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Purified Intercellular Adhesion Molecule-1 (ICAM-1) Is a Ligand for Lymphocyte Function-Associated Antigen 1 (LFA-1)

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Summary

Lymphocyte function-associated antigen 1 (LFA-1) is a leukocyte cell surface glycoprotein that promotes intercellular adhesion in immunological and inflammatory reactions. It is an αβ complex that is structurally related to receptors for extracellular matrix components, and thus belongs to the integrin family. ICAM-1 (intercellular adhesion molecule-1) is a distinct cell surface glycoprotein. Its broad distribution, regulated expression in inflammation, and involvement in LFA-1-dependent cell-cell adhesion have suggested that ICAM-1 may be a ligand for LFA-1. We have purified ICAM-1 and incorporated it into artificial supported lipid membranes. LFA-1$^+$ but not LFA-1$^-$ cells bound to ICAM-1 in the artificial membranes, and the binding could be specifically inhibited by anti-ICAM-1 treatment of the membranes or by anti-LFA-1 treatment of the cells. The cell binding to ICAM-1 required metabolic energy production, an intact cytoskeleton, and the presence of Mg^{2+} and was temperature dependent, characteristics of LFA-1- and ICAM-1-dependent cell-cell adhesion.

Introduction

The specificity of cell–cell interactions in embryogenesis, histogenesis, and the immune system is thought to be mediated by cell surface adhesion molecules. These interactions may be between two cells or between a cell and the extracellular matrix. Recently, a family of ten different structurally related cell surface αβ complexes (the integrins) has been defined that appear to link the extracellular environment with the cytoskeleton (Hynes, 1987). There are three integrin subfamilies. Each subfamily has a unique β subunit, designated integrin β_1, integrin β_2, and integrin β_3, which can associate with multiple α subunits. The β subunits are 37% to 49% identical to one another (Hynes, 1987), and the α subunits are 25% to 46% identical (Corbi et al., 1987; Poncz et al., 1987; Argraves et al., 1987; Suzuki et al., 1987). This strong structural homology suggests a high degree of functional relatedness. The integrin family includes receptors for extracellular matrix components such as fibronectin, which recognize the sequence RGD within their ligands (Ruoslahti and Pierschbacher, 1986) and utilize the β_1 or β_3 subunits; and three leukocyte adhesion glycoproteins: lymphocyte function–associated antigen 1 (LFA-1), Mac-1, and p150,95, which utilize the β_2 subunit. In contrast to the extracellular matrix receptors, the leukocyte adhesion receptors promote cell–cell interactions, and little is known about how they function.

LFA-1 is a leukocyte cell surface glycoprotein widely expressed on cells of hematopoietic lineage. It contains noncovalently associated α and β subunits of 180,000 and 95,000 M_r (CD11a and CD18, respectively). The function of LFA-1 as an intercellular adhesion molecule has been defined by the ability of anti-LFA-1 monoclonal antibodies (MAbs) to inhibit adhesion-dependent leukocyte functions specifically, and by the clinical and in vitro consequences of an inherited defect in cell surface expression of LFA-1 (Springer et al., 1987; Martz, 1986; Anderson and Springer, 1987). The mechanism by which LFA-1 promotes intercellular adhesion has not been determined. Formally, LFA-1 could either participate directly in adhesion by binding to a ligand, or participate indirectly by regulating (or catalyzing) interactions mediated by other receptor–ligand systems. LFA-1 does not act as its own ligand via like–like interactions, since LFA-1$^+$ cells can interact with LFA-1$^-$ cells (Rothlein et al., 1986; Springer et al., 1987).

We have previously identified a cell surface glycoprotein of M_r = 90,000, ICAM-1 (intercellular adhesion molecule-1), which is an attractive candidate for an LFA-1 ligand. MAbs to ICAM-1 inhibit a number of LFA-1-dependent cellular interactions including phorbol ester–stimulated lymphocyte aggregation (Rothlein et al., 1986), the adhesion of T-lymphocytes to fibroblasts (Dustin et al., 1986) and endothelial cells (M. D. Dustin and T. A. Springer, unpublished data), and the interactions between cytotoxic T-lymphocytes and certain target cells (M. W. Makgoba, M. E. Sanders, G. E. Ginther Luce, E. A. Gugel, M. L. Dustin, T. A. Springer, and S. Shaw, submitted). The binding of T-lymphocytes to fibroblasts can be inhibited by anti-ICAM-1 treatment of the fibroblasts or by anti-LFA-1 treatment of the lymphocytes, suggesting a possible receptor–ligand interaction between the two molecules. The wide distribution of ICAM-1, its high expression at inflammatory sites, and its regulated expression by inflammatory cytokines have also suggested that ICAM-1 could be a ligand for LFA-1 (Dustin et al., 1986; Pober et al., 1986; Cotran et al., 1987).

