by antiserum against isologous immunoglobulins regulates the proliferation *in vivo* of AFC producing IgG antibodies is not yet known. Since this antiserum interacts *in vitro* with immune rosette-forming B cells, containing aggregated antibodies on their surface [3], and since it eliminates such RFC *in vivo* (Table 1), this suggests that in the course of the immune response it is the RFC, on the surface of which immune complexes are adsorbed, which inhibit the antigen-dependent activation of those immunocompetent B lymphocytes which are the precursors of the plasma cells producing IgG antibodies.

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ULTRASTRUCTURE OF CONJUGATES OF CYTOLYTIC T LYMPHOCYTES AND TARGET CELLS

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Conjugates of target cells and cytolytic T lymphocytes, isolated on the 11th day after alloimmunization, were investigated. Conjugates were formed by small and medium-sized lymphocytes, in the cytoplasm of which mature secretory granules, crystalloid structures, and lipids were found. The lymphocyte was spherical in shape and its area of contact with the target cell did not exceed 5-15%. Cytolysis of the target cells was observed after incubation for 30-60 min. The lymphocyte became flatter, its nucleus became oval in shape, and the area of its contact with the target cell increased. Meanwhile, hypertrophy and a change in the orientation of the Golgi complex were found in the zone of contact with the target cell, fusion of the secretory granules with the lipids and crystalloid structures took place, and immature secretory granules and vacuolar degeneration of the mitochondria appeared. "Peeling" of the lymphocyte membrane was observed, and structures connected with it and called "membranosomes" are described. It is suggested that secretory processes are activated in the cytoplasm of cytolytic T lymphocytes during their interaction with target cells.

KEY WORDS: *cytolytic T lymphocytes; Golgi complex; secretory granules.*

The study of the mechanisms of action of cytolytic T lymphocytes lies at the basis of the solution of the problem of transplantation and antitumor immunity. Immune T lymphocytes can be specifically adsorbed *in vitro* on target cells (TC) carrying stimulating antigen on their surface and can cause their lysis [7]. This model provides wide opportunities for the study of the mechanisms of cytolysis and the causes of death of the TC.

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In the investigation described below the ultrastructure of the effector T lymphocyte was studied during interaction with TC.

EXPERIMENTAL METHODS

Mice of strains C3H and DBA/2 aged 8-12 weeks were used. Ascites lymphoid leukemia L-1210 was transplanted into DBA/2 mice by weekly intraperitoneal injection of $5 \cdot 10^5$ cells suspended in 0.5 ml medium No. 199.

C3H mice were immunized with L-1210 cells in a single intraperitoneal injection of $25 \cdot 10^6$ tumor cells suspended in 3 ml of medium No. 199. C3H-anti-L-1210 cytolytic T lymphocytes (CTL) were isolated from the peritoneal exudate on the 11th day after immunization, by the method of Berke et al. [6].

L-1210 cells, used as TC, were incubated at the rate of $6 \cdot 10^6$ cells in 1 ml medium RPMI-1629 with 10% calf serum and with ^{51}Cr in a dose of 75 μCi/ml for 60 min, then washed three times with medium No. 199.

To obtain L-1210-CTL conjugates [6] suspensions of $4 \cdot 10^7$, $2 \cdot 10^7$, and $1 \cdot 10^7$ lymphocytes were centrifuged with $4 \cdot 10^6$ L-1210 cells in 1 ml medium RPMI-1629 with 10% calf serum at 250g and at room temperature for 4 min. The residue was resuspended with a Pasteur pipet and diluted with the same medium to a cell concentration of $1 \cdot 10^6$ cells/ml in four samples. The cells were incubated for 15, 30, 90, and 180 min at 37°C to determine the percentage of cytolysis of TC [3, 7].

For electron-microscopic investigation a cell suspension containing CTL and L-1210 (in the ratio of 2:1) was centrifuged for 10 min at 250g in 4 ml medium RPMI-1629 with 10% calf serum, the supernatant was removed, and the cells were fixed with 1% glutaraldehyde, or the residue was resuspended in the same medium, the cell concentration was adjusted to $1 \cdot 10^6$ cells/ml, and the sample was incubated at 37°C for 5, 15, 30, 60, and 180 min; 1% glutaraldehyde was added, centrifugation was repeated at 250g for 10 min, the supernatant was removed, and the material was subsequently treated by the method described earlier [1].

Suspensions of cells containing conjugates that were incubated for 30 min at 4°C served as the control.

