

Motility and Ultrastructure of Large Granular Lymphocytes on Lipid Bilayers Reconstituted with Adhesion Receptors LFA-1, ICAM-1, and Two Isoforms of LFA-3

Olli Carpén,^{*‡} Michael L. Dustin,^{*‡} Timothy A. Springer,^{*‡} James A. Swafford,^{||} Laurel A. Beckett,[§] and John P. Caulfield^{‡||}

*The Center for Blood Research and ‡the Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115; and §Channing Laboratories and ||the Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, Massachusetts 02115

Abstract. Large granular lymphocytes, mediators of NK activity, bind to other cells using both the LFA (lymphocyte function-associated)-1-ICAM and the CD2-LFA-3 adhesion pathways. Here we have studied the motility and ultrastructure of large granule lymphocyte (LGL) on lipid bilayers containing purified LFA-1, ICAM-1, and the transmembrane and glycosphosphatidylinositol isoforms of LFA-3. LGLs moved at 8 $\mu\text{m}/\text{min}$ on ICAM-1 but poorly (<1 $\mu\text{m}/\text{min}$) on its receptor pair LFA-1. TM-LFA-3 promoted locomotion at a rate close to ICAM-1, whereas the cells were less motile on GPI-LFA-3. The difference in the rates of locomotion on the two isoforms of LFA-3 is presumably attributable to their difference in anchoring and lateral mobility in the bilayer. In spite of the variation in motility the ultrastructure of the adhering cells was similar on all four ligands. LGLs contacted the membrane variably, i.e., cells adhering only in a few small areas or in larger areas were detected on each ligand. The relative percentage of the plasma membrane facing the lipid bilayer was greatest on ICAM-1 and least on the transmembrane isoform of LFA-3, demonstrating no correlation with motility. The ratio of adjacent plasma

membrane to lipid bilayer was virtually constant for all four ligands. Activation of the LGLs with a combination of CD2 mAb T11₂ and T11₃ (T11_{2/3} mAb) reduced the movement on ICAM-1 and virtually immobilized the cells on the other bilayers. In the presence of T11_{2/3} mAb, the area of cell membrane attaching to bilayers containing ICAM-1 and GPI-LFA-3 was decreased and the percentage of plasma membrane facing other cells was increased. No preferential orientation of the Golgi apparatus or degranulation was detected in the absence or presence of T11_{2/3} mAb, but a significantly lower percentage of LGLs on ICAM-1 contained a profile of the Golgi apparatus after exposure to T11_{2/3} mAb. The results demonstrate that the motility of LGLs depends on the type of receptor in the opposing bilayer, the receptor mobility in the bilayer, and the activation of the cells. The ultrastructure of LGLs binding to any of the adhesion molecules does not have the characteristics of LGLs in cytolytic contact with target cells, suggesting that the mediation of an attack on a target requires more complex stimulus than any one of the single adhesion proteins tested here.

MANY lymphocyte functions, such as binding to target cells during cytolytic events, antigen presentation and helper functions, and extravasation and migration to sites of inflammation are dependent on adhesion receptors (35). Large granular lymphocytes (LGLs),¹

John P. Caulfield's present address is Syntex Research, Department of Molecular Immunology, Electron Microscopy Unit, 3401 Hillview Ave., P.O. Box 10850, Palo Alto, CA 94303.

1. *Abbreviations used in this paper:* GPI, glycosphosphatidylinositol; ICAM, intercellular adhesion molecule; LFA, lymphocyte function-associated; LGL, large granular lymphocyte; NK, natural killer; TM, transmembrane.

mediators of natural killer (NK) activity, are a subpopulation of lymphocytes that may serve as a first line of defense against tumor cells and virus-infected cells (41). LGLs in contact with target cells undergo characteristic ultrastructural changes (6, 17, 31, 46). In early phases of contact, the LGL membrane forms prominent interdigitations with the target cell membrane. Subsequently, this develops into a wide contact area, where the cell membranes are closely opposed at the periphery like a gasket and more widely separated to form a pocket in the center. The cytoskeleton and the Golgi apparatus, centrioles, and granules reorient toward the contact area and granules discharge into the pocket. In addition to their cytolytic ability, LGLs are highly motile as

has been shown *in vitro* and *in vivo* (5, 34, see 41). Both motility and cytolytic interactions are dependent in part on adhesion molecules in the plasma membrane of the LGL and the cell to which it attaches.

LGLs express the constituents of two important adhesion pathways. One of them involves an interaction between leukocyte-integrin lymphocyte function-associated molecule-1 (LFA-1, CD11a/CD18) and intercellular adhesion molecules (ICAM) and the other between CD2 and LFA-3. Of the three different leukocyte-integrins, all LGL express LFA-1 (CD11a) and a fraction of the cells additionally expresses Mac-1 (CD11b) and p150/95 (CD11c) (28, 40). Both LFA-1 and Mac-1 bind to ICAM-1 (9, 29); LFA-1 interacts with another ligand, ICAM-2 (37), and also with additional unidentified ligands (8). CD2, the receptor for the other adhesion pathway is expressed on most LGL (28, 41). It is a signal transducing glycoprotein that significantly enhances immune responses (36). Both the LFA-1-ICAM-1 interaction and the CD2-LFA-3 pathways are involved in LGL adherence to tumor cells (28) and certain mAb against CD2 induce increased NK activity against resistant target cells (4, 30, 33). Immunomodulators, such as IL-2, increase the expression of LFA-1, CD2, and ICAM-1 on LGLs (28). *In vitro*, IL-2 enhances LGL adherence to tumor cells and endothelial cells (2, 3), and *in vivo* leads to increased LGL extravasation (24). The receptors and counter-receptors that LGLs use during extravasation and migration to tissues have not been characterized.

In contrast to the expression of LFA-1, which is limited to leukocytes and CD2, which is limited to LGLs and T cells, their counter-receptors, i.e., ICAMs and LFA-3, respectively, are expressed on both hematopoietic and non-hematopoietic cells. LGLs express low amounts of ICAM-1, ICAM-2, and LFA-3, but are likely to express higher levels of yet unidentified ICAM(s) (8, 28). ICAM-1 and LFA-3 are present on endothelial cells and the expression of ICAM-1 on several cell types is increased by mediators secreted during inflammation, such as IL-1, TNF α and IFN γ (12). Thus, the receptors are of potential importance during margination and extravasation of lymphocytes. ICAM-1 is a transmembrane protein whereas LFA-3 exists in two different isoforms (36). The transmembrane isoform of LFA-3 (TM-LFA-3) contains a hydrophobic membrane-spanning region and a 12 amino acid cytoplasmic domain while glycosylphosphatidylinositol-linked LFA-3 (GPI-LFA-3) is anchored in the outer leaflet of the plasma membrane (14, 32, 45). The functional significance of the two isoforms is not known but both forms are fully able to mediate CD2-dependent adhesion (18).

LFA-1, ICAM-1, and both isoforms of LFA-3 can be immunoaffinity purified from cellular lysates. The molecules can be incorporated into liposomes, which, on glass coverslips form planar lipid bilayers. The bilayers, reconstituted with adhesion molecules at physiological site densities, promote lymphocyte adherence (10). This model system permits the study of receptor-counter-receptor interactions of each adhesion pathway separately. Cells do not attach to planar bilayers that do not contain adhesion proteins. Thus, adhesion of cells to bilayers with incorporated adhesion proteins must be mediated solely by these proteins (16). On the other hand, adhesion molecules attached to solid supports to which cells can also adhere independently do not represent pure systems.

Planar lipid bilayers offer a second advantage over other

types of solid supports, the possibility of lateral diffusion of incorporated molecules. Lateral diffusion of phospholipid analogues in glass supported planar bilayers above the transition temperature of the bilayer is rapid and similar to diffusion rates of phospholipids in biological membranes (26). However, transmembrane proteins are immobile in planar bilayers (26). Therefore, glycolipid-anchored adhesion proteins should diffuse freely in the supported bilayers like untethered transmembrane or glycolipid anchored proteins in natural membranes. On the other hand, transmembrane adhesion proteins in the planar membranes should be immobile.

In this study we have investigated how the adhesion receptor-ligand pairs support LGL locomotion. ICAM-1, LFA-1, and two forms of LFA-3 were purified and incorporated into glass supported planar bilayers. The counter-receptors LFA-1 and ICAM-1 allowed us to investigate the function of these molecules in a reciprocal manner. Membrane anchor isoforms of LFA-3 with identical CD2-binding sites but either transmembrane or glycolipid membrane anchors allowed us to investigate the influence of counter-receptor lateral diffusion on LGL movement. Since especially ICAM-1 and LFA-3 are present on target cell surfaces and these molecules are involved in the cytolytic events, we examined whether the morphology of cells binding to any of the reconstituted adhesion molecules would resemble LGL contacts with target cells. Finally, cells adherent to bilayers were activated with T11_{2/3} mAb directed against CD2 to see how activation affected motility and whether the LGLs would orient and degranulate against the bilayers.

