Annals of Biomedical Engineering, Vol. 25, pp. 1072–1080, 1997
Printed in the USA.

0090-6964/97 $10.50 + .00

Controlling Receptor-Ligand Contact to Examine Kinetics of T Cell Activation

S. M. PATRICK,* H. AN,* M. B. HARRIS,† I. B. IVANOV,‡ N. S. BRAUNSTEIN,† and E. F. LEONARD*

*Department of Chemical Engineering and †Department of Medicine, Columbia University, NY, NY, and ‡Laboratory of Thermodynamics and Physiochemical Hydrodynamics, University of Sofia, Sofia, Bulgaria

Abstract—A method for controlling the contact of cell-surface receptors with immobilized ligands has been developed. Cells are trapped in an asymmetric liquid film that can be quantitatively thinned by reducing the film's capillary pressure. Ligands adsorbed to the liquid–solid interface are forced into increasingly tighter contact with the cells as the air–liquid interface is drawn down. Controlling the degree of thinning allows study of repulsive forces, and controlling its time course produces a definite time 0 for analyzing signal transduction. This system was tested by examining the time course of calcium mobilization in T cells upon activation with anti-CD3 antibody at different dilutions and ionic strengths. The averaged calcium transient of the responding cells was essentially the same for each condition. However, the fraction of responding cells decreased with anti-CD3 dilution, and indicated that the critical ligand density for T cell activation lies between ~35 and 70 molecules of anti-CD3 per μm^2. Decreasing the medium's ionic strength from the normal value of 157 mM to 57 mM did not affect either the average calcium response profile or the fraction of responding cells, but strongly affected receptor-ligand contact, decreasing the percent of spontaneous activation from 38% to 5%. Such an imposed decrease sets the stage for film thinning to impose much greater control of receptor-ligand contact.

Keywords—T cell activation, Signal transduction, Biophysical forces, Receptor-ligand interaction.

INTRODUCTION

A new method has been developed to control receptor-ligand contact. Briefly, cells are trapped in a liquid film over a solid surface to which ligands of interest have been attached. The film is asymmetric in that it is bounded on one side by a solid and on the other by gas. When the film is thinned, cell receptors are brought into contact with the ligands. The apparatus used to facilitate this thinning is adaptable to the microscope stage and can be used to examine forces opposing receptor-ligand contact, as well as the kinetics of signal transduction events resulting from receptor-ligand binding. In this study, the apparatus is used to investigate the activation of T lymphocytes by anti-CD3.

Initiation of an immune response normally requires cooperation between antigen presenting cells (*e.g.*, macrophages, B lymphocytes, or dendritic cells) and T lymphocytes. Although various surface molecules on these cells interact and thereby contribute to both signaling and cell adhesion (23), the specificity of the T lymphocyte response is a function of a relatively low-affinity interaction of the clonotypic T cell receptor (TCR) molecule with its ligand, an MHC-peptide complex (6,24). Signal transduction through the TCR and the associated CD3 complex (18,20) leads to the induction of various cellular messaging systems, including a rise in intracellular free calcium (4).

Intracellular calcium mobilization, an early event in T cell signal transduction, has been monitored in a number of studies examining activation of T cells by APCs. Early studies recorded intracellular calcium at the single-cell level using calcium-sensitive fluorescent dyes and fluorescence microscopy, and demonstrated that calcium was mobilized as a result of specific antigen presentation by an APC (15,22). More recent studies have provided valuable information on the time course of the intracellular calcium response and its dependence on APC stimulatory peptide concentration (1,19), as well as on the accompanying morphological changes of the T cell (8). These single-cell studies found considerable variability among individual T cell responses, an observation that had been obscured by the analysis of average responses in population studies. Population studies necessarily reveal an average response, but often one that loses important details of the individual responses, even when they are manifested in every cell of the population because activation is asynchronous. In many of these studies, it was difficult for the investigators to initiate APC-T cell contact at all. For example, Donnadieu *et al.* (8) sometimes centrifuged the two cell types together to initiate contact.

Acknowledgment—We thank Dr. Edwardo Macagno for allowing us to use his calcium imaging system and Dr. Huajun Wang for his assistance with its use. This work was supported by the Artificial Organs Research Fund at Columbia University.

Address correspondence to E. F. Leonard, Department of Chemical Engineering, Columbia University, Mudd Building, Room 814, 500 West 120th Street, NY, NY 10027.

(Received 17Apr97, Revised 14Jul97, Revised, Accepted 17Jul97)

The experimental difficulty of initiating APC-T cell contact may result from the biophysical forces that exist between all cells. The formation of specific bonds between two cells or a cell and a surface takes place against a background of nonspecific repulsive forces (3). These forces, which prevent aggregation in the bloodstream, include electrostatic, steric, and hydration interactions and arise largely from the layer of negatively charged polysaccharides attached to proteins and lipids in the cell membrane. This layer extends up to 150 nm from the cell surface (14). In low ionic strength solutions, the range of the repulsive electrostatic force increases because charge shielding by counterions in solution is reduced (25). The hydration layer surrounding cells is also increased in solutions of low ionic strength (25). Nonspecific attractive forces between cells, van der Waals and hydrophobic, also exist; however, the repulsive forces dominate through the range where specific recognition occurs (14). The existence of this repulsive barrier was demonstrated by Capo *et al.* (5), who showed that cells often must be forced together for strong specific bonds to be formed.

