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Immunology

# Direct evidence for a receptor–ligand interaction between the T-cell surface antigen CD2 and lymphocyte-function-associated antigen 3

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ABSTRACT **The recognition of foreign antigen by T lymphocytes requires direct contact with cells expressing the antigen. It has recently become clear that T lymphocytes can form conjugates with other cells in the absence of foreign antigen expression. Studies using monoclonal antibodies (mAbs) to inhibit conjugate formation have suggested that a portion of the antigen-dependent adhesion is mediated by T lymphocytes interacting with cells expressing lymphocyte-function-associated antigen 3 (LFA-3), a widely distributed cell surface protein. We have investigated antigen-independent adhesion by incorporating affinity-purified LFA-3 into the lipid membrane of an artificial target cell (ATC; a nylon-matrix vesicle with a lipid membrane). These vesicles are similar in size and density to intact cells, so that conjugates between cells and ATCs may be seen by light microscopy. ATCs expressing a density of LFA-3 similar to that on intact cells were found to form conjugates with T cells, but only if the T cells expressed the sheep erythrocyte receptor, CD2 (T11; LFA-2). Previous studies using mAbs have implicated the CD2 molecule in both adhesion and T-cell activation. ATCs prepared without surface protein or with purified HLA class I protein failed to interact with the CD2-positive T cells, indicating that the adhesion found was mediated by the LFA-3 molecule. Furthermore, mAb against LFA-3 or CD2 was able to block the LFA-3-mediated vesicle–cell interaction, whereas mAb against LFA-1 or HLA failed to inhibit the interaction. These results provide direct evidence that LFA-3 functions as an adhesion molecule by serving as a ligand for the CD2 molecule on T cells.**

Normal activation of T lymphocytes requires direct binding to a stimulator or target cell and recognition of antigen on the surface of the bound cell (1). Adhesion of a T lymphocyte to another cell, however, does not require that cell to express antigen (2, 3). This antigen-independent adhesion may be a prerequisite for the interaction of the antigen-specific receptor on the T cell with antigen on the target cell and subsequent triggering (1–3).

The T-cell surface molecule CD2 appears to be involved in both binding and activation (2–10). CD2 has been implicated in adhesion by the ability of anti-CD2 monoclonal antibody (mAb) to block antigen-independent adhesion between T cells and other nucleated cells (2, 3). Furthermore, mAb against CD2 has been shown to block T-cell adhesion with sheep erythrocytes (7, 8), and soluble affinity-purified CD2 will also block the interaction between sheep erythrocytes and human T cells (11). Other experiments suggest that CD2-mediated adhesion may be involved with regulating T-cell activation. Certain mAbs directed against CD2 apparently can activate T cells by induction of interleukin 2 receptor expression and release of interleukin 2 (9, 10). In contrast, another mAb against CD2 has been shown to inactivate T cells, rendering them refractory to stimulation (1, 12). Thus the role of the CD2 molecule in normal T-cell function is unclear.

The surface protein LFA-3 (lymphocyte-function-associated antigen 3) was initially defined by mAb blocking of cytotoxic-T-lymphocyte-mediated lysis and inhibition of mitogen- or alloantigen-induced proliferation (4–6). LFA-3 is a non-major histocompatibility complex (MHC)-encoded protein (13) that is broadly distributed on both lymphoid and nonlymphoid cells (5). Although expressed on T cells, the molecule appears to function via its expression on stimulator or target cells (5, 6, 14). Studies based on mAb blocking have suggested that LFA-3 is involved in antigen-independent adhesion and may be the ligand for CD2 (2, 3, 15). Recent data showing that anti-LFA-3 can block the binding of erythrocytes to T cells support the idea that LFA-3 is the ligand for erythrocyte receptor (16).

Given the number of different cell surface molecules, experiments using intact cells to investigate the proteins involved in T-lymphocyte adhesion will seldom be definitive. In the present study, we investigated receptor–ligand interactions by using an approach that circumvents the problem of target cells expressing many different ligands. Cell-size vesicles that have a continuous lipid membrane surrounding a semipermeable nylon matrix were prepared (17). These spherical vesicles, termed artificial target cells (ATCs), are similar in density to intact cells and can be easily sedimented by centrifugation and visualized with a light microscope. In another study, we found that ATCs expressing anti-CD3 mAb can trigger cytolytic T lymphocytes, resulting in the permeabilization of the sealed lipid membrane surrounding the ATC (S. J. Mentzer, M.L.R., S.J.B., and S.H.H., unpublished observations). In the present study, we purified LFA-3, incorporated the affinity-purified protein into ATC membranes, and demonstrated direct binding of these ATCs to T cells expressing CD2.