The results show that LGLs can adhere to bilayers reconstituted with ICAM-1, LFA-1, or either form of LFA-3. Motility is dependent on the ligand in the bilayer and the state of activation of the cells. The ultrastructure of the LGLs adhering to membranes reconstituted with different adhesion molecules is similar. The LGLs do not flatten towards the bilayers either in the presence or absence of T11_{2/3} mAb but increase their contact areas with other cells after T11_{2/3} mAb treatment.

Materials and Methods

Cells

LGLs were enriched from platelet leukopheresis as described earlier (39). Briefly, platelets were first washed out by repeated centrifugation of the cells in PBS/10 mM EDTA and aspiration of the platelet-rich supernatant until the supernatant was clear. After removal of the plastic and nylon-wool adherent cells LGLs were enriched by Percoll density gradient centrifugation. The fractions containing LGLs were further depleted of CD3⁺ cells by coating the cells with CD3-specific mAb OKT3 and absorbing out the antibody-bearing cells with magnetic beads (Dyna; Robbins Scientific, Mountain View, CA) coated with F(ab)₂ goat anti-mouse IgG and IgM (Tago Inc., Burlingame, CA) according to the manufacturer's instructions. The cell population in all experiments consisted of 85–95% of cells with LGL morphology as judged by Giemsa-stained cytocentrifuge smears and >75% CD16⁺ cells by FACS[®] analysis.

Before the experiments the cell population was activated with rIL-2 (100 ng/ml) (Genetics Institute, Cambridge, MA) for 18 h at +37°C. During cell purification, rIL-2 activation, and motility experiments the cells were incubated in complete medium (RPMI 1640, supplemented with 10% FCS, 5 mM glutamine, and gentamycin).

Preparation of Protein-containing Lipid Bilayers

LFA-1 and ICAM-1 were immunoaffinity purified from lysates of the EBV-transformed B-cell line JY. TM-LFA-3 was purified from a JY-clone

deficient in synthesis of GPI-anchored molecules and GPI-LFA-3 from human erythrocytes which only express the GPI-linked form of LFA-3. The purification of these molecules in functionally active form and the preparation of liposomes containing adhesion proteins has been described previously (11, 13, 18). Planar bilayers were formed from the liposome preparations on 12-mm glass coverslips (Bellco Glass Inc., Vineland, NJ) as described earlier (10). Site densities of the reconstituted membranes were determined by radioimmunoassay (11). In preliminary experiments, adhesion receptor densities in the planar membrane yielding optimal efficiency of LGL attachment for each purified counter-receptor were determined and used subsequently. All experiments were done under optimal binding conditions since a relatively high density of adhering cells was essential for adequate samples sizes in EM. Site densities of each ligand that promoted comparable LGL binding to the bilayer were used as follows: ICAM-1, 300 sites/ μm ; LFA-1, 500 sites/ μm ; TM-LFA-3, 1,400 sites/ μm ; and GPI-LFA-3, 750 sites/ μm . No bound LGLs were detected on control lipid bilayers, which lacked adhesion proteins. Previously we have shown that lymphocytes do not adhere to planar membranes containing glycophorin (13, 25) and that mAb to relevant epitopes of LFA-3, ICAM-1, and LFA-1 block adhesion of lymphocytes to planar bilayers bearing the respective purified ligands (11, 13, 25).

Attachment of LGLs to Reconstituted Bilayers

LGLs (0.2×10^6) were plated on planar bilayers on coverslips in the wells of 24-well tissue culture plates. After an attachment period of 20 min at 37°C the plate containing coverslips was placed in Pyrex 190 \times 100 crystallizing dishes filled with medium. The coverslips were inverted to remove unbound cells and placed on glass slides. Silicone grease sealed a fluid chamber between the coverslip and the slide. During the mounting procedure the coverslips were kept submerged in medium to prevent the exposure of the bilayers to air.

A combination of two CD2 specific mAbs, T11₂ and T11₃ (27), was used to activate LGL through CD2. Both mAbs were added to the adherent cells in a 1:500 dilution of ascites. After 5 min of incubation with the mAb the cells were examined microscopically.

Recordings and Data Analysis

The motility of LGLs isolated from five different donors was measured with a Leitz Orthoplan microscope equipped with a 25 \times interference contrast objective. During the observation period the coverslips were kept at 37°C with an air stream stage incubator (model ASI 400; Nicholson Precision Instruments, Bethesda, MD). Cell motility was recorded for 8 min using a time-lapse video imaging system consisting of NuVicon camera (Dage MTI Inc., Wabash, MI) and Sony TVO 9000 tape recorder. Fields containing 10–20 cells were selected for recording. The individual cell paths were traced from the recordings on clear plastic placed on the monitor screen. The length of the movement of individual cells was measured using a digitizing tablet (The Morphometer, Woods Hole Educational Associates) attached to an IBM PS2. All recorded cells ($n > 10$ /condition) including non-motile cells were measured and averaged. Since different numbers of cells were measured in different experiments the data were combined as an arithmetic average of all cells measured. The precision of the mean motility dependent both on the number of cells averaged and on the variability from cell to cell, which varied considerably for different experiments. Thus, the sample size divided by sample variance was used as a weight for the experiment mean in comparative analyses.

Electron Microscopy

Cells on marked coverslips, prepared as described above, were allowed to adhere for 40 min at 37°C and were fixed in the culture medium with an equal volume of mixed aldehydes consisting of 1% glutaraldehyde and 3% formaldehyde in 0.1 M cacodylate, pH 7.4. Cells were washed twice in acetate veronal buffer containing 7% sucrose, post-fixed in 1% OsO₄ in veronal for 30 min, washed twice again, stained in block with uranyl acetate in veronal, washed twice more, and dehydrated in ethanol and propylene oxide. All fixation, staining, and washings were performed at room temperature. After an overnight infiltration at 4°C in a 1:1 mixture of resin and propylene oxide, the cells were embedded in pure resin by placing the coverslips on the edges of the wells of flat silicon molds so that the plastic cured beneath the coverslip. The coverslip was separated from resin by plunging into liquid N₂. The resin was then cut into pieces that were re-embedded with two pieces facing one another so that the sectioning plane passed through both pieces perpendicular to the plane of the coverslip. Sections

with silver grey interference colors, 50-nm thick, were cut with Swiss Diatome knives on LKB ultramicrotomes, picked up on naked 300 mesh copper grids, stained with uranyl acetate and lead citrate, carbon coated, and examined with a JEOL 100C/ASID electron microscope.

Stereology

All cells found on the bilayers were photographed at primary magnifications between 6,000 and 8,300 \times and printed with an enlarger magnification of 2 \times . Most cells were completely contained within the micrographs. Three sets of measurements were performed. First, the degree of apposition of the plasma membrane to the artificial bilayer was measured with a digitizer to estimate the degree of flattening against the artificial bilayer. The ends of the region of the plasma membrane attached to the substrate were marked and the length of both the plasma membrane in the attached region and the underlying bilayer were measured. In cases where the bilayer could not be seen its position was estimated to be a straight or curvilinear line that touched the tips of the plasma membrane at their greatest extensions. The ratio of these measurements was then calculated for each micrograph.

The second set of measurements was originally designed to measure cell orientation toward the bilayer. However, the orienting structure, the Golgi apparatus, was seen in too few cells to establish statistical significance so the data were analyzed for the presence of Golgi profiles in the cells. Each cell in each micrograph was scored for the presence or absence of the Golgi apparatus.

The third set of measurements was designed to measure the percentage of adherent cell plasma membrane that faced either the artificial bilayer, other cells, or the culture medium. Each micrograph was overlaid with a Mertz grid with a point spacing of 2.2 cm and a line length of 3.45 cm/point. The line intercepts with the plasma membrane were scored as substrate if the intercepts occurred on membrane that faced the bilayer, as other cells if the intercept occurred on membrane that faced another cell, or medium if the intercept occurred on membrane that faced the culture medium. The data for each micrograph was expressed as a percentage of the total number of line intercepts with the plasma membrane.

The mean responses under differential conditions were compared by analysis of variance, using Fisher's protected least significant difference test to control for multiple comparisons. Since the precision of the sample mean motility varied substantially across experiments, weighted analysis of variance was used. The percentages of cells showing Golgi profiles were compared across cells by chi-square tests.

Results

LGL Adhesion to Planar Bilayers Reconstituted with Adhesion Receptors

The relative efficacy of LGL binding to different adhesion receptors in planar bilayers was assessed qualitatively. Greater numbers of LGL attached to LFA-1 bearing planar bilayers than attached to ICAM-1 or either form of LFA-3. Treatment of LGL with a combination of mAb to the T11₂ and T11₃ epitopes of CD2 resulted in an increase in LGL adhesion to ICAM-1, GPI-LFA-3, and TM-LFA-3 bilayers and also led to an increase in cell–cell interaction. No attachment of LGL occurred to planar bilayers lacking adhesion receptors.

