Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 3909–3913, April 1997
Immunology

Antigen receptor engagement delivers a stop signal to migrating T lymphocytes

(locomotion/inflammation/reconstitution/polarity/integrins)

MICHAEL L. DUSTIN*, SHANNON K. BROMLEY, ZHENGYAN KAN, DANIEL A. PETERSON, AND EMIL R. UNANUE

Center for Immunology and Department of Pathology, Washington University School of Medicine, Box 8118, 660 South Euclid Avenue, St. Louis, MO 63110

Contributed by Emil R. Unanue, February 7, 1997

ABSTRACT **We investigated the role of the T cell antigen receptor (TcR) in control of T cell migration in an *in vitro* system. We used T cells from transgenic mice bearing a TcR for the lysozyme peptide 48–62 bound to I-A^k (3A9). T cells from the 3A9 TcR transgenic mice crawled on purified intercellular adhesion molecule-1 substrates, but strikingly, stopped upon interaction with the physiological ligand, i.e., the mouse I-A^k with covalently attached hen egg white lysozyme peptide residues 48–62 complex. TcR-triggered stopping was reversible by treatment with adhesion-strengthening phorbol esters. The microtubule organizing center of stopped cells was positioned adjacent to the site of stable cell anchorage. Direct conversion of lymphocyte function associated-1 to the high-affinity conformation with antibodies also stopped T cells in a similar manner to antigen. Thus, physiological TcR engagement triggers a stop signal through lymphocyte function associated-1. We propose that the stop signal is an early and essential event in T cell activation that also will play an important role in control of T cell migration.**

Antigen-specific T cells are enriched in the draining lymph nodes and antigen-containing tissue sites during an immune response (1–4). The mechanism of this enrichment is not known, but it has been proposed that extended interaction of antigen-specific T lymphocytes with antigen-presenting cells (APC) may account for this effect (5). Consistent with this proposal, the T cell must interact with the APC for several hours to initiate cytokine production and to enter the cell cycle (6–8). However, this requirement to remain in contact with the APC is contrary to the natural tendency of lymphocytes to actively crawl through lymphoid and nonlymphoid tissues (9–12). Thus, antigen-specific mechanisms to suppress locomotion and ensure extended contact of T cells and APC should exist.

Here, we tested the hypothesis that antigen receptor engagement can alter T lymphocyte migration on a model cell surface containing purified adhesion molecules without and with purified antigenic major histocompatibility complex (MHC)-peptide complexes. Our results demonstrate that T cell antigen receptor (TcR) engagement by physiological ligands generates a long-lived stop signal. This result is discussed in terms of an integrated model for T cell migration and activation.

METHODS

T Cell Culture. Spleen cells from 3A9 transgenic mice (B10.BR background) were stimulated with 1 μM hen egg white lysozyme (HEL) peptide 48–62 for 3 days, and then the nonadherent cells were cultured with 50 units/ml mouse interleukin 2 (IL-2) or EL-4 cell supernatant (50 units/ml IL-2 activity) for 3–4 days before use. T cell blasts from B10.BR mice were prepared by culture with 5 μg/ml concanavalin A for 3 days followed by expansion of nonadherent cells with 50 units/ml mouse IL-2 or EL-4 supernatant for 3–4 days. Human T cell blasts were prepared by treating peripheral blood T cells with 2 μg/ml phytohemagglutinin for 3 days followed by culture for 4 days with 50 units/ml IL-2.