## MATERIALS AND METHODS

**Cell Lines and mAbs.** The following cell lines were used: JY, an Epstein–Barr virus-transformed B cell line; Jurkat, a T-cell line expressing CD2; HPB-MLT.2, a T-cell line expressing very low levels of CD2; Hut-78, HL60, K562, and DAP, cell lines not expressing CD2. These cell lines were passaged *in vitro* in RPMI 1640 supplemented as described (3). The following mAbs were used: anti-framework HLA, W6/32 (IgG2a) (American Type Culture Collection); anti-LFA-3, TS2/9 (IgG1) (4–6); anti-CD2, TS2/18 (IgG1) (4–6); anti-LFA-1 $\beta$ chain, TS1/18 (IgG1) (4–6). mAbs were produced *in vitro* as described or *in vivo* as ascites in pristane-primed BALB/c mice. mAb W6/32 was purified on a protein A-Sepharose column as described (18). The anti-LFA-3 mAb TS2/9 (4–6) was purified from ascites by preparative ion-exchange high-pressure liquid chromatography column (Dynamax 30-nm Ax, Rainin, Woburn, MA).

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Abbreviations: ATC, artificial target cell; LFA-3, lymphocyte-function-associated antigen 3; mAb, monoclonal antibody.

**Conditions for Affinity Purification.** Purified antibody was coupled to Sepharose 4B (Pharmacia) as described (18, 19). We used the human B-cell line JY (HLA-A2, B7; DRw4, w6) as a source of LFA-3, since these cells have been shown to express functional LFA-3 by mAb blocking studies (4–6, 14).

Anti-LFA-3 Sepharose was prepared and elution conditions were determined using the detergent lysate of $^{125}$I-labeled JY cells as a source of $^{125}$I-labeled LFA-3. Surface labeling, detergent solubilization, and immunoprecipitation were as described (18). After precipitation the anti-LFA-3 beads were divided into equal aliquots, each of which was subjected to a different elution condition. Eluted samples were analyzed by $NaDodSO_4$/polyacrylamide gradient gel (5–15%) electrophoresis under reducing conditions, using the buffer system of Laemmli (20). The $^{125}$I-labeled precipitated protein was determined by autoradiography. This approach was used to select the mildest conditions that eluted the protein, as well as to select conditions that did not elute LFA-3 but were sufficiently harsh to pre-elute nonspecifically bound proteins. This approach resulted in selection of 1 M NaCl for washing the anti-LFA-3 affinity column and a buffer with a pH of 3 for elution.

**Affinity Purification of LFA-3 and HLA.** JY cells ($2.5 \times 10^9$) were washed three times with phosphate-buffered saline (PBS: 10 mM sodium phosphate/140 mM NaCl, pH 7.2) containing 0.2 mM phenylmethylsulfonyl fluoride and solubilized by a 60-min incubation on ice with 2% (vol/vol) Triton X-100 in PBS containing 0.2 mM phenylmethylsulfonyl fluoride, 5 mM iodoacetamide, and 0.2 trypsin-inhibitor units of aprotinin per ml (2 ml per $10^8$ cells). Lysate was centrifuged at $200 \times g$ for 10 min to remove nuclei and at $100{,}000 \times g$ for 60 min to remove insoluble material and then filtered through a 0.45-$\mu$m filter (Millipore); this filtered material was referred to as soluble lysate. Affinity columns, each with a 5-ml bed volume and attached in series, consisted of a pre-column, human IgG-Sepharose 4B; an anti-HLA column, W6/32-Sepharose 4B; and an anti-LFA-3 column, TS2/9-Sepharose 4B. The lysate was added across the columns at 20 ml/hr. The columns were washed in series with 50 ml of 0.5% Triton X-100/PBS. The anti-HLA and anti-LFA-3 columns were individually washed with 50 ml of 0.5% Triton X-100/1 M NaCl/20 mM phosphate buffer, pH 7.2, and then with 50 ml of 9 mM taurocholic acid/150 mM NaCl/20 mM phosphate buffer, pH 7.2. The LFA-3 was eluted from the affinity column by adding 9 mM taurocholic acid/150 mM NaCl/20 mM acetate buffer, pH 3. Fractions (2 ml) were collected in tubes containing sufficient Hepes buffer to raise the pH of the eluted samples to 7.0. The HLA was eluted under conditions similar to those given in a previous report (21), with 9 mM taurocholic acid/150 mM NaCl/20 mM carbonate buffer, pH 11.5.