Motility of LGLs on Various Adhesion Molecules

The majority of the LGLs moved on lipid bilayers reconstituted with ICAM-1, LFA-1, and TM-LFA-3 whereas most of the cells were non-motile on GPI-LFA-3 (Fig. 1). The cells moved randomly on the bilayers and frequently changed direction during the eight minutes of recording. The motile cells moved at a constant rate but stationary cells remained immobile during the observation period. The motile cells had the polarized morphology typical of other motile cell types with leading edges and uropods. However, the cells attached to GPI-LFA-3 were rounder than the cells leading edges and uropods. However, the cells attached to GPI-LFA-3

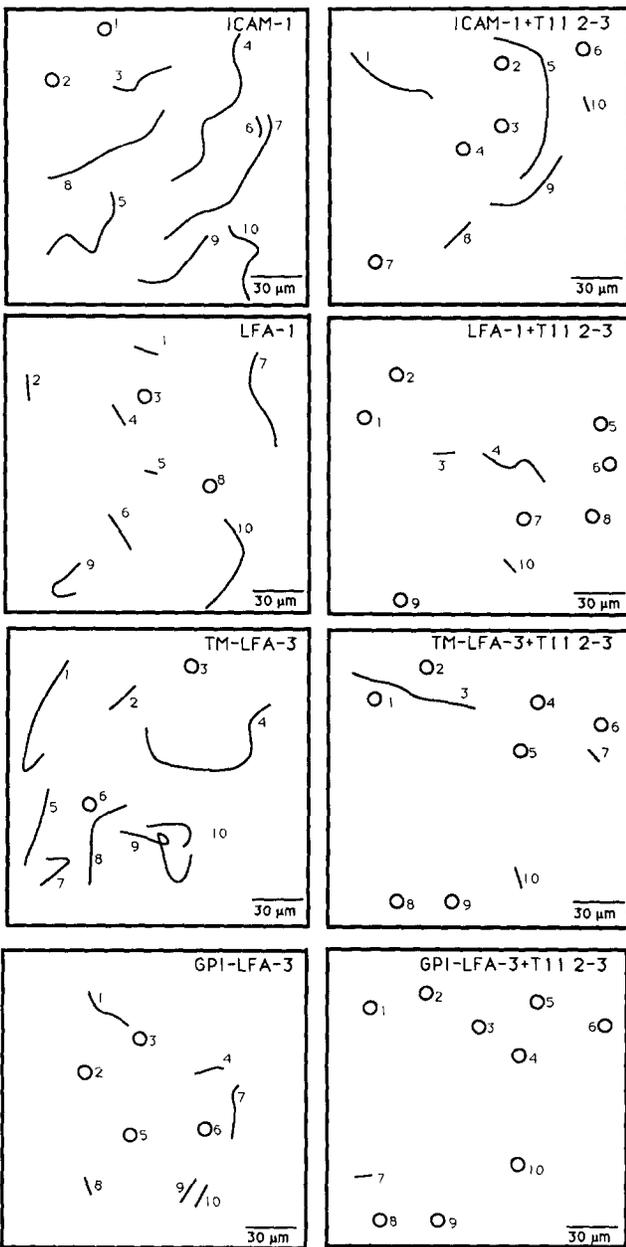


Figure 1. Paths of LGLs moving on various adhesion receptors during an 8-min period. LGLs were allowed to attach to glass supported lipid bilayers reconstituted with various adhesion molecules. Before recording, a combination of T11₂ and T11₃ mAb ascites (1:500) was added to cells in the right panels. Each line and number represents the path of an individual cell traced from the monitor screen after a time-lapse video recording. O, a stationary cell.

were rounder than the cells on other ligands. Sometimes the cells, especially on ICAM-1, appeared to be tethered to a spot and sent long extensions from the cell body. The cell body would then move suddenly into the extension, in other cases, the cells changed direction or the movement was halted. Occasionally neighboring cells conjugated with each other, moved as a pair for a short distance and usually dissociated.

LGLs moved at different rates on the reciprocal molecules of the LFA-1-ICAM-1 adhesion pair. The average rate of migration was over 17 times higher on ICAM-1 than on LFA-1 (8.23 $\mu\text{m}/\text{min}$ vs. 0.48 $\mu\text{m}/\text{min}$) (Table I). Rates up to 30 $\mu\text{m}/\text{min}$ were measured on ICAM-1. LGL moved at different rates on the lipid bilayers reconstituted with the two different forms of LFA-3. Lipid bilayers containing TM-LFA-3 supported motility almost as well as ICAM-1, the average being 7.31 $\mu\text{m}/\text{min}$. On the other hand, only few cells showed significant movement on GPI-LFA-3 (average rate 1.75 $\mu\text{m}/\text{min}$) and in one out of four experiments no motile cells were detected. Thus, the ligands fell into two groups, a high motility group, ICAM-1 and TM-LFA-3, with an average rate of ~ 8 $\mu\text{m}/\text{min}$ and a low motility group, LFA-1 and GPI-LFA-3 (<2 $\mu\text{m}/\text{min}$). The difference in the motility induced by a given ligand was statistically significant ($p < 0.05$) when compared with the two ligands in the other group but not when compared with the other ligand in its own group.

The Effect of T11₂ and T11₃ mAb on LGL Motility

To test how triggering of cells through CD2 would alter the motility of LGLs, the T11_{2/3} mAb combination was added to LGL adherent on lipid bilayers. Treatment of the cells with T11_{2/3} mAb slowed the cells on bilayers reconstituted with ICAM-1 and virtually immobilized the cells on the other bilayers (Table I). The decrease in motility promoted by CD2 activation was statistically significant for each ligand except LFA-1 (Table I). The magnitude of the decreased motility was greatest for both isoforms of LFA-3, $\sim 95\%$, and was 62% for ICAM-1, and 69% for LFA-1. Comparison of the combined values with T11_{2/3} mAb to the values without T11_{2/3} mAb showed that the effect of CD2 activation on motility was highly significant, $p = 0.0002$.

Ultrastructure of Cells on Lipid Bilayers

The cells adherent to artificial bilayers containing any of the four adhesion molecules had ultrastructural characteristics of LGLs, such as low nucleocytoplasmic ratio and osmiophilic cytoplasmic granules. There was no pattern of preferential orientation of the granules, Golgi apparatus, or centrioles regardless of the ligand in the bilayer. Degranula-

Table I. Rate of LGL Locomotion on Reconstituted Lipid Membranes

Ligand	Without T11 mAb ($\mu\text{m}/\text{min}$)*	With T11 mAb ($\mu\text{m}/\text{min}$)*	Significance of T11 mAb effect (p)
ICAM-1	8.23 \pm 1.72 (5)	3.08 \pm 1.38 (5)	0.0009
LFA-1	0.48 \pm 4.56 (5)	0.15 \pm 1.20 (2)	NS
TM-LFA-3	7.31 \pm 1.56 (4)	0.26 \pm 1.67 (4)	0.0002
GPI-LFA-3	1.75 \pm 3.03 (4)	0.11 \pm 0.35 (3)	0.026

* Each value represents mean \pm SD of the number of experiments given in parenthesis.

