Thymocytes stimulated in vitro in mixed culture were adsorbed by centrifugation on to the surface of target cells for an electron-microscopic study of the cytology of immune T lymphocytes and of the early stages of cytolysis. A well-developed Golgi apparatus and clusters of tubular structures 50-60 nm in diameter, communicating with the cisternae of the granular endoplasmic reticulum, with "emptied" vesicles, and with the plasma membrane of the lymphocyte, were found in the cytoplasm of the lymphocytes. Over a wide area the plasma membrane formed numerous contacts with the membrane of the target cells, so that closed slit-like spaces were formed. With these data and also modern views regarding interconversion of membranes and intracellular transport in mind, a hypothetical scheme for the mechanism of cytolysis of the target cell by the immune T lymphocyte is suggested.

KEY WORDS: T lymphocytes; cytolysis; ultrastructure.

The final stage of differentiation of B lymphocytes is known to be the plasma cell, the cytoplasm of which is filled with cisternae of the granular endoplasmic reticulum, where specific immunoglobulins are synthesized and accumulate. Antibodies are secreted by the plasma cell into the blood stream. Conversely, for the T lymphocyte to exert its cytolitic action, direct contact between it and the target cell is essential [15]. Under these circumstances, depending on the time of immunization, cytolitic activity may be possessed by lymphoblasts and by small and middle-sized lymphocytes [4]. However, the mechanism whereby cytolysis takes place remains unknown.

This paper describes an electron-microscopic investigation of the cytology of T lymphocytes obtained as a result of stimulation of thymus cells in vitro, in which the character of the contacts formed by the T lymphocytes when specifically adsorbed on to the surface of L cells was studied, and a schematic model of their interaction is suggested.

EXPERIMENTAL METHOD

To obtain cytolytic T lymphocytes, thymus cells from BALB/c (H-2^d) mice, stimulated by spleen cells from C3H (H-2^k) mice aged 8-12 weeks by the method described previously [1], were used.

The target cells were L cells, seeded on the day before the experiments in plastic flat-bottomed tubes measuring 10-45 mm at the rate of $3 \times 10^5$ cells in 0.5 ml medium 199 with 10% bovine serum. On the day of the experiments the L cells were washed and treated with cytolitic thymus lymphocytes obtained on the fifth day of stimulation in vitro in the proportion of $3 \times 10^5$ cells in 0.5 ml medium RPMI 1629 with 10% calf embryonic serum (in a ratio of 20 lymphocytes to 1 L cell). A 5-day monoeulture of thymus cells from BALB/c mice was used as the control. Immediately after addition of the lymphocytes the tubes were centrifuged for 7 min at 1000 rpm, incubated, and fixed 30 min after the beginning of incubation. For electron-microscopic investigation the cells were fixed first with 1% glutaraldehyde solution and then for 30 min by Dalton's method [8], after which they were embedded in a mixture of Epon and Araldite by the usual method. Ultrathin sections were obtained on the LKB-8800 Ultratome, stained with a 1% aqueous solution of uranyl acetate and lead citrate, and examined in the
JEM-100B electron microscope with an instrumental magnification of 5000×, 30,000×, and 50,000×.

EXPERIMENTAL RESULTS

The principal data on the submicroscopic organization of the cytolytic T lymphocytes are summarized in Fig. 1. Mainly lymphoblasts and large lymphocytes were adsorbed on the L cells, but some middle-sized and small lymphocytes also were found. At the point of contact between the T lymphocyte and target cell (Figs. 1 and 2) the plasma membranes could be parallel to one another (at a distance apart of 15-17 nm) or not parallel (in that case the distance between them varied from 10 to 150 nm); in some areas the plasmalemmas of two cells were evidently in contact for a distance of 20-40 nm. Outgrowths from the lymphocyte pressed deeply into the cytoplasm of the target cell (Fig. 1), but in that case also the plasma membranes of both cells remained intact.

In the mitochondria of the T lymphocytes the cristae were well developed and the mitochondrial matrix possessed considerable electron-optical density. Many lipid granules were found, some of them over 1 μ in diameter. The centrioles had the usual structure. The cytoplasmic microtubules and microfilaments were well developed and often arranged in clusters. The lysosomal apparatus of the T lymphocytes consisted of both primary lysosomes (multivesicular bodies) and secondary (true) lysosomes and residual postlysosomes, in which "myelin figures" were frequently found.

Numerous collections of ribosomes and polysomes were found in the cytoplasm of the T lymphocytes. In some areas parallel cisternae of the granular endoplasmic reticulum were identified. The agranular endoplasmic reticulum was well developed (Figs. 1 and 3) and consisted mainly of tubular structures 50-60 nm in diameter and up to 1 μ in length (in the plane of the ultrathin section). The tubular structures could communicate with the cisternae of the granular endoplasmic reticulum and plasma membrane of the cell (Fig. 1). Inside the tubular structures an osmiophilic material could be seen, and so-called "emptied" vesicles formed at their ends (Fig. 3). These formations were 70-100 nm in diameter and their outer membrane was covered with processes about 10 nm long. The structures described could perhaps also have been formed from cisternae of the Golgi complex. Cisternae and vesicles of the Golgi complex were well developed in the T lymphocytes; as a rule no vacuoles of the Golgi apparatus could be discovered.

Only small- and middle-sized lymphocytes with pale cytoplasm, containing no structures of the granular endoplasmic reticulum, were found in cells of a monoculture of thymus lymphocytes.

The cytotoxic effect of the immune T lymphocytes can be divided into three stages: 1) specific binding; 2) programing for lysis; 3) independent lysis of the target cell [11]. The workers cited postulated that in the second stage there is either the transfer of informationally-active structures from the immune lymphocyte into the target cell [2] or activation of lysosomal enzymes of the lymphocyte membrane [13], or a high concentration of negatively charged ions develops at the point of contact, with the result that the membrane of the target cell is injured [5], or finally, a lymphotoxin exerts its action [9]. However, none of the hypotheses has yet been properly confirmed.

It was accordingly very important to study the cytology of the immune T lymphocytes, the character of the contacts formed with target cell, and the integrity of the latter's cytoplasmic membrane. During the electron-microscopic study of lymph node T immunoblasts labeled with antiserum no distinguishing features could be found. The workers concerned found a dark matrix, on account of clusters of microfilaments, cisternae of the ergastoplasmic reticulum, and secretion-filled vacuoles in the cytoplasm of the medium-sized and small T lymphocytes [12]; attempts to find the place of fusion of the two cells or lysis of the cytoplasmic membrane of the target cell at the point of contact were unsuccessful. No reliable injury to the plasmalemma of the target cells could be found [10].

A mixed culture of thymus cells with a high content of cytolytic T lymphocytes, in which the proportion of blast cells was about 35% on the fourth to fifth day of stimulation in vitro, was obtained previously by the writers [1]. Since lymphocytes added to the suspension and monolayer of the target cells could not be adsorbed on their surface simultaneously, in order to study the early periods of killing the cells were sedimented by centrifugation.