Preparation of Substrates. Human intercelluar adhesion molecule-1 (ICAM-1) (13), mouse ICAM-1 (14), I-A^k and mouse I-A^k with covalently attached HEL peptide residues 48–62 (I-A^k–HEL48–62) (15) were purified by immunoaffinity chromatography and reconstituted in liposomes by detergent dialysis (16). Planar bilayers were formed in parallel plate flow cells (Bioptechs, Butler, PA) on glass coverslips (17). Double bilayers were prepared by first forming a circular bilayer in the flow cell. The flow cell was rapidly flushed with Hepes buffered saline (devoid of any protein), and a second liposome suspension was injected to form a bilayer on areas not covered by the first bilayer. Bilayers were blocked with 0.2 μm of filtered 5% nonfat dry milk. Rhodamine-phosphatidylethanolamine (0.2 mol %) was placed in either the first or second bilayer. Site densities were determined by iodinated antibody binding (18). Experiments with murine T cells were performed in 20 mM Hepes/137 mM NaCl/1.7 mM KCl/0.7 mM Na_2PO_4/5 mM glucose/2 mM $MgCl_2$/1 mM $CaCl_2$, pH 7.2. Experiments with human T cells were performed in RPMI medium 1640/10% fetal calf serum. The flow cell was maintained at 37°C. "Low Ca" conditions used Hepes buffered saline without added Ca^{2+}, and the 3A9 T cells were treated with 10 μM BAPTA-AM [1,2-bis(2-aminophenoxy)ethane-*N*′,*N*′,*N*′,*N*′-tetraacetate acetoxymethyl ester] (Molecular Probes) for 20 min at room temperature or with 500 nM thapsigargin for 15 min at 37°C (19).

Image Acquisition and Analysis. Images were acquired with a cooled charged-coupled device camera (Photometrics, Tucson, AZ) on an inverted microscope (Yona Microscopes, Silver Spring, MD). Cell tracking was performed by summing 20 successive images and measuring path lengths using IP Lab software (Signal Analytics, Vienna, VA). Interference reflection microscopy was performed with a 100× Zeiss Neofluar objective.

Fura-2 Ratio Imaging (20). Cells were incubated with Fura-2-AM (Molecular Probes) at 1 μg/ml for 10 min at 37°C. Fura-2 was excited with alternating 340 and 380 nm light, and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

0027-8424/97/943909-5$2.00/0
PNAS is available online at **http://www.pnas.org**.

Abbreviations: APC, antigen-presenting cell; HEL, hen egg white lysozyme; I-A^k–HEL48–62, mouse I-A^k with covalently attached hen egg white lysozyme peptide residues 48–62; ICAM-1, intercellular adhesion molecule-1; LFA-1, lymphocyte function associated-1; MHC, major histocompatibility complex; MTOC, microtubule organizing center; PMA, phorbol 12-myristate 13-acetate; TcR, T cell antigen receptor.

*To whom reprint requests should be addressed. e-mail: dustin@pathbox.wustl.edu.

emission at 510 nm was imaged with the cooled charge-coupled device camera. The ratio of 340 over 380 was computed and calibrated using cells clamped to low and high Ca^{2+} levels with ionophore 4-Br-A23187 (Molecular Probes).

Immunofluorescence Staining. Cells were incubated on blocked bilayers with the indicated molecules for 30 min at 37°C and then were fixed with 1% paraformaldehyde for 20 min at room temperature. The cells were permeabilized for 3 min with 0.1% Triton X-100 and then stained with YOL-1 rat-anti-tubulin mAb (21) (Harlan Labs, Crawley Down, UK) and fluorescein isthiocyanate-goat anti-rat IgG (Zymed).

RESULTS

Our experiments consisted of examining the migratory behavior of T cells on planar phospholipid bilayers containing murine ICAM-1 and the MHC molecule, I-A^k. The T cells were obtained from a TcR transgenic mouse, 3A9, which has specificity for the 48–62 peptide of HEL complexed to I-A^k (22). Many of the experiments used purified I-A^k containing a covalently bound peptide HEL48–62 (I-A^k–HEL48–62) (15). The T cells were harvested and stimulated in culture with the peptide and an activated T cell supernatant containing IL-2. Many of the 3A9 T cells (42%) adhered to ICAM-1 bilayers. The adherent cells crawled with an average velocity of 10 μm/min. (Fig. 1*a*, left side of line, and *b*).

The effect of antigen receptor engagement on T cell migration was tested by coreconstituting ICAM-1 and I-A^k–HEL48–62 (0.2–200 molecules per μm^2). Adhesion was increased 2-fold (to 83%) when I-A^k–HEL48–62 was present and all adhesion was blocked by anti-ICAM-1 mAb YN1/1 (14). 3A9 cells adherent to ICAM-1 and I-A^k–HEL48–62 displayed a dramatic decrease in crawling velocity and an increase in the proportion of stopped cells (Fig. 1*a* , left side of line, and *c*). In contrast, crawling of nontransgenic T cells was not altered by I-A^k–HEL48–62 (Fig. 1*c*). The stopping effect was dose-dependent for I-A^k–HEL48–62 down to 0.2 molecule per μm^2 or ≈20 molecules per contact (Fig. 1*b*), similar to the sensitivity limit of antigen recognition estimated to be on the order of 100 MHC-peptide complexes per APC (23). Stopping was specific for the peptide bound to I-A^k, because 3A9 crawling on ICAM-1 was not altered by high levels of purified I-A^k alone (Fig. 1*c*).