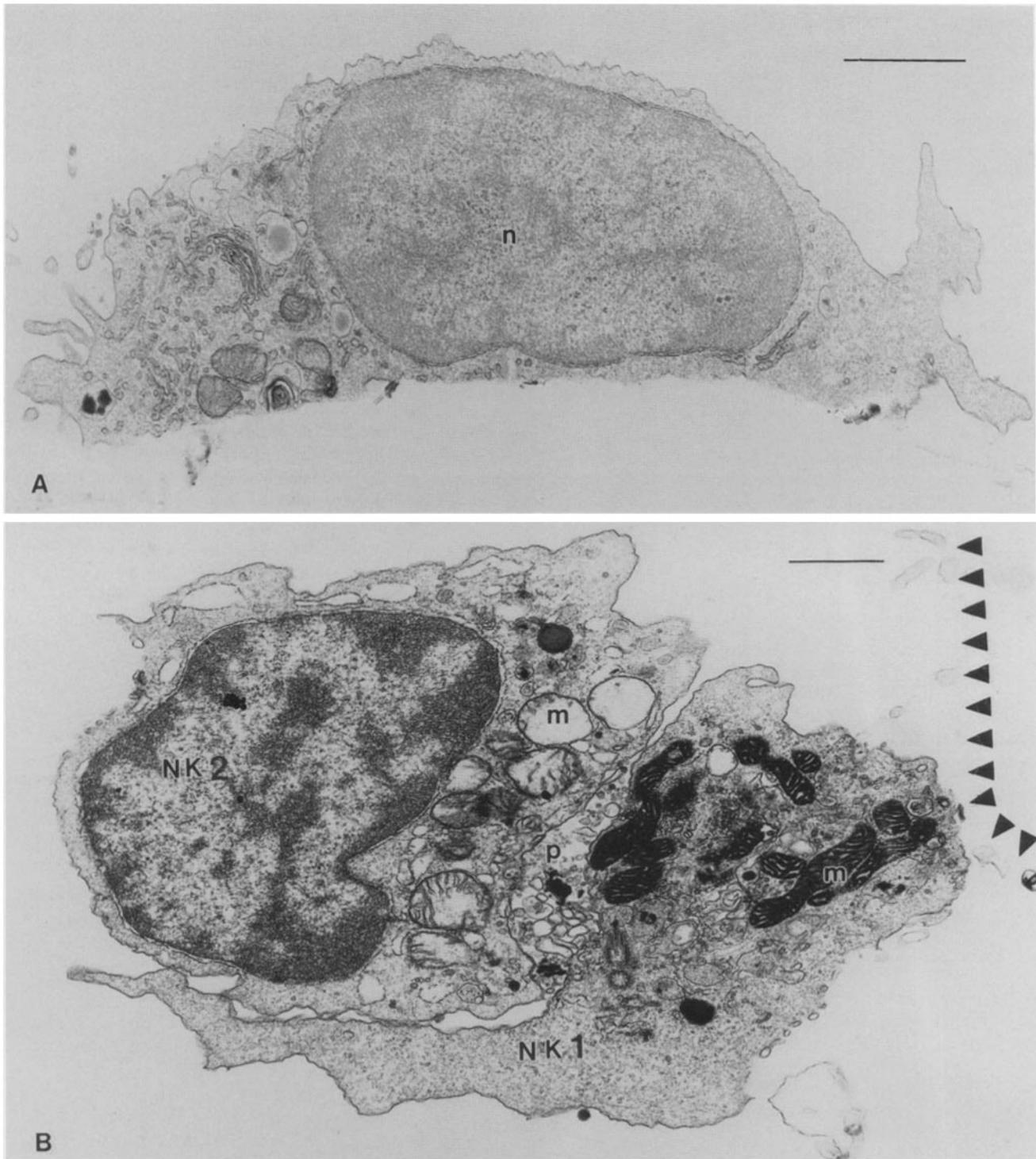
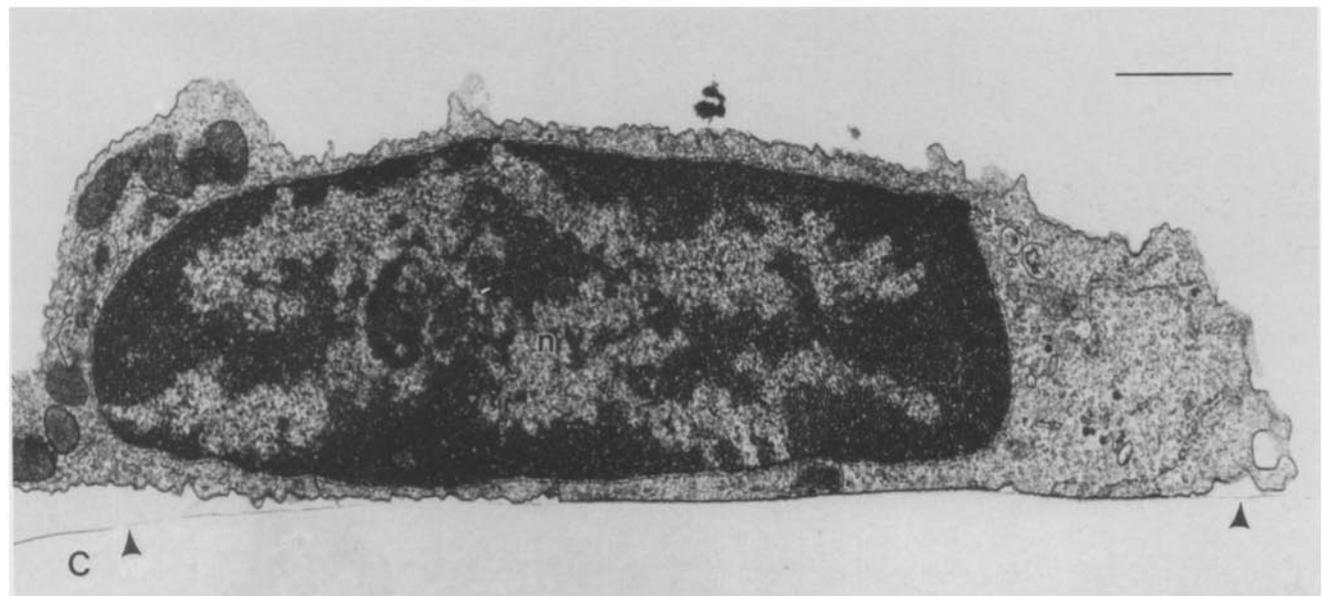
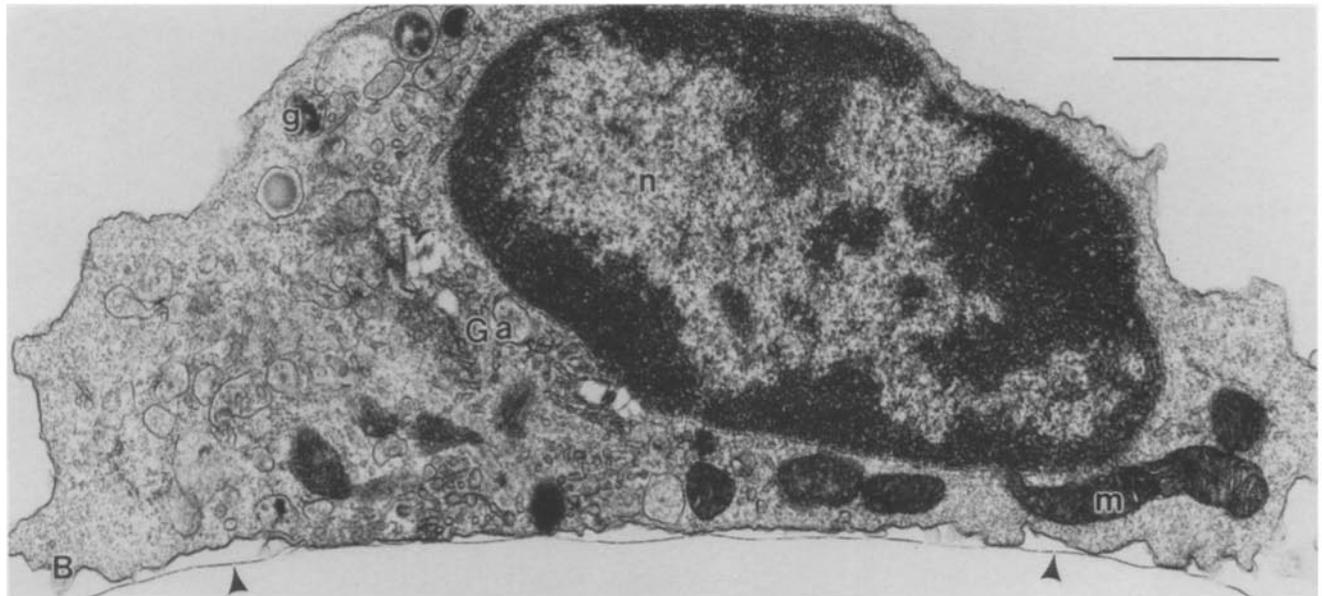
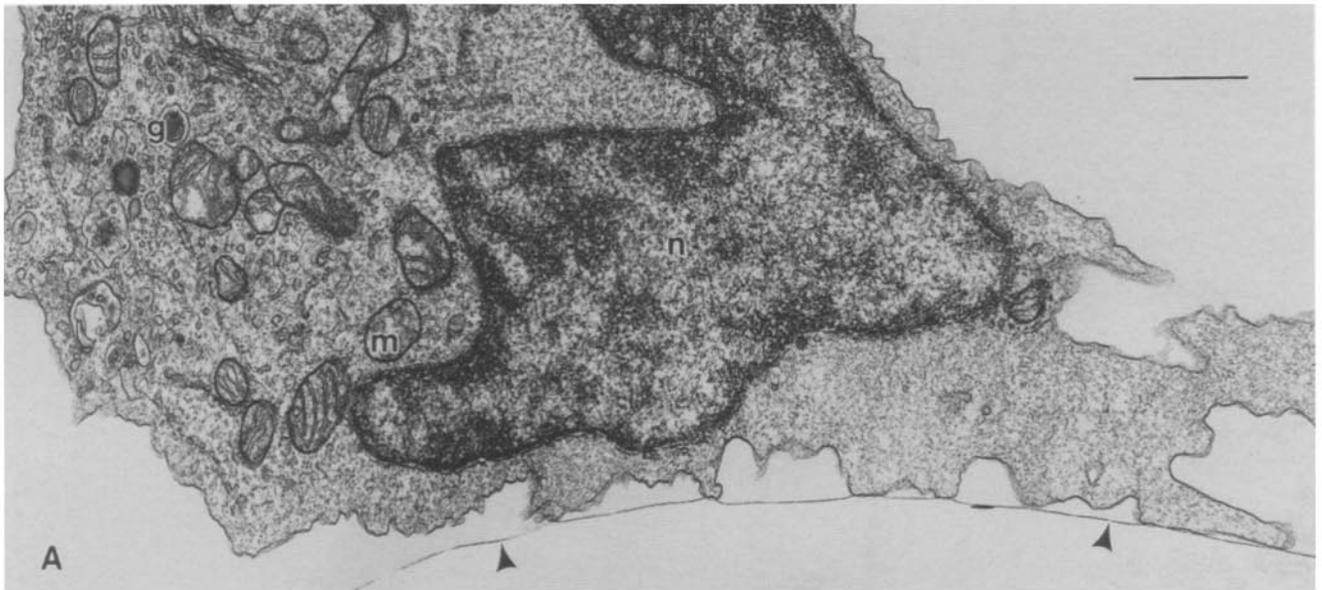


