ELECTRON-MICROSCOPIC AND AUTORADIOGRAPHIC STUDY OF INTERACTION BETWEEN LYMPHOCYTES AND TARGET CELLS IN TISSUE CULTURE

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Autoradiographic studies using labeled uridine-H^3 have shown that the numerous granules of DNA-containing material found in the cytoplasm of target cells are inactive. During interaction between lymphocytes and target cells, intimate contact between the cytoplasmic membranes of these cells was demonstrated electron-microscopically. Many immune lymphocytes underwent destruction. In some cases direct contact between nuclear material of the lymphocytes and the cytoplasmic membrane of the target cell was possible. The DNA-containing bridge is a projecting process of chromatin reaching from the destroyed lymphocyte into the phasgosome of the target cell. Clasmatosis of target cells, a specialized mechanism by means of which the cells get rid of excesses of phagocytosed material, was demonstrated.

Cytoplasmic and nuclear DNA-containing bridges, found during interaction between lymphocytes and target cells in tissue culture, were described in a previous paper [2]. To examine the nature of these structures, which are visible under the ordinary optical microscope, an electron-microscopic study was made of interaction between lymphocytes and target cells, and an attempt was made to discover whether the numerous granules of DNA-containing material in the cytoplasm of target cells are functioning. For this purpose, an autoradiographic study was carried out using labeled uridine-H^3.

EXPERIMENTAL METHOD

The experimental system of Rosenau and Moon [11], with transplanted L-cells and lymphocytes of immune BalB/c mice, was used. The method was described previously [11]. For the electron-microscopic investigation, L-cells were seeded in Petri dishes, 5 cm in diameter, at the rate of 500,000 cells in 5 ml medium No. 199 and 10% bovine serum inactivated at 56°. After incubation at 37° in an atmosphere of 5% CO_2 for 10 h, the medium was decanted and a suspension of lymph gland cells in medium No. 199, with concentration adjusted to 4 million cells ml, was added to the culture. Altogether three experiments were carried out. The cells were fixed 3 and 9 h after addition of the lymphocytes with 1% glutaraldehyde, made up in phosphate buffer (pH 7.2), for 5 min and then with 1% OsO_4, made up in phosphate buffer, for 40 min. Subsequent treatment was carried out by the method described previously [1]. After the end of polymerization, plane-parallel disks of polymethacrylate were studied in the JEM-7 microscope, with instrumental magnifications of 5000, 30,000, 50,000, and 70,000.

For the autoradiographic investigation, cultures of L-cells were grown in flasks with cover slips in concentrations of 100,000 L-cells ml medium No. 199 and 10% bovine serum, inactivated at 56°. After incubation at 37° for 18 h, the medium was decanted and a suspension of spleen cells in medium No. 199, with

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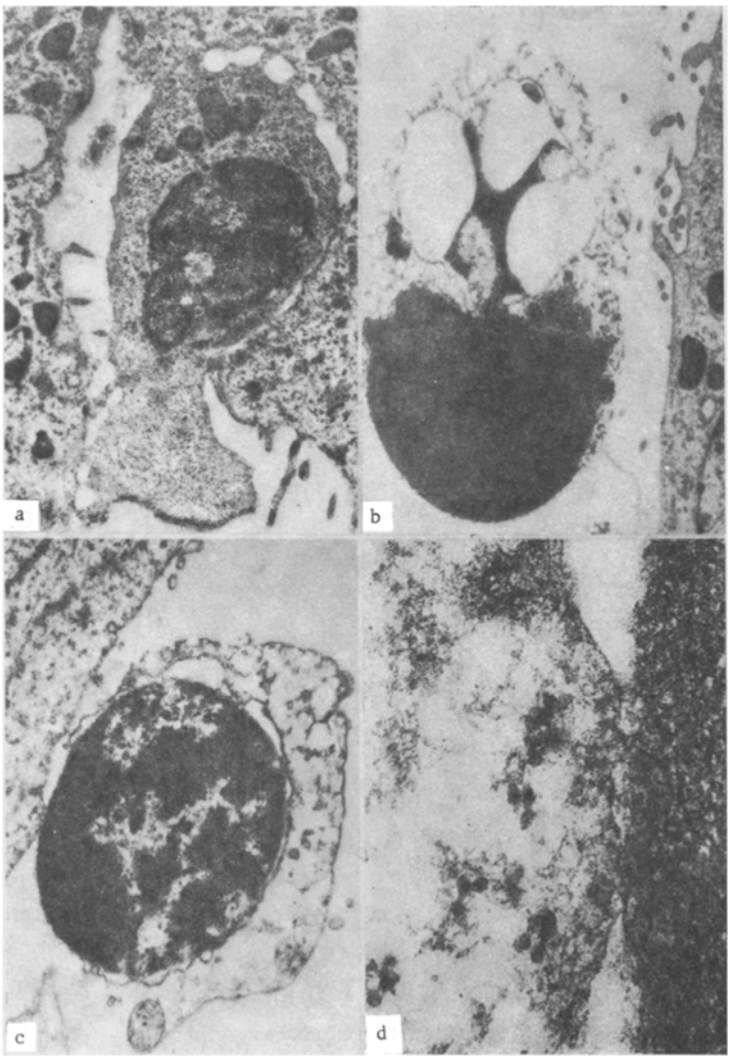