EXPERIMENTAL RESULTS

The results of the study of cytolysis of L-1210 cells depending on the incubation time and the ratio of lymphocytes to TC are given in Table 1.

Ultrastructure of CTL 0-5 min after Formation of Conjugates with TC. The small and medium-sized lymphocytes were spherical in shape and their contact with TC did not exceed 5-15% of the total surface of the lymphocyte. The CTL were easily differentiated from TC by their size, their submicroscopic organization, and by the presence of accumulations of nucleoids of type A oncornaviruses in the cytoplasm of TC.

Vacuoles 200-300 nm in diameter, containing a high-contrast osmiophilic component, were found in the cytoplasm of the lymphocytes; morphologically these structures were similar to mature secretory granules [12]. The crystalloid structures were characterized by low electron-optical density; in its ultrastructure, the limiting membrane was identical to the membrane of the ergastoplasmic reticulum. Lipids, cytolysomes containing accumulations of ribo-

TABLE 1. Lysis of L-1210 Cells Labeled with ^{51}Cr during Interaction with Cytolytic T Lymphocytes*

Lymphocytes/TC ratio	Incubation time, min		
	30	90	180
10:1	7,1%	27%	58%
5:1	5%	22%	51,3%
2,5:1	0,7%	7,8%	36%

*Averaged results of three experiments

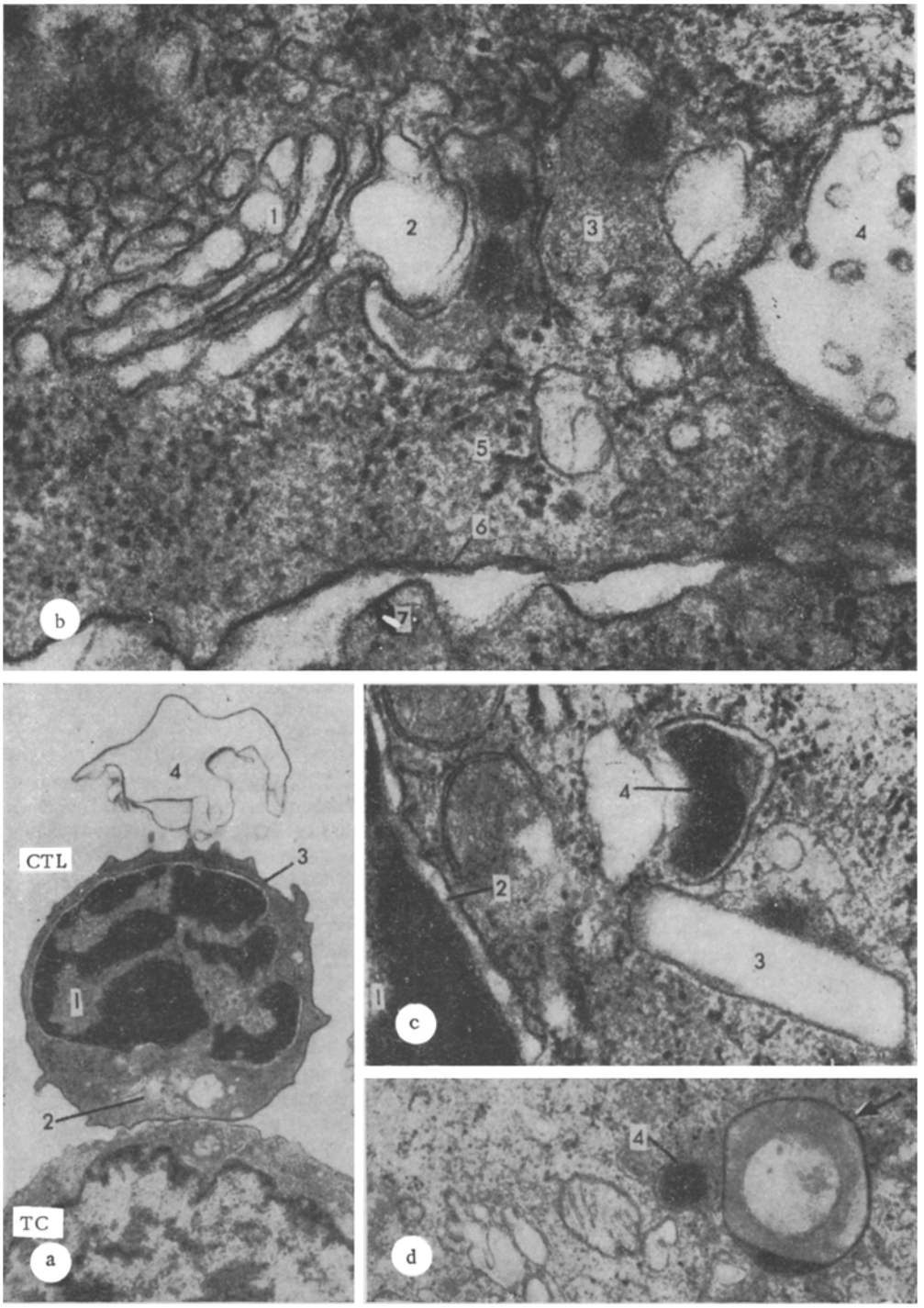

Fig. 1. Ultrastructure of cytolytic T lymphocytes and L-1210 target cells 30 min after formation of conjugates: a) adsorption of cytolytic T lymphocyte (CTL) on target cell (TC): 1) nucleus of lymphocyte; 2) Golgi complex; 3) plasma membrane (plasmalemma) of lymphocyte; 4) area of plasmalemma separated from surface of lymphocyte (9000×). b) Hypertrophy of Golgi complex: 1) cisterns of Golgi complex; 2) vacuoles of Golgi complex; 3) "immature" secretory granules; 4) multivesicular body; 5) ribosomes; 6) plasmalemma of lymphocyte; 7) plasmalemma of TC (110,000×). c, d) Areas of cytoplasm of CTL; 1) nucleus of lymphocyte; 2) nuclear membrane of lymphocyte; 3) crystalloid structure; 4) mature "secretory" granule. Arrow indicates structure formed evidently as a result of fusion of mature "secretory" granule and lipid vacuole. Magnification: c) 65,000×; d) 30,000×.