**Analysis of Purified Protein.** Protein concentration was assessed by subjecting an aliquot of the purified protein to hydrolysis in a sealed glass tube containing 6 M HCl for 12 hr at 100°C. The concentration of alanine in the hydrolysate was determined by enzymatic analysis (22); bovine serum albumin was used as the protein standard. An aliquot of affinity-purified protein (5–12 $\mu$g) was acetone-precipitated as described (18), heated in Laemmli sample buffer (20) under reducing conditions, and analyzed by $NaDodSO_4$/polyacrylamide gel elctrophoresis as described above. The location of the protein bands was determined by Coomassie blue or silver staining (Bio-Rad).

Serological activity of the purified proteins was determined by dot blot analysis. Aliquots (50 $\mu$l) of fractions eluted from the affinity columns were applied to nitrocellulose paper (Schleicher & Schuell) and reactive sites were blocked using "blotto" (5% Carnation low-fat dry milk/0.005% Tween 20/150 mM NaCl/50 mM Tris buffer, pH 8). LFA-3 was detected by the addition of anti-LFA-3; following a 60-min incubation the nitrocellulose was washed in blotto and an indicator antibody, horseradish peroxidase-conjugated goat anti-mouse IgG heavy and light chains (Miles), was added. The peroxidase was then located using 4-chloro-1-naphthol (Bio-Rad) as substrate. Dot blots were also done to indicate the position of eluted HLA, using W6/32 as first antibody.

**Preparation of ATCs.** These spherical vesicles consist of a porous nylon matrix surrounding an aqueous compartment containing a trapped polymer. The nylon-matrix vesicles were prepared by a modification of the method of Chang (17). The vesicles were prepared by polymerization of nylon monomer (1,6-hexanediamine) around a microdroplet of buffer also containing hemoglobin and Ficoll. Polymerization was accomplished using an acid dichloride to crosslink amines at the water/organic solvent interface. This type of interfacial polymerization was initially described by Wittbecker and Morgan (23). The polymer provides colloidal osmotic pressure and keeps the vesicles spherical. The hemoglobin is trapped within the vesicle as well as crosslinked with nylon at the vesicle surface (17). Vesicles prepared without hemoglobin are much more susceptible to rupture.

The nylon vesicles were prepared as follows. 1,6-Hexanediamine (230 mM) (Aldrich) in carbonate buffer (800 mM) at pH 10.2 was mixed with hemoglobin (300 $\mu$M) and Ficoll 400 (350 $\mu$M) (Pharmacia). The solution (5 ml) was mixed with 5 volumes of 4:1 cyclohexane (Aldrich)/chloroform (Fisher) containing 5% (vol/vol) sorbitan trioleate (Fluka) in a 150-ml Pyrex beaker. The mixture was stirred 2 min (3-cm magnetic stirring bar) to form an emulsion and then was sonicated briefly ($\approx$10 sec in an ultrasonic bath) to form microdroplets. To crosslink the nylon present at the interface between the aqueous and organic solvents, sebacoyl chloride (19 mM) and terephthaloyl chloride (9.5 mM) (Aldrich) in an additional 25 ml of organic solvent were rapidly added and the solution was stirred for another 2 min. The mixture was then transferred to a 50-ml glass-stoppered Pyrex tube and the vesicles were allowed to settle at 4°C. Vesicles were washed twice with cyclohexane and once with tetradecane, letting the vesicles sediment at room temperature. Vesicles were suspended in 20 ml of tetradecane and added to 60 ml of 0.2% Triton X-100 (Fluka) in PBS in a 100-ml capped bottle (Bellco Glass, no. 5637). After vigorous mixing for 20 sec, either the vesicles were allowed to sediment into the aqueous phase overnight or the bottles were centrifuged briefly at $300 \times g$.