Fig. 1. Adsorption of lymphocytes on surface of target cell: a) cytoplasmic processes of immune lymphocyte form close contact with cytoplasm of L-cell, 13,500×; b) destruction of immune lymphocyte, escape of nucleoplasm into intercellular medium, 15,500×; c, d) contact between nucleus of destroyed immune lymphocyte and cytoplasm of L-cell, 13,500 and 155,000×.

concentration adjusted to 4 million cells/ml, was added to the culture. After contact for 6, 9, and 12 h, the incubation medium was poured off, and medium No. 199 containing 10 μCi uridine-H^3 (specific activity 2700 μCi/mg) was added. The cells were then incubated for 10 min on a water bath at 37°, and after incubation the medium was decanted, and the cells were washed three times and fixed by Carnoy's method.

The specimens were treated with trichloroacetic acid for 20 min at 4° and washed three times with water, cooled to the same temperature. They were then coated with type M NIKFI emulsion and exposed for 2 weeks.

EXPERIMENTAL RESULTS

Labeling with uridine-H^3 showed that after contact between the L-cells and immune lymphocytes for 6, 9, and 12 h, granules of label were found only in the nuclear region of the target cells. The cytoplasm of the L-cells was not labeled. It was impossible to find labeled granules of DNA-containing material in the cytoplasm of the L-cells.

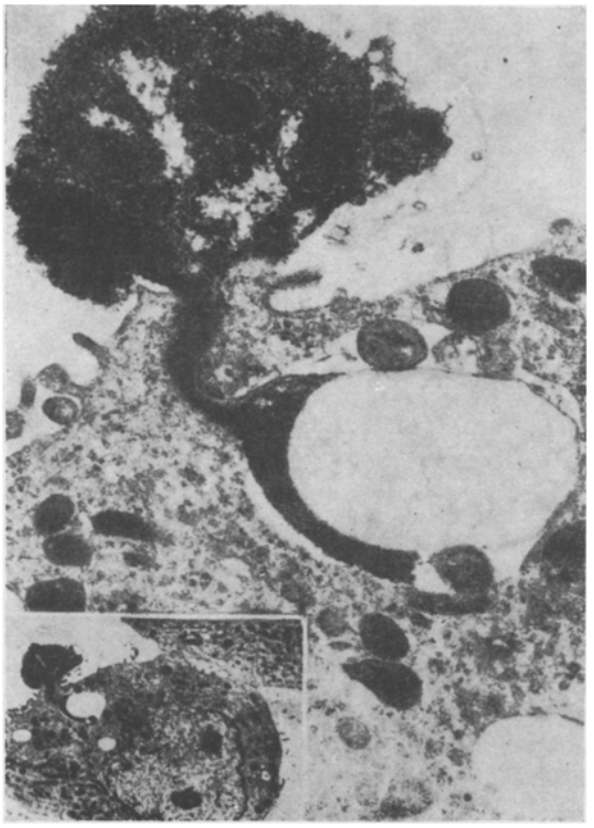

Fig. 2. Phagocytosis of chromatin elements of a destroyed immune lymphocyte, 4500 and 31,000×.

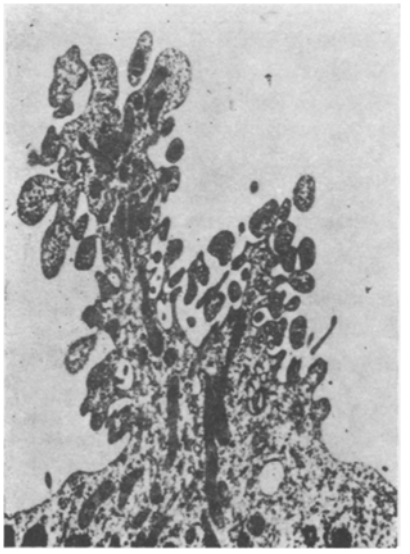

Fig. 3. Peripheral area of target cell during active plasmatosis 9 h after addition of immune lymphocytes, 17,000×.

Electron-microscopic investigation revealed increased adsorption of immune lymphocytes on the surface of the target cells. Adsorption of morphologically unchanged and evidently living lymphocytes took place by means of multiple cytoplasmic processes, or through direct contact between the cytoplasmic membranes of the cells (Fig. 1a).