The action of antigen receptor engagement on a crawling 3A9 T cell was directly observed when T cells crawled from regions containing ICAM-1 to an adjacent region containing ICAM-1 + I-A^k–HEL48–62 (Fig. 2). The 3A9 T cells stopped immediately upon hitting this boundary. The stopping effect was accompanied by an increase in cytosolic free Ca^{2+} followed by cytoplasmic Ca^{2+} oscillations. In these experiments the cells were monitored for up to 4 h and remained stopped over this period.

The stop signal could be a direct consequence of adhesion strengthening (24). Phorbol ester treatment of lymphocytes induces prolonged adhesion strengthening (13) and therefore was tested for ability to stop T cell migration on ICAM-1. Treatment of 3A9 T cells with phorbol ester increased the proportion of adherent cells (95%), but actually increased migration (Fig. 3*a*). In fact, the effect of phorbol ester was dominant over antigen receptor engagement in that pretreating T cells with phorbol ester prevented stopping on ICAM-1 + I-A^k–HEL48–62. More importantly, treatment with phorbol 12-myristate 13-acetate (PMA) restarted migration of 3A9 cells that were previously stopped on ICAM-1 + I-A^k–HEL48–62. Thus, there are two modes of adhesion strengthening for T cells; one leads to cell stopping and the other to cell locomotion.

Cytoplasmic Ca^{2+} increases can trigger changes in lymphocyte shape and stop crawling of T cell hybridomas (19, 25). Cytoplasmic Ca^{2+} increases were prevented in 3A9 T cells by use of low Ca^{2+} media and by loading the T cells with the Ca^{2+} chelator BAPTA-AM [1,2-bis(2-aminophenoxy)ethane-

