Force contribution of the LFA-1/ICAM-1 complex to T cell adhesion

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Summary

Little is known in quantitative terms about forces between cells generated during adhesion and recognition, or about the contribution of any one set of molecular associations to the development of these forces. To determine the forces involved in adhesion dependent on lymphocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1), we have measured the junctional avidity between single cell pairs consisting of a cloned T cell that expresses LFA-1 and a fibroblast cell that expresses MHC class II molecules and ICAM-1 after transfection. Micromanipulation was used to induce conjugation of cell pairs and to determine the force required to separate the conjugate. T cell adhesion to three related fibroblast cell lines was compared: the parent line that does not express ICAM-1 or other LFA-1 counter-receptors, and two transfectants that have high and moderate levels of surface ICAM-1 expression. The force needed to separate the conjugates varied with the fibroblast ICAM-1 expression levels. The T cell adhesion to ICAM-1-expressing fibroblasts was strong, and the critical separation stresses measured for the three cell lines were $1.4 \times 10^3$ dyn/cm$^2$ (1 dyn=10$^{-5}$ N) for the ICAM-1-negative fibroblast, $4.98 \times 10^3$ dyn/cm$^2$ for the fibroblast with a moderate level of ICAM-1 expression, and $6.25 \times 10^3$ dyn/cm$^2$ for the fibroblast line with the highest ICAM-1 expression. The dependence of adhesion strength on the LFA-1/ICAM-1 complex was confirmed by the use of blocking antibodies, which showed the contribution from the interaction of CD4/MHC class II to be negligible.

Key words: lymphocyte function-associated antigen 1, intercellular adhesion molecule 1, adhesion, micromanipulation, T lymphocyte, CD11a, CD18, CD54, integrin.

Introduction

Lymphocyte function-associated antigen 1 (LFA-1) is a $\beta_2$ integrin (Sanchez-Madrid et al. 1983) that plays an important role in the function of cytolytic and helper T cells (Davignon et al. 1981; Tedder et al. 1986; Tohma et al. 1991; Owens, 1991) and in lymphocyte recirculation (Hamann et al. 1988; Pals et al. 1988). LFA-1 has three cell-surface ligands (de Fougerolles and Springer, 1992; Rothlein et al. 1986; Staunton et al. 1989), and of these intercellular adhesion molecule 1 (ICAM-1) is the best characterized. Antibodies against ICAM-1 have been shown to block immune responses in vitro that depend on cell-cell contact (Boyd et al. 1988). The avidity with which T cells adhere to surfaces coated with purified ICAM-1 is increased upon cross-linking of the antigen receptor or CD2, or stimulation with phorbol esters (Dustin and Springer, 1989; van Kooyk et al. 1989), indicating that LFA-1-mediated adhesion is regulated according to the activation status of the T cell. LFA-1 is also expressed on B cells, and studies have shown that its avidity is increased on cross-linking of surface immunoglobulin (Dang and Rock, 1991), class II major histocompatibility complex (MHC)-encoded molecules (Mourad et al. 1990), or CD40 (Barrett et al. 1991).

Although it is recognized that the interaction of adhesion receptors contributes to stable cell-cell association, little is known in quantitative terms about adhesive forces between cells or about the contribution of any one set of molecular associations to the development of these forces. Model systems have been described which permit detailed analysis of cell and molecular cross-bridge detachment energies (Evans et al. 1991a, b), but adhesion forces dependent on the association of physiological adhesion receptor pairs are for the most part uncharacterized. One approach that has been used to quantitate adhesive forces due to the interaction of a particular receptor pair is the reconstruction of one of the pair in purified form in lipid bilayers and the application of micromanipulation techniques to measure the strength of adhesion between cells and the model membrane (Tozeren et al. 1992). This approach removes the receptor from the environment and
molecular context of the cell membrane, usually with the use of harsh conditions. Reconstitution provides a degree of control over membrane composition, but does not permit the comparison of specific receptor-dependent adhesive forces with adhesive contributions from other molecular species in the cell membrane.