T cell activation by APCs involves a large number of variables, including efficiency of antigen processing and presentation by APCs, ligation of a variety of surface molecules, and cytokine production. However, engagement of the TCR or CD3 complex with a specific antibody can directly, if artificially, stimulate T cells and leads to an observable calcium response (27). A number of studies have been done to examine more directly signals mediated by the TCR complex (13,16). The delay in calcium mobilization observed in these studies is caused in part by a ligand-receptor recognition-binding event and in part by signal transduction from the TCR complex. Since it is not possible to distinguish completely between these two steps, studies like these cannot give precise information on the time course of T-cell signal transduction. A method to generate a more accurately controlled time 0 for ligand-receptor contact would help to separate these steps.

The method developed in this laboratory allows ligands to be brought into tight, controlled contact with cell surface receptors, overcoming repulsive forces and creating an accurately controlled time 0. An open chamber is attached to a capillary tube allowing the thickness of a liquid film to be adjusted (Fig. 1A). A ligand-coated coverslip at the base of the chamber forms the solid–liquid interface and allows real-time microscopic observation of the cells. Medium is added to the well, forming a thick film to which cells are added (Fig. 1B). The film is then quantitatively thinned, pulling the liquid–air interface down, and forcing cells into contact with the adsorbed ligands on the air–solid interface (Fig. 1C). The essential quality of the apparatus is its capacity to impose a known, uniform force on a group of cells in a setting where they can be observed individually.

When the film is quantitatively thinned, the cells are deformed. The shapes of the deformed cells can be determined by interference microscopy. When cells entrapped in the thin film are illuminated from a point source of monochromatic light, interference rings are produced by the curvature of the liquid film covering the cell. Using the interferograms and equations describing the film, the profile of the deformed cell can be reconstructed. The pressure acting on the cell surface can be determined from the deformed cell shape (2,10).

In the experiments reported here, anti-CD3 IgG molecules were adsorbed via a Protein A interlayer to the solid boundary of the film-thinning apparatus. Using anti-CD3 antibodies allowed the examination of initiation of receptor-ligand binding with a simplified ligand-coated surface. Murine $CD4^+$ T cell hybridomas were loaded with a calcium-sensitive fluorescent dye and added to the chamber. After film thinning, T cell activation was examined by monitoring intracellular free calcium levels using fluorescence microscopy.

Using this system, the kinetics of T cell activation was examined by monitoring the calcium response profiles of single T cells activated at a precisely controlled time 0. Using surfaces of varying anti-CD3 density, the critical ligand density for T cell activation was examined. In addition, by varying the ionic strength of the medium, the role of repulsive electrostatic forces in preventing receptor-ligand binding was investigated.

METHODS

Materials

Click's Medium (EHAA) was obtained from Irvine Scientific (Santa Ana, CA, USA). Fetal calf serum (FCS) was obtained from Hyclone (Logan, UT, USA). The monoclonal antibody used (145-2C11), directed to the ϵ chain of the CD3 complex on T cells, was obtained from Pharmingen (San Diego, CA, USA). Goat anti-human albumin antiserum, Protein A, and RPMI 1640 were obtained from Sigma (St. Louis, MO, USA). Fura-2/AM was obtained from Molecular Probes (Eugene, OR, USA). Chromic-sulfuric acid was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were of reagent grade or better.

Cell Culture

Murine 7E12 CD4(+) T hybridomas were developed in the laboratory of Dr. Ned Braunstein. The cell line was maintained in Clicks Medium (EHAA) with 10% (v/v) FCS, and 0.5% (v/v) of 0.1 M β-mercaptoethanol at 37°C and 5% CO_2.

Asymmetric Film Thin Apparatus

The apparatus, shown in Fig. 1A, was composed of a plastic ring containing a hole 10 mm in diameter and 6 mm