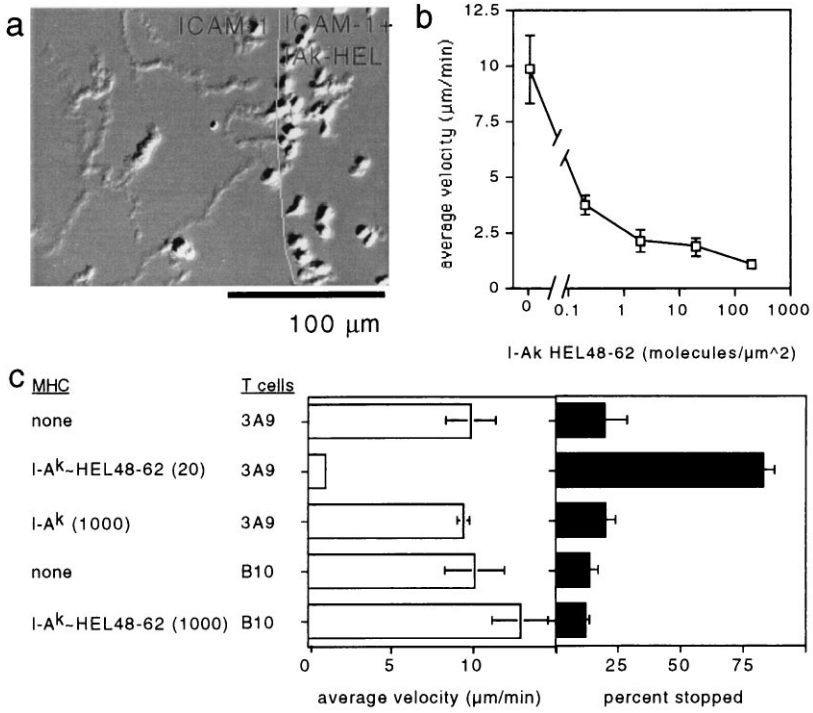

FIG. 1. Effect of antigen receptor engagement on T cells crawling. (*a*) Visualization of T cell stopping upon antigen recognition. 3A9 T cells on 800 molecules per μm^2 ICAM-1 adjacent to 800 molecules per μm^2 ICAM-1 + 20 molecules per μm^2 I-A^k–HEL48–62 bilayers over 12-min period. The line indicates the boundary between bilayers. (*b*) T cell migration velocity on bilayers with ICAM-1 and different densities of I-A^k–HEL48–62. (*c*) Specificity of the stop signal. The average velocity of crawling including stopped cells (*Left*) and percentage of cells that did not move 10 μm in 12 min—i.e., stopped cells (*Right*). Conditions are indicated on left axis. The number in parentheses is the density of the MHC molecules in molecules per μm^2. All bilayers contain ICAM-1 at 800 molecules per μm^2. T cells were maintained in EL-4 supernatant.

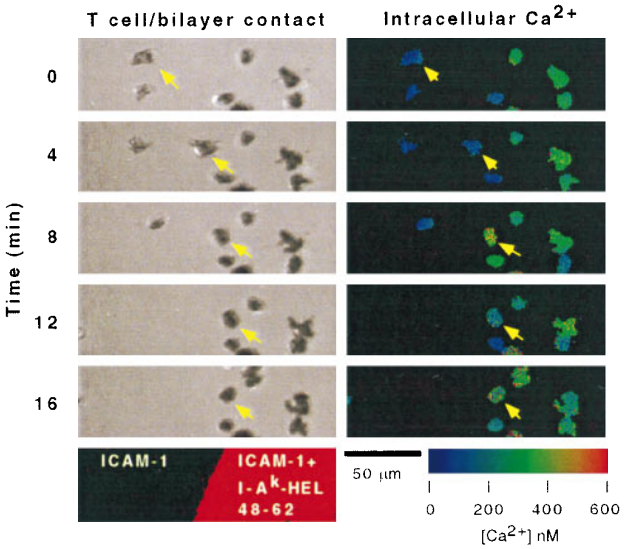

FIG. 2. Visualization of 3A9 cell stopping by antigen and Ca^{2+} mobilization. (*Left*) Interference reflection microscopy. (*Right*) Pseudocolor Fura-2 ratio. (*Bottom Left*) Fluorescence image indicating ICAM-1 secondary bilayer (unlabeled) and ICAM-1 + I-A^k–HEL48–62 containing primary bilayer (labeled). The arrow indicates a cell that crawled from the 800 molecules per μm^2 ICAM-1 bilayer onto the 800 molecules per μm^2 ICAM-1 + 20 molecules per μm^2 I-A^k–HEL48–62 bilayer, stopped at the boundary, and initiated cytoplasmic Ca^{2+} oscillations.

N′,N′,N′,N′-tetraacetate acetoxymethyl ester] or treating the cells with thapsigargin (19). Neither the lymphocyte function associated-1 to ICAM-1 nor 3A9 TcR to I-A^k–HEL48–62 interactions required physiological levels of extracellular Ca^{2+}

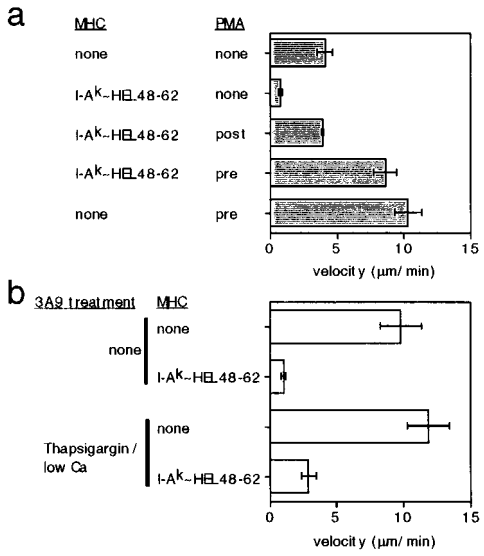

FIG. 3. The stop signal is reversible by adhesion strengthening phorbol esters, but does not require increased cytoplasmic Ca^{2+}. (*a*) Effect of PMA. 3A9 T cells were treated with 50 ng/ml PMA as indicated: pre indicates 15-min pretreatment before exposure to bilayers, post indicates addition of PMA after cells were exposed to bilayers for 30 min. 3A9 T cells treated with PMA for 1 h had 75% as much TcR on the surface as control cells. Cells were maintained in recombinant IL-2 so the basal migration was slower. Data from three experiments. (*b*) Effect of cytoplasmic Ca^{2+} increase. 3A9 T cell migration was examined in normal Ca^{2+} media or in low Ca^{2+} media with 500 nM thapsigargin treatment as indicated. These cells had been maintained in the EL-4 supernatant. Data from two experiments. All bilayers have 800 molecules per μm^2 of ICAM-1 with or without 20 molecules per μm^2 of I-A^k–HEL48–62.