Destruction of the lymphocytes was most commonly expressed as the partial or total loss of their cytoplasm. Cells whose nucleus in certain parts was completely without its nuclear membrane, and whose nucleoplasm was in direct contact with the surface of the cytoplasmic membrane of the L-cell, could be observed (Fig. 1c, d). Often the nucleoplasm escaped into the extracellular medium and resembled processes of chromatin, equal in length to the diameter of the lymphocyte. Fragments of nuclear material of lymphocytes, in the form of processes of chromatin, underwent phagocytosis by the cytoplasm of the target cells. The nucleus of a damaged lymphocyte is shown in Fig. 2. The cytoplasm is absent, and on one side a fragment of detached nuclear membrane can be seen. The nucleus is destroyed, and granules of chromatin are distributed irregularly over the region of the nucleus. In the center of the nucleus, in some places chromatin is completely absent. However, the nucleolus is intact, and at its center a condensation can be distinguished, and the nuclear band, undergoing phagocytosis by the target cell, ends in a phagocytic vacuole. This distinctive process of chromatin is about 10 μ long. The internal membrane of the phagocytic vacuole is evidently formed by the internal layer of the nuclear membrane of the lymphocyte. Inside the vacuole two mitochondria are clearly visible and are probably remnants of the phagocytosed cytoplasm of a lymphocyte. Around the phagocytic vacuole in the cytoplasm of the L-cell large lysosomes can be seen. In some cases complete lymphocytes have undergone phagocytosis by L-cells. After contact for 9 h, the number of lysosomes in the cytoplasm of the L-cells is considerably increased.

In the late stages of contact, 9-12 h after addition of lymphocytes, distinctive cytoplasmic processes appear on the surface of the L-cell. As a rule, on each L-cell one process is formed, several tens of microns in length and a few microns in width (Fig. 3). Numerous phagosomes, mitochondria, lysosomes,

collections of ribosomes and polysomes, and also peripheral parts of the cytoplasm of the L-cell can be seen in these processes. A study of serial ultrathin sections through these processes from different angles showed that they are evidently formed as a result of local plasmatosis, by the detachment of individual intracellular components from the target cells during their interaction with the lymphocytes.

Cytoplasmic bridges between lymphocytes and histiocytes in tissue culture were first described by Aronson [3]. They have repeatedly been observed by McFarland [7, 8] in mixed cultures of leukocytes from unrelated donors. The formation of intimate cytoplasmic contacts between lymphocytes and target cells has also been observed in electron-microscopic studies by Biberfeld et al. [4] and Weiss [12]. Immune lymphocytes, during contact with target cells, form finger-like cytoplasmic processes. The impression is gained that at some areas of contact, the cytoplasmic membrane may pass from one cell to the other (Fig. 1a). Microfilming data [10] suggest that the cytoplasmic bridges, described by the writers previously, and visible under the optical microscope [2], are an elongation of the lymphocyte cytoplasm: the "tail or uropod by means of which the lymphocyte attaches itself to other cells" [7, 10].

In the present experiments many destroyed immune lymphocytes were observed. Destruction of the lymphocytes evidently began with loss of the cytoplasm. When the nuclear membrane was ruptured, the integrity of the nucleus was destroyed, and the nucleoplasm escaped into the extracellular medium in the form of distinctive processes of chromatin which could be phagocytosed by the the target cell. In some cases, phagocytosis of nuclear fragments, having lost their nuclear membrane and lying in the immediate vicinity of the target cell, could take place. The DNA-containing bridges described previously and visible under the optical microscope [1] can therefore be regarded as processes of chromatin emerging from the destroyed nucleus of the lymphocyte.

It was shown previously [2] that DNA passes into the cytoplasm of L-cells from lymphocytes. To discover whether the granules of DNA-containing material present in the cytoplasm of the L-cells possess functional activity, a radioactive labeling method was used. Experiments showed that after incubation for 10 min with uridine-H^3, only the nuclei of the target cells were labeled.

The extent to which destruction of the nuclear material of the lymphocytes in the cytoplasm of the L-cells can take place is not known. Can individual intact DNA molecules penetrate into the nucleus of the target cell? The ability of exogenous intact DNA to be incorporated by nuclei of mammalian cells has been demonstrated by biochemical methods.

Reutilization of DNA of lymphocytes by other cells has been the subject of several investigations [5, 6]. In particular, Hill and Spurna [9], using a culture of L-cells and lymphocytes from a mouse lymphoma, labeled with thymidine-H^3, demonstrated autoradiographically that granules of label were present in the nucleoli of L-cells. These workers also described a number of hybrid cells, the nuclei of which were partially or half labeled.

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