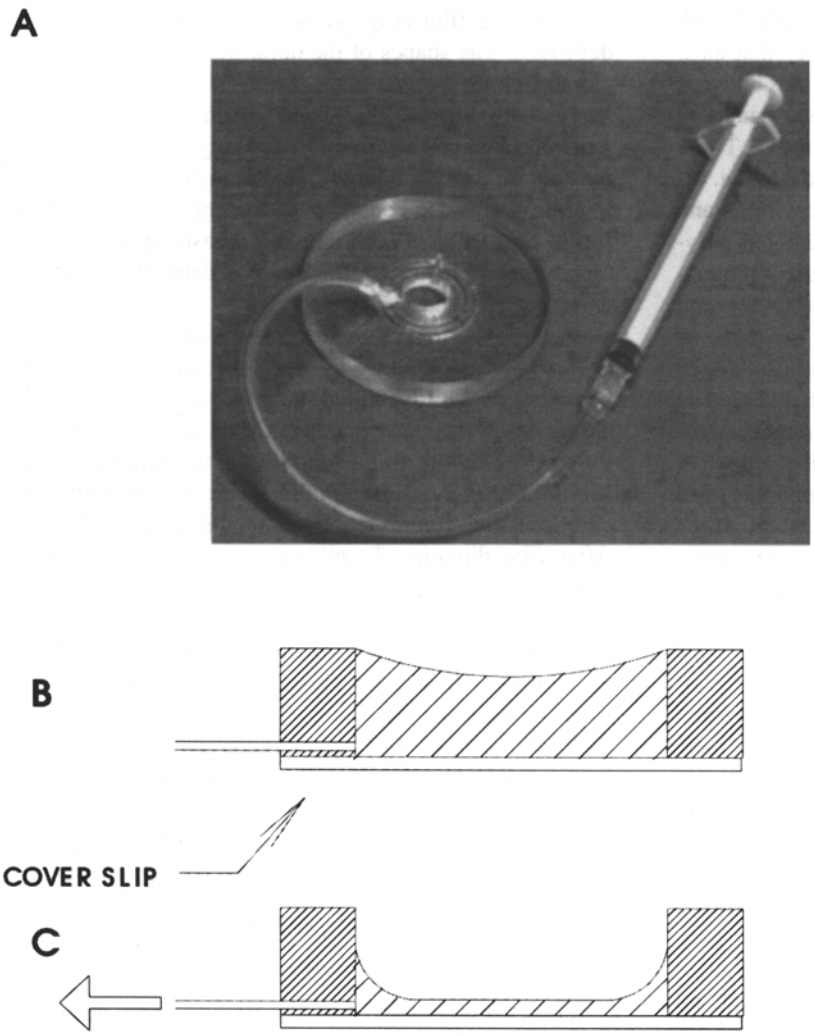

FIGURE 1. Asymmetric film thinning apparatus. (A) Chamber with attached syringe. (B) Thick film. (C) Thin film.

deep. An antibody-coated coverslip was attached to the bottom of the apparatus, forming a well. The well was filled with medium, forming a thick film (Fig. 1B). A small hole at the base of the well was attached to a 1 ml syringe that allowed the film to be thinned (Fig. 1C). For these experiments, the capillary pressure was reduced to a value ~0.0004 atm below ambient. From asymmetric film analysis (2), this pressure was calculated to apply an average pressure of 0.054 atm to the top surface of the cell.

Fluorescence Imaging System

The imaging system consisted of a Photometrics (Tucson, AZ, USA) series 200 cooled CCD videocamera mounted on a Nikon (Melville, NY, USA) Diaphot-inverted microscope equipped with 40×/0.70 LWD and 40×/1.3 objectives, a super high-pressure mercury lamp, a 400 nm dichroic mirror, a 480 nm long-pass emission filter, and a computer-controlled filter wheel with 340 and 380 nm excitation filters and a shutter. The exposure time for images collected under both 340 and 380 nm excitation was 0.2 sec. The images were created using a 2 × 2 bin on the CCD chip, resulting in a final image size of 150 × 150 pixels. The camera output was collected and analyzed by a Macintosh Quadra 950 Apple computer (Cupertino, CA, USA) using custom calcium imaging software provided by Photometrics.

Antibody Immobilization

Chromic sulfuric acid-washed round coverslips were incubated with an excess of protein A IgG binding fragment (100 μg ml^{-1}) for 30 min, then washed twice with saline. Then, the coverslips were incubated with an excess of antibody solution (100 μg ml^{-1}) for 30 min. The antibody solutions contained different ratios of anti-CD3 antibody with anti-albumin antiserum. Protein A was used to orient the IgG molecules on the coverslip and minimize the possibility that the antibodies might induce spurious activation by engagement of the T cell Fc receptors. The

TABLE 1. Experimental conditions.

	Ionic Strength (mM)[a]	Anti-CD3 Dilution
1	157	1:25
2	157	1:100
3	157	1:200
4	157	1:400
5	57	1:25

[a]Medium Debye lengths: 157 mM = 0.77 nm, 57 mM = 1.27 nm.

coverslips were washed twice with saline and attached to the chamber using a thin layer of vacuum grease applied to a boss on the bottom surface.

Intracellular Calcium Measurements in Asymmetric Film Thin Apparatus

Cell Preparation. 7E12 cells (~2 × 10^6 cells ml^{-1}) were incubated in culture medium with 5 μM Fura-2/AM for 30 min at 37°C. Then, the cells were washed twice by centrifugation at 280*g* for 7 min, and resuspended in experimental medium at 5 × 10^6 cells ml^{-1}. The experimental medium, RPMI 1640, was similar to the culture medium, except it lacked phenol red, FCS, and β-mercaptoethanol, and contained 20 mM HEPES buffer and an additional 0.6 mM calcium.

Chamber Preparation. Antibody-coated coverslips at different anti-CD3 dilutions were attached to the chamber. 180 μl of either experimental medium of normal ionic strength (157 mM) or a mixture of normal and low (14 mM) ionic strength medium (57 mM final ionic strength) was added to the well (Table 1). The Debye length of each solution was calculated from the ionic strength using a formula for 1:1 electrolytes (12). The medium of low ionic strength was a 1 mM calcium-supplemented phosphate-buffered saline solution adjusted with sucrose to an osmotic pressure of 295 mOsm. 20 μl of Fura-loaded T cells (5 × 10^6 cells ml^{-1}) were added to the well using a pipette.

Calcium Imaging. Pairs of images were taken of the cells to monitor their intracellular calcium levels. The ratios of the intensity at 340 nm excitation (from free Fura-2) over the intensity at 380 nm excitation (from calcium bound Fura-2) were calculated by the software program, and from these the intracellular calcium levels were calculated using the method of Grynkiewicz *et al.* (9). The average ratio was determined for each cell at each time point. The ratios of the cells were monitored for 1 to 2 min to determine their baseline calcium levels. Then, the film was thinned by reducing the capillary pressure by 0.0004 atm. The cell ratios were monitored after film thinning. A T cell was considered activated when its ratio increased to at

