

LOCALIZATION OF AGGREGATED CELL SURFACE ANTIGENS
OF TARGET CELLS BOUND TO CYTOTOXIC T LYMPHOCYTES*

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The binding of cytotoxic T lymphocytes (CTL) and target cells is an immunologically specific reaction (1-3). Binding is a rapid process which requires Mg^{++} and Ca^{++} (4), an active system of microfilaments (5, 6), and intact metabolic activity of the interacting cells.¹ Because reagents such as local anesthetics which are known to induce membrane disorganization (7) interfere reversibly with CTL-target cell interaction (8), and because soluble membrane components of target cells did not (9-11), we have previously proposed that cell membrane organization plays a role in binding of CTL and target cells (9).

de Petris and Raff (12) have observed that cap formation induced by anti-immunoglobulin sera always occurs toward the posterior region of cells. Rutishauser et al. (13) have reported that cap formation in cells bound to nylon fibers was found to localize opposite to the point of binding. In the present study we have examined the localization of capped surface antigens of target cells bound to CTL and found that cap formation always occurs toward the site of binding. It is suggested that the polar localization of capped target cell surface determinants is directed by the CTL-target cell interaction.

Materials and Methods

Animals, Tumor, Immunization, and Preparation of Cytotoxic Lymphocytes. 2-mo old BALB/c, C57BL/6, AKR/J, and C3H/eb mice were provided by the Weizmann Institute Animal Breeding Center. Leukemia EL4 of C57BL/6 (EL4) was maintained in ascites form in C57BL/6 mice. 2-mo old male BALB/c mice were injected intraperitoneally with 25×10^6 EL4 cells. BALB/c anti-EL4 peritoneal exudate cytotoxic lymphocytes (PEL) were prepared 11 days after tumor injection as previously described (14).

*Formation of Cytotoxic Lymphocyte (PEL)-Target Cell (EL4) Conjugates.*² PEL-EL4 conjugates were formed by centrifuging 1×10^6 PEL with 1×10^6 EL4 cells in 1 ml phosphate-buffered saline supplemented with 10% fetal calf serum (PBS-FCS) at 250 g for 10 min at room temperature.

Sera and Fluorescent Reagents. Hyperimmune antiserum to EL4 (50% cytolytic titer of 256-512 in the presence of complement) was prepared by immunizing BALB/c mice with 6 weekly intraperitoneal injections of 25×10^6 EL4 cells. The BALB/c anti-EL4 serum has previously been shown to react with antigens which are common to leukemia EL4 and normal C57BL/6 tissues (15). Anti- θ C3H serum was prepared by immunizing AKR/J mice with 14 weekly intraperitoneal injections of 1×10^6 C3H/eb thymocytes. Fluorescein-conjugated antimouse IgG (7S) globulins produced in goats (GAMG-FI) were obtained from Meloy, Springfield, Va.

* Supported by the U. S.-Israel Binational Science Foundation.

¹ Berke, G., and D. Gabison. Energy requirements of the binding and lytic steps of T-lymphocyte-mediated cytotoxicity of leukemic cells in vitro. *Eur. J. Immunol.* In press.

² Quantitative aspects and the specificity of PEL-EL4 conjugation have been presented in full detail elsewhere. Berke, G., D. Gabison, and M. Feldman. The frequency of effector cells in populations containing cytotoxic T-lymphocytes. *Eur. J. Immunol.* In press.

Fluorescent Staining of Cell Surface Determinants. 1×10^6 EL4 cells were incubated with 0.1 ml antiserum (BALB/c anti-EL4 or AKR/J anti-C3H/eb) for 30 min at 0°C. The EL4 cells were washed twice with PBS-FCS and 1×10^6 PEL in 1 ml PBS-FCS were added. The suspension was left for 10 min at room temperature and then centrifuged 250 g for 10 min at this temperature. 0.1 ml of GAMG-FI diluted 1:10 was added to the cell mixture for 30 min at room temperature. Finally the cells were washed and resuspended in 0.1 ml PBS-FCS.

Microscopy and Photography. Cell-bound fluorescence was observed in a Zeiss reflected-light microscope equipped with a high pressure mercury lamp (HBO-200), exciter filter I-BG 12 and barrier filters 44 and 50 (Carl Zeiss, Oberkochen, Wuerttemberg, West Germany). Photographs were taken with a 35 C Zeiss camera and Tri-X 400 ASA film (Eastman Kodak Co., Rochester, N. Y.). Exposure time for fluorescence was 2 min.

