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ISBN
9780819420527

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Publication Date
1996-05-10

DOI
10.1117/12.239501

Polarized T-cell Sensitivity to Antigen Revealed with an Optical Trap

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ABSTRACT

T-cell contact with antigen-presenting B cells initiates an activation cascade which includes an increase in T-cell $[Ca^{2+}]$ and leads to T-cell differentiation and proliferation. We evaluated cell-cell contact requirements for T-cell activation by using an optical trap to control the orientation of T-cell/B-cell pairs and fluorescence microscopy to measure subsequent T-cell $[Ca^{2+}]$ responses. B cells were trapped with a titanium-sapphire laser tuned to 760 nm and placed at various locations along the T cell, which had a polarized appearance defined by shape and the direction of crawling. T-cell intracellular $[Ca^{2+}]$ was detected as an emission shift from the combination of fura-red and fluo-3, two cytoplasmic $[Ca^{2+}]$ indicators. T cells which were presented antigen at the leading edge had a higher probability of responding (84% vs. 31%) and a shorter latency of response (42 s vs. 143 s) than those contacting B cells with their trailing end. Similar results were obtained using beads coated with antibodies to the T-cell receptor. These findings demonstrate a role for initial T-cell/B-cell contact geometry in T-cell activation by showing that the T cell is a polarized antigen sensor.

Keywords: lymphocyte, Ca^{2+} signaling, confocal microscopy, optical trap, antigen presentation.

INTRODUCTION

T-cells are activated when they contact antigen-presenting cells (APCs) such as B cells. Part of the activation cascade involves a rise of $[Ca^{2+}]_i$. Within hours of activation, structural changes occur within the T-cell which orient it toward the APC. This T-cell polarity *following* contact with an APC has been well-characterized on the basis on plasma membrane protein clustering, cytoskeletal and organellar reorganization and cytokine secretion These changes promote the activation of specific cells in crowded environments such a lymph nodes, where most antigen is detected (Poo et al., 1988; Kupfer et al., 1994).

We had previously found that T-cell hybridomas warmed to 37°C and placed on glass or plastic substrates assumed a polarized shape which correlated with their ability to crawl. In addition, observation of 68 random T-B interactions suggested that T cells which contacted B cells with their leading edge usually generated $[Ca^{2+}]_i$ responses, while T cells contacting B cells with their tails had only a 17% chance (3/17) of progressing past the contact phase (Negulescu et al, submitted). The purpose of the present study was to directly determine whether morphological polarity and the ability of T cells to detect antigen were related. The laser-based optical trap was used to control T-cell B-cell contact geometry and T-cell $[Ca^{2+}]_i$ was measured as an indicator of successful T-cell receptor (TCR) activation. We found that T cells were preferentially responsive to contact with B cells at their leading edge. T-cell/B cell contact during antigen presentation involves intercellular interactions between a number of molecular pairs, any of which could

contribute to the observed polarity (Clark and Ledbetter, 1994). In order to begin exploring the molecular basis for this polarity, we have developed a paradigm in which antibody-coated beads are used to mimic interactions between specific molecular pairs. Initial results indicate that increased TCR density at the leading edge of the T cell may account for the polarized response to antigen.

METHODS

Cell Culture. The murine hen egg lysozyme (HEL)-restricted, $CD4^+$ T cell (IE5) (Adorini et al., 1988) and MHC II-restricted B cell (2PK3) hybridomas (a kind gift of A. Sette, Cytel) were grown in RPMI 1640 containing 10% fetal bovine serum (RPMI/FBS) 10 mM HEPES and 1% NEAA, glutamine, and Na^+ pyruvate. Cells were maintained in a humidified incubator at 37°C with 5% CO_2/95% air. IE5 cells were moderately adherent to plastic flasks at 37°C and were resuspended for collection by gentle shaking at room temperature. Antigen-presenting 2PK3 cells were incubated with 10 μg/ml HEL for between 3 and 12 h. This protocol produced a maximal response from IE5 T cells as judged by a contact-dependent $[Ca^{2+}]_i$ response in about 70% of cells. T cells were also probed with antibody-coated microspheres. We used 6 μm diameter polystyrene microspheres stabilized with sulfate charges (IDC. Portland Or). 100μg/ml goat-α rat IgG in 10% PBS was adsorbed to beads (0.5% solids) for 8 hrs at room temperature, centrifuged and washed twice with 10% PBS and then conjugated with 50μg/ml rat α-mouse CD3ε for 3 hr. Beads were centrifuged and washed twice before use. Beads prepared in this way were active (i.e. able to stimulate T cells) for up to 8 weeks when stored at 4°C.