Figure 2. LGL adhering to artificial bilayers containing ICAM-1. In *A*, the cell is apparently motile with a classic leading edge to the right and the trailing edge to the left. Note the cell is not flat on the bilayer (*bottom*) which is because of warping during reembedding and that the bilayer itself is poorly visualized. In *B*, the cells were treated with T11_{2/3} mAb. The bilayer is to the right and is indicated with dotted line and the adherent cell (*NK1*) is attached across a small area. The adherent cell faces a second cell (*NK2*) that is not adherent to the bilayer. The pocket (*p*) between the two cells contains membrane vesicles typical of LGL exocytosis (31). *NK2* appears damaged since the mitochondria (*m*) are disrupted whereas those of *NK1* are intact. Bars, 1 μm .

tion against the bilayer was also not observed. Some cells appeared to be motile with the classic filamentous ruffled edge on one side of the cell and nucleus and organelles on the other (Fig. 2 *A*). This was observed particularly in cells on

bilayers containing ICAM-1. The morphology of the attachment area of LGLs on each of the adhesion proteins generally appeared similar to one another in the absence of T11_{2/3} mAb. The structure of cells on bilayers with any single



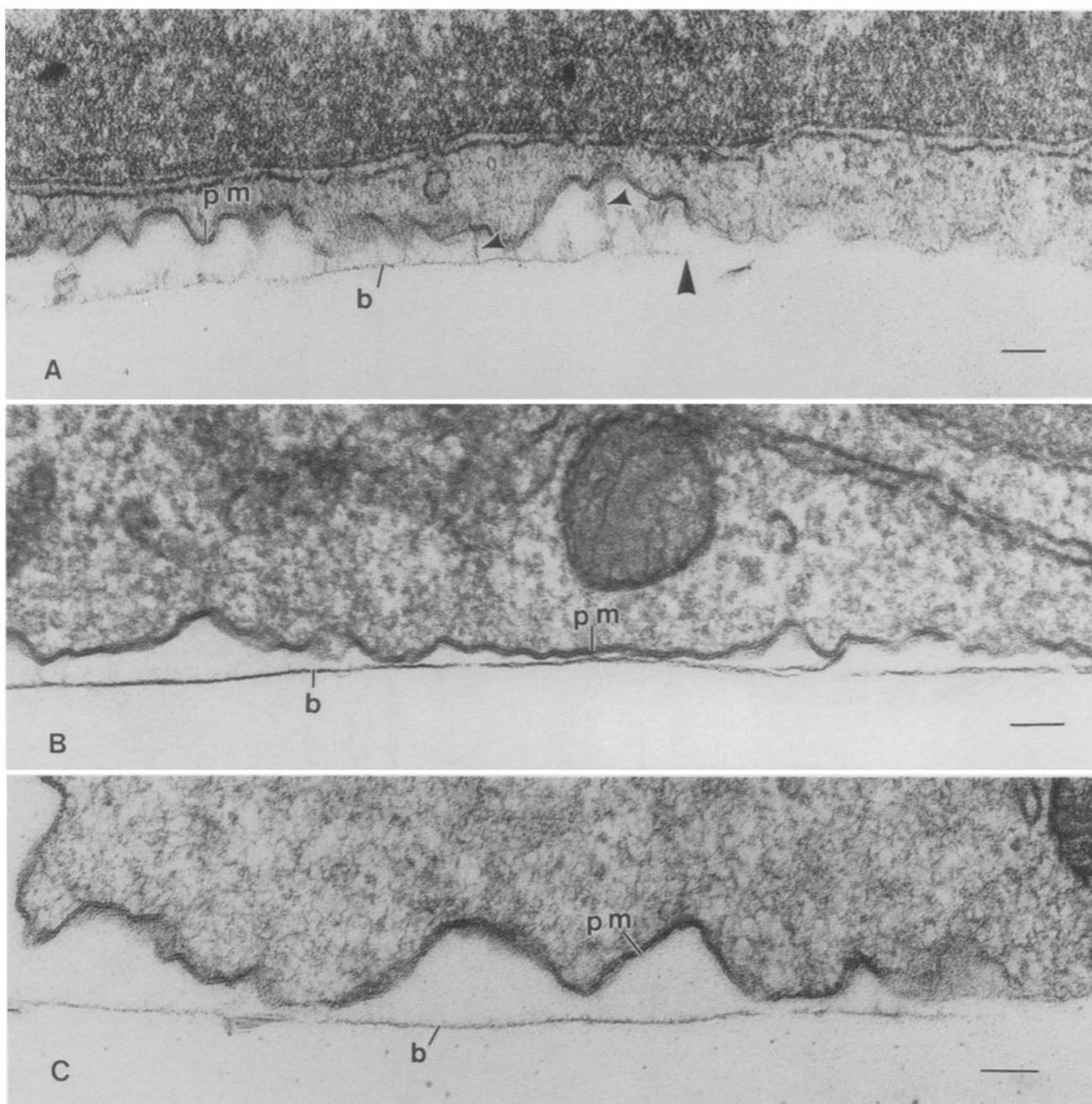


Figure 4. High magnification of the adhesion of cells to artificial bilayers containing different adhesion molecules. In *A* the bilayer (*b*) contained ICAM-1; note the undulations in the plasma membrane (*pm*), the electron-dense strands (*arrows*) running from the plasma membrane to the bilayer, and the absence of the bilayer on the right (*bracket*). In *B*, the bilayer contained LFA-1 and in *C*, TM-LFA-3. Bars, 0.1 μm .

ligand varied within each experiment as much as the structure of cells adhering to different ligands. This is illustrated in Fig. 3 in which all three micrographs are taken from a single plating of LGLs on a bilayer containing GPI-LFA-3. The plasma membrane of the cells contacted the bilayer in a few

small areas (Fig. 3 *A*) or in larger areas (Fig. 3 *B*); rarely, the plasma membrane was opposed to the bilayer across most of the cell-bilayer interface (Fig. 3 *C*). These patterns of contact were similar with all four ligands. None of the ligands induced the formation of the characteristic contacts

Figure 3. Three LGL profiles from a culture on a bilayer containing GPI-LFA-3. In each micrograph the bilayer is marked by arrows. Note that the plasma membrane contacts the bilayer at a few points in *A*, more extensively in *B*, and along most of the length of the interface in *C*. These micrographs are near the magnification for which the measurements in Table III were made. *Ga*, Golgi apparatus; *n*, nucleus; *m*, mitochondria; *g*, granules. Bars, 1 μm .

Table II. Ratio of the Length of the Basal Plasma Membrane to the Length of the Substrate under the Cell

Ligand	T11 mAb	Experiment 1	Experiment 2
		<i>n</i>	<i>n</i>
ICAM-1	-	1.17 ± 0.11 (9)	1.09 ± 0.06 (22)
	+	1.05 ± 0.07 (3)	1.18 ± 0.25 (13)
LFA-1	-	1.10 ± 0.08 (10)	1.22 ± 0.19 (11)
	+	1.22 ± 0.19 (9)	1.18 ± 0.11 (9)
TM-LFA-3	-	1.20 ± 0.10 (6)	1.13 ± 0.06 (6)
	+	1.22 ± 0.22 (7)	1.74* ± 0.78 (9)
GPI-LFA-3	-	1.31 ± 0.23 (8)	1.12 ± 0.08 (9)
	+	1.26 ± 0.29 (7)	1.11 ± 0.05 (10)

All values represent the mean ± SD with the numbers of cells measured given in parenthesis. *, Is the only value that is significantly different from all other values, approximately 4 standard errors above the next largest mean value. The means of the other ratios do not differ significantly as determined by Fisher's protected least significant difference multiple comparison procedure.

that LGLs form against target cells or produced flattening of all of the adherent cells.

High magnification (>80,000×) examination of the structural relationship between the cell membranes and the bilayers was difficult to perform because the artificial bilayers often did not pick up osmium underneath the cell. However, the outer leaflet of the plasma membrane of the cell was separated by ~20 nm from the artificial bilayer at the closest point of apposition for all conditions examined (Fig. 4).

T11_{2/3} mAb caused the LGLs on the bilayer to adhere to other cells, most often on their surface away from the bilayer. This was particularly notable with ICAM-1 (Fig. 2 B). The adherent cells often oriented toward one another and degranulated into the space between the cells. LGLs adhering to ICAM-1 and activated with T11_{2/3} mAb were less spread on the bilayer than unactivated cells and appeared to lift off.

