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Authors

Wei, Xunbin
Krasieva, Tatiana B
Negulescu, Paul A
et al.

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Antigen recognition by T-lymphocyte studied with an optical trap

Xunbin Wei^{1,2}, Tatiana B. Krasieva², Paul A. Negulescu¹, Zhanxiang Zhang^{2,3}, Chung-Ho Sun²
Michael W. Berns², Gregory J. Sonek^{2,3}, Michael D. Cahalan¹, Bruce J. Tromberg^{1,2}

Department of Physiology and Biophysics¹, Beckman Laser Institute and Medical Clinic², and

Department of Electrical and Computer Engineering³

University of California, Irvine, CA 92697, USA

ABSTRACT

T-cell contact with antigen-presenting B cells initiates an activation cascade which includes an increase in T-cell intracellular calcium and leads to T-cell proliferation and differentiation. We studied cell-cell contact requirements for T-cell activation using an optical trap to control the orientation of T-cell/B-cell pairs and fluorescence microscopy to measure subsequent T-cell $[Ca^{2+}]_i$ response. B cells or beads coated with antibodies to the T-cell receptor (TCR) are trapped with a titanium-sapphire laser and placed at different locations along the T-cell, which has a polarized appearance defined by the shape and direction of crawling. T-cell $[Ca^{2+}]_i$ is detected as an emission shift from the combination of fura-red and oregon-green, two cytoplasmic $[Ca^{2+}]_i$ indicators. T cells which are presented antigen at the leading edge have a higher probability of responding and a shorter latency of response than those contacting B-cells or beads with their trailing end.

Keywords: Optical laser trapping, fluorescence detection, optical manipulation, T-cell activation, T-cell receptor, calcium imaging, antibody-coated bead

1. INTRODUCTION

Since Ashkin et al.¹ first described the optical trapping of micrometer-sized dielectric particles in a single beam gradient force trap, optical laser traps (optical tweezers) have been successfully used in a variety of biological applications,² ranging from DNA stretching and the study of cell-signaling mechanisms to microchemistry and materials engineering.

When a laser beam is focused to a diffraction-limited spot using a high-numerical-aperture lens, it creates a single-beam gradient force trap. The trap can be used to confine and manipulate microscopic objects: dielectric particles, such as latex microspheres; and biological specimens, such as bacteria, viruses and cells.

In this paper, we report the most recent results from our laboratory in the use and application of laser trapping microscopy to study T-cell activation dynamics.

T helper lymphocytes are the cells responsible for the adaptive immune response and activation of other cells of the immune system including macrophages and B lymphocytes. In the body, individual T helpers need to be activated first by physical contact with antigen-presenting cells (APCs), such as B cells, to develop into effector cells. T-cell contact with antigen-presenting B cells initiates an activation cascade which includes an increase in T-cell intracellular calcium and leads to T-cell proliferation and differentiation.^{3,4} 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 as lymph nodes, where most antigen is detected. Although T-cell/B-cell physical contact is required for an immune response, little is known about the patterns of cellular interaction and their relation to activation.

In our study, fluorescence spectroscopy and imaging have been successfully combined with optical micromanipulation to investigate B lymphocyte-T lymphocyte interactions via site-selective placement and orientation of cells.^{5,6,7} Here we demonstrate that T cells are effectively polarized antigen sensors, a result which should further our understanding of cell activation, signal processing and the human immune response.

2. METHODS

Cell Culture. The murine hen egg lysozyme (HEL)-restricted, CD4⁺ T cell (IE5) and MHC II-restricted B cell (2PK3) hybridomas were grown in RPMI 1640 containing 10% fetal bovine serum (RPMI/FBS) 10 mM HEPES and 1% NEAA, glutamine, and sodium pyruvate. Cells were maintained in a humidified incubator at 37°C with 5% CO₂/95% 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 hours. This protocol produced a maximal response from IE5 T cells as judged by a contact-dependent [Ca²⁺]_i response about 70% of cells. T cells were also probed with antibody-coated latex microspheres. We used 6 µm diameter polystyrene microspheres stabilized with sulfate charges (IDC, Portland Or). 100 µg/ml mouse-α hamster IgG in 10% PBS was adsorbed to beads for 8 hours at room temperature, centrifuged and washed twice with 10% PBS and then conjugated with 50 µg/ml hamster α-mouse CD3ε for 3 hours. Beads were centrifuged and washed twice before use.