The sedimented vesicles were washed several times by suspension in 0.2% Triton X-100/PBS followed by centrifugation at $300 \times g$. Because the vesicles were heterogeneous in size (up to several hundred micrometers), it was necessary to isolate the smaller vesicles by velocity sedimentation. Vesicles were resuspended in 10 ml and centrifuged at $50 \times g$ for 5 min; the supernatant was removed and centrifuged at $300 \times g$ for 5 min. The vesicles that sedimented at $300 \times g$ were found to have the following average size distribution: 2% were $\geq$40 $\mu$m; 11% were $<$40 $\mu$m and $>$12.5 $\mu$m; 87% were $\leq$12.5 $\mu$m and $>$5 $\mu$m.

The lipid membrane was constructed by covalent attachment of phosphatidylethanolamine to the nylon vesicles followed by detergent dialysis in the presence of added lipid. Nylon spheres ($10^8$) were incubated with 10 mM iminothiolane (Pierce) in pH 9 buffer with 8 mM dithiothreitol for 15 min at 24°C. Then the spheres were washed with PBS (pH 7), and *N*-[4-(*p*-maleimidophenyl)butryl] egg phosphatidylethanolamine (200 nmol) (Avanti Polar Lipids, Birmingham, AL) in 0.5 ml of 100 mM octyl $\beta$-D-glucoside was added at 24°C and incubated for 12 hr. After several washes the nylon spheres were suspended in PBS containing the following lipids solubilized with taurocholic acid: phosphatidylcholine

(16 μmol), phosphatidylethanolamine (12 μmol), phosphatidylserine (4 μmol), phosphatidylglycerol (1 μmol), and cholesterol (10 μmol). After dialysis against three changes of PBS over a 72-hr period, the ATCs were harvested, washed with PBS, and reconstituted with purified proteins.

**Preparation of ATCs Expressing Membrane Proteins.** Reconstitution of purified surface protein into the ATC membranes was accomplished as follows. The affinity-purified protein (10 μg of LFA-3 or 6 μg of HLA) in 9 mM taurocholic acid was mixed with PBS containing lipid-sealed ATCs ($10^7$ vesicles), diluting the final detergent concentration to 1 mM. This mixture was dialyzed against two changes of PBS (1000 volumes each) over a 48-hr period to remove the taurocholic acid. The ATCs were harvested from the dialysis tubing, and any purified protein not associated with the ATC was removed by three cycles of centrifugation at 300 × *g*, followed by suspension in PBS/0.1% bovine serum albumin. Protein addition to ATCs by dilution and dialysis minimizes solubilization of the lipid associated with the ATC. As a control, ATCs with a lipid membrane but lacking purified proteins were prepared in parallel.

**Flow Cytometry.** Surface expression of membrane proteins on intact cells and ATCs was analyzed with a Coulter Epics V cell sorter. Cells or vesicles ($10^5$) were incubated with 50 μl of culture supernatant containing ≈2.5 μg of the indicated mAb plus 50 μg of human IgG (Sigma) for 40 min on ice in medium with 0.02% azide. Samples were washed twice, incubated with fluorescein-conjugated goat $F(ab')_2$ anti-mouse IgG (Tago, Burlingame, CA) for another 30 min on ice, and washed twice. Cells were fixed with 1% paraformaldehyde before flow cytometry.

**Adhesion Assay.** To discriminate intact cells from the ATCs, the human tumor cells were labeled by incubation with fluorescein diacetate (10 μg/ml; Molecular Probes, Junction, OR) for 10 min; followed by three washes. ATCs reconstituted with LFA-3 or HLA, or without added protein, were incubated with Jurkat cells or HPB-MLT.2 cells at a ratio of 1:1 ATCs and T cells (both at 2 × $10^5$ per ml). Cells and vesicles (≈50 μl each) were mixed in RPMI 1640/5% fetal bovine serum and centrifuged at 40 × *g* in a 0.5-ml microcentrifuge tube for 2 min. To the indicated samples, mAb was added when the ATCs and Jurkat cells were mixed. Samples were incubated on ice for 60 min, gently resuspended five times with a disposable plastic pipette (tip i.d. 0.5 mm) and observed with a light microscope, using fluorescence to locate the cells and phase contrast to determine the location of the ATCs.