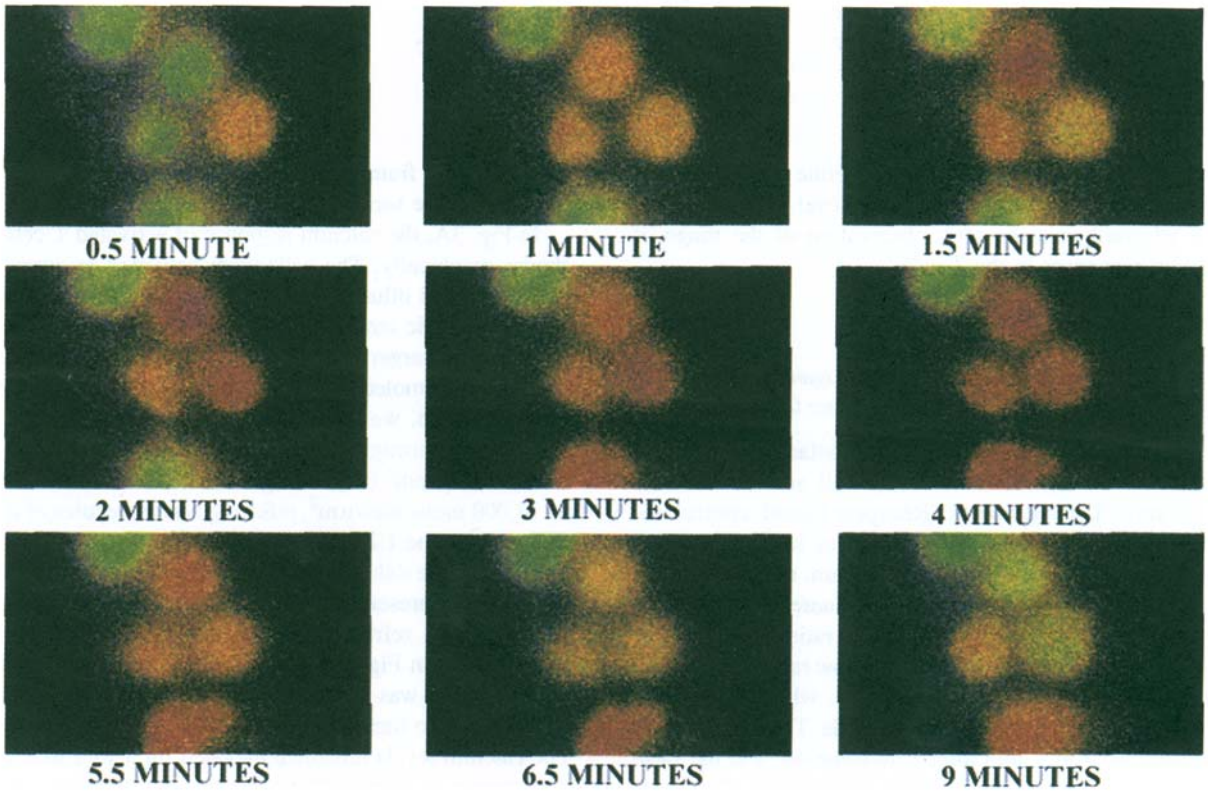

FIGURE 2. Ratioed images shown in false color. Calcium mobilization in T cells activated by anti-CD3 diluted 1:25 with anti-albumin.

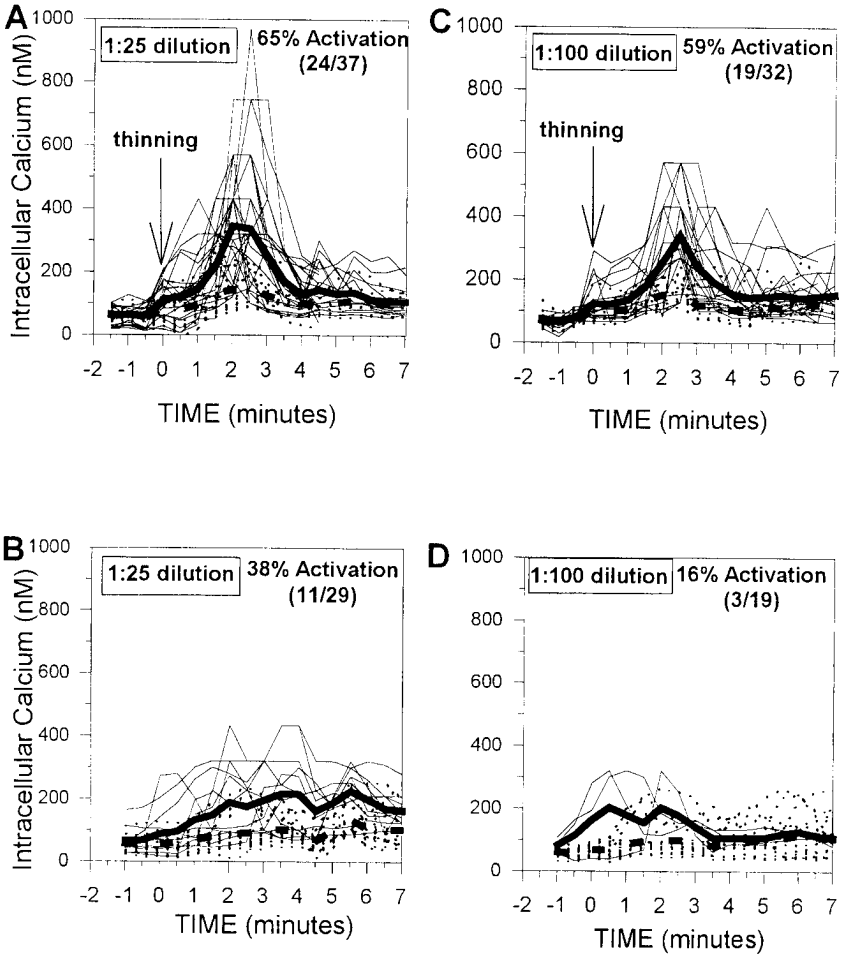

FIGURE 3. Calcium response of 7E12 cells activated by anti-CD3 diluted with anti-albumin in medium of normal (157 mM) ionic strength. Thin solid and dashed lines represent responses of individual activated and refractory cells, respectively. Thick lines represent average responses of activated and refractory populations. Anti-CD3 was diluted 1:25 with anti-albumin in (A) and (B), 1:100 in (C) and (D), 1:200 in (E) and (F), and 1:400 in (G) and (H). In (A), (C), (E), and (G), the film was thinned to initiate receptor-ligand contact. In (B), (D), (F), and (H) [the controls], the films were not thinned.

least 50% above its unstimulated baseline ratio. This corresponds to an intracellular calcium level of 270 nm and was arbitrarily chosen after observation of the range of calcium responses in the T cells.