Electron Microscopy Measurements

Three sets of measurements were made on the electron micrographs taken from the two different experiments. First, the degree of apposition of the plasma membrane to the lipid bilayer was determined by measuring with a digitizer the ratio of the length of the plasma membrane facing the bilayer to the length of the bilayer beneath the cell. The ratio would be 1 for cells that were completely flat against the bilayer and would increase in value for cells attached by punctate sites with folds in between the attachment sites. The means ± SD of the measurements are shown in Table II. The weighted grand mean of all measurements was 1.20 and a pooled SD of 0.20. In the second experiment, the ratio for the cells on TM-LFA3 treated with T11_{2/3} mAb was significantly different from all the other values (1.74, *p* < 0.01). This result was not obtained in the first experiment, however. The data, exclusive of the exceptional value, suggest that there is a constant ratio of plasma membrane to underlying bilayer for all ligands and that this ratio is not altered by treatment with T11_{2/3} mAb. Further, it shows that no ligand is inducing closer apposition of the cell to the bilayer, such as that shown in Fig. 3 C, than another ligand.

In the second set of measurements, micrographs were evaluated for the presence of Golgi profiles within the cells.

Because there were no differences between the two experiments the data from both were pooled (Table III). On bilayers containing ICAM-1, the addition of T11_{2/3} mAb caused a drop in the percentage of the cells with a Golgi profile from 53% (16/30) to 22% (4/18), *p* < 0.05. Addition of T11_{2/3} mAb to cells on bilayers containing the other three ligands produced no significant changes. These results suggest that T11_{2/3} mAb alters the Golgi apparatus in cells on ICAM-1 containing bilayers.

The third set of measurements determined the relative surface area of the plasma membrane that faced either the bilayer or other cells with the data expressed as a percentage of the total area of the plasma membrane (Table IV). The mean percentage of the plasma membrane facing the bilayer was 32% for ICAM-1, 27% for GPI-LFA-3, 23% for LFA-1, and 20% for TM-LFA-3. The differences between ICAM-1 and both LFA-1 and TM-LFA-3 were statistically significant, *p* < 0.05, as were the differences between GPI-LFA-3 and TM-LFA-3. There was no correlation with the motility data above. The high motility group ligands ICAM-1 and TM-LFA-3 were at opposite ends of the range of measurements.

For bilayers containing either ICAM-1 or GPI-LFA-3, T11_{2/3} mAb produced a decrease in the relative surface area facing the bilayer, *p* < 0.05 and *p* < 0.01, respectively. T11_{2/3} mAb produced no change in the relative surface area facing the bilayer in cells on TM-LFA-3 or LFA-1 containing bilayers. Thus, T11_{2/3} mAb causes a decrease in the relative area of the cell membrane in the contact with bilayers containing ICAM-1 or GPI-LFA-3 but little change in this parameter in cells on the other two ligands.

In the absence of T11_{2/3} mAb, a small percentage of the plasma membrane faced other cells with no statistical differences between the ligand types. For cells on bilayers containing either form of LFA-3 or ICAM-1, T11_{2/3} mAb caused an increase in the relative area of the plasma membrane oriented toward other cells which was statistically significant for ICAM-1 and GPI-LFA-3 (*p* < 0.05). Cells on bilayers containing LFA-1 had a decrease in the relative surface area facing other cells after the addition of T11_{2/3} mAb, even though these cells had higher values in the absence of T11_{2/3} mAb than cells on bilayers containing the other three ligands. This change, however, was not significant nor was the increase in surface area facing other cells measured on TM-LFA-3 bilayers. Thus, after exposure to T11_{2/3} mAb

Table III. Presence of the Golgi Apparatus in LGLs on Artificial Lipid Membranes

Ligand	T11 mAb	Experiment 1	Experiment 2
ICAM-1	-	0.60 (6/10)	0.50 (10/20)
	+	0 (0/4)	0.29 (4/14)
LFA-1	-	0.56 (5/9)	0.45 (5/11)
	+	0.75 (6/8)	0.67 (6/9)
TM-LFA-3	-	0.40 (2/5)	0.17 (1/6)
	+	0.14 (1/7)	0.40 (4/10)
GPI-LFA-3	-	0.22 (2/9)	0.33 (3/9)
	+	0.43 (3/7)	0.78 (7/9)

Combining the data from the two experiments and analyzing by χ^2 tests only the shift in values for ICAM-1 after the addition of T11 mAb is significant (*p* < 0.05). However, if the data for both forms of LFA-3 is compared to ICAM-1 in the absence of T11 mAb, there is also a significant difference, *p* < 0.05.

Table IV. Orientation of the Plasma Membrane of LGLs Adhering to Reconstituted Adhesion Molecules

Ligand	T11 mAb	Experiment 1				Experiment 2			
		Substrate	Other cells	Medium	<i>n</i>	Substrate	Other cells	Medium	<i>n</i>
				%				%	
ICAM-1	-	29.4 ± 2.8	5.2 ± 3.0	65.3 ± 3.0	(9)	34.1 ± 2.3	0.4 ± 0.3	65.5 ± 2.3	(17)
	+	22.7 ± 5.7	8.8 ± 7.0	68.4 ± 2.3	(4)	24.1 ± 3.7	17.2 ± 5.3	58.7 ± 6.9	(13)
LFA-1	-	27.6 ± 4.0	5.9 ± 3.2	66.5 ± 5.5	(9)	19.4 ± 3.2	10.3 ± 4.6	70.2 ± 5.5	(10)
	+	29.8 ± 3.2	0	70.2 ± 3.2	(8)	25.4 ± 3.8	4.9 ± 3.4	69.7 ± 6.7	(9)
TM-LFA-3	-	23.4 ± 0.4	0	76.6 ± 0.4	(5)	17.1 ± 2.9	3.5 ± 3.5	79.4 ± 3.1	(6)
	+	22.0 ± 2.6	7.6 ± 5.0	70.4 ± 4.2	(7)	15.3 ± 2.1	15.2 ± 7.0	69.5 ± 6.8	(7)
GPI-LFA-3	-	27.9 ± 2.1	0.5 ± 0.4	71.7 ± 1.7	(9)	29.0 ± 2.2	3.3 ± 3.3	67.6 ± 2.9	(9)
	+	15.7 ± 3.6	28.3 ± 12.1	56.0 ± 12.2	(8)	20.8 ± 2.2	9.1 ± 4.6	70.1 ± 4.9	(9)

Each number represents the mean ± SE with the number of micrographs that were counted given in parenthesis. The values for substrate or cells for individual micrographs were treated as replicates. The effect of T11 mAb on cells adherent to various ligands were assessed using analysis of variance where the experimental unit was a micrograph and the micrographs from the experiments were pooled.

cells on ICAM-1 and GPI-LFA-3 increased the area of plasma membrane in contact with other cells whereas the changes with the other two ligands were not significant.

Discussion

This study shows that LGLs move at a rate of ~8 μm/min on lipid bilayers containing either ICAM-1 or TM-LFA-3. They move poorly (<2 μm/min) on bilayers containing LFA-1 or GPI-LFA-3. Ultrastructurally the cells adhere similarly to bilayers containing the four ligands with a constant ratio of the plasma membrane to bilayer of 1.2 in the attachment site. Activation of the cells through perturbation of CD2 with T11_{2/3} mAb reduces cell motility on all ligands. LGLs on bilayers containing ICAM-1 or GPI-LFA-3 decrease their relative surface area in contact with other cells. LGLs on any of the bilayers, whether treated with T11_{2/3} mAb or not, do not degranulate against the bilayer or form contacts with the bilayers similar to those seen between LGLs and cytolytic targets.

LGLs express all three forms of leukocyte integrins, however, only LFA-1 has been shown to significantly contribute to adhesion to other cells (41). Thus, the binding of LGLs to ICAM-1 on lipid bilayer probably is primarily mediated by LFA-1. The binding to TM-LFA-3 occurs through CD2. The fact that both LFA-1 and CD2 generate considerable movement is of special interest since the receptors are biochemically and evolutionally different. Leukocyte-integrin molecules belong to the integrin superfamily of surface receptors (19) whereas CD2 as well as LFA-3 and ICAM-1 belong to Ig-superfamily (44). Moreover, the avidity of LFA-1 can be upregulated by stimuli, such as activation through the T cell receptor for antigen (TCR), CD2, or with PMA, whereas the avidity of CD2 is not regulated on resting T lymphocytes (11, 43). These findings demonstrate that receptors with different extracellular domain organization and avidity regulation can serve a similar function. Other studies have shown that LGLs can move using fibronectin receptors (34) and that lymphocytes that apparently account for the LGL population are motile on antibody complexes using Fc receptors (1). Thus, at least four different membrane proteins have the ability to support motility of LGLs.