Optical Trapping. The geometry of T cell-B cell contact was manipulated using a tunable, near infra-red titanium:sapphire laser (Coherent) producing a trapping beam at about 760 nm (Berns et al., 1992). The laser was directed through the TV port of a modified Axiovert 135 microscope, past a custom short-pass (720 nm) dichoic reflector and a x63 Neofluor objective and produced about 60 mW trapping power at the focal plane. The microscope was also equipped with a Zeiss Laser Scanning Confocal (LSM 410) and an argon laser set at 488 nm. This arrangement allowed trapping and $[Ca^{2+}]_i$ measurements on the same cells.

$[Ca^{2+}]_i$ imaging. To measure T-cell $[Ca^{2+}]_i$ on the LSM, IE5 cells were co-loaded with a combination of fura-red/AM (5 μM) and fluo-3/AM (2 μM), two long-wavelength Ca^{2+} indicators which respond to the 488-nm excitation line of the argon laser. Cells loaded for 1 h at 25°C produced a red to green emission shift when $[Ca^{2+}]_i$ was elevated (Diliberto et al., 1994). This shift was quantified by scanning cells with the argon laser and dividing the fluorescence intensity signals from two photomultipliers with emission bands of 535-585 nm (green) and <610 nm (red). In these experiments a single, 2PK3 cell was held in the trap on a heated stage and positioned so that it made contact with a particular region of a dye-loaded T cell. Once the cells were positioned, the trapping beam was cut off and 488 nm laser excitation scans were performed. A third photomultiplier collected a Ca^{2+}-insensitive blue emission band (400-480 nm) from incandescent illumination which was used to produce a brightfield image. 30-40 scans at 10-s intervals were made to determine whether a $[Ca^{2+}]_i$ increase occurred in the T cell following contact with the APC. T cells not responding within 400 s were scored as unresponsive. Because the B cell was between 1/3 to 1/2 the size of the T cell, the spatial resolution of mapping was about a third of the T cell's length (~5 μm).

Immunofluorescence. IE5 cells were fixed in PBS containing 4% paraformaldehyde and stained with 5μg/ml FITC-conjugated hamster α-mouse CD3ε (PharMingen, San Diego CA) in a PBS blocking solution containing 1% BSA. Mouse NIH 3T3 cells were used as negative controls. Cell fluorescence images (100x) were grabbed by an image processor (Videoprobe) using a SIT camera.

RESULTS

T-cell sensitivity to antigen localized by optical trapping. We tested the hypothesis that T-cell sensitivity to B cells was polarized by using a laser-based optical trap to control the point of initial cell-cell contact while measuring T-cell $[Ca^{2+}]_i$. With the B cell placed at the T-cell tail, no response occurred (Figure 1*Aa,B*), and the B cell detached from the T cell within 2 min. Trapping the loose B cell and placing it at the leading edge of the same T cell rapidly elicited a T-cell $[Ca^{2+}]_i$ increase (Figure 1*Ab,B*). The $[Ca^{2+}]_i$ responses correlated with morphological changees in the T cell. For example , T-cells would orient toward, increase contact with and engulf B cells placed at the leading edge. In contrast, T cells making contact with B cells *via* the trailing edge showed little morphology change.