To quantitate the percentage of human cells conjugated with ATCs, a small aliquot of sample was placed on a glass slide, and a 12-mm coverslip was placed over the sample and held in place with fingernail polish. Cells were observed in suspension; a cell was scored as a conjugate if tightly associated with at least one ATC. For each replicate, 100 cells chosen at random were scored as conjugates or nonconjugates. The percentage of conjugates was calculated as the total number of cell–ATC conjugates observed divided by the total number of cells observed.

## RESULTS

**Purification of LFA-3 and HLA.** LFA-3 was purified from the soluble detergent lysate of JY cells by means of an anti-LFA-3 affinity column. Conditions found to give the best yield of highly purified LFA-3 retaining serological activity were determined as described in *Materials and Methods*. The fractions containing the eluted protein were determined by dot blot analysis (data not shown). Control dot blots showed that anti-LFA-3 mAb was not eluted from the affinity column under these conditions. HLA class I proteins were also purified from JY cells, using an anti-HLA (W6/32) column attached in series with the anti-LFA-3 column. The position of the eluted HLA was determined by dot blot analysis (data not shown).

Purified LFA-3 represented 0.05% of the initial protein in the soluble detergent lysate, and an 800-fold purification was achieved as judged by dot blot analysis (data not shown). By comparison, purified HLA represented 0.1% of the initial protein in the soluble lysate, and dot blot analysis indicated a 400-fold purification (data not shown). Analysis of microgram amounts of purified protein by gel electrophoresis revealed a broad band at $M_r$ 60,000–80,000 for LFA-3 and two major bands, at $M_r$ 44,000 (heavy chain) and 12,000 ($\beta_2$-microglobulin), for HLA (Fig. 1). Even though similar amounts of LFA-3 and HLA gave dot blots of similar density (data not shown), purified LFA-3 stained more weakly with Coomassie blue than did HLA; silver staining was necessary to visualize LFA-3 (Fig. 1). Poor staining with Coomassie blue would be consistent with a high level of carbohydrate on the LFA-3 molecule.

**Membrane Protein Expression by ATCs.** Cell-size spherical nylon vesicles were prepared by a modification of the method of Chang (17). A lipid membrane was associated with the nylon vesicles by first covalently attaching lipid to the nylon surface, using phosphatidylethanolamine modified with a bifunctional cross-linker. This covalently attached lipid served as a template for the formation of the lipid membrane added subsequently by detergent dialysis. The outer membrane was prepared using commercial lipid selected to give a lipid composition similar to that of an intact cell. The resulting spherical vesicles (ATCs) were similar in size and density to intact cells and were easily centrifuged and viewed with a microscope.

Lipid-sealed ATCs were reconstituted with purified LFA-3 or HLA. Once associated with the ATC membrane, the reconstituted proteins were not displaced by repeated washing. ATCs labeled by indirect immunofluorescence and examined under a microscope displayed a uniform surface fluorescence when the appropriate first antibody was used (data not shown). Flow cytofluorometry showed that ATCs reconstituted with LFA-3 expressed the same level of LFA-3 surface fluorescence as JY cells (Fig. 2 *e* vs. *c*). ATCs reconstituted with HLA gave fluorescence levels comparable with HLA found on JY cells (Fig. 2 *f* vs. *d*).

**Conjugate Formation Between T Cells and ATCs with Surface LFA-3.** To determine whether affinity-purified LFA-3 reconstituted onto ATCs could bind to T lymphocytes, we used Jurkat, a human T-cell-tumor line that expresses CD2 (Fig. 2*a*). To aid in visualizing T-cell–ATC conjugates, Jurkat cells were first labeled with fluorescein diacetate. ATCs and T cells were mixed at a ratio of 1:1, centrifuged at 40 × *g* for 2 min, and incubated on ice for 60 min. Samples were gently