We have directly tested this hypothesis using an artificial lipid membrane cell adhesion assay. We have purified ICAM-1, reconstituted it into artificial membranes, and shown that it is a ligand for the LFA-1-dependent adhesion system.

Results

Immunoaffinity Purification of ICAM-1

ICAM-1 was purified by monoclonal antibody immunoaffinity chromatography from JY lymphoblastoid cells. We

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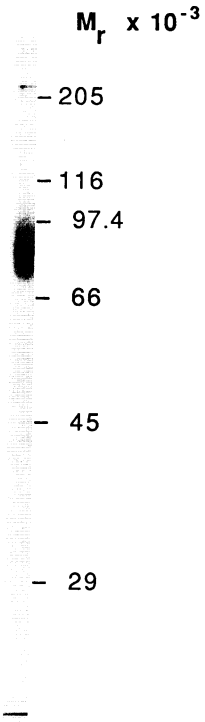

Figure 1. SDS–PAGE of Purified ICAM-1

Approximately 0.5 μg of ICAM-1 purified from JY B-lymphoblastoid cells by immunoaffinity chromatography was electrophoresed under nonreducing conditions on an SDS–8% polyacrylamide gel and visualized by silver staining.

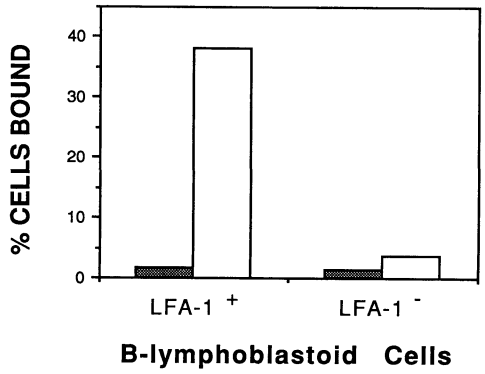

Figure 2. LFA-1$^+$ but Not LFA-1$^-$ B-Lymphoblastoid Cells Bind to Purified ICAM-1 Incorporated into Artificial Planar Membranes

Purified ICAM-1 (open bars) or glycophorin from human erythrocytes as a negative control (shaded bars) was incorporated into artificial planar membranes supported on glass coverslips. ^{51}Cr-labeled JY (LFA-1$^+$) or patient (LFA-1$^-$) B-lymphoblastoid cells were centrifuged (25 × g, 3 min) onto the planar membranes and incubated at 37°C for 1 hr. After extensive washing, cells bound to the planar membrane were quantitated by counting of the remaining cell-associated radioactivity.

predicted that ICAM-1 from this source would be functional in promoting adhesion since JY cells homotypically aggregate, both spontaneously (Mentzer et al., 1985; Rothlein and Springer, 1986) and to a much greater extent after phorbol ester stimulation (Rothlein and Springer, 1986), in a manner that is both ICAM-1- and LFA-1-dependent as evidenced by specific inhibition with monoclonal antibodies (Rothlein et al., 1986). JY cells, with or without prior stimulation with phorbol ester, were lysed with the detergent Triton X-100, and ICAM-1 was purified from the lysate by binding to an RR1/1 MAb column. Weakly bound contaminating proteins were removed by washing with a pH 11 buffer. Triton X-100 was then displaced and exchanged with the dialysable detergent octylglucoside prior to elution of the bound ICAM-1 at pH 12.5. ICAM-1 purified by this procedure was substantially pure, and migrated on SDS–PAGE as a broad band of 80,000–90,000 M_r (Figure 1), consistent with our previous observations from immunoprecipitation experiments (Rothlein et al., 1986; Dustin et al., 1986). Approximately 5 μg of purified ICAM-1 was recovered from 5 g of JY cells.

Binding of Cells to Purified ICAM-1 in Supported Lipid Membranes

To test whether ICAM-1 could mediate cellular adhesion, the purified glycoprotein was reconstituted into artificial lipid vesicles. Purified ICAM-1 was added to lipids solubilized in octylglucoside, and the detergent was removed by dialysis. The resulting ICAM-1-containing vesicles were fused to glass coverslips (Brian and McConnell, 1984) to produce supported planar membranes on which cell binding could be measured. The human erythrocyte membrane glycoprotein glycophorin was similarly incorporated as a control. When ^{51}Cr-labeled JY cells were gently centrifuged onto planar membranes, incubated, and then washed, a significant percentage of the cells specifically bound to membranes containing ICAM-1, but not to membranes containing glycophorin (Figure 2). Furthermore, LFA-1$^-$ EBV-transformed B-lymphoblastoid cells derived from a patient with leukocyte adhesion deficiency (Anderson et al., 1985) failed to bind, demonstrating that the binding was specific and required the presence of LFA-1 on the binding cells. ICAM-1 was active in promoting adhesion whether or not the JY cells from which it was isolated received prior stimulation with phorbol esters.