RESULTS

Film Thinning Initiates Cell Receptor-Ligand Contact and Produces a Controlled Time 0

The chamber was used to initiate contact between CD3 complexes on the surface of the T cell and the immobilized anti-CD3 antibodies. Receptor-ligand contact resulted in T cell activation, observed as intracellular calcium mobilization. In Fig. 2, the calcium rise in activated T cells is shown in false color by the fluorescence imaging system. The false color represents the ratio of the pairs of images. Green and yellow cells have low ratios, and, therefore, low intracellular calcium levels, whereas red cells have high ratios and high calcium levels. The cells became activated ~0.5 to 1 min after film-thinning, and the false color changed to red, indicating a rise in intracellular calcium levels. After a few minutes, the calcium level began to return to baseline. The presence of cells of different colors in each frame indicates that the responses of individual cells are somewhat different.

In Fig. 3A, the calcium response of activated T cells is shown graphically. The cells were activated by immobilized anti-CD3 diluted 1:25 with anti-albumin in medium of normal ionic strength. Since the molecular weight of IgG is much larger than that of the protein A used, and both of these molecules were adsorbed using a large excess of protein, we assumed that the IgG density can be approximated using its vertical cross-sectional area. Using a value of 70 nm^2 (21), the IgG density was calculated to be 14,000 molecules/μm^2, inferring 560 molecules of anti-CD3/μm^2 at the 1:25 dilution point. The thin lines in Fig. 3 represent the calcium response of individual cells. The thick lines represent average calcium profiles of the responding and refractory populations. The average response curve in Fig. 3A shows that, upon receptor-ligand binding, there was a 0.5 to 1 min lag before the calcium levels began to increase to at least 100% above baseline. The calcium levels remained elevated for 1 to 2 min, and then began to decay. Some heterogeneity among individual cell responses is seen. 65 ± 8% of the cell population became activated upon film thinning, whereas the rest

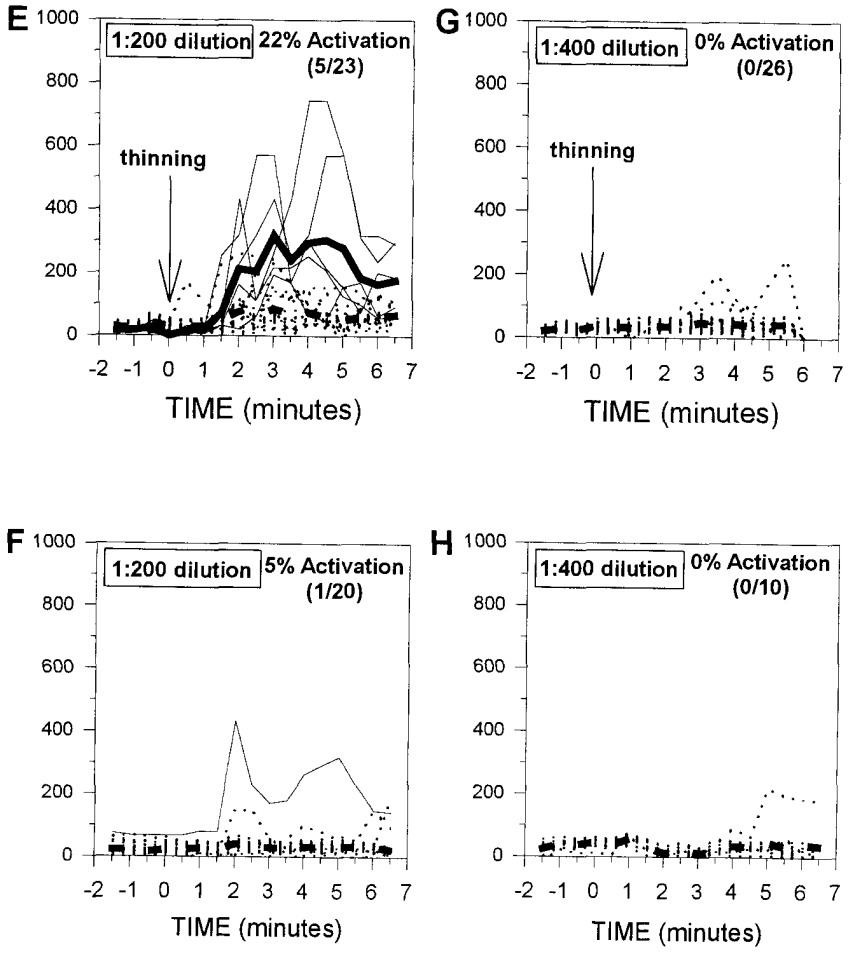

FIGURE 3. Continued

were refractory. (FACS analysis using the anti-CD3 antibody showed that the 7E12 population was 99.8% $CD3^+$ with near-uniform levels of CD3 expression.) The activation upon film thinning was synchronous, as evidenced from the averaged curve.