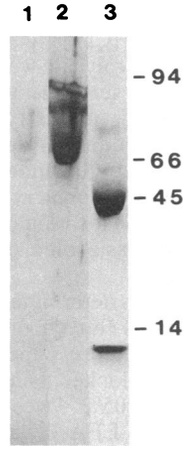


FIG. 1. Affinity-purified LFA-3 and HLA, analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Lane 1: LFA-3 (5 μg) stained with Coomassie brilliant blue. Lane 2: LFA-3 (5 μg), subjected to two cycles of silver staining (Bio-Rad). LFA-3 is the broad band at $M_r$ 60,000–80,000. The two bands toward the top of the silver-stained gel are background bands present in all lanes and likely to be a result of 2-mercaptoethanol. Lane 3: HLA (12 μg) stained with Coomassie brilliant blue. HLA class I heavy chain migrated at $M_r$ ≈44,000, while $\beta_2$-microglobulin migrated at $M_r$ ≈12,000. Positions of standards run in parallel are shown at right ($M_r$ × $10^{-3}$).

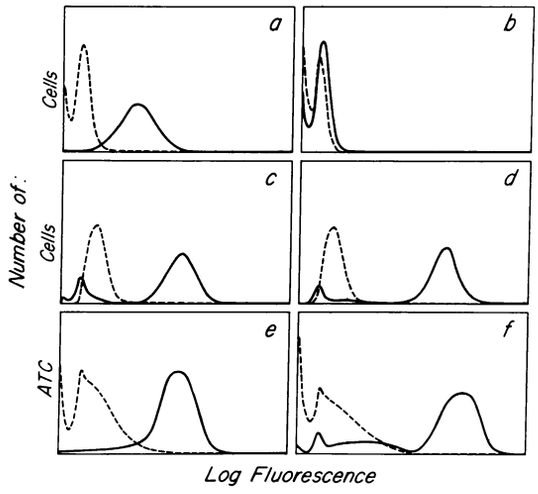


FIG. 2. Expression of surface molecules on intact cells and ATCs, shown by indirect immunofluorescence staining followed by flow cytofluorometry. (*a*) Jurkat cells. (*b*) HPB-MLT.2 cells. (*c* and *d*) JY cells. (*e*) ATCs reconstituted with LFA-3. (*f*) ATCs reconstituted with HLA class I proteins. Samples were incubated with 50 μl of culture supernatant containing 50 μg of human IgG (Sigma) and ≈2.5 μg of the following mAbs (solid traces): anti-CD2 mAb TS2/18 (*a* and *b*); anti-LFA-3 mAb TS2/9 (*c* and *e*); anti-HLA mAb W6/32 (*d* and *f*). Dashed lines represent background staining determined with fluorescein-conjugated goat anti-mouse IgG only.

suspended and the percentage of T cells bound to ATCs was determined by fluorescence and phase-contrast microscopy.

ATCs reconstituted with LFA-3 readily formed conjugates with the CD2-expressing human T-cell line Jurkat (Fig. 3 *a–d*). In contrast, ATCs reconstituted without cell surface protein formed very few conjugates with T cells (Fig. 3 *e* and *f*). To quantitate conjugate formation, three to six replicates for each condition were prepared and a total of 100 T cells for each replicate were scored for conjugate formation with

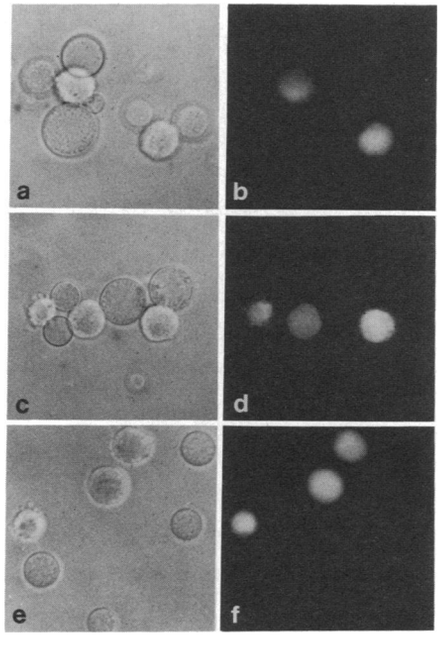


FIG. 3. Photomicrograph of adhesion between T cells and LFA-3 ATCs. Fluorescein diacetate-labeled Jurkat cells were incubated with ATCs reconstituted with LFA-3 (*a–d*) or with control ATC lacking surface protein (*e* and *f*). Matched light and fluorescence photomicrographs (*a* and *b*, *c* and *d*, and *e* and *f*) are shown for samples of ATCs and T cells viewed in suspension.