LGLs moved significantly slower on lipid membranes

reconstituted with LFA-1 than on membranes reconstituted with its counter-receptor, ICAM-1. This difference could arise from quantitative or qualitative differences in molecules on the cell surface and in the bilayer. Quantitative differences between the level of LFA-1 and ICAMs on the surface of the LGL are difficult to assess since LGL may express additional ICAMs (8). In fact, the presence of high levels of other ICAMs on LGL is likely since ICAM-1 and ICAM-2 are expressed at relatively low levels on LGL (8), yet a large proportion of these cells adhere to planar bilayers with a moderate density of LFA-1. Thus, the difference in the ability of these molecules to support locomotion is probably not explained by different receptor site densities on the cell surface. LFA-1 and ICAM-1 differ in avidity regulation and cytoskeletal associations which may affect their functioning in locomotion. ICAM-1 is constitutively in a high avidity state on both reconstituted membranes and cell surface. On the other hand, reconstituted LFA-1 is constitutively active in binding ICAMs whereas the avidity of LFA-1 on cell membrane can be regulated (11, 43). An avidity regulated occupation of cell surface LFA-1 receptors may benefit cell motility compared to cell surface ICAMs. Both LFA-1 and ICAM-1 are apparently linked to the cytoskeleton (20), (Carpén, O., P. Pallai, D. Staunton, and T. Springer, manuscript in preparation). However, the subcellular distribution of LFA-1 in B cells is different from ICAM-1 suggesting a difference in the membrane protein-cytoskeleton linkage (Dustin M., O. Carpén, and T. Springer, manuscript submitted for publication; Carpén, O., P. Pallai, D. Staunton, and T. Springer, manuscript in preparation). The membrane protein interactions with cytoskeleton that are crucial for locomotion are not well characterized, but they may account for the differences between LFA-1 and ICAM-1.

LGLs moved on TM-LFA-3 at the higher rate than on GPI-LFA-3. These two isoforms have identical extracellular domains; differential splicing at the transmembrane-cytoplasmic domain junction signals reanchoring of one isoform onto GPI (32, 45). The LFA-3 isoforms were present in the membranes at site densities that provide similar levels of lymphocyte binding (Chang, P.-Y., M. Lawrence, M. Dustin, L. Ferguson, D. Golan, and T. Springer, manuscript submitted for publication). The difference in cell motility on the two isoforms most likely reflects the membrane anchoring of the

two forms, which accounts for their different lateral diffusion capacity in lipid bilayers (Chang, P.-Y., M. Lawrence, M. Dustin, L. Ferguson, D. Golan, and T. Springer, manuscript submitted for publication). As an immobile ligand, TM-LFA-3 can form a stable substrate, on which the cells can exert traction. On the other hand, GPI-LFA-3, when bound to CD2, is free to move within the artificial bilayer following the movement of CD2, which makes it poorly suited to support cell motility. Some level of movement was detected even in cells on GPI-LFA-3. However, the GPI-LFA-3 preparations used here had a variably small fraction of immobile protein (~25%, Chang, P.-Y., M. Lawrence, M. Dustin, L. Ferguson, D. Golan, and T. Springer, manuscript submitted for publication), which might account for the observed movement.

Triggering of the adherent LGLs with the anti-CD2 mAb pair T11_{2/3} reduced motility on all bilayers. Interaction with purified LFA-3 alone is not able to activate CD2-bearing cells (38); thus cells bound to LFA-3 also respond to T11_{2/3} mAb treatment. The results indicate that the rate of movement is strongly regulated by the state of activation of the cells. It is known that activation of T cells through CD2 (43) increases the avidity of LFA-1 and cell adhesion. However, there is no evidence CD2 and ICAMs undergo avidity regulation (11). The universal effect of T11_{2/3} mAb suggests alternative or additional mechanisms for regulating movement. Most probably the signalling between plasma membrane and the cytoskeleton or the cytoskeleton itself is affected by CD2 stimulation. Interestingly, triggering of B lymphoblasts with PMA does not decrease the ability of the cells to migrate on ICAM-1 (Dustin M., O. Carpén, and T. Springer, manuscript submitted for publication), unlike the effect of T11_{2/3} mAb on LGLs.

Both quantitatively and qualitatively there was little variation in the ultrastructure of the cells adherent to the bilayers in the absence of T11_{2/3} mAb, despite the differences in motility described above. The ratio of plasma membrane in the contact site with the bilayer is nearly a constant 1.2. The value suggests, that the adhesion process is highly regulated and involves limited regions of the plasma membrane. Features characteristic of interactions with target cells, such as long LGL membrane protrusions or wide contact areas with preferential polarization of cytoplasmic organelles and discharge of cytoplasmic material against the bilayer (6, 7, 21), were not seen. In light of the EM results it thus seems unlikely that receptor-ligand binding through any of the membrane proteins reconstituted on glass supported lipid bilayer mimics the events during cytolytic LGL/target cell contact. Apparently a combination of several adhesion ligands or other characteristics of a native membrane are required to induce a full cytolytic response.

There are differences between the artificial bilayer system used here and cytolytic contacts formed by LGLs. For example, T11_{2/3} mAb trigger CD2 on the entire cell surface whereas in LGL/target contact CD2 perturbation occurs at the site of adhesion. However, when NK cell clones adhering to resistant target cells were stimulated with T11_{2/3} mAb, they showed cellular polarization indistinguishable from that described for LGLs in cytolytic contacts with target cells (31). Further, the glass that supports bilayers does not deform like a cell membrane in response to the extension of LGL pseudopods and thus prevents interdigitation of mem-

brane surfaces. Such interdigitations are seen during early events of effector/target cell contacts and have been postulated to be important for later events leading to wide contact areas and redistribution of cellular organelles (6).

After treatment of cells on ICAM-1 containing bilayers with T11_{2/3} mAb, there was a significant decrease in the percentage of cells containing a Golgi profile. This result can be interpreted in two ways. First, there could be a preferred orientation of the Golgi apparatus in a plane parallel to the plane of the bilayers. The plane of orientation then shifts after T11_{2/3} mAb treatment. The decrease in profiles would occur because the cell and Golgi apparatus are anisotropic structures that are being cut non-randomly, i.e., only orthogonal to the plane of adherence to the bilayer. Further, this interpretation would also correlate with the T11_{2/3} mAb induced decrease in the percentage of the plasma membrane facing the bilayer. Second, the decrease in the Golgi profiles could indicate a decrease in the volume of the Golgi apparatus. The Golgi apparatus is known to alter its volume in response to drugs (22, 42), during cell division (23), and after vital infection (15). Perhaps, T11_{2/3} mAb can cause a similar alteration. In any case, these hypotheses must be tested experimentally.

Only recently, the possibility has been appreciated that leukocyte adhesion receptors may be involved in biological processes in ways other than providing adherence between cells. This study, along with other recent findings, demonstrates that the adhesion molecules can independently support functions, the specificity of which depends on the adhesion pathways used. The adhesive interactions formed between LFA-1-ICAM-1 and CD2-TM-LFA-3 generate LGL locomotion and may thus be important in screening for potential target cells and in the process of extravasation and migration in tissues.

This work was supported by National Institutes of Health grants (CA31798 to T. A. Springer, AI23083, AM36308 and AR35907 to J. P. Caulfield) and by grants from the Emil Aaltonen Foundation, the Finnish Cultural Foundation and the Wihuri Foundation to O. Carpén. O. Carpén is a National Institutes of Health Fogarty Fellow.

Received for publication 10 May 1991 and in revised form 12 July 1991.

References

- Alexander, E., and P. Henkart. 1976. The adherence of human Fc receptor-bearing lymphocytes to antigen-antibody complexes. II. Morphologic alterations induced by the substrate. *J. Exp. Med.* 143:329-347.
- Alavena, P., C. Paganin, I. Martin-Padura, G. Peri, M. Gaboli, E. Dejana, P. C. Marchisio, and A. Mantovani. 1991. Molecules and structures involved in the adhesion of natural killer cells to vascular endothelium. *J. Exp. Med.* 173:439-450.
- Aronson, R., P. Libby, E. Brandon, M. Janicka, and J. Mier. 1988. IL-2 rapidly induces natural killer cell adhesion to human endothelial cells: a potential mechanism for endothelial injury. *J. Immunol.* 141:158-163.
- Bolhuis, R. L. H., R. C. Roozmond, and R. J. van de Griend. 1986. Induction and blocking of cytotoxicity in CD2+, CD3- NK and CD2+, CD3+ cytotoxic T lymphocytes via CD2 50 kD sheep erythrocyte receptor. *J. Immunol.* 136:3939-3944.
- Bottazzi, B., M. Introna, P. Allavena, A. Villa, and A. Mantovani. 1985. In vitro migration of human large granular lymphocytes. *J. Immunol.* 134:2316-2321.
- Carpén, O., I. Virtanen, and E. Saksela. 1982. Ultrastructure of human natural killer cells: nature of the cytolytic contacts in relation to cellular secretion. *J. Immunol.* 128:2691-2697.
- Carpén, O., I. Virtanen, V. Lehto, and E. Saksela. 1983. Polarization of NK cell cytoskeleton upon conjugation with sensitive target cells. *J. Immunol.* 131:2695-2698.
- de Fougerolles, A. R., S. A. Stacker, R. Schwarting, and T. A. Springer. 1991. Characterization of intercellular adhesion molecule-2 (ICAM-2)