(26, 27). Prevention of the I-A^k–HEL48–62 induced Ca^{2+} increase was confirmed by Fura-2 imaging (not shown). Blocking the cytoplasmic Ca^{2+} increase did not alter crawling on ICAM-1 containing bilayers, nor did it impair the ability to stop on ICAM-1 + I-A^k–HEL48–62 (Fig. 3*b*). Therefore, increases in cytoplasmic Ca^{2+} may reinforce the stop signal, but are not required.

Cell polarity plays an important role in locomotion (28). Cell polarity was directly examined by staining fixed cells with anti-tubulin mAb to determine the position of the microtubule organizing center (MTOC) (29). The MTOC was located in the trailing uropod of 3A9 T cells on ICAM-1 (Fig. 4*a*). Large bundles of microtubules projected forward from the MTOC, cupping the nucleus, and extending toward the leading lamellipodia. I-A^k–HEL48–62 (20–2,000 molecules per μm^2) induced striking repolarization of the MTOC to a central position just over the contact area such that the microtubule bundles now projected directly away from the substrate (Fig. 4*b*). This is analogous to the position of the MTOC in T cell-APC conjugates (29). Thus, proximity of the MTOC to the site of TcR engagement is a characteristic of stopped cells. This was true even when intracellular Ca^{2+} increases were prevented (not shown).

The nature of the adhesive interaction of crawling and stopped cells was examined by interference reflection microscopy, a method for visualizing close contact areas between cells and substrates (30). 3A9 T cells on ICAM-1 formed a large contact area that advanced along the substrate, led by a broad lamellipodium (Fig. 4 *c–e*). The cells detached from the substrate without membrane shedding. This suggests that the LFA-1/ICAM-1 bonds release at or before the trailing edge. 3A9 cells on ICAM-1 + I-A^k–HEL48–62 formed large dynamic contacts with periodic extension and collapse of lamellipodia (Fig. 2 *f-h*). The light areas in the interference-reflection images may represent abundant vesicles focused

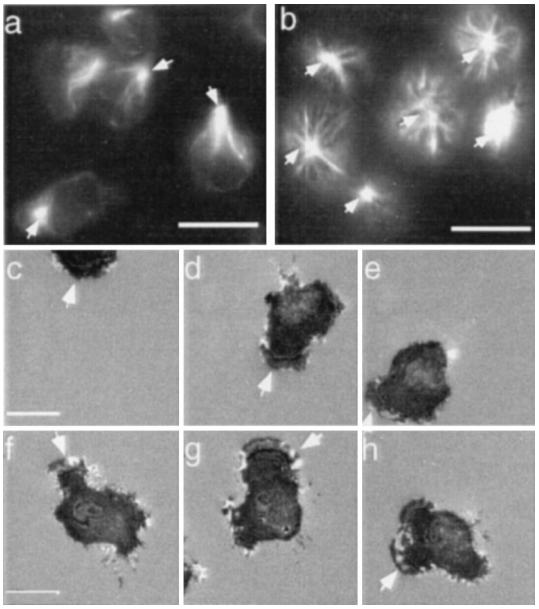

FIG. 4. Polarity and contact morphology of 3A9 T cells on physiological ligands. Tubulin immunofluorescence of 3A9 T cells on ICAM-1 at a focal plane 3 μm above the contact (*a*) or ICAM-1 + 20 molecules per μm^2 of I-A^k–HEL48–62 at a focal plane 1 μm above the contact (*b*). The focal plane for this display was chosen from a series of optical sections for optimal focus of MTOC. Arrowheads indicate MTOC. Interference reflection microscopy on 3A9 cell crawling on ICAM-1 at 30-sec intervals (*c–e*) or 3A9 cells stopped on ICAM-1 + 20 molecules per μm^2 of I-A^k–HEL48–62 (*f–h*). Arrowheads indicate lamellipodia. (Scale bars = 10 μm.)

around the MTOC. These images suggest that the stop signal blocks release of ICAM-1 by LFA-1.