Results

To examine the distribution of aggregated cell surface antigens of EL4 cells bound to PEL, EL4 cells were incubated with BALB/c anti-EL4 serum diluted 1/20. Antibody-treated EL4 cells were then mixed with PEL and PEL-EL4 conjugates were formed by centrifugation. The conjugates were then treated with GAMG-FI, washed, and examined for cell-bound fluorescence. In Fig. 1, various types of PEL-EL4 conjugates are shown: PEL are small- to medium-sized lymphocytes, EL4 are lymphoblastoid. Examination of the distribution of fluorescence on EL4 cells shows cap formation at the site of the conjugation. In some cases the fluorescent cap concentrates exclusively between the cells and in others it is in close proximity to the conjugation area. The kinetics of the redistribution of fluorescence on EL4 cells conjugated with PEL and on free EL4 cells is presented in Table I. 60 min after conjugation, cap formation was observed in 70% of the conjugated EL4 cells, 85–95% of which (mean of 10 experiments) were localized at the site of conjugation. A patchy distribution of fluorescence was seen in 30% of the conjugates.

Since EL4 cells possess the θ -antigen, it was of interest to examine the distribution of the aggregated antigen in PEL-EL4 conjugates. EL4 cells were reacted with AKR/J anti- θ C3H serum diluted 1/10. PEL-EL4 conjugates were then formed and labeled with GAMG-FI. Examination of the distribution of fluorescence showed cap formation in 55% of conjugated EL4 cells; 90% of the caps were localized at the site of PEL-EL4 conjugation. In 10% of the conjugated cells of the cap was opposite the site of the conjugation. A patchy distribution was seen in 45% of the conjugated EL4 cells.

Discussion

Immunogenetic evidence supports the theory that target cell surface histocompatibility antigens are involved in the interaction of CTL and target cells (1). The finding that alloantisera, prepared against appropriate cells, inhibit the interaction of CTL and target cells (10) is compatible with this theory. However, attempts to specifically block CTL-target cell interaction by solubilized target cell antigens, which possess full serological activity, have been unsuccessful (9–11). Because the binding of CTL and target cells is an energy-dependent process² which requires Mg^{++} and Ca^{++} , and because it is totally inhibited by cytochalasin B and local anesthetics but not by soluble membrane components of target cells, we have suggested that cell membrane organization plays a role in the binding process (8, 9).