Optical Trapping. The geometry of T cell-B cell contact was manipulated using a tunable, near infrared titanium:sapphire laser producing a trapping beam at about 760 nm (Berns et al., 1992). The trapping laser was introduced via the TV port of a Zeiss Laser Scanning Confocal microscope (LSM 410). A short-pass (720 nm) dichroic reflector was used to separate trapping and fluorescence excitation beams. A 100X 1.3 NA Neofluor objective and focused the near infrared and visible beams, resulting in 60 mW trapping power at the focal plane. This arrangement allowed trapping and fluorescence-based [Ca²⁺]_i measurements on the same cells.

[Ca²⁺]_i imaging. To measure T-cell [Ca²⁺]_i on the LSM, IE5 cells were co-loaded with a combination of fura-red/AM (5 µM) and oregon-green/AM (2 µM), two long-wavelength Ca²⁺ indicators which respond to the 488-nm excitation line of the argon laser. Cells loaded for 1.5 hr at 37°C produced a red to green shift when [Ca²⁺]_i was elevated. This shift was quantified by scanning cells with the argon laser and dividing the fluorescence intensity signals from two photomultipliers with emission bands of 520-570 nm (green) and >610 nm (red). In these experiments a single, 2PK3 cell or antibody-coated bead 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 were performed. A third photomultiplier collected a Ca²⁺-insensitive blue emission band (400-480) from incandescent illumination which was used to produce a brightfield image. 30-40 scans at 10 s intervals were made to determine whether a [Ca²⁺]_i increase occurred in the T cell following contact with APC. T cells not responding within 400 s were scored as unresponsive.

Antibody coating on beads. Various sizes of sulfate polystyrene beads were coated with 100 µg/ml of anti-hamster IgG monoclonal antibodies (mAbs) first and then incubated with various densities of hamster anti-murine TCR:CD3 mAbs. The anti-hamster mAbs bind the Fc portion of hamster anti-TCR:CD3 mAbs so that all of the binding sites of the hamster anti-murine TCR:CD3 mAbs are free (figure 1). Well-coated beads are manipulated with an optical trap and placed at different sites of T cells (leading edge, mid-section, or tail region).

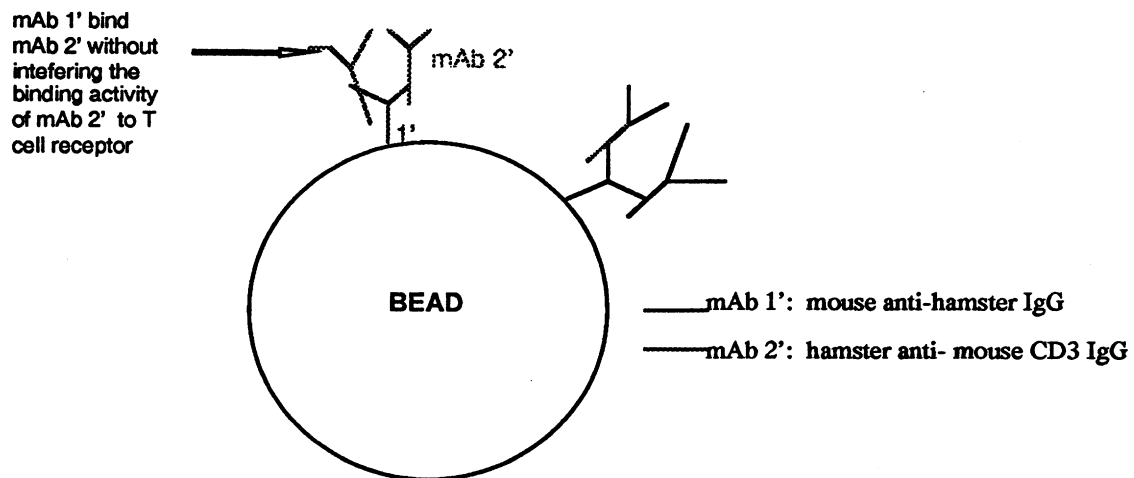


Figure 1. Antibody coating on bead diagram

Notice that mAb 1' bind mAb 2' without interfering the binding activity of mAb 2' to T cell receptor so that all of the binding sites of mAbs 2' are free .