During the course of experiments using planar membranes supported on glass coverslips, we found that vesicles will bind directly to the plastic surface of multi-well tissue culture plates to provide a form of supported lipid membrane more convenient than glass coverslips. Similar preparations were recently described by others (Quill and Schwartz, 1987). We refer to these preparations as plastic-bound vesicles (PBV), since the nature of the membrane bound to the plastic has not been determined. However, preliminary immunofluorescence experiments with ICAM-1 in PBV and planar membranes indicate that the PBV form a layer of large, packed cobblestone-like vesicles instead of a continuous single membrane (M. Dustin; data not shown). Although most results reported below are from experiments with PBV, we have obtained similar results with planar membranes supported on glass.

The binding of cells to ICAM-1 in PBV was as specific as to ICAM-1 in planar membranes: JY cells, SKW-3

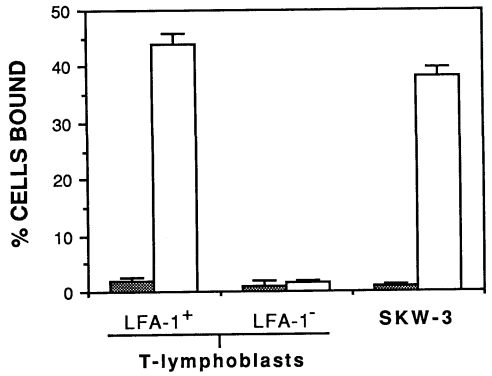

Figure 3. Binding of T-Lymphoblasts and SKW-3 T-Lymphoma Cells to ICAM-1 Incorporated into Plastic-Bound Vesicles

Purified ICAM-1 (open bars) or glycophorin (shaded bars) was incorporated into lipid vesicles and then bound to the wells of a 96-well tissue culture plate. LFA-1$^-$ T-lymphoblasts were derived from a leukocyte adhesion deficiency patient (patient #1, (Anderson et al., 1985)), while LFA-1$^+$ T-lymphoblasts were derived from a related, healthy control. Results are representative of three experiments.

T-lymphoma cells, and LFA-1$^+$, but not LFA-1$^-$ T-lymphoblasts, bound to ICAM-1 in PBV (Figures 3 and 4). Only background binding was observed with glycophorin-containing PBV.

Both the specificity of cell binding and the dependence on cellular LFA-1 were confirmed in MAb blocking experiments. MAbs reactive with the α or β subunits of LFA-1 and MAbs to ICAM-1 inhibited the binding of T-lymphoblasts to ICAM-1 (Figure 4). In contrast, MAbs to the α subunit of the p150,95 adhesion molecule, the unrelated LFA-3 adhesion molecule, or HLA, present in high density on JY cells, did not significantly inhibit the cell binding.

To identify the site of inhibition, either cells or planar membranes were pretreated with MAb prior to the binding assay. The specific binding of JY cells and T-lymphoblasts was completely inhibited when the ICAM-1-containing PBV were pretreated with anti-ICAM-1 MAb RR1/1 (Figure 5). Pretreatment of the cells with the same antibody had little effect. Conversely, the anti-LFA-1 monoclonal antibody TS1/18 could completely inhibit cell binding, but only when the cells, not the PBV, were pretreated. The control antibody TS2/9 reactive with LFA-3 had no significant inhibitory effect when either cells or PBV were pretreated. Additionally, an anti-HLA MAb, W6/32, did not inhibit the cell binding (data not shown). These results strongly suggest that LFA-1 binds to ICAM-1, and that this interaction is sufficient to mediate cell–cell adhesion.

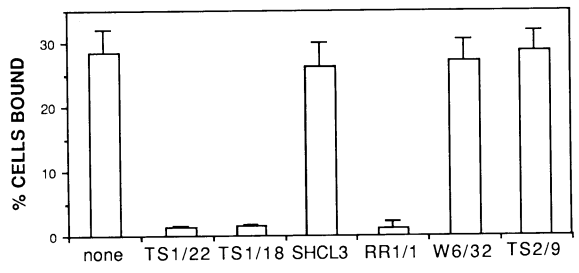

Figure 4. Monoclonal Antibodies to ICAM-1 or LFA-1 Inhibit the Adhesion of T-Lymphoblasts to ICAM-1 Incorporated into Plastic-Bound Vesicles

MAbs (20 μg/ml of purified IgG, final concentration) were added to the cell binding assay and were present throughout the experiment. Specificity of MAbs: TS1/22, anti-LFA-1 α subunit; TS1/18, anti-LFA-1 β subunit; SHCL3, anti-p150,95 α subunit; RR1/1, anti-ICAM-1; W6/32, anti-HLA; TS2/9, anti-LFA-3.