- reveals the presence of a novel ligand for lymphocyte function associated antigen-1 (LFA-1). *J. Exp. Med.* 174:253-268.
9. Diamond, M. S., D. E. Staunton, A. R. de Fougerolles, S. A. Stackel, J. Garcia-Aguilar, M. L. Hibbs, and T. A. Springer. 1990. ICAM-1 (CD54)—A counter-receptor for Mac-1 (CD11b/CD18). *J. Cell Biol.* 111:3129-3139.
 10. Dustin, M. L., and T. A. Springer. 1988. Lymphocyte function associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. *J. Cell Biol.* 107:321-331.
 11. Dustin, M. L., and T. A. Springer. 1989. T cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature (Lond.)*. 341: 619-624.
 12. Dustin, M. L., R. Rothlein, A. K. Bhan, C. A. Dinarello, and T. A. Springer. 1986. Induction by IL-1 and interferon, tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). *J. Immunol.* 137:245-254.
 13. Dustin, M. L., M. E. Sanders, S. Shaw, and T. A. Springer. 1987. Purified lymphocyte function-associated antigen-3 (LFA-3) binds to CD2 and mediates T lymphocyte adhesion. *J. Exp. Med.* 165:677-692.
 14. Dustin, M. L., P. Selvaraj, R. J. Mattaliano, and T. A. Springer. 1987. Anchoring mechanisms for LFA-3 cell adhesion glycoprotein at membrane surface. *Nature (Lond.)*. 329:846-848.
 15. Griffiths, G., S. D. Fuller, R. Back, M. Hollinshead, S. Pfeiffer, and K. Simons. 1989. The dynamic nature of the Golgi complex. *J. Cell Biol.* 108:277-287.
 16. Hafeman, D. G., L. M. Smith, D. T. Fearon, and H. M. McConnell. 1982. Lipid monolayer-coated solid surfaces do not perturb the lateral motion and distribution of C3b receptors on neutrophils. *J. Cell Biol.* 97:224-227.
 17. Henkart, M. P., and P. A. Henkart. 1982. Lymphocyte mediated cytotoxicity as a secretory phenomenon. In *Mechanisms of cell-mediated cytotoxicity*. W. R. Clark, and P. Goldstein, editors. Plenum Publishing Corp., New York. 227-300.
 18. Hollander, N., P. Selvaraj, and T. A. Springer. 1988. Biosynthesis and function of LFA-3 in human mutant cells deficient in phosphatidylinositol anchored proteins. *J. Immunol.* 141:4283-4290.
 19. Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell*. 48:549-554.
 20. Kupfer, A., and S. J. Singer. 1989. Cell biology of cytotoxic and helper T cell functions. Immunofluorescence microscopic studies of single cells and cell couples. *Annu. Rev. Immunol.* 7:309-337.
 21. Kupfer, A., G. Dennert, and S. J. Singer. 1983. Polarization of the Golgi apparatus and the microtubule-organizing center within cloned natural killer cells bound to their targets. *Proc. Natl. Acad. Sci. USA.* 80:7224-7228.
 22. Lippincott-Schwartz, J., L. C. Yuan, J. S. Bonifacino, and R. D. Klausner. 1989. Rapid redistribution of golgi proteins into the ER in cells treated with Brefeldin A: evidence for membrane cycling from golgi to ER. *Cell*. 56:801-813.
 23. Lococo, J. M., E. G. Berger, and G. Warren. 1989. Mitotic Golgi fragments in HeLa cells and their role in the reassembly pathway. *J. Cell Biol.* 109:463-465.
 24. Lotze, M. T., L. W. Frana, S. O. Sharrow, R. J. Robb, and S. A. Rosenberg. 1985. In vivo administration of human interleukin 2. I. Half-life and immunologic effects of the Jurkat cell line-derived interleukin 2. *J. Immunol.* 134:157-163.
 25. Marlin, S. D., and T. A. Springer. 1987. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell*. 51:813-819.
 26. McConnell, H. M., T. H. Watts, R. M. Weis, and A. A. Brian. 1986. Supported planar membranes in studies of cell-cell recognition in the immune system. *Biochim. Biophys. Acta.* 864:95-106.
 27. Meuer, S. C., R. E. Hussey, M. Fabbi, D. Fox, O. Acuto, K. A. Fitzgerald, J. C. Hodgdon, J. P. Protentis, S. F. Schlossman, and E. L. Reinherz. 1984. An alternative pathway of T-cell activation: A functional role for the 50 kd T11 sheep erythrocyte receptor protein. *Cell*. 36:897-906.
 28. Robertson, M. J., M. A. Caligiuri, T. J. Manley, H. Levine, and J. Ritz. 1990. Human natural killer cell adhesion molecules. Differential expression after activation and participation in cytotoxicity. *J. Immunol.* 145: 3194-3201.
 29. Rothlein, R., M. L. Dustin, S. D. Marlin, and T. A. Springer. 1986. A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *J. Immunol.* 137:1270-1274.
 30. Schmidt, R. E., J. M. Michon, J. Woronicz, S. F. Schlossman, E. L. Reinherz, and J. Ritz. 1987. Enhancement of natural killer function through activation of the T11 E rosette receptor. *J. Clin. Invest.* 79:305-308.
 31. Schmidt, R. E., J. P. Caulfield, J. Michon, A. Hein, M. M. Kamada, R. P. MacDermott, R. L. Stevens, and J. Ritz. 1988. T11/CD2 activation of cloned human natural killer cells results in increased conjugate formation and exocytosis of cytolytic granules. *J. Immunol.* 140:991-1002.
 32. Seed, B. 1987. An LFA-3 cDNA encodes a phospholipid-linked membrane protein homologous to its receptor CD2. *Nature (Lond.)*. 329:840-842.
 33. Siliciano, R. F., J. C. Pratt, R. E. Schmidt, J. Ritz, and E. L. Reinherz. 1985. Activation of cytolytic T lymphocyte and natural killer cell function through the T11 sheep erythrocyte binding protein. *Nature (Lond.)*. 317: 428-430.
 34. Somersalo, K., and E. Saksela. 1991. Fibronectin facilitates the migration of human natural killer cells. *Eur. J. Immunol.* 140:991-1002.
 35. Springer, T. A. 1990. Adhesion receptors of the immune system. *Nature (Lond.)*. 346:425-433.
 36. Springer, T. A., M. L. Dustin, T. K. Kishimoto, and S. D. Marlin. 1987. The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system. *Annu. Rev. Immunol.* 5:223-252.
 37. Staunton, D. E., M. L. Dustin, and T. A. Springer. 1989. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature (Lond.)*. 339:61-64.
 38. Tiefenthaler, G., T. Hünig, M. L. Dustin, T. A. Springer, and S. C. Meuer. 1987. Purified lymphocyte function-associated antigen-3 and T11 target structure are active in CD2-mediated T cell stimulation. *Eur. J. Immunol.* 17:1847-1850.
 39. Timonen, T., C. W. Reynolds, J. R. Ortaldo, and R. B. Herberman. 1982. Isolation of human and rat natural killer cells. *J. Immunol. Meth.* 51: 269-277.
 40. Timonen, T., M. Patarroyo, and C. Gahmberg. 1988. CD11a-c/CD18 and GP84 (LB-2) adhesion molecules on human large granular lymphocytes and their participation in natural killing. *J. Immunol.* 141:1041-1046.
 41. Trinchieri, G. 1989. Biology of natural killer cells. *Adv. Immunol.* 47:187-376.
 42. Turner, J. R., and A. M. Tartakoff. 1991. The response of the Golgi apparatus to microtubule alterations: the roles of metabolic energy and membrane traffic in Golgi complex organization. *J. Cell Biol.* 109:2081-2090.
 43. van Kooyk, P. van de Wiel-van Kemenade, P. Weder, T. W. Kuijpers, and C. G. Figdor. 1989. Enhancement of LFA-1-mediated cell adhesion by triggering through CD2 or CD3 on T lymphocytes. *Nature (Lond.)*. 342: 811-813.
 44. Williams, A. F., and A. N. Barclay. 1988. The immunoglobulin superfamily: domains for cell surface recognition. *Annu. Rev. Immunol.* 6:381-405.
 45. Wallner, B. P., A. Z. Frey, R. Tizard, R. J. Mattaliano, C. Hession, M. E. Sanders, M. L. Dustin, and T. A. Springer. 1987. Primary structure of lymphocyte function associated antigen-3 (LFA-3): the ligand of the T-lymphocyte CD2 glycoprotein. *J. Exp. Med.* 166:923-934.
 46. Young, J. D. E., and Z. A. Cohn. 1986. Cell-mediated killing: a common mechanism. *Cell*. 46:641-650.