Another approach to the characterization of specific adhesion receptors is offered by the use of cell lines transfected with adhesion receptor cDNAs. Comparison of the transfected and parent cell lines can provide insight into the relationship between adhesion strength and other functional properties. To quantify the strength of cell adhesion mediated by the LFA-1/ICAM-1 binding interaction, we have applied micromanipulation techniques to measure the force of adhesion between a cloned murine helper T cell line, CF.6, specific for pigeon cytochrome c and the MHC class II-encoded molecule Eκ, and fibroblast cell lines that express Eκ and varying levels of murine ICAM-1 after transfection. In this system only the T cell expresses LFA-1 and ICAM-1 is present only on the fibroblast. Furthermore, functional characterization of the fibroblasts indicates that the ICAM-1 molecule expressed after transfection is the only LFA-1 ligand expressed by these cells. Thus, in contrast to previous studies (Sung et al. 1986), the interaction of LFA-1 with ICAM-1 can be examined in isolation from its interaction with alternative ligands and can be further isolated by subtracting the "background" force of adhesion between the T cell and the untransfected parent fibroblast. Furthermore, functional characterization of the transfected fibroblasts indicates that the ICAM-1 molecule expressed after transfection is the only LFA-1 ligand expressed by these cells. Thus, in contrast to previous studies (Sung et al. 1986), the interaction of LFA-1 with ICAM-1 can be examined in isolation from its interaction with alternative ligands and can be further isolated by subtracting the "background" force of adhesion between the T cell and the untransfected parent fibroblast line that does not express ICAM-1. The conjugation area and strength.

Materials and methods

Cell lines

The cloned murine helper T-cell line C.F6 is specific for pigeon cytochrome c in the context of Eκ. It was maintained by periodic antigen stimulation, followed by expansion and rest in cultures containing interleukin 2 (IL-2). Only resting cells (14 to 21 days post-IL-2) were used for adhesion measurements. The antigen-presenting cell, DCEK.Hi7, is a murine fibroblast cell line which expresses the murine class II MHC genes Eκ and Eδ after transfection (J. Miller and R. N. Germain, unpublished observations). DCEK.Hi7 was transfected with the murine ICAM-1 cDNA (Siu et al. 1989) as described previously (Kuhlman et al. 1991). ICAM-1 expression levels on transfected cells were determined by flow cytometric analysis of cells stained with fluorescein-labeled BE29G1 (rat anti-murine ICAM-1) (Kuhlman et al. 1991) and comparison with standards. The ICAM-1-transfected DCEK cell lines were designated DCEK.ICAM (high expression) and DCEK.Imod (moderate expression).

Adhesion measurements

The system used is the same as previously described (Sung et al. 1986). Micropipettes with an internal radius (Ri) of 2.0 to 2.8 μm were prepared with the use of a micropipette puller and filled with culture medium. The micropipette was mounted on a hydraulic micromanipulator, with the wide end of the pipette connected to a pressure regulation system. The pressure regulation system used had an accuracy of better than 5 dyn/cm² (1 dyn=10⁻⁵ N) and a time constant of approximately 20 ms. These studies were performed at room temperature. The experiment was recorded on video tape and the time course of conjugate formation and the radius of the cell-cell interface were measured from the recorded video images. The arrangement has been described in detail elsewhere (Sung et al. 1988; Tozeren et al. 1992). The minimum aspiration pressure that led to the total separation of the two cells is referred to as the critical separation pressure (Pc). The critical separation stress (Sc) was calculated as (Sung et al. 1986):

\[ Sc = 2(R_p/R_i)^2 P_c, \]

where \( R_p \) is the radius of the C.F6-holding pipette and \( R_i \) is the radius of the interface of conjugation: \( 2(R_p/R_i)^2 \) gives the ratio of the surface area of the portion of the cell held within the holding pipette (a hemispherical cap with area=\( \pi R_i^2 \)) to the conjugation area (\( \pi R_p^2 \)).