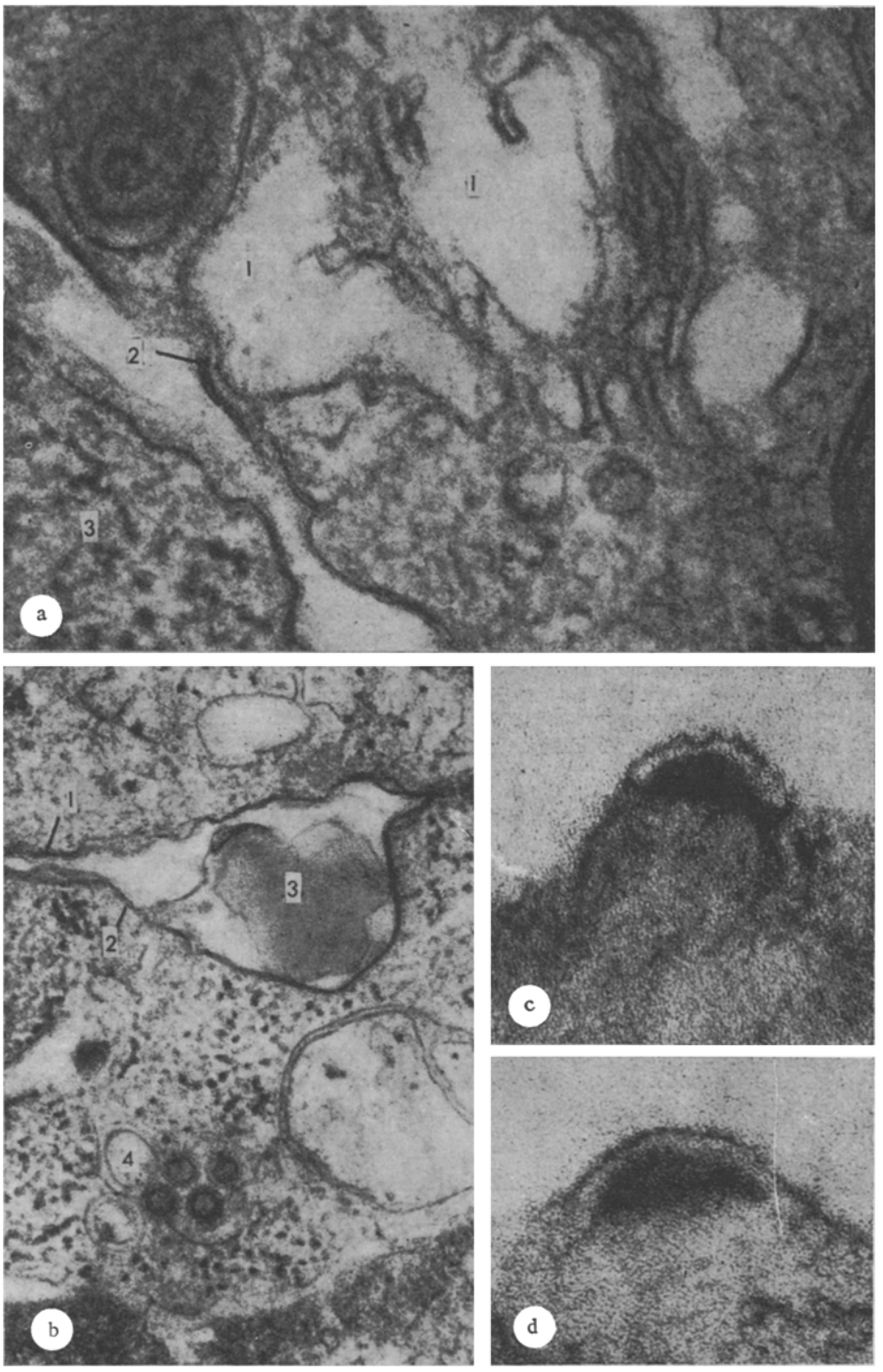

Fig. 2. Ultrastructure of zone of contact between CTL and TC 30-60 min after formation of conjugates: a) localization of hypertrophied vacuoles of Golgi complex (1) near plasma membrane of lymphocyte (2) in zone of its contact with TC (2) (210,000×). b) Zone of contact of CTL and TC 60 min after formation of conjugates: 1) plasmalemma of CTL; 2) plasmalemma of TC; 3) osmiophilic component located between cells in zone of contact of CTL and TC; 4) accumulation of nucleoids of type A oncornavirus in cytoplasm of TC (70,000×); c, d) "membranosomes" located on surface of CTL (760,000×).

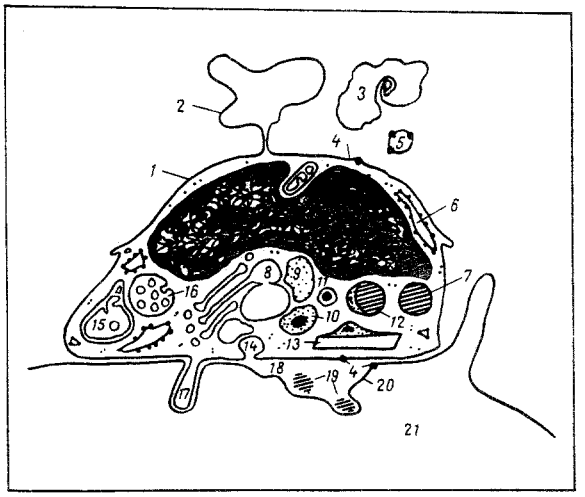

Fig. 3. Diagram of CTL 30-60 min after contact with TC. 1) Plasmalemma of CTL; 2) area of plasmalemma separated from lymphocyte; 3) separated area of plasmalemma; 4) "membranosomes"; 5) "membranosomes" separated from lymphocyte; 6) granular endoplasmic reticulum; 7) lipids; 8) hypertrophied Golgi complex; 9, 10) immature "secretory" granules; 11) mature "secretory" granules; 12) fusion of "secretory" granules with lipids and crystalloid structures (13); 14) hypothetical fusion of membranes of vacuoles of Golgi complex with plasmalemma of CTL in zone of its contact with TC; 18) intercellular space in zone of contact of CTL and TC; 19) "secretion"; 20) plasmalemma of TC; 21) cytoplasm of TC.

somes, lysosome-like structures, multivesicular bodies, and mitochondria also were observed. The Golgi complex was poorly differentiated and its location was unconnected with the zone of contact between lymphocyte and TC. The CTL after incubation for 30 min at 4°C and also after culture for 30-60 min separately from TC at 37°C had a similar submicroscopic organization.