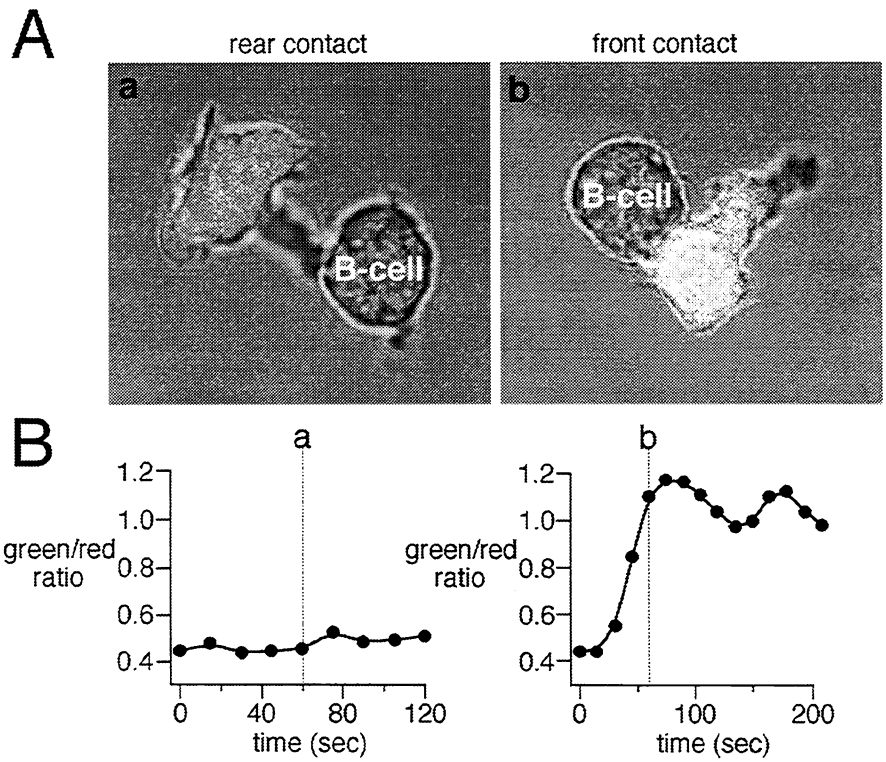

Figure 1. Optical trapping of B cell reveals functional polarity of T-cell.
(*A*) Bright-field, fluorescence intensity overlays of T-B pairs with B cell (labelled) trapped at either the tail (a) or leading edge (b) of the T cell. (a) and (b) represent the same cell pair. T-cell intensity ratio generated from fluo-3 and fura-red co-loaded into IE5 cells (low intensity=low $[Ca^{2+}]_i$; bright =high $[Ca^{2+}]_i$ -see Methods). (*B*) Time course of fluo-3/fura-red intensity ratios for cells shown in (*A*). Experiment is representative of 15 runs). Ratio is proportional to $[Ca^{2+}]_i$.

The results of 29 different T-B pairs are summarized in Table 1, which shows that successful signal transduction depends on the proper orientation of the cell pair. T cells which were presented antigen at the leading edge (contact zone 1) had a higher probability of responding (84% vs. 31%) and a shorter latency of response (42 s vs. 143 s) than those contacting with their tail (contact zone 3). Indeed, the long latency seen in the tail responders was mostly due to the T cell reorienting its leading edge around to contact the B cell. Cells were not very successful at such maneuvering however, and about 50% of B cells placed at the rear of the T cell eventually detached from the T cell. Table 1 also shows that similar polarity and response kinetics were observed using αCD3-coated polystyrene beads to stimulate T cells. Polarity was restricted to T cells since B cells were generally round and could stimulate 85% of T cells when presented at the leading edge.

Table 1. Localized T-cell response to TCR stimulation.

Contact zone (on T-cell)	cells responding (%)		latency (sec)	
	B cell	αCD3-bead	B cell	αCD3-bead
1	84	92	42±16	31±12
2	66	75	72±23	40±14
3	31	12	146±33	88±32

To determine the molecular basis for the polarity, cells were fixed and stained with FITC-labelled α-CD3ε antibodies. This antibody is a marker for T-cell receptor (TCR) complex. The staining pattern in Figure 2b reveals a highly enriched region containing TCR at the leading edge of the T cell. In general, staining was ~ 5-10x brighter at the leading edge than at the tail.