ATCs. The ATCs reconstituted with LFA-3 formed conjugates with about 30% of the Jurkat cells, while the ATCs reconstituted with HLA or without membrane proteins formed conjugates with 3% and 4% of the Jurkat cells, respectively (Fig. 4). ATCs containing purified murine H-2 antigens also failed to form conjugates with Jurkat. ATCs prepared with or without membrane proteins did not form ATC–ATC aggregates (data not shown). In parallel experiments, ≈30% of Jurkat cells formed rosettes with sheep erythrocytes (data not shown), in agreement with previous work (11). These data suggest that the conjugate formation between Jurkat cells and LFA-3-bearing ATC was specific for LFA-3.

**Adhesion Requires CD2 Expression on the T Cell.** The role of CD2 in the formation of conjugates with LFA-3 ATCs was demonstrated by comparing LFA-3 ATC binding to Jurkat cells, which express high levels of CD2, with binding to HPB-MLT.2 cells, which express very low levels of CD2 (Fig. 2 *a* and *b*). Less than 5% of HPB-MLT.2 cells formed conjugates with LFA-3 ATCs (Fig. 4). Conjugate formation was also <5% for LFA-3 ATCs incubated with cell lines (Hut-78.1, JY, K562, HL60, DAP) that either do not express CD2 or express very low levels of CD2 (data not shown).

Data similar to those presented in Fig. 4 were found in four separate experiments with three different preparations of affinity-purified LFA-3. Mixing the Jurkat cells at a ratio of 1 cell per 4 ATCs or at a 1:1 ratio resulted in the same percent conjugate formation. Thus the ATCs were not limiting. In some experiments the percentage of conjugates formed between LFA-3 ATCs and Jurkat cells was >30%; however, in these experiments the background conjugate formation was also increased. In all experiments the ratio of specific or LFA-3-mediated conjugates to background conjugates was about 10, similar to that in Fig. 4.

**Inhibition of Jurkat Interaction with LFA-3 ATCs.** Blocking studies using mAbs support the hypothesis that LFA-3 is bound by the CD2 molecule. Anti-CD2 or anti-LFA-3 mAb blocked conjugate formation between Jurkat cells and LFA-3 ATCs to background levels (i.e., by ≈90%; Fig. 4). mAbs to other T cell proteins (LFA-1 or HLA) failed to inhibit conjugates (Fig. 4). The inability of mAb against LFA-1 or HLA to block conjugates argues against nonspecific blocking

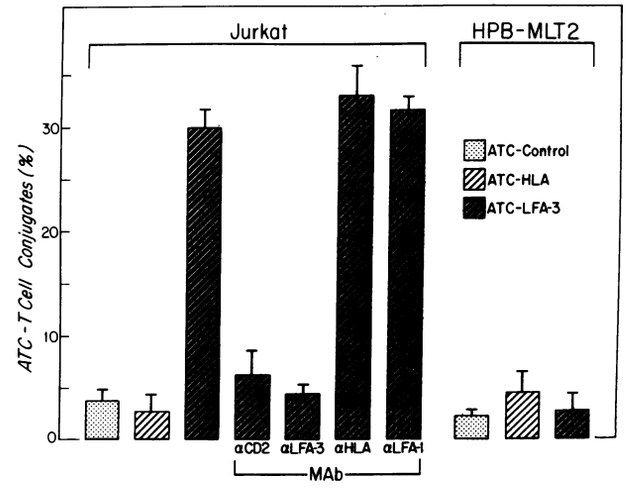


FIG. 4. Quantitation of T-cell adhesion to ATCs. Data are expressed as percent binding of Jurkat or HPB-MLT.2 cells to ATCs. As indicated the experiments were carried out in the absence or presence of mAb. Conjugate formation was determined for ATCs lacking surface proteins (▨), ATCs expressing LFA-3 (■), and ATCs expressing HLA class I proteins (▨). Data represent mean ATC–T-cell conjugates (± SEM) for triplicate samples, except for Jurkat incubated with LFA-3 ATCs (mean ± SEM for six replicates).

by the anti-CD2 mAb, since LFA-1 and HLA class I proteins are expressed at levels equal to or greater than the level of CD2 on the Jurkat cells. Thus mAb inhibition of conjugates provides further evidence that conjugate formation is a result of a receptor–ligand interaction between CD2 and LFA-3.