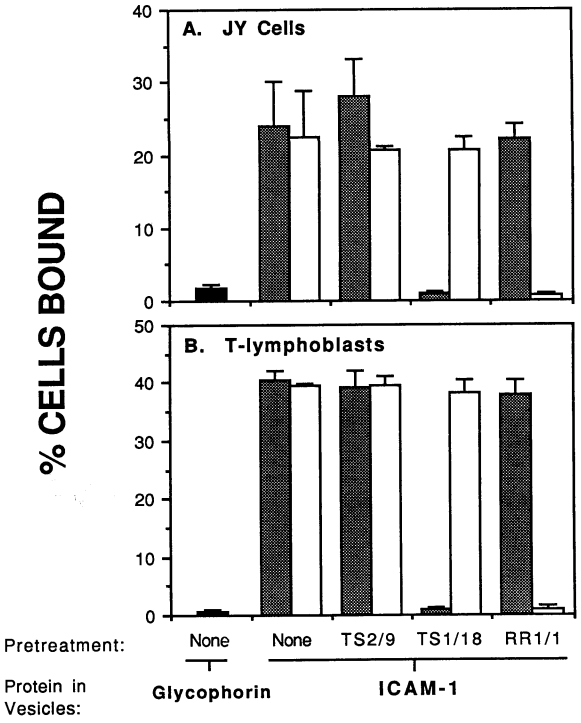

Figure 5. Monoclonal Antibodies Inhibit Cell Adhesion by Binding to ICAM-1 in Plastic-Bound Vesicles or to LFA-1 on Cells

Cells (shaded bars) or plastic-bound vesicles (open bars) were pretreated with 20 μg/ml of purified IgG, then washed extensively to remove unbound antibody. Solid bars represent binding of untreated cells to glycophorin incorporated into plastic-bound vesicles. Specificity of MAbs: TS2/9, anti-LFA-3; TS1/18, anti-LFA-1 β subunit; RR1/1, anti-ICAM-1.

Characteristics of Cell Binding to ICAM-1

The LFA-1-dependent adhesion steps in CTL-mediated killing and phorbol ester–stimulated leukocyte homotypic aggregation (self-adhesion) have similar characteristics that most likely reflect fundamental aspects of the adhesion process itself. Both require the presence of divalent cations (Martz, 1980; Rothlein and Springer, 1986), metabolic energy production (Martz, 1977; Patarroyo et al., 1983b), and a cytoskeleton with functional microfilaments (Martz, 1977; Rothlein and Springer, 1986; Patarroyo et al., 1983a), and both are temperature dependent (Martz, 1977; Patarroyo et al., 1983b). It was of interest to determine whether these characteristics also applied to the supported membrane model where an intact, living cell contributed only one of the two adhesion components.

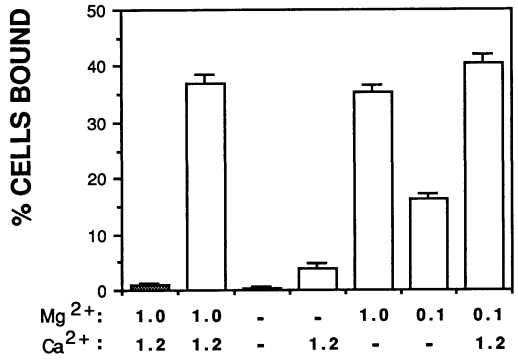

Figure 6. Adhesion of T-Lymphoblasts to ICAM-1 in Plastic-Bound Vesicles Requires the Presence of Divalent Cations

Cells and plastic-bound vesicles containing glycophorin (shaded bar) or ICAM-1 (open bars) were washed with Ca^{2+}- and Mg^{2+}-free HBSS/10% dialyzed FCS preequilibrated with assay buffer containing the indicated concentrations of $MgCl_2$ or $CaCl_2$ for 15 min at 4°C, incubated with the ICAM-1-containing PBV, and washed in the same buffer. Results are representative of three experiments.

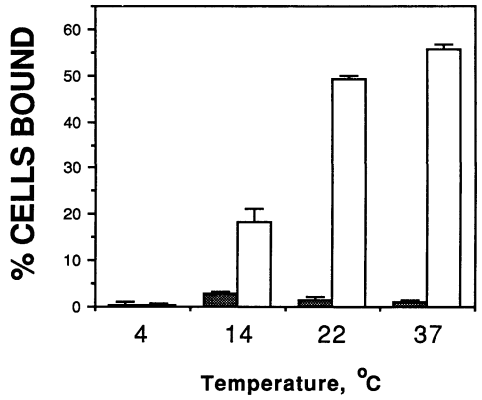

Figure 8. Effect of Temperature on the Adhesion of T-Lymphoblasts to ICAM-1 in Plastic-Bound Vesicles

Cells and plastic-bound vesicles containing glycophorin (shaded bars) or ICAM-1 (open bars) were preequilibrated at the indicated temperature before use in the adhesion assay. After binding 1 hr at the indicated temperatures, unbound cells were washed out with medium at the same temperature. Results are representative of two experiments performed at all temperatures, and three experiments at 4°C, 22°C, and 37°C.