LFA-1 is capable of avidity cycling toward ICAM-1 (13). Lymphocytes crawl rapidly on ICAM-1 substrates (18, 31), but cannot crawl on substrates of anti-LFA-1 antibodies that bind with a fixed high affinity (32, 33). These results suggests that LFA-1 affinity cycling is required for locomotion, but this has not been shown directly. Soluble anti-human LFA-1 monoclonal antibodies can be used to force LFA-1 into the high-affinity conformation. KIM185 promotes LFA-1 conversion to the high-affinity conformation, and mAb 24 holds LFA-1 in the high-affinity conformation (34). These antibodies were used to test the hypothesis that locking LFA-1 in the high-affinity conformation produces a stop signal on ICAM-1 (see Table 1). Human T cell blasts treated with mAb 24 or KIM185 showed a reduction in migration on ICAM-1 and fully stopped when KIM185 and mAb 24 were combined. The functionally neutral anti-LFA-1 mAb TS2/4 was used to show that the dramatic effect of the mAb 24 + KIM185 combination was not a nonspecific effect of using two antibodies. Thus, locking LFA-1 in the high-avidity state stops locomotion on ICAM-1. This result strengthens our conclusion that the TcR-mediated stop signal operates by blocking deadhesion.

Table 1. Requirement of LFA-1 affinity cycling for T cell locomotion

Cell treatment	% adhesion	Average velocity,* μm/min	% stopped
TS2/4	41 ± 4.6	11.6 ± 2.5	6.7 ± 2.5
KIM185	92 ± 6.1	7.7 ± 1.3	43 ± 3.8
TS2/4 + mAb 24	35 ± 3.7	5.1 ± 0.83	43 ± 8.1
mAb 24 + KIM185	94 ± 8.1	—	100

*Average velocity is for moving cells only. ICAM-1 density is 500 molecules per μm^2; KIM 185 and mAb 24 at 2 μg/ml; TS2/4 at 10 μg/ml. Stimulation of adhesion by KIM185 is a common observation (34). Data are presented as mean and range of two experiments in which 200 cells were measured for each value.

DISCUSSION

We have shown that TcR engagement by purified MHC-peptide complexes delivers a stop signal for T cell migration on purified ICAM-1, a major cell-cell adhesion ligand of lymphocytes. It was critical to use the physiological MHC-peptide complexes because the unique low-affinity interaction of the TcR with MHC-peptide complexes is not well duplicated by anti-TcR mAb (35, 36). The stop signal is likely to have an essential role in T cell activation and localization.

We propose a model in which activation of a resting T cell can be divided into a series of molecular events separated by distinct thresholds or checkpoints (37). These steps are initiation of locomotion, initial TcR engagement, and generation of a stable contact cap between the T cell and the APC. In our model, initial TcR engagement delivers the stop signal (checkpoint 1) that is essential for the formation of the contact cap (checkpoint 2).

A common step for both nonantigen-activated and activated T cells is the initiation of locomotion. Naive, memory, and recently activated T cells must become motile to recirculate and/or enter into inflamed tissues. After all, the act of crossing the endothelium requires active locomotion (10, 12). The series of signals required to induce locomotion of resting or activated cells is not known. Once a cell migrates through tissues it may stop spontaneously; for example, if it lacks a substrate for interaction with its integrin adhesion receptors. The issue, however, that calls our attention is the case of engagement of the TcR with its ligand that results, as indicated, in marked inhibition of migration. The stop signal is essential to T cell activation and introduces a significant pause in migration. This temporary suspension of migration may lead to accumulation of antigen-specific cells by blocking egress of T cells from antigen-containing tissue sites. We propose that the stop signal is the first checkpoint in activation because subsequent signaling requires that the T cell remains with the APC to produce a contact cap—the fundamental signaling unit for full T cell activation.