To account for the "roughness" of the cell surface in calculations of contact area, a correction factor was derived as follows: cell conjugates were incubated in a swelling solution consisting of 3 x 10⁻² M cytochalasin B in medium and the osmolarity was decreased by step-wise addition of water at 30 minute intervals. After each addition and incubation at room temperature, the diameter of the area of conjugation was measured by light microscopy. The diameter increased with each decrease in osmolarity, reached a maximum value, then decreased with subsequent additions. The ratio of the maximum conjugation diameter to the unw swollen diameter was used as a correction factor in subsequent area calculations. The correction factors were 1.24, 1.18 and 1.25 for DCEK.Hi7, DCEK.Imod and DCEK.ICAM, respectively.

Results

Flow cytometric analysis of ICAM-1 expression by the three DCEK cell lines is shown in Fig. 1. Comparison of fluorescence of cells stained with fluorescein-labeled anti-ICAM-1 antibody to standards indicated that the DCEK.ICAM cell line expresses \( 2 \times 10^5 \) ICAM-1 molecules/cell; the DCEK.Imod cell line expresses \( 3 \times 10^4 \) molecules/cell. ICAM-1 expression by DCEK.Hi7 was virtually undetectable at < 200 ICAM-1 molecules/cell, and has been shown to be undetectable by RNA hybridization (Siu et al. 1989).

The effect of anti-LFA-1 mAb on the response of C.F6 to antigen presented by DCEK.Hi7 and DCEK.ICAM is shown in Fig. 2. As demonstrated previously (Kuhlman et al. 1991) DCEK.ICAM presents antigen significantly more efficiently to C.F6 than does DCEK.Hi7. The anti-LFA-1 antibody, FD441.8, had no effect on antigen presentation by DCEK.Hi7 and blocked presentation by DCEK.ICAM so that the antigen dose-response curves for the two cell lines coincide. This result indicates that the only ligand for LFA-1 expressed by any of the cells is ICAM-1.
Fig. 1. Surface expression of ICAM-1 by DCEK.Hi7 and the ICAM-1-transfected daughter lines DCEK.imod and DCEK.ICAM. The negative control shows background staining with FITC-labeled goat anti-rat alone. Cells (1×10^6) were incubated for 60 minutes on ice with the anti-murine ICAM-1 monoclonal antibody BE29G1 (15). After washing away unbound antibody, cells were incubated an additional 60 minutes with FITC-labeled goat anti-rat IgG (Zymed), washed, and fixed. DCEK.imod is a subclone of DCEK.ICAM.

The distribution of LFA-1 molecules on the C.F6 cell was analyzed by immunofluorescence confocal scanning microscopy (Fig. 3) and was found to be uniform with no evidence for the presence of large aggregates. The distribution of ICAM-1 over the DCEK.ICAM cell surface also appeared uniform (not shown).

A typical adhesion experiment is shown in Fig. 4 (the sequence is left to right, top to bottom). DCEK cells were seeded into a round micropipette chamber (1.7 cm × 0.7 cm) with a total volume of 0.8-1.2 ml and incubated at 37°C for one hour. After this period the DCEK cells adhered strongly to the bottom of the chamber, yet did not spread out. The C.F6 cells were added to the chamber, and the micropipette was used to select and hold a single C.F6 cell by application of a small aspiration pressure of 100-500 dyn/cm². The C.F6 cell was brought close to the DCEK cell and aligned by manipulating the holding pipette. The aspiration pressure in the C.F6-holding pipette was removed, and the C.F6 cell was allowed to interact freely with the DCEK cell. The length of the conjugation region between the two cells was measured using confocal microscopy.

Fig. 2. Activation of C.F6 by pigeon cytochrome c peptide presented by DCEK.Hi7 and DCEK.ICAM: functional evidence that alternate LFA-1 ligands are not expressed on DCEK cell lines. Fifty thousand mitomycin C-treated fibroblast cells and 1×10^5 to 2×10^5 T cells were cocultured in 96-well plates (200 μl per well) with various amounts of antigen (synthetic analog of pigeon cytochrome c 81-104). The anti-LFA-1 mAb FD441.8 was added at 40 μg/ml at the initiation of culture. After 48 hours of culture, 50 μl of supernatant were removed from each well and assayed for IL-2 by using the lymphokine-dependent cell line NK. NK cells were cultured with supernatants (200 μl total volume) for 24 hours, including a 6- to 8-hour pulse with [3H]thymidine. Data are representative of three experiments.