The binding of cells to ICAM-1 in artificial membranes required divalent cations. While T-lymphoblasts bound specifically to ICAM-1 in PBV in the presence of physiological concentrations of Mg^{2+} plus Ca^{2+}, no binding occurred in the absence of both cations (Figure 6). Mg^{2+} alone produced binding equivalent to the combination of both cations, while Ca^{2+} alone produced only about one-tenth of this binding. Even at 0.1 mM, one-twentieth of the normal physiological concentration, Mg^{2+} alone was sufficient to promote nearly half-maximal binding, and when combined with Ca^{2+}, acted more than additively to produce maximal binding.

The binding of T-lymphoblasts to ICAM-1 also required metabolic energy production and, at least in part, functional microfilaments (Figure 7). The combination of sodium azide and 2-deoxy-D-glucose inhibits energy production and completely inhibited T-lymphoblast binding to ICAM-1. However, cytochalasin B, an inhibitor of microfilaments, only partially inhibited the binding (88% in this experiment, 49%–84% in other experiments), although at the same concentration cytochalasin B inhibited the phorbol ester–stimulated aggregation of these cells by greater than 95%.

Cellular adhesion to ICAM-1 in PBV was temperature dependent (Figure 8). T-lymphoblasts bound very effectively at 37°C, but not at 4°C. Intermediate levels of binding occurred at 22°C and 14°C.

Three of the matrix receptor integrins, the vitronectin, fibronectin, and platelet IIb–IIIa receptors, bind to regions within their ligands that contain the sequence RGD, and peptides containing this sequence competitively inhibit binding. Neither the hexapeptide GRGDSP nor a control peptide, GRGESP, inhibited T-lymphoblast binding to ICAM-1 (Figure 9). This is in agreement with the observation that RGD peptides do not inhibit lymphocyte homotypic adhesion, which is dependent on the interaction between LFA-1 and ICAM-1 (Rothlein and Springer, unpublished data). Although these findings suggest that the core recognition sequence within fibronectin is distinct from that within ICAM-1, it remains possible that the recognition sequences are closely related.

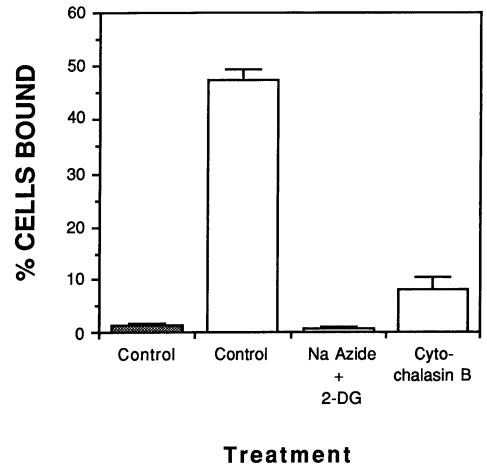

Figure 7. Binding of T-Lymphoblasts to ICAM-1 in Plastic-Bound Vesicles Requires Metabolic Energy and an Intact Cytoskeleton

Cells were pretreated for 1 hr at 37°C with a mixture of 50 mM 2-deoxy-D-glucose plus 10 mM sodium azide, or with 20 μM cytochalasin B dissolved in DMSO. Control cells were pretreated with DMSO at the same final concentration as cells treated with cytochalasin B. All inhibitors remained present during the 1 hr adhesion assay. Cells were not centrifuged, but were allowed to settle at 1 × g onto plastic-bound vesicles. Shaded bar represents binding to plastic-bound vesicles containing glycophorin; open bars represent binding to ICAM-1-containing vesicles. Results are representative of two experiments without centrifugation, and two with centrifugation.

Discussion

We have proven that ICAM-1 is a ligand for LFA-1 by demonstrating that purified ICAM-1 incorporated into ar-

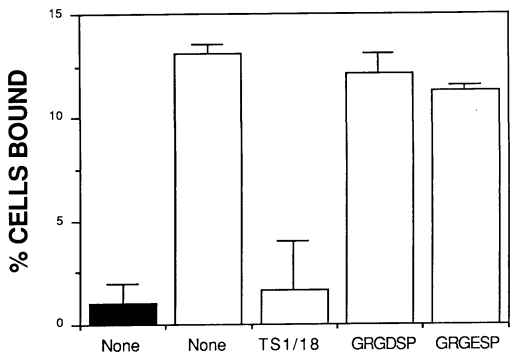

Figure 9. Binding of T-Lymphoblasts to ICAM-1 Is Not Inhibited by RGD Peptides

The binding of cells to glycophorin (solid bar) or ICAM-1 (open bars) incorporated into PBV was tested in the presence of LFA-1 β MAb (TS1/18), the hexapeptide GRGDSP, or the control peptide GRGESP. Antibody was present at 20 μg/ml, and peptides were present at 1 mg/ml. Identical results were obtained with 0.1 mg of peptide per ml.