Figure 3B shows the control for the experiments in Fig. 3A. Calcium mobilization was monitored in cells under the same conditions, but without film thinning to initiate receptor-ligand contact. In this case, 38 ± 6% of the cell population became activated. Since these experiments were conducted in medium of normal ionic strength, the electrostatic repulsive forces were reduced, so some cells became activated as a result of simple settling under the influence of gravity onto the antibody-coated surface. However, without precise control of receptor-ligand contact, the initiation of activation occurred at different times, and the average response curve does not represent the calcium response of a typical cell.

The Percent of Activated Cells Decreases with Anti-CD3 Dilution

The anti-CD3 antibody density was further reduced with anti-albumin to determine the critical ligand density for T cell activation. These experiments were done at normal ionic strength, and are shown in Fig. 3C–3H. Data shown in each graph were collected in 2 or 3 repeated experiments of 10 to 15 cells each, and the standard deviations indicate the range around the averages. In Fig. 3D,F,H, data were collected in one experiment, because the number of cells in the field of view was larger. The shape of the calcium response curve of the responding cells was not changed by anti-CD3 dilution to 1:100. Increasing the dilution of anti-CD3 from 1:25 to 1:100 reduced the anti-CD3 average density from ~560 to 140 molecules/μm^2, but did not significantly reduce the percent of activation upon film thinning, the percent of responding cells falling only to 59 ± 17% from 65% (Fig. 3C). However, in the control (Fig. 3D), the percentage of spontaneous activation was reduced to less than half when the anti-CD3 dilution was increased from 1:25 to 1:100.

When the anti-CD3 density was diluted 1:200 and 1:400, for an approximate anti-CD3 density of 70 and 35 molecules/μm^2, the percent of cells activated upon film thinning was reduced to 22 ± 11 and 0%, respectively (Fig. 3E,G). At 1:200, the magnitude of the averaged calcium response curve was the same as at the 1:25 and 1:100

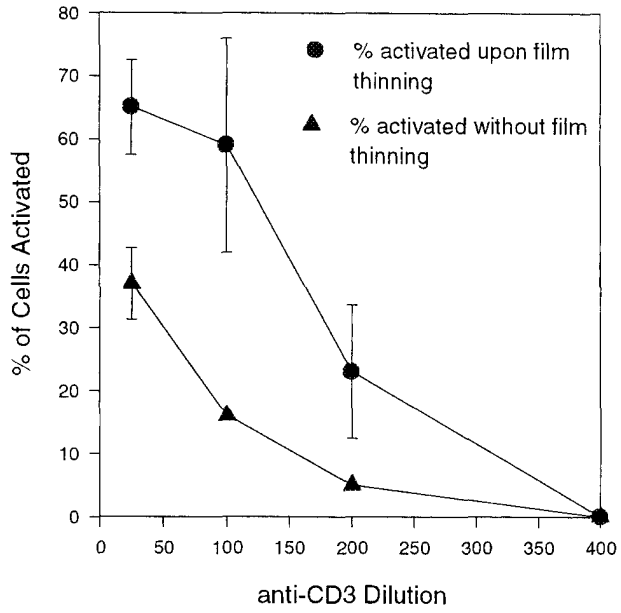

FIGURE 4. Percent of cells responding at each antibody dilution.

dilution points, but the duration was longer. The low anti-CD3 density at this dilution point caused activation to become somewhat asynchronous, despite the controlled receptor-ligand contact, leading to a longer duration of the averaged calcium mobilization curve. Spontaneous activation for the corresponding controls (Fig. 3F,H) was reduced to 5% at 1:200 dilution and 0% at 1:400. Thus, the critical ligand density for activation of T cells with anti-CD3 seems to lie between the 1:200 and 1:400 dilution points where there is an approximated average of 70 and 35 anti-CD3 molecules/μm^2, respectively. Figure 4 summarizes the percents of controlled and spontaneous activation seen at each antibody dilution.

Spontaneous Activation Decreases When Ionic Strength Is Decreased

The ionic strength of the medium was lowered to examine the shielding of repulsive electrostatic forces, as well as to gain better control of receptor-ligand contact. Data were collected in 2 to 3 repeated expeirments of 4 to 11 cells for each condition shown in Fig. 5. Comparison of Figs. 3A and 5A shows that reducing the ionic strength from 157 to 57 mM does not significantly affect the shape of the calcium response or the percent of activation upon film thinning. The percent of activated cells at 1:25 anti-CD3 dilution was 65% at 157 mM ionic strength, and 74 ± 25% at 57 mM. However, reducing the ionic strength to 57 mM greatly reduced spontaneous activation. At 57 mM ionic strength, there was essentially no spontaneous activation (6 ± 8%) (Fig. 5B), as compared with 38% of the cells activating spontaneously at an ionic strength of 157 mM (Fig. 3B).

DISCUSSION

The asymmetric film thinning apparatus provided a precisely controlled time 0 for analyzing the kinetics of T cell signal transduction. Since the interaction of the cell surface CD3 complexes with the immobilized antibodies was