The contact cap is formed by cooperation of the TcR and costimulatory adhesion molecules such as CD4, CD8, CD2, and CD28. The genesis of the contact cap is likely to involve the MTOC, a critical organizing point within the cell for membrane traffic, and myosin II, which is required for specific capping (38). Our results show that the stop signal and MTOC movement to the contact area are closely associated (Fig. 4). It has been suggested that MTOC movement to the contact area also may have a role in directing secretion (29). However, the more fundamental role of the MTOC proximity to the contact may be to establish a central point for condensation of TcR and costimulatory molecules into a stable cap. The important feature of the contact cap is that the relatively low-affinity TCR/MHC-peptide interaction can be driven forward by packing the TcR with adhesion molecules of similar size. This reduces the two-dimensional K_d to a point where a very small number of MHC-peptide complexes can be captured efficiently in the contact cap (36, 39, 40). Thus, the stop signal sets the stage for later events in T cell activation.

Immune responses are initiated in highly organized lymphoid tissues. Recently, elements of this organization have been attributed to the action of chemokines and their receptors (41). It is likely that multiple environmental signals shape the complex structure of lymphoid tissues and that stop signals will play an important role in antigen-driven changes in lymphoid tissue organization. We show here that the stop signal is reversible by a chemokinetic signal from phorbol esters (Fig. 3). This finding is important because it shows that adhesion-strengthening agents can lead to stopping or moving of T cells, and furthermore, that these signals may be arranged in a hierarchy. We propose that the dominance of the stopping or chemokinetic signals may change during T cell activation to orchestrate cellular movements. For example, the receptor for the potent T cell chemoattractant stromal cell derived factor-1 (42), CXCR4, is strongly up-regulated after T cell activation (43). We speculate that the TcR stop signal may be dominant in a resting T cell where CXCR4 is low, but the chemokinetic signal from SDF-1 may gain dominance when CXCR4 on the T cell blasts is up-regulated, leading to restoration of migration.

The stop signal demonstrated here is likely to be essential for T cell activation and to have a significant impact on T cell migration by punctuating activation-dependent changes in T cell tropism. It will be critical to directly establish the hierarchy of chemotactic, chemokinetic, and stop signals involved in controlling T cell migration.

We thank M. Davis for 3A9 TcR transgenic mice, N. Hogg for mAb 24, M. Robinson for KIM 185, R. Rothlein for suggesting the use of activating LFA-1 antibodies for locomotion studies, and T. A. Springer for stimulating discussion of unpublished results. We thank S. Petzold, J. Miller, and R. Houdei for technical assistance. This work was supported by grants from the National Institutes of Health, the Howard Hughes Medical Institute, and Boehringer Ingelheim.

1. Sprent, J., Miller, J. F. A. P. & Mitchell, G. F. (1971) *Cell. Immunol.* **2,** 171–181.
2. Mondino, A., Khorutus, A. & Jenkins, M. K. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 2245–2252.
3. Rowley, D. A., Gowans, J. L., Atkins, R. C., Ford, W. L. & Smith, M. E. (1972) *J. Exp. Med.* **136,** 499–513.
4. Kalish, R. S. & Johnson, K. L. (1990) *J. Immunol.* **145,** 3706–3713.