tificial supported membranes can directly mediate LFA-1-dependent lymphocyte adhesion. The binding of cells to purified ICAM-1 in artificial membranes was highly specific, required the presence of LFA-1 on the binding cell membrane, could be reciprocally inhibited by treatment of the cells with anti-LFA-1 MAb or of the membranes with anti-ICAM-1 MAb, and retained the distinct functional characteristics normally exhibited in LFA-1-dependent adhesion between cells. That is, the binding of cells to ICAM-1 required metabolic energy, a functional cytoskeleton, and the presence of extracellular Mg^{2+}, and was dependent on temperature. The interaction between LFA-1 and ICAM-1 is one of few documented examples of heterophilic (not like–like) binding between defined integral surface membrane proteins in cell biology. Another recently described example is the interaction between the CD2 and LFA-3 membrane glycoproteins (Selvaraj et al., 1987).

The tissue distribution of ICAM-1 is consistent with its role as a ligand of LFA-1. Although ICAM-1 is widely expressed on cells of both hematopoietic and non-hematopoietic origin including lymphocytes, monocytes, fibroblasts, epithelial cells, and endothelial cells, its expression in vivo is low in normal tissue but high in inflamed tissue or in lymph nodes draining sites of inflammation (Dustin et al., 1986; Cotran et al., 1987), where afferent or efferent immune interactions would be expected to take place. In vitro, the normally low basal expression of ICAM-1 on dermal fibroblasts, keratinocytes, and endothelial cells can be dramatically increased by treatment with the inflammatory mediators interferon-γ, IL-1, TNF, or lymphotoxin, and the increased ICAM-1 expression directly correlates with increased LFA-1-dependent adhesion of lymphocytes to the induced cells (Dustin et al., 1986; Pober et al., 1986, 1987).

The data presented here demonstrate that ICAM-1 alone, in the absence of other associated proteins, is sufficient to mediate lymphocyte adhesion directly. One proposed mechanism by which MAbs could inhibit cell–cell adhesion is by induction of "negative signals", or by inhibition of functions required for normal physiology such as membrane transport (Martz, 1986; Golstein et al., 1982). Our results argue strongly against the hypothesis that anti-ICAM-1 MAbs inhibit adhesion between cells by the induction of a negative signal. It is difficult to conceive of such a mechanism operating within the artificial, defined lipid membranes. Although our data do not directly discount the theory that MAb binding to LFA-1 induces intracellular signals, our evidence that ICAM-1 is a direct adhesion ligand supports the concept that MAbs to LFA-1 and ICAM-1 inhibit by steric hindrance of receptor–ligand interactions. Furthermore, the hypothesis that anti-LFA-1 MAbs inhibit by interference with ligand-binding functions is consistent with observations that cells genetically deficient in LFA-1 expression are defective in both intercellular adhesion (Rothlein and Springer, 1986; Springer et al., 1987) and adhesion to purified ICAM-1 as shown here. In those LFA-1$^-$ cells, LFA-1 would not be present to deliver an inhibitory signal.

Although ICAM-1 alone is sufficient to mediate adhesion, the characteristics of cell binding to purified ICAM-1 suggest that the overall lymphocyte adhesion process itself may be complex. The requirement for an intracellular supply of energy (based on inhibition by sodium azide plus 2-deoxy-D-glucose) demonstrates that adhesion to ICAM-1 is an active process that requires more than the presence of receptor and ligand on opposing membranes. The inhibition of cell binding with cytochalasin B suggests that LFA-1 on the cell surface may be functionally linked to the cytoskeleton. Both LFA-1 and the cytoskeletal component talin have been found to redistribute to sites of T-lymphocyte adhesion to cells presenting specific antigen (Singer and Kupfer, 1987). The extracellular matrix receptor homologs of LFA-1, i.e., the fibronectin receptors, are closely associated with the cytoskeleton, and especially closely with talin (Hynes, 1987; Horwitz et al., 1986).

The temperature dependence of cell binding to purified ICAM-1 is also consistent with LFA-1-dependent adhesion as an active, dynamic process. The reduced cell binding observed at 14°C and the absence of binding at 4°C could be due to either effects on metabolic energy production or changes in membrane fluidity. This temperature dependence is not an inherent feature of the artificial membrane adhesion model, since at 4°C, T-lymphoblasts can bind efficiently to the unrelated LFA-3 molecule reconstituted into planar membranes by using the CD2 glycoprotein as a receptor (Dustin et al., 1987). These findings with artificial membranes are in agreement with observations on CTL adhesion to target cells, which showed that the LFA-1 pathway is temperature dependent while the CD2/LFA-3 pathway functions efficiently at 4°C (Shaw et al., 1986).