## DISCUSSION

T lymphocytes can adhere to cells in the absence of antigen recognition (2, 3). The cell surface molecules involved with antigen-independent adhesion appear to be of fundamental importance for immune function and antigen recognition (1, 3). To date all investigations of the receptors and ligands involved in this process have relied upon blocking T-lymphocyte function or adhesion with mAbs. This approach has indicated that the following T-cell surface proteins may act as receptors involved in antigen-independent adhesion: CD2, CD4, CD8, and LFA-1 (1). This is not an inclusive list and it is likely that other receptors may play a role in adhesion. There are several problems with relying solely on mAb blocking to define receptor–ligand interactions. It is possible that several receptor–ligand pairs of low avidity are involved in cell–cell adhesion. Blocking of one of these may disrupt the adhesion if the remaining pairs are unable to maintain the cell–cell binding. It is also possible that the binding of mAb to the cell surface may induce cellular effects unrelated to blocking of receptor–ligand interaction. Two reports (1, 12) have suggested that the ability of mAb to block function may be due to the delivery of a negative signal to the T cell rather than due to steric inhibition of receptor–ligand interaction.

The expression of many different cell surface proteins makes it difficult to use intact cells to examine the involvement of one receptor or ligand in cell–cell adhesion. We approached this problem by using spherical vesicles the size and density of intact cells. These vesicles (ATCs) consist of an outer nylon matrix covered by a lipid membrane. Isolated cell surface proteins were reconstituted into the ATC lipid membrane. LFA-3, as well as HLA class I proteins, were purified by use of mAb affinity column. Since the ATCs can be handled like cells, surface expression of incorporated protein can be readily analyzed by flow cytofluorometry. We have been successful in preparing ATCs expressing a wide range of membrane protein densities. The ATCs used in these studies expressed surface protein at a density similar to that found on JY, a human Epstein–Barr virus-transformed cell line (Fig. 2).

The ability of ATCs expressing surface LFA-3 to form conjugates with T cells was investigated. These vesicles are sedimented readily by centrifugation and can be observed easily under the light microscope, allowing one to visually record conjugates between ATCs and intact cells. Using these vesicles, we obtained data indicating that LFA-3 is a ligand for the T-cell surface protein CD2. This was demonstrated by two independent methods. First, ATCs with LFA-3 bound only to T cells expressing high amounts of CD2. ATCs with a high density of class I HLA protein formed conjugates with only 3% of Jurkat cells, compared to 30% for ATCs containing LFA-3 (Fig. 4). The second method examined the ability of mAb against LFA-3 to block ATC conjugates with Jurkat cells. We found that mAb against either LFA-3 or CD2 could block the interaction between T cells and LFA-3 ATCs, whereas mAb against either HLA or LFA-1 had no effect on conjugate formation. On average, LFA-3 ATCs formed conjugates with 30–50% of Jurkat cells. Recent studies by Dustin *et al.* (24) have also demonstrated binding of Jurkat cells to liposomes containing LFA-3.

In this assay system, formation of stable conjugates between T cells and ATCs depends on several variables, including the density of receptor and ligand, the relative affinity and avidity of this interaction, and experimental conditions such as the length of the incubation and the shear force exerted to mix the samples. Failure of the LFA-3 ATCs to form 100% conjugates may be due to any one or a combination of these variables. It should be possible to determine how the rate of conjugate formation is affected by individual variables, such as the density of ligand.

The finding that LFA-3 can mediate ATC binding to T cells expressing CD2, and the ability of anti-CD2 to block this interaction, strongly suggests that CD2 and LFA-3 are a receptor–ligand pair involved in cell–cell adhesion. ATCs, designed to mimic the size, lipid membrane, and protein surface density of an intact cell, provide a method for direct examination of the molecules involved in cell–cell adhesion and T-cell activation. While this report shows that LFA-3 is a ligand able to mediate cell adhesion, it is possible that the interaction of LFA-3 with its receptor may have other effects as well. Based on the ability of appropriate anti-CD2 mAbs to activate T cells, it is likely that the LFA-3–CD2 interaction may also be involved in T-cell activation.

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