Fig. 3. Confocal microscope images of a C.F6 cell. Six consecutive optical sections along the z-axis from a single cell treated with a low osmolarity cytochalasin B solution (112 mosM, 3×10^-7 M cytochalasin B), then stained with fluorescein-labeled M17/4.2 (anti-CD11a) and fixed with 2% formalin. Each section has a thickness of 2 μm and was obtained with a Bio-Rad Microscience MRC-600 confocal microscope using a Nikon ×60 oil immersion lens.
Fig. 4. Sequence of photographs taken from the TV monitor during an adhesion measurement. The numbers on the top of the screen represent in order; month-day, hour, minute, second and tens of milliseconds. The pipette radius is 2.1 μm. First row, left to right: conjugation of C.F6 and DCEK.ICAM. Second row: recapture of C.F6 and cell separation ($P_c=1.37 \times 10^4$ dyn/cm²). Separation of the two cells took about 40 seconds. Third row: C.F6 is shown moving back to the DECK.ICAM cell after release of aspiration pressure.

Table 1. Summary of adhesion measurements

<table>
<thead>
<tr>
<th></th>
<th>$R_*$ (μm)</th>
<th>$P_c \times R_*$ (dyn/cm)</th>
<th>$S_c$ ($10^3$ dyn/cm²)</th>
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</thead>
<tbody>
<tr>
<td>DCEK.Hi7 (n=19)</td>
<td>2.28±0.122</td>
<td>0.22±0.011</td>
<td>0.141±0.012</td>
</tr>
<tr>
<td>DCEK.Imod (n=45)</td>
<td>3.14±0.15</td>
<td>1.05±0.11</td>
<td>0.408±0.047</td>
</tr>
<tr>
<td>DECK.ICAM (n=64)</td>
<td>3.56±0.14</td>
<td>1.87±0.15</td>
<td>0.625±0.047</td>
</tr>
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*R*, radius of conjugation area. Values shown are corrected by the cytochalasin, osmotic swelling procedure. Uncorrected values are given in parenthesis. *n* is number of cell pairs studied. *Values are means±s.e.m.*

two cells as seen on the video monitor increased with time until reaching average plateau levels of 5.70 μm for the DCEK.ICAM-C.F6 pairs, 5.32 μm for the DCEK.Imod-C.F6 pairs, and 3.68 μm for the DCEK.Hi7-C.F6 pairs (Table 1). The conjugation area increased as soon as the cells came into contact and was maximal within 5-10 min. The increase was approximately linear with time, with similar slopes for DCEK.ICAM and DCEK.Imod until steady-state levels were achieved (Fig. 5). This time course was confirmed in separate adhesion assays measuring the binding of C.F6 cells to tissue culture plates coated with purified ICAM-1 (not shown).

The area of conjugation was calculated by assuming its shape to be that of a disk seen edge on in the video images. At steady state, this area averaged 39.8 μm², 31.0 μm² and 16.3 μm² (values corrected as described above) for the DCEK.ICAM-C.F6 pairs, the DCEK.Imod-C.F6 pairs and the DCEK.Hi7-C.F6 pairs, respectively. In the resting state, the mean diameters of DCEK.ICAM, DCEK.Imod and DECK.Hi7 were 15.36 μm, 14.35 μm and 14.05 μm, respectively. The C.F6 cells had a mean diameter of approximately 8.8 μm (uncorrected values).