Ultrastructure of CTL 30-60 min after Formation of Conjugates with TC. CTL adsorbed on target cells were flattened, their nuclei were shifted toward the pole opposite to TC, and they were oval in shape; the area of contact with TC was increased to 20-30% (Fig. 1a). Numerous cytoplasmic appendages were formed, but throughout the area of contact between CTL and TC the plasmalemmas of the two cells did not approach closer than 6-7 nm and their fusion was never observed. Enlargement of the parallel cisterns and vacuoles of the Golgi complex took place after 15 min. After 30 min, giant vacuoles up to 300-700 nm in diameter were seen in the composition of the Golgi apparatus (Fig. 1b). The hypertrophied Golgi complex moved toward the surface of the lymphocyte in the zone of its contact with TC (Fig. 2a).

Immature secretory granules appeared, together with polymorphic vacuoles filled with an osmiophilic component of moderate electron-optical density, measuring up to 400-600 nm (Fig. 1b, c, d). Accumulations of an osmiophilic component, similar in electron-optical density to the contents of secretory granules, were located in the intercellular space in the zone of contact between CTL and L-1210 cells (Fig. 2b). Fusion of these accumulations with lipids and crystalloid structures also was found (Fig. 1c, d). Vacuolar degeneration of mitochondria in the zone of the hypertrophied Golgi complex was observed. The mitochondria became round, increased in diameter, and were converted into giant vacuoles 1500-2000 nm in diameter.

"Peeling" of the cytoplasmic membrane of the lymphocyte was found, mainly opposite to the zone of its contact with TC (Fig. 1a).

Directly beneath the plasmalemma of the lymphocyte oval structures of high electron-optical density and measuring up to 20-40 nm were seen: These were conventionally described as "membranosomes" (Fig. 2c, d).

On electron-microscopic investigation of the cytoplasm of CTL isolated from the peritoneal exudate no signs of secretion were found [10, 13]. However, in the present investigations considerable changes were found to take place in CTL during contact with TC, evidence of activation of secretory processes (Fig. 3): The lymphocyte became flattened, its nucleus became oval in shape, and it was shifted to the side opposite the zone of contact with TC. Besides mature granules, immature secretory granules appeared and could be seen to fuse with lipids and the crystalloid structures; hypertrophy and displacement of the vacuoles of the Golgi complex took place toward the zone of contact with TC. Fusion of the hypertrophied vacuoles of the Golgi complex with the plasmalemma could lead to an increase in the surface area of the lymphocyte, as a result of which fragments of the cytoplasmic membrane of CTL separated from the lymphocyte in the course of "peeling." It is perhaps at this moment that new receptors of CTL were formed. The importance of formation of the "membranosomes" is difficult to explain at present. They must evidently be differentiated from the minimal forms of oncornaviruses [2], and from decomposition products of lymphocyte membrane enzymes [8] or receptors of T lymphocytes.

The process of interaction between CTL and TC has been subdivided into three stages: 1) specific binding; 2) programming for lysis (or lethal shock); 3) disintegration of TC [9, 11]. It has been shown that 10 min is long enough for the second stage. These results were obtained by the use of lymphocytes stimulated *in vitro* or *in vivo*, where the effector cells were chiefly lymphoblasts [4, 5, 9, 11]. Previously the writers studied the ultrastructure of T lymphoblasts and found in their cytoplasm tubular formations packed with secretion, which evidently was discharged through these structures and the system of emptied vesicles. Deposition of secretion in these cells has not been observed [1]. On the 11th day after alloimmunization *in vivo*, the effector cells were small and medium lymphocytes [4, 6]. On the addition of an excess of these cells to TC, some adsorption of CTL in whose cytoplasm the Golgi complex was well developed and already oriented toward the zone of contact with TC is possible, and this would explain the early cytolysis of TC. Evidence in support of this is also given by the results of a study of cytolysis of TC: A fivefold difference in the lymphocytes:TC ratio (2:1 and 10:1) was observed only during the first hour of incubation. The difference was much reduced after 3 h.

It can be concluded from the results described above that during "maturation" of the lymphoblast and its conversion into a small lymphocyte, secretion becomes concentrated and deposited in the form of mature secretory granules and crystalloid structures. Contact with TC is the signal for activation of secretory processes and the discharge of the secretion. It is thus evident that after the stage of binding, a stage of "mobilization" arises for the small and medium lymphocyte, and this can be represented as follows: 1) the signal to the cytoplasm (or nucleus) of CTL; 2) hypertrophy and a change in the orientation of the Golgi complex; 3) activation of CTL organoids participating in deposition and synthesis of the specific product; 4) discharge of the secretion into the slit-like intercellular space bounded by the surface of the lymphocyte and TC.

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