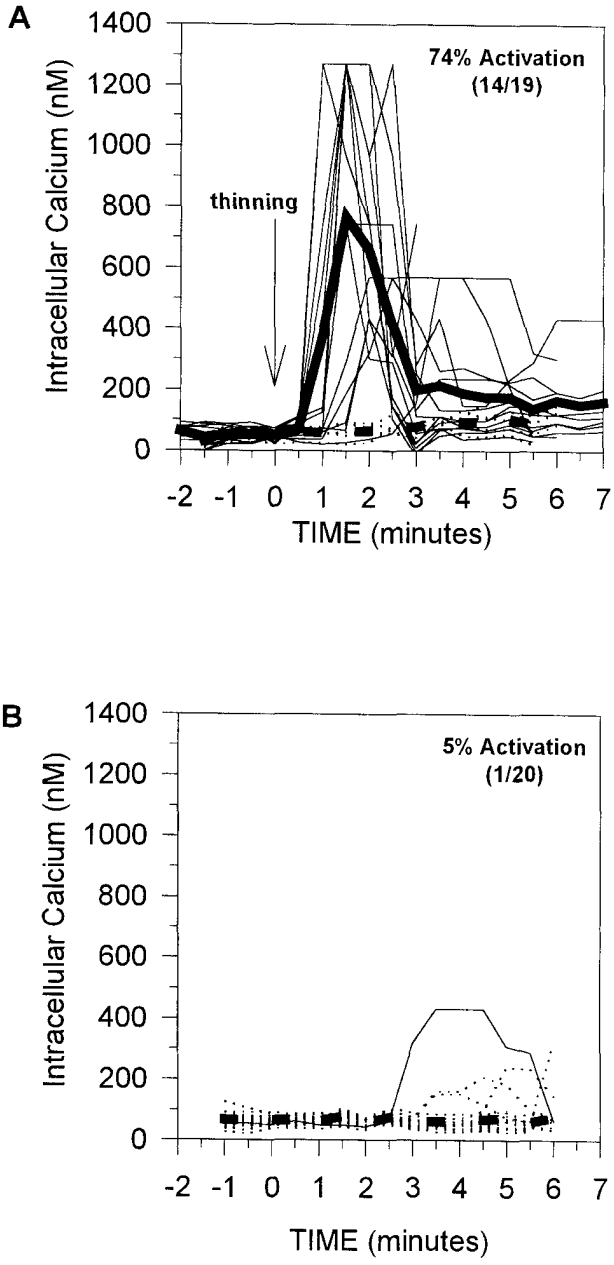

FIGURE 5. Calcium response of 7E12 cells activated by anti-CD3 diluted with anti-albumin in medium of low (57 mM) ionic strength. In (A), the film was thinned to initiate receptor-ligand contact. (B) Control.

controlled by film thinning, the time course of calcium mobilization reflected delays caused almost entirely by signal transduction events. Controlling the initiation of T cell activation also allowed a more representative average response profile to be observed. The average response curves showed that upon ligand-receptor binding, there was a 0.5 to 1 min lag before the calcium levels increased to at least 100% above baseline. Even with a controlled time 0, however, some heterogeneity in calcium responses was observed. The heterogeneity in calcium response among the responding cells probably represents cell-to-cell variation in the time constants for signal transduction events leading to calcium mobilization. Thus, despite uniformity in their level of cell surface CD3 expression, as heterokaryons, the individual hybridoma cells in the population likely vary in the levels of expression of other molecules that couple the CD3-calcium signal.

From the anti-CD3 antibody dilution experiments, the critical ligand density for T cell activation was found to lie between the 1:200 and 1:400 dilution points. Combinatorial analysis based on independent probabilities (to determine the likelihood of 2 or 3 anti-CD3 molecules binding to the protein A-coated surface adjacent to each other) suggests that there is an average of 280 antibody pairs and 14 triplets under each cell at the 1:200 dilution point, and 140 pairs and 1.8 triplets at the 1:400 dilution point. It has been observed that deaggregated soluble anti-CD3 does not lead to calcium mobilization or T cell activation unless cross-linked with secondary anti-IgG, suggesting that aggregation of multiple TCR/CD3 complexes is necessary for activation (17). Therefore, antibody pairs or triplets may be necessary to produce a calcium response. That the critical ligand density falls between the points where 14 and 1.8 triplets are inferred suggests that triplets are more effective than less aggregated antibody in initiating receptor aggregation. The critical number of triplets found here agrees with the number of MHC/peptide complexes (1 to 10), estimated to be needed in the initial T cell/APC contact area to produce a calcium response (1). This number is also consistent with experimental data reporting the total number of complexes needed on an APC for T cell activation (7,11). It should be emphasized that these data address initial events in TCR-mediated signaling and not later features of T cell activation, such as cytokine production, which may require the serial engagement of thousands of TCRs (26).

Figure 3 shows that the magnitude of the calcium mobilization profile of the responding cells was not changed significantly by dilution of the anti-CD3 molecules. This finding suggests that calcium mobilization is an all-or-none phenomenon in T cells activated by anti-CD3 antibody. This phenomenon was also observed by Agrawal and Linderman (1), in T cells activated by APCs displaying decreasing numbers of peptide-loaded MHC complexes on their surfaces.

The ionic strength of the medium was varied to examine the role of repulsive electrostatic forces in preventing receptor-ligand binding. At higher ionic strengths, there are more counterions present, so the negatively charged surfaces of the cells and immobilized ligands will be more effectively shielded from each other. The amount of charge shielding is related to the Debye length of a solution, with solutions of longer Debye lengths experiencing less shielding. The 157 and 57 mM solutions had Debye lengths of 0.77 and 1.27 nm, respectively (12). Figures 3B and 5B show that the amount of spontaneous activation occurring at 57 mM was much less than at 157 mM for the same antibody dilution. This indicates that the repulsive forces have a longer reach in the 57 mM solution and is consistent with the Debye lengths of the two solutions. Lowering the ionic strength did not reduce the percent of activation upon film thinning (Figs. 3A and 5A). Therefore, low ionic strength does not cause the cells to become refractory, but rather seems only to alter the repulsive forces, making this maneuver a useful tool for controlling receptor-ligand contact.

