tunicamycin was $M_r = 55,000$. Estimating approximately 2,000 to 3,000 M_r U per high mannose oligosaccharide on the intracellular precursor of $M_r = 73,000$, ICAM-1 would contain approximately 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[2] (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. We used the natural attachment assay in which activated but not resting 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 binding 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 dermal fibroblasts with IL 1 natural attachment was significantly increased in an IL 1 concentration-dependent manner that was correlated with ICAM-1 surface expression.

The importance of ICAM-1 and LFA-1 and the regulation by IL1 suggest natural attachment is a functionally significant assay. Natural attachment may represent part of the non-antigen-specific component of leukocyte adhesion in functions such as antigen presentation (50), and cytotoxic 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 of afferent 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 endothelial 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 extravasation 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 endothelial cells by IL 1 is rapid[4], increased adhesion of lymphocytes mediated by ICAM-1 might be an event mediating lymphocyte influx into inflammatory loci. Subsequent production of IFN-γ by activated lymphocytes at the inflammatory site might mediate additional amplification of the local inflammatory immune response.

ICAM-1 has some properties in common with the papain 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). Furthermore, ICAM-1 appears to be required in two LFA-1-dependent adhesion systems, leukocyte self-aggregation[2] and natural attachment. These results suggest that ICAM-1 could be a cell surface molecule that interacts with LFA-1 on other cells.[2]

The work reported here suggests that ICAM-1 is important in leukocyte adhesion and is regulated in a manner consistent with its being an important molecule in inflammatory 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.

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