5. Kosaka, H., Smith, C. D. & Sprent, J. (1991) *J. Exp. Med.* **176,** 1291–1302.
6. Goldsmith, M. A. & Weiss, A. (1988) *Science* **240,** 1029–1031.
7. Lipkowitz, S., Greene, W. C., Rubin, A. L., Novogrodsky, A. & Stenzel, K. H. (1984) *J. Immunol.* **132,** 31–37.
8. Stenzel, K. H., Rubin, A. L. & Novogrodsky, A. (1978) *Exp. Cell Res.* **115,** 285–291.
9. Chang, T. W., Celis, E., Eisen, H. N. & Solomon, F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2917–2921.
10. Parrott, D. M. V. & Wilkinson, P. C. (1981) *Prog. Allergy* **28,** 193–284.
11. Miura, S., Tsazuka, Y., Fukumura, D., Serizawa, H., Suematsu, M., Kurose, I., Imaeda, H., Kimura, H., Nagata, H., Tsuchiya, M. & Ishii, H. (1995) *Gastroenterology* **109,** 1113–1123.
12. Springer, T. A. (1995) *Annu. Rev. Physiol.* **57,** 827–872.
13. Dustin, M. L. & Springer, T. A. (1989) *Nature (London)* **341,** 619–624.
14. Welder, C. A., Lee, D. H. S. & Takei, F. (1993) *J. Immunol.* **150,** 2203–2210.
15. Viner, N. J., Nelson, C. A., Deck, M. B. & Unanue, E. R. (1996) *J. Immunol.* **156,** 2365–2368.
16. Mimms, L. T., Zampighi, G., Nozaki, Y., Tanford, C. & Reynolds, J. A. (1981) *Biochemistry* **20,** 833–840.
17. McConnell, H. M., Watts, T. H., Weis, R. M. & Brian, A. A. (1986) *Biochim. Biophys. Acta* **864,** 95–106.
18. Dustin, M. L., Miller, J. M., Ranganath, S., Vignali, D. A. A., Viner, N. J., Nelson, C. A. & Unanue, E. R. (1996) *J. Immunol.* **157,** 2014–2021.
19. Negulescu, P. A., Kraieva, T., Khan, A., Kerschbaum, H. H. & Cahalan, M. D. (1996) *Immunity* **4,** 421–430.
20. Poenie, M., Tsien, R. Y. & Schmitt-Verhulst, A. (1987) *EMBO J.* **6,** 2223–2232.
21. Stowers, L., Yelon, D., Berg, L. J. & Chant, J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5027–5031.
22. Babbitt, B. P., Allen, P. M., Matsueda, G., Haber, E. & Unanue, E. (1985) *Nature (London)* **317,** 359–361.
23. Harding, C. V. & Unanue, E. R. (1990) *Nature (London)* **346,** 574–576.
24. Martz, E. (1987) *Human Immunol.* **18,** 3–37.
25. Pasternak, C. & Elson, E. L. (1985) *J. Cell Biol.* **100,** 860–872.
26. Stewart, M. P., Cabanas, C. & Hogg, N. (1996) *J. Immunol.* **156,** 1810–1817.
27. Rothlein, R. & Springer, T. A. (1986) *J. Exp. Med.* **163,** 1132–1149.
28. Bretscher, M. S. (1996) *Cell* **85,** 465–467.
29. Singer, S. J. & Kupfer, A. (1986) *Annu. Rev. Cell Biol.* **2,** 337–65.
30. Izzard, C. S. & Lochner, L. (1976) *J. Cell Sci.* **21,** 129–159.
31. Dustin, M. L., Carpén, O. & Springer, T. A. (1992) *J. Immunol.* **148,** 2654–2663.
32. Bretscher, M. S. (1992) *EMBO J.* **11,** 405–410.
33. Bretscher, M. S. (1992) *EMBO J.* **11,** 283–289.
34. Ortlepp, S., Stephens, P. E., Hogg, N., Figdor, C. G. & Robinson, M. K. (1995) *Eur. J. Immunol.* **25,** 637–643.
35. Matsui, K., Boniface, J. J., Steffner, P., Reay, P. A. & Davis, M. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12862–12866.
36. Alam, S. M., Travers, P. J., Wung, J. L., Nasholds, W., Redpath, S., Jameson, S. C. & Gascoigne, N. R. (1996) *Nature (London)* **381,** 616–620.
37. Shaw, A. S. & Dustin, M. L. (1997) *Immunity*, in press.
38. Braun, J., Fujiwara, K., Pollard, T. D. & Unanue, E. R. (1978) *J. Cell Biol.* **79,** 409–418.
39. Dustin, M. L., Ferguson, L. M., Chan, P. Y., Springer, T. A. & Golan, D. (1996) *J. Cell Biol.* **132,** 465–474.
40. van der Merwe, P. A., McNamee, P. N., Davies, E. A., Barclay, A. N. & Davis, S. J. (1995) *Curr. Biol.* **5,** 74–84.
41. Förster, R., Mattis, A. E., Kremmer, E., Wolf, E., Brem, G. & Lipp, M. (1996) *Cell* **87,** 1037–1047.
42. Bleul, C. C., Fuhlbrigge, R. C., Casasnovas, J. M., Aiuti, A. & Springer, T. A. (1996) *J. Exp. Med.* **184,** 1101–1109.
43. Bleul, C. C., Wu, L., Hoxie, J. A., Springer, T. A. & Mackay, C. R. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 1925–1930.