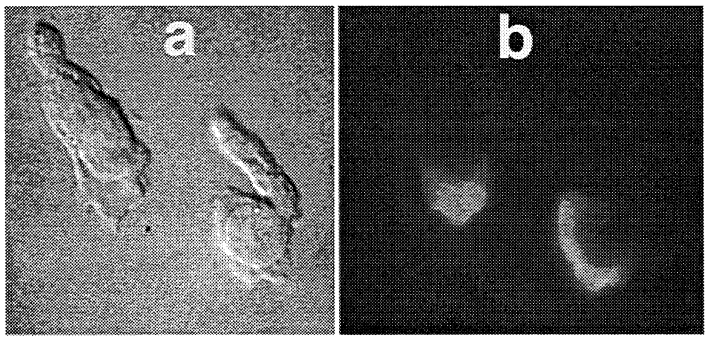

Figure 2. Polarized distribution of aCD3e staining ina single IE5 cell.
(a) DIC image of two cells with polarized appearance .(b) 5μg/ml αCD3 staining. Note highly stained region at the spreading, leading edge.

DISCUSSION

In this paper, we placed antigen-presenting cells or antibody-coated beads in an optical trap and probed the T-cell surface for local responsiveness to antigen. Fluorescence detection of T-cell $[Ca^{2+}]_i$ signal provided a convenient and sensitive functional assay for successful TCR engagement.. These experiments show that cells held in and around such traps are viable and responsive. In addition, cell-cell or cell-bead contacts produced highly consistent results. In the present study we describe a localized sensitivity to antigen which exists prior to contact with the APC and is therefore distinct from activation-induced polarity. Functional mapping of the T-cell surface with either B-cells or beads reveal an essential role for T-B orientation during the contact phase. When initial T-cell contact was made with the leading edge, the latency between contact and $[Ca^{2+}]_i$ signaling was as little as 25 s. This interval is similar to the lag between the addition of TCR antibodies and the $[Ca^{2+}]_i$ rise and probably represents a minimum time required for receptor engagement and the biochemical cascade leading to the Ca^{2+} release from internal stores (Crabtree and Clipstone, 1994). The short latency indicates that, under optimal contact conditions, a T cell need only scan the B cell for a few seconds before transducing adequate information to produce a $[Ca^{2+}]_i$ rise. However, as shown by experiments in which B cells were placed at the trailing edge of the T cell, improper orientation increased this latency and greatly reduced the chance for successful engagement. The fact that both beads and cells had approximately the same latency is significant. Although we did not measure it, the density of antibody on the bead is probably higher than the density of MHC-peptide on the B cell. Therefore, under the conditions of these experiments, the B cell is probably saturating the T cell's detection ability. Decreasing antibody density on the bead will allow us to determine the minimal number of receptors which must be engaged in order to transmit a positive signal. In addition, because this experimental system quantitatively links ligand binding to a functional response, we should also be able to determine the relation between stimulus intensity, response latency and intracellular signaling patterns.

The molecular basis for localized T-cell sensitivity to antigen could reside at either receptor or post-receptor steps in the signal transduction cascade which leads to $[Ca^{2+}]_i$ elevation. The results from experiments using αCD3-coated beads and FITC-conjugated αCD3 suggest that polarity exists at the receptor level. It remains to be seen whether accessory receptors such as CD4 or post-receptor signaling machinery such as kinases are also segregated near the leading edge. What factors could determine inherent T-cell polarity? It is likely that the mechanisms controlling cell motility also maintain the zone of high sensitivity to antigen presentation at the leading edge. It will be of interest to investigate how specific molecular elements responsible for crawling are co-localized with those transducing signals *via* TCR engagement.

ACKNOWLEDGEMENTS

Supported by NIH grants GM41514 and NS14609 to MDC. Optical trap experiments were performed at the Laser Microbeam and Medical Program and the Optical Biology Shared Resource Facility of the Clinical Cancer Center supported by NIH grants RR-01192 and CA-62203. We thank Alessandro Sette for providing IE5 and 2PK3 cell hybridomas and Luette Forrest and Diana Cooper for tissue culture support.

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