At 5-15 minutes after the beginning of conjugation, the C.F6 cell-holding pipette was manipulated to recapture the C.F6 cell and the aspiration pressure in the C.F6 cell-holding pipette was increased stepwise in increments on the order of 100-500 dyn/cm² (Fig. 4). At each pressure level, the C.F6 cell-holding pipette was
lymphoblastoid target JY were readily observable in the cytotoxic T lymphocyte (CTL) clone Fl and its visible by light microscopy (x8000). In a previous study remained connected by thin tethers that were barely conjugation shape and position, suggesting that the cells from the DCEK cell, which remained attached to the conjugated with the DCEK cell. With sufficiently high light microscope. (Sung et al. 1986) tethers that formed between the holding pressure was released, the C.F6 cell became attached to its holding pipette. After separation, if the bottom of the chamber, while the C.F6 cell remained separated as the C.F6-holding pipette was pulled away gradually (at a rate of approximately 0.25 dyn/cm²). For low pressures, such pulling caused the C.F6 cell to slip out of its holding pipette while remaining conjugated with the DCEK cell. With sufficiently high pressure, the conjugated cells became completely separated as the C.F6-holding pipette was pulled away from the DCEK cell, which remained attached to the bottom of the chamber, while the C.F6 cell remained attached to its holding pipette. After separation, if the holding pressure was released, the C.F6 cell became reattached to the DCEK cell and resumed its original conjugation shape and position, suggesting that the cells remained connected by thin tethers that were barely visible by light microscopy (×8000). In a previous study (Sung et al. 1986) tethers that formed between the cytotoxic T lymphocyte (CTL) clone Fl and its lymphoblastoid target JY were readily observable in the light microscope. The results of measurements on 64 DCEK.ICAM-C.F6 pairs, 45 DCEK.Imod-C.F6 pairs and 19 DCEK.Hi7-C.F6 pairs are summarized in Table 1. The radius of the conjugation area and the critical separation stress (force/area) were higher for cell pairs involving the ICAM-1-expressing cells. For DCEK.ICAM-C.F6 pairs the $S_c$ was found to be $6.25 \times 10^4$ dyn/cm². This is 4.5 times the critical separation stress for DCEK.Hi7-C.F6 and 1.25 times that for DCEK.Imod-C.F6 conjugates.

To determine whether other cell-surface molecules on C.F6 contributed to the strength of adhesion, we repeated measurements on DCEK.ICAM-C.F6 pairs in the presence of monoclonal antibodies (mAbs). The effect of the presence of the anti-ICAM-1 mAb BE29G1 on adhesion strength can be seen in Fig. 6 by comparing the deformation of the C.F6 cell membrane within the pipette with that seen in Fig. 4. The diminished critical separation pressure due to the presence of the anti-ICAM-1 mAb is clearly evident in the reduced deformation of the segment of the cell membrane in the holding pipette during separation. Other mAbs used were FD441.8 (LFA-1), Y17 (Eκ), GK1.5 (CD4), 30-H12 (Thy 1) and 11-4.1 (H2Kk), all at 0.40–400 µg/ml. The results from these experiments were normalized to those obtained in the absence of mAbs (Fig. 7). In the presence of BE29G1 or FD441.8, the values of $R_i$ were reduced by 20–30%. The force resultants $(P_c \times R_p)$ for separation were much smaller for the pairs under antibody treatment and were the same as measured for DCEK.Hi7-C.F6 pairs. The BE29G1 and FD441.8 mAbs reduced the $S_c$ 84% and 79%, respectively, accounting for virtually all of the difference in $S_c$ between DCEK.ICAM and DCEK.Hi7. With the further addition of mAbs against CD4 and Eκ, the adhesion strength between the cells was diminished somewhat further, with a total reduction in force resultant of 93% and in the $S_c$ of 87%. Antibodies had similar effects on the DCEK.Imod-C.F6 pairs (not shown). No significant reduction in the strength of adhesion occurred between ICAM-1-negative DCEK.Hi7 and C.F6 in the presence of the antibodies. The above results indicate that most of the adhesive force generated between the ICAM-1-bearing DCEK cells and C.F6 is due to the LFA-1-ICAM-1 interaction with relatively little contribution from the T cell antigen receptor and CD4.