We have recently determined the complete primary structure of the LFA-1 α subunit (Larson and Springer, unpublished data). The requirement for Mg^{+2} in the interaction of LFA-1 with ICAM-1 correlates with the presence of putative divalent cation–binding sites within the LFA-1 α subunit. LFA-1 α is highly homologous to p150,95 α (Corbi et al., 1987) and other integrin α subunits. Divalent cation–binding sites within the α subunits and a cation requirement for ligand binding appear to be general properties of the integrin family (Hynes, 1987; Corbi et al., 1987;

Poncz et al., 1987; Argraves et al., 1987; Suzuki et al., 1987). Although an RGD-containing hexapeptide based on the fibronectin sequence does not inhibit the interaction between LFA-1 and ICAM-1, it is possible that LFA-1 recognizes an RGD-like sequence within ICAM-1.

By analogy with the RGD receptors, it might be expected that LFA-1 could bind to more than one ligand. We have previously suggested that ICAM-1 is not the sole ligand for LFA-1 since anti-ICAM-1 MAb does not inhibit the LFA-1-dependent aggregation of some cell types (Rothlein et al., 1986), does not inhibit LFA-1-dependent CTL interactions with some target cells (Makgoba et al., submitted), and only partially inhibits the adhesion of some lymphocytes to endothelial cells (M. D. Dustin and T. A. Springer, unpublished data). The ability of LFA-1 to bind to alternative ligands, perhaps with different cellular distribution and regulation of expression compared to ICAM-1, would lend a fine degree of discrimination and flexibility to the adhesive interactions of leukocytes with other cells. Although such alternative ligands have not been identified, the artificial membrane cell-binding system described here provides a means of testing the hypothesis.

The finding that LFA-1 functions by binding to a specific ligand, as do the members of the integrin family of extracellular matrix receptors, provides a functional correlate to the structural homology between these receptors. It is interesting that the integrin family has evolved to function in both cell–cell and cell–matrix interactions.

Experimental Procedures

Monoclonal Antibodies

The mouse anti-human MAbs TS1/22 and TS1/18 (anti-LFA-1 α and β subunit, respectively; Sanchez-Madrid et al., 1983), TS2/9 (anti-LFA-3; Sanchez-Madrid et al., 1982), and RR1/1 (anti-ICAM-1; Rothlein et al., 1986) have been previously described and were used as purified IgG1 preparations (Ey et al., 1978).

Purification of ICAM-1 by Monoclonal Antibody Affinity Chromatography

ICAM-1 was purified from detergent lysates of the EBV-transformed B-lymphoblastoid cell line JY by affinity chromatography using the anti-ICAM-1 monoclonal antibody RR1/1 (Rothlein et al., 1986). All chromatography steps were performed at 4°C, and all buffers contained 0.2 U/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 50 μM sodium vanadate. RR1/1 was purified from the ascites fluid of hybridoma-bearing mice, or from hybridoma culture supernatants by ammonium sulfate precipitation and protein A affinity chromatography (Ey et al., 1978). The purified IgG, or rat IgG (Sigma Chemical Co., St. Louis, MO), was covalently coupled to Sepharose CL-4B (Pharmacia, Piscataway, NJ) at a concentration of 4 mg of IgG per ml of gel (March et al., 1974). JY cells were grown to approximately 1 × 10^6 cells per ml in RPMI-1640 containing 10% fetal bovine serum and 10 mM HEPES (complete medium). To increase the cell surface expression of ICAM-1, phorbol 12-myristate 13 acetate (PMA) was added at 25 ng/ml for 6–8 hr before cells were harvested. For one preparation, PMA was omitted. Sodium vanadate (50 μM) was also added to the cultures during this time. The cells (approximately 6 g per 5 liters of culture) were washed in Hank's Balanced Salt Solution (HBSS) and lysed in 100 ml of lysis buffer (0.14 M NaCl, 50 mM Tris, pH 8.0; 1% Triton X-100) by stirring at 4°C for 30 min. Unlysed nuclei and insoluble debris were removed by centrifugation at 10,000 × g for 15 min, followed by centrifugation of the supernatant at 150,000 × g for 1 hr and filtration through 0.45 uM membrane filters. A 1 ml pre-column of rat IgG-Sepharose attached in series to a 1 ml column of RR1/1-Sepharose was extensively prewashed with pH 12.5/Triton buffer (50 mM triethylamine, 0.1% Triton X-100 [pH 12.5] at 4°C), and then equilibrated with lysis buffer. After loading of the detergent lysate at 1 ml per min, the RR1/1-Sepharose column was disconnected and washed sequentially at a flow rate of 1 ml per min with 20 ml of each of the following: lysis buffer; 20 mM Tris (pH 8.0), 0.14 M NaCl, 0.1% Triton X-100; 20 mM glycine (pH 10.0), 0.1% Triton X-100; 50 mM triethylamine (pH 11.0), 0.1% Triton X-100; and 50 mM triethylamine (pH 11.0), 1% n-octyl-β-D-glucopyranoside (octylglucoside, OG). The bound ICAM-1 was eluted with 50 mM triethylamine (pH 12.5), 1% octylglucoside, at a flow rate of 1 ml per 3 min. Fractions (0.5 ml) were collected and immediately neutralized by the addition of 0.1 volume of 1 M Tris, pH 6.7. Fractions containing the eluted ICAM-1 were identified by SDS–polyacrylamide electrophoresis (Springer et al., 1984), followed by silver staining (Morrissey, 1981). The fractions containing ICAM-1 were pooled and concentrated approximately 20-fold by using Centricon-30 microconcentrators (Amicon, Danvers, MA), and reanalyzed by SDS–8% PAGE. As estimated by silver staining and comparison to standard proteins, approximately 5 μg of ICAM-1 was recovered.