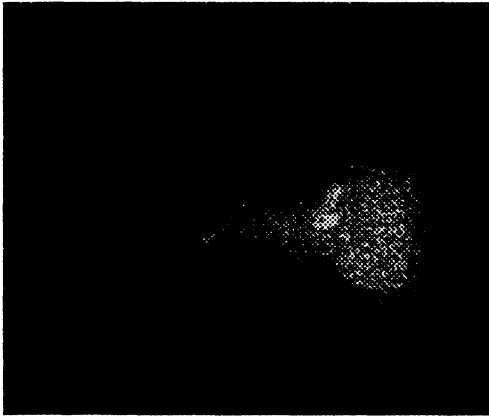
3. RESULTS AND DISCUSSION

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. 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 activation. With the B cell placed at the T-cell tail, no response occurred, and the B cell detached from the T cell within two minutes. 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. We found that T cells were preferentially responsive to contact with B cells at their leading edge. T cells which were presented antigen at the leading edge ("head contact") 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 ("tail contact").

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. To investigate whether polarity could be observed via TCR engagement alone, we used beads coated with antibodies to the CD3 subunit of the TCR complex to mimic TCR engagement in the absence of any coreceptors and got similar results (figure 2 and table 1). Initial results showed more dramatic polarity was observed using 6 μ m diameter anti-TCR: CD3 mAb coated polystyrene beads to stimulate T cells (87% for leading edge contact vs. 6% for tail contact) and implied increased TCR density at the leading edge of the T cell might account for the polarized response to antigen.

A

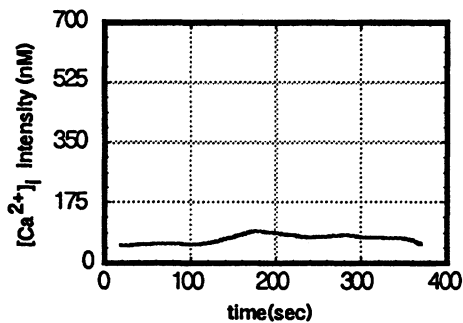
a. rear contact



b. front contact

**B**

a



b

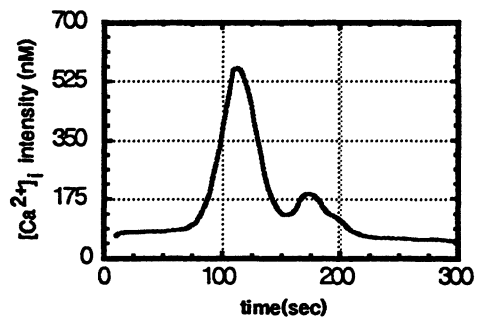
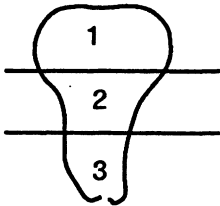


Figure 2. Optical trapping of antibody-coated bead reveals functional polarity of T cell.

(A) Bright field, fluorescence intensity overlays of T-cell-bead pairs with antibody-coated bead (round shape) trapped at either the tail (a) or leading edge (b) of the T cell. T cell intensity ratio generated from oregon-green and fura-red co-loaded into IE5 cells (dark, red=low $[Ca^{2+}]_i$; bright, green=high $[Ca^{2+}]_i$; --see Methods). (B) Time course of $[Ca^{2+}]_i$ for cells shown in (A).

The results of 29 different T-B cell pairs and 109 bead-T cell pairs are summarized in Table 1. T cells which were presented either with antigen or anti-CD3 mAb at the leading edge (contact zone 1) had a higher probability of response and shorter latency of response than those contacting with their tail (contact zone 3).

Table 1. Polarized T-cell Response to TCR Stimulation

contact zone (on T cell)	cells responding (%)		latency (sec)	
	B cell	anti-CD3 bead	B cell	anti-CD3 bead
	82 (14/17)	87 (77/87)	42 ± 16	52 ± 15
	80 (4/5)	82 (14/17)	60 ± 22	78 ± 39
	31 (4/13)	6 (1/15)	146 ± 29	340

T cells were stimulated by either antigen-presenting B cells or anti-CD3-coated beads at the region indicated. Region 1 is defined as the leading edge. Latency indicates the delay between contact and a detectable $[Ca^{2+}]_i$ increase in the responding population.

These findings show that T-cells are effectively polarized antigen sensors, a result which should further our understanding of cell activation, signal processing and the human immune response.

4. SUMMARY

Optical laser trapping microscopy has emerged as a powerful tool not only for the optical manipulation of cells and macromolecules, but also for the study of cellular physiological responses via force transduction and fluorescence imaging. We describe here the most recent results from our laboratory in the use and application of laser trapping microscopy to study activation of T cells using receptor-specific microspheres delivered to different cellular regions via an optical trap. Here we show not only that T-cell is a polarized antigen sensor, but also that receptor stimulation can be directly correlated to a functional response. Such studies are expected to aid in the design of new therapies for promoting or inhibiting immune response within the human body.

5. ACKNOWLEDGMENTS

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