Fig. 8 compares results (uncorrected values) obtained in this study with those obtained in a previous study of adhesion between the lymphoblastoid cell line JY and the alloreactive CTL line F1 (Sung et al. 1986). The radius of the conjugated area between DCEK.ICAM-C.F6 pairs was about twice as large as that of JY-F1 pairs. The rate at which the conjugation area increased before reaching steady state values was also faster in the present study. The separating force resultant for the DCEK.ICAM-C.F6 pairs was 1.9 times higher than that for the JY-F1 pairs, whereas the separation force resultant for the DCEK.Imod-C.F6 pair was similar to that for the JY-F1 pair. Because of the smaller area of conjugation, the JY-F1 pairs had an adhesion strength, as measured by the critical separation stress, twice that of the DCEK.ICAM-C.F6 pairs.

**Discussion**

As the goal of this study was to quantitate the ICAM-1/LFA-1 interaction, we sought to determine whether other LFA-1 ligands are expressed on DCEK cells. Direct proof of their presence or absence cannot be achieved, however, because suitable reagents are not yet available. Two pieces of indirect evidence support the conclusion that ICAM-1 is the only LFA-1 ligand involved in the interaction of C.F6 with DCEK transfectants. First, as shown in Fig. 2, antibodies to LFA-1 have no significant effect on the response of C.F6 to antigen presented by DCEK.Hi7, indicating that LFA-1 ligands are absent from the antigen-presenting cell. In contrast, anti-LFA-1 mAbs inhibit antigen presentation by the ICAM-1 transfectant,
Fig. 6. Effect of anti-ICAM-1 mAb on adhesion of DCEK.ICAM and C.F6. The experiment shown was carried out in the presence of mAb BE29G1 (45 μg/ml) on the same day as the sequence shown in Fig. 3. Top row: conjugation of DCEK.ICAM and C.F6 with the same size pipette as in Fig. 3. Bottom row: separation of the cells ($P_c=686$ dyn/cm$^2$).

Fig. 7. Normalized histogram of the radius of conjugated area ($R_1$), critical force resultant ($P_c \times R_p$), and critical separation stress ($S_c$) for DCEK.ICAM-C.F6 cell pairs in the presence of mAbs. All values have been normalized to those for C.F6-DCEK.ICAM cell pairs in the absence of antibodies.

DCEK.ICAM to the level of the ICAM-1-negative cell line, but not below, indicating that the only LFA-1 ligand is ICAM-1. Second, as shown in Fig. 7, antibodies to LFA-1 and antibodies to ICAM-1 have a similar impact on the adhesion of C.F6-DCEK conjugates.

Our results show that ICAM-1 expression by the fibroblast antigen-presenting cells resulted in an in-
crease in the area of contact with the C.F6 cell and an increase in the force per unit area, the critical separation stress, required to separate the conjugates. The magnitude of these parameters reflected the level of fibroblast ICAM-1 expression. Evidently, other adhesion mechanisms also contributed to conjugate formation, as C.F6 freely formed conjugates with the ICAM-1-negative cell line, DCEK.Hi7. The contribution of these other mechanisms was, however, relatively weak and accounted for 1/5 or less of the critical separation stress for C.F6-DCEK.ICAM cell pairs. The greater strength with which C.F6 binds to DCEK.ICAM cells compared to DCEK.Hi7 is consistent with the relative efficiencies of these cell lines in presenting antigen to C.F6. It is difficult to determine whether all of the enhancement in the function of DCEK.ICAM relative to its parent cell line is due to the adhesion-stabilizing contributions of the LFA-1/ICAM-1. LFA-1 has also been shown to have costimulatory functions in addition to its function as an adhesion receptor (van Seventer et al. 1990; Kuhlman et al. 1991).