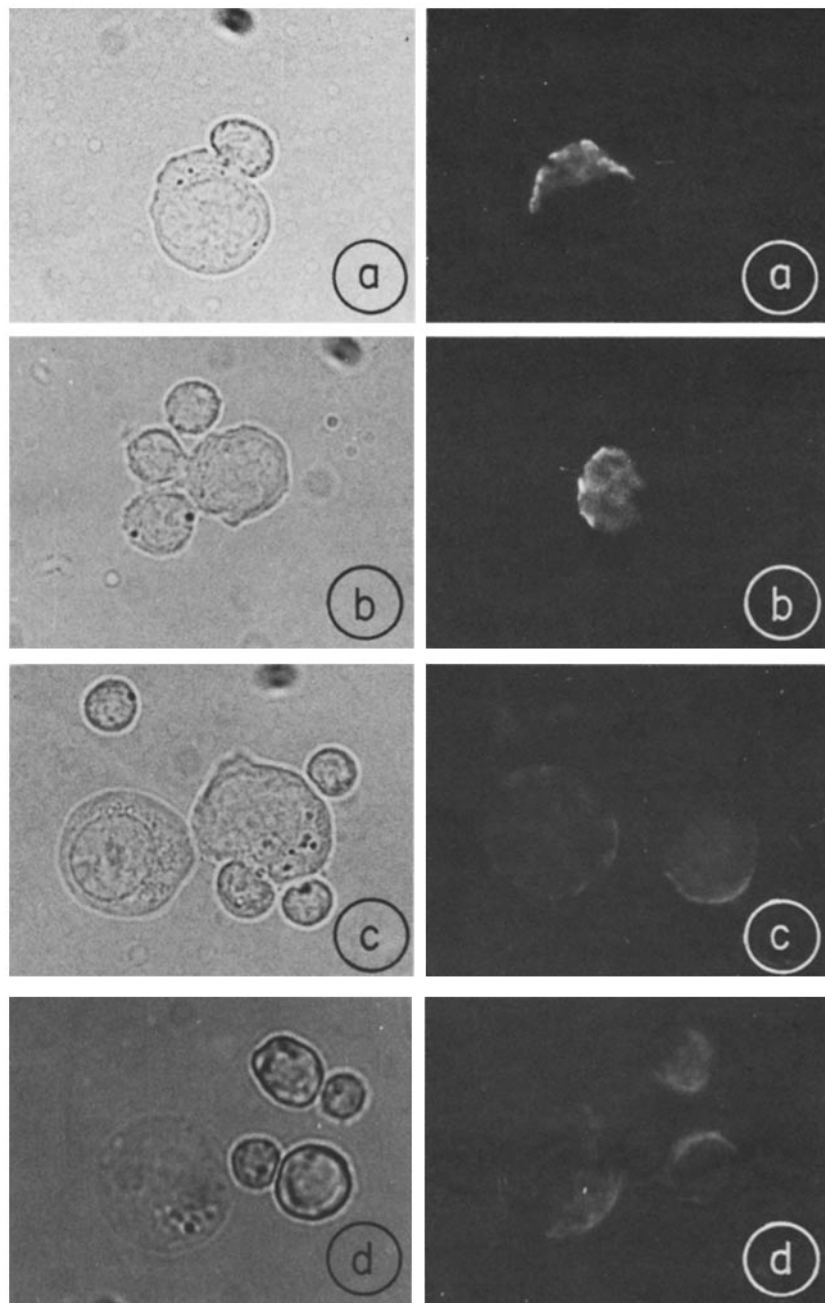







FIG. 1. Localization of aggregated target cell surface antigens at the binding site of cytotoxic lymphocytes and target cells. PEL-EL4 conjugates are presented in bright field (left) and dark field (right): (a), a conjugate of one PEL and one EL4; (b), a conjugate of three PEL and one EL4; and (c), a conjugate of three PEL and one EL4 to which a second EL4 is attached. A nonpolar distribution of fluorescence is shown on the second EL4 cell; (d), a conjugate of one PEL and one EL4 (upper right) and one PEL and two EL4, one of which is undergoing lysis and whose contour is ill defined (center).

TABLE I
Distribution of Fluorescence on PEL-Conjugated EL4 Cells and on Free EL4 Cells

Time*	PEL-EL4 conjugates			EL4 alone	
					
min	% †			% §	
0	0	0	100	0	100
30	47	6	47	30	70
60	59	11	30	55	45

*Incubation at room temperature (22-26°C) after conjugation.

†Percent of all conjugated EL4 cells.

§Percent of all free EL4 cells.

Treatment of cells with a bivalent antibody directed against cell surface antigens frequently results in aggregation of the specific cell surface component, cap formation and internalization of the aggregated complex (16). Movement of the cell influences the localization of the cap by directing it towards the trailing portion of the cell (16, 17). Interesting similarities exist between CTL binding to target cells and the phenomenon of cap formation. The two processes occur at the cell surface. They require temperature and metabolic energy and are influenced by reagents that affect microfilaments and microtubules (5, 16, 17) and by local anesthetics (8, 18).

The results reported in this paper have shown that aggregated membrane antigens of target cells conjugated to CTL cap almost exclusively at the site of CTL-target cell conjugation. Additional experiments have shown that when precapped target cells are reacted with CTL, the caps are not initially restricted to the conjugation area but localized there after a brief incubation period. The finding of cap formation at the site of attachment of target cells to CTL is clearly different from the opposite localization of cap formation found by Rutishauser et al. (13) in lymphocytes attached specifically or nonspecifically to nylon fibers. The localization of the cap at the binding site of CTL and target cells may indicate that binding occurs through a unique area of the target cell where the cap tends to localize. Alternatively, the localization of the cap may be a consequence of CTL-target cell interaction, which attracts a high local concentration of target cell surface determinants to the site of the binding. Such a mechanism could provide stable bond formation between CTL and target cells after their initial interaction.

Summary

The redistribution of aggregated cell surface antigens of target cells bound to cytotoxic T lymphocytes was investigated. It was found that cap formation induced by antibody always occurred toward the site of binding. It is suggested

that the polar localization of capped target cell surface determinants is a consequence of cytotoxic T-lymphocyte target cell interaction.

We wish to thank Miss Barbara Tanenbaum for skilful technical assistance, and Professor M. Feldman for his interest in this work.

Received for publication 16 June 1975.

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