This system can be used to control receptor-ligand binding, to examine forces opposing this process, and to study signal transduction kinetics. It provides a means for examining interactions of cell surface receptors in a system that is physiologically relevant. To initiate receptor-ligand contact, the cells are pushed through a repulsive force field created largely by the negative charges existing on both the cell surface and on the adsorbed ligands. This system can be modified to include a variety of biologically relevant receptor-ligand pairs on the opposing planar surfaces. Then, it should make possible direct study in a controlled manner of the effects of different receptor-ligand interactions in lowering the force required to induce T cell activation.

REFERENCES

1. Agrawal, N. G. B., and J. J. Linderman. Calcium response of T helper lymphocytes to antigen-presenting cells in a single-cell assay. *Biophys. J.* 69:384–395, 1995.
2. An, H., S. M. Patrick, I. B. Ivanov, and E. F. Leonard. Deformation of cells in an asymmetric thin liquid film. Manuscript in preparation, 1997.
3. Bell, G. I., M. Dembo, and P. Bongrand. Competition between nonspecific repulsion and specific bonding. *Biophys. J.* 45:1051–1064, 1984.
4. Cantrell, D. T cell antigen receptor signal transduction pathways. *Ann. Rev. Immunol.* 14:259–274, 1996.
5. Capo, C. et al. Concanavalin-A-mediated thymocyte agglutination: a model for a quantitative study of cell adhesion. *J. Cell Sci.* 56;21–48, 1982.
6. Corr, M. et al. T cell receptor-MHC class I peptide interactions: affinity, kinetics, and specificity. *Science* 265:946–949, 1994.

7. Demotz, S., H. M. Grey, and A. Sette. The minimal number of class II MHC-antigen complexes needed for T cell activation. *Science* 249:1028–1030, 1990.
8. Donnadieu, D. et al. Imaging early steps of human T cell activation by antigen-presenting cells. *J. Immunol.* 148: 2643–2653, 1992.
9. Grynkiewicz, G., M. Poenie, and Y. Tsien. A new generation of $Ca(^{2+})$ indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260:3440–3450, 1985.
10. Hadjiiski, A., R. Dimova, D. Denkov, B. Ivanov, and R. Borwankar. Film trapping technique: precise method for the three-phase contact angle determination of solid and fluid particles of micrometer size. *Langmuir* 12:6665–6675, 1996.
11. Harding, C. V., and E. R. Unanue. Quantitation of antigen-presenting cell MHC class II/peptide complex necessary for T-cell stimulation. *Nature* 346:574–576, 1990.
12. Israelachvili, J. Intermolecular and Surface Forces: With Applications to Colloidal and Biological Systems. San Diego: Academic Press, 1991.
13. Kanner, S. B., J. P. Deans, and J. A. Ledbetter. Regulation of CD3-induced phospholipase C-gamma (PLC gamma 1) tyrosine phosphorylation by CD4 and CD45 receptors. *Immunology* 75:441–447, 1992.
14. Luckham, P. F., and P. G. Hartley. Interactions between biosurfaces. *Adv. Colloid Interface Sci.* 49:341–386, 1994.
15. Nisbet-Brown, E., R. K. Cheung, J. W. W. Lee, and E. W. Gefland. Antigen-dependent increase in cytosolic free calcium in specific human T-lymphocyte clones. *Nature* 316: 545–547, 1985.
16. Oettgen, H. C., C. Terhorst, L. C. Cantley, and P. M. Rosoff. Stimulation of the T3-T1 cell receptor complex induces a membrane-potential sensitive calcium influx. *Cell* 40:583–590, 1985.
17. Ratcliffe, J. H., K. M. Coggeshall, M. K. Newell, and M. H. Julius. T cell receptor aggregation, but not dimerization, induces increased cytosolic calcium concentrations and reveals a lack of stable association between CD4 and the T cell receptor. *J. Immunol.* 148:1643–1651, 1992.
18. Reinherz, E. et al. Antigen recognition by human T lymphocytes is linked to surface expression of the T3 molecular complex. *Cell* 3:735–743, 1982.
19. Rotnes, J. S., and B. Bogen. Ca^{2+} mobilization in physiologically stimulated single T cells gradually increases with peptide concentration (analog signaling). *Eur. J. Immunol.* 24:851–858, 1994.
20. Samelson, L. E., J. B. Harford, and R. D. Klausner. Identification of the components of the murine T cell antigen receptor complex. *Cell* 43:223–231, 1985.
21. Sarma, V. R., E. W. Silverton, D. R. Davies, and W. D. Terry. The three-dimensional structure at 6 A resolution of a human gamma G1 immunoglobulin molecule. *J. Biol. Chem.* 246:3753–3759, 1971.
22. Shapiro, D. N., B. S. Adams, and J. E. Niederhuber. Antigen-specific T cell activation results in an increase in cytoplasmic free calcium. *J. Immunol* 135:2256–2261, 1985.
23. Springer, T. A. Adhesion receptors of the immune system. *Nature* 346:425–434, 1990.
24. Sykulev, Y. et al. Kinetics and affinity of reactions between an antigen-specific T cell receptor and peptide-MHC complexes. *Immunity* 1:15–22, 1994.
25. Trommler, A., D. Gingell, and H. Wolf. Red blood cells experience electrostatic repulsion but make molecular adhesions with glass. *Biophys. J.* 48:835–841, 1985.
26. Viola, A., and A. Lanzavecchia. T cell activation determined by T cell receptor number and tunable thresholds. *Science* 273:104–106, 1996.
27. Weiss, A., and J. B. Imboden. Cell surface molecules and early events involved in human T lymphocyte activation. *Adv. Immunol.* 41:1–38, 1997.