Preparation of Artificial Supported Lipid Membranes

Artificial lipid vesicles containing ICAM-1 or the control protein glycophorin (Sigma Chemical Co., St. Louis, MO) were prepared as previously described (Dustin et al., 1987). Purified ICAM-1 or glycophorin was incorporated at a final concentration of 2.5 μg/ml in the vesicle suspension. In some experiments, HBSS lacking Mg^{2+} and Ca^{2+} was used in the final dialysis steps of the preparation. Planar membranes were prepared on glass coverslips (Brian and McConnell, 1984). To prepare supported lipid membranes bound to plastic surfaces, 30 μl of vesicle suspension was added directly to the bottom of wells in 96-well tissue culture plates (Falcon), followed by incubation and washing as described for planar membranes on glass coverslips. These preparations are referred to as plastic-bound vesicles (PBV).

Cell Adhesion Assays

Cell adhesion assays using planar membranes or PBV were both done in essentially the same way, except that the cell numbers and volumes for PBV assays were reduced to one-fifth that used in planar membrane assays.

T-lymphoblasts were prepared (Dustin et al., 1987) from normal controls and from a leukocyte adhesion deficiency (LAD) patient whose cells fail to express LFA-1 (Anderson et al., 1985), and were used between 10 and 22 days after the start of culture.

To detect cell binding, cells were radiolabeled by incubation of 10^7 cells in 1 ml of complete medium with 100 μCi of $Na_2{}^{51}CrO_4$ for 1 hr at 37°C, followed by four washes in complete medium. In MAb blocking experiments, purified MAbs (20 μg/ml) were added to the assay, or cells and PBV were pretreated with 20 μg/ml of antibody in complete medium at 4°C for 30 min, followed by four washes with complete medium to remove unbound antibody. Synthetic peptides were provided by Dr. M. Pierschbacher, La Jolla Cancer Research Foundation, San Diego, CA. In experiments on the effects of divalent cations on cell binding, the cells were washed once with HBSS lacking Ca^{2+} and Mg^{2+} plus 5 mM EDTA, followed by three washes and final resuspension in Ca^{2+}- and Mg^{2+}-free HBSS plus 10% dialyzed FCS. CaCl and MgCl were added back to concentrations indicated in the text. In all experiments, cells and planar membranes or PBV were preequilibrated at the appropriate temperature (4°C, 14°C, 22°C, or, in most experiments, 37°C) in the appropriate assay medium.

To measure cell binding to purified ICAM-1 in supported lipid membranes, ^{51}Cr-labeled cells (5 × 10^5 EBV transformants in planar membrane assays; 1 × 10^5 EBV transformants or SKW-3 cells, 2 × 10^5 T-lymphoblasts in PBV assays) were centrifuged for 2 min at 25 × g onto the supported membranes, followed by incubation at 4°C, 14°C, 22°C, or 37°C for 1 hour. Where indicated, the centrifugation was omitted and cells were allowed to settle at 1 × g. After incubation, unbound cells were removed by eight cycles of filling and aspiration with buffer preequilibrated to the appropriate temperature. Bound cells were solubilized with 0.1 N NaOH/1% Triton X-100 and were gamma counted. Percent of cell binding was determined by dividing cpm from bound cells by input cell–associated cpm (cpm released into the medium during incubation were subtracted from total input cpm). In planar membrane assays, input cpm were corrected for the ratio of the surface area of coverslips compared to the surface area of the culture wells.

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