We have found that T cell lines typically express $10^5$ to $10^6$ LFA-1 molecules/cell by Scatchard analysis of mAb binding, with C.F6 at the high end of this range (B. A. Lollo, unpublished). Thus, a large number of LFA-1/ICAM-1 molecules are available in this system for adhesion stabilization. The high expression levels for LFA-1 may indicate that the LFA-1/ICAM-1 interaction is of low affinity. The inverse relationship between receptor affinity and required receptor number for stable adhesion has been discussed by Hammer and Lauffenburger (1987). The adhesion experiments described here were carried out under static conditions, in the absence of a shear force. Thus, the strength of adhesion is most likely to be determined by the number of receptors involved and the reverse rate for bond formation under stress (Bell, 1978; Hammer and Lauffenburger, 1987). Slow rate constants for formation of the LFA-1/ICAM-1 interaction are suggested by the observation that activated neutrophils under shear stress in a parallel plate flow chamber do not adhere to ICAM-1 in lipid bilayers unless CD62 is also present (Lawrence and Springer, 1991). CD62, on the other hand, promotes neutrophil rolling but not stable adhesion under shear stress.

After initial contact, the conjugation area for cell pairs containing ICAM-1-expressing fibroblasts increased steadily over several minutes until achieving a constant value. This process may correspond to the time-dependent strengthening of adhesion observed by Lotz et al. (1989) in their measurements of adhesion to substrata coated with fibronectin and tenasin. In that study the area of cell-substratum contact also increased with time and was correlated with increased adhesive force. Under critical aspiration pressure, the C.F6 cells separated smoothly from the fibroblasts. In cases where tether formation was observed we do not know from which of the cells the tethers originated. In their analysis of detachment of red cells held by one or a few agglutinin molecule cross-bridges, Evans et al. (1991a) observed that bond failure was caused by the extraction of receptor molecules from the cell membrane. It is possible that tether formation is the result of such an event. Both LFA-1 and ICAM-1 have been reported to interact with the actin cytoskeleton (Vogt and Derich, 1991; Pardi et al. 1992), although for T cells the association was dependent on activation. As noted above, tether formation and deformation of the C.F6 cell was much less pronounced than observed in the detachment of the CTL clone F1 from its target cell JY.

A number of differences were observed between the present system and the JY-FI system. The time course of growth of the area of conjugation between the DCEK.ICAM-C.F6 cell pairs showed no noticeable time lag, whereas the curve for the JY-FI pairs typically had an s-shape, showing a brief latent period before the conjugation area increased. The area of conjugation at steady state for DCEK.ICAM-C.F6 is about 4 times that of the F1-JY cell pairs, but the adhesion strength per unit area for DCEK-C.F6 conjugates is only about one-half of that of JY-FI pairs. The stronger adhesion between the JY-FI pairs may reflect the involvement of molecules other than LFA-1 and ICAM-1, particularly the T cell antigen receptor and CD4 on the F1 cells and DR molecules on the JY cell, with the further possible contribution of LFA-1 avidity modulation on both cells.

The experiments described here were carried out on single cell pairs between a resting T cell line and Eβ-expressing fibroblasts in the absence of antigen. In the previous study, the CTL clone F1 was capable of recognizing allogeneic MHC class II-encoded molecules expressed constitutively by the JY cell line. Thus, it was not possible to separate the contributions to adhesion of the T cell antigen receptor and the effect of T cell receptor occupation on LFA-1 avidity. Furthermore, JY expresses ICAM-1, ICAM-2, ICAM-3 and LFA-1 (de Fougerolles and Springer, 1992). In the present system, the antigen, an exogenous peptide, was not present in these experiments, and the DCEK cell lines do not express LFA-1. Thus, in future studies it will be possible to determine the effect in this system of providing antigen, with the consequent involvement of the C.F6 antigen receptor.

We are grateful to Dr. Robert Hoffman for the use of his confocal microscope, and to Dr. Richard Dutton, Dr. Aydin Tozeren and Dr. Richard Skalak for their critical review of the manuscript. We thank Mr. Simon Telian, Mr. Jason Haga and Ms. Elaine Hanson for their technical assistance, and Ms. Jackie Richardson and Ms. Allison Phillips for their secretarial assistance. This work was supported by U.S. Public Health Service grants RO1-CA37955 and HL43026, American Cancer Society grants IM-552, IM-648, and a Rita Allen Foundation Scholarship (A.A.B.).

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(Received 2 December 1991 - Accepted, in revised form, 19 May 1992)