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CYTOCHEMICAL STUDY OF INTERACTION BETWEEN LYMPHOCYTES AND TARGET CELLS IN TISSUE CULTURE

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It has been suggested [2, 6–10] that the effect of immune lymphocytes is due to migration of nucleic acids or ribosomes from lymphocytes to target cells. Nuclei of immune lymphocytes were found to destroy target cells as did intact immune lymphocytes [2, 7].

Histochemical and autoradiographic studies as described subsequently demonstrated the formation of cytoplasmic bridges (connections) containing DNA and RNA between lymphocytes and target cells, and the appearance in the cytoplasm of target cells of numerous granules of DNA-containing material and previously labelled RNA of lymphocytes.

Materials and Methods.—Experiments were performed following the system [5] tissue culture of continuous L cells plus lymphocytes from immune and non-immune BALB/c mice. The latter were immunized twice intraperitoneally with 2.5 million L cells during one month's interval. One month later the mice were inoculated into the spleen with 2.5 million L cells in 0.1 ml.

Four days after inoculation the spleens were removed aseptically and cell suspensions were prepared.

This method has previously been described in detail [2, 7]. Coverslips in penicillin vials were seeded with 100,000 L cells in 1 ml of medium 199 with 10 per cent inactivated bovine serum. After 18 h of incubation at 37°C the medium was removed and a suspension of lymphocytes in medium 199 without serum with a concentration

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of 4 million/ml was added to the culture. The preparations were fixed in Carnoy's fluid or with methyl alcohol at 1, 2, 3, 6, 9, 12, and 24 h after addition of lymphocytes.

DNA was detected by Feulgen's method and by staining with acridine orange (0.012 per cent solution of acridine orange in 0.1 *N* acetate buffer at pH 4.5) after

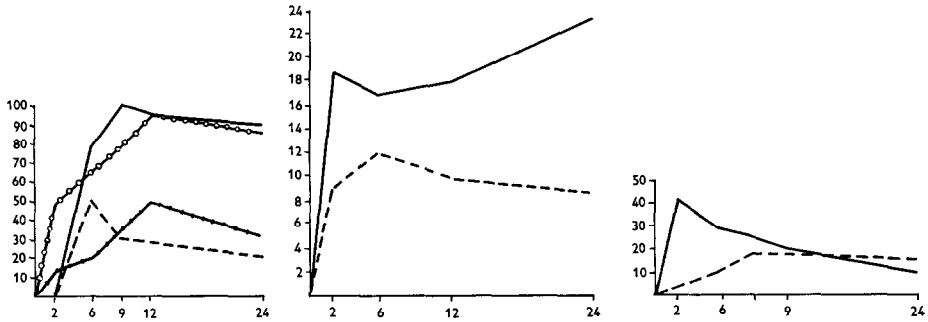

Fig. 1.—The dynamics of DNA and ^{3}H-uridine labelled RNA transition from lymphocytes in the cytoplasm of L-cells in the course of their interaction. *Abscissa*: h after addition of immune and normal lymphocytes to L-cells. *Ordinate*: % L-cells in the cytoplasm, —, DNA after addition of immune lymphocytes; – – –, normal lymphocytes; ●—●, labelled RNA after addition of normal lymphocytes; ○—○, immune lymphocytes.

Fig. 2.—Labelling of L-cells in the course of the interaction with lymphocytes. *Abscissa*: h; *Ordinate:* number of label granules. Average number of label granules per 1 labelled L-cell after addition —, of immune; – – –, of normal lymphocytes.

Fig. 7.—The dynamics of the appearance of DNA-containing bridges between lymphocytes and L-cells in the course of their interaction. *Abscissa*: h after addition of immune and normal lymphocytes to L-cells. *Ordinate*: % cells; – – –, % bridges after addition of normal lymphocytes; —, of immune lymphocytes.

preliminary hydrolysis of the preparations for 5 min in 1 *N* HCl at 60°C according to the method described elsewhere [3]. RNA was detected by staining preparations with methyl green-pyronine according to Brachet's procedure [1]. DNA and RNA were also detected by staining the preparations with toluidine blue and the molybdate ammonium method according to procedures of Love and Rabotti [3, 4]. For control purposes the preparations were treated with deoxyribonuclease (in a solution buffered with tris (hydroxymethyl) aminomethane-HCl 0.02 *M* to pH 7.3, containing 0.5 m*M* $CaCl_2$ and 45 m*M* $MgCl_2$ and ribonuclease *in vitro* in a concentration of 200 μg/ml for 2 h at 37°C, respectively.

Immune and normal lymphocytes were incubated with ^{3}H-uridine (specific activity 2.7 mC/mmole) in a concentration of 5 μC/ml or with ^{3}H-alanine (sp.a. 310 μC/g) in a concentration of 30 μC/ml for 1 h in medium 199. Thereafter the lymphocytes were washed three times and added to L cell culture. To remove precursors, the preparations were treated in 5 per cent TCA for 15 min at 4°C, washed with distilled water and covered with photo emulsion of M type, exposure time: 15 days. After development the preparations were stained with methyl-green-pyronine.

Results and Discussion.—At 1–2 h after addition of lymphocytes to tissue culture

cytoplasmic bridges were seen between these and L cells. They were observed by phase contrast microscopy of unstained preparations, by staining with hematoxylin and eosin and also by means of all the above procedures of DNA staining. The length of the bridge is approximately equal to lymphocyte diameter. Staining of preparations by Brachet's method with control by ribonuclease revealed RNA-positive material in bridges. In two experiments normal and immune lymphocytes previously labelled with ^{3}H-uridine were added to L-cells. The average number of granules above immune lymphocytes 2 h after addition to L cells was 10.4 per cell and above normal lymphocytes 3 per cell. In L cells the label was detected early, 1–2 h after addition of lymphocytes (Fig. 1, 2). After 2 h mainly those cells were labelled which had been pasted all over with lymphocytes. By this time the label was mostly detected in the cytoplasm, and in 6 h granules of the label were seen above the nucleus as well as above the cytoplasm. Sometimes the label was found in the cytoplasmic bridges stretching from labelled lymphocyte to still unlabelled target cell (Fig. 3). When normal lymphocytes were added to the culture, L cells were also labelled, but to a considerably lesser degree (Fig. 1).

In order to exclude synthesis of ribosomal RNA, the culture of L cells was, in the next experiment, treated with actinomycin D in a concentration of 1.5 μg/ml in medium 199 for 20 min. The culture was washed twice and labelled normal and immune lymphocytes were added. This treatment had no effect on the appearance of the label in L cell cytoplasm.

Staining by Feulgen's technique, as well as with toluidine blue after Love and Rabotti [4] and with acridine orange according to the procedure described in [3] revealed DNA transfer from lymphocytes to target cells (Fig. 5). By means of these methods dense tortuous DNA-containing strands (or spherical structures) may be seen to stretch from the nuclei of some lymphocytes to the cytoplasm or nuclei of L cells. These structures are not homogeneous, but reveal a different density. They are seen after the first 3–6 h of contact between lymphocytes and target cells. Then their quantity is gradually decreasing. 6, 9, 12 h respectively in the cytoplasm of L cells there appear numerous small granules of DNA-containing material of lymphocyte nuclei fragments (Fig. 5). In Fig. 1 curves are given of the number of L cells in the cytoplasm of which DNA granules were found. The number of bridges in cultures with immune lymphocytes was twice as large as that in cultures with normal lymphocytes and reached its maximum by 2–3 h. At the same time DNA granules began to appear in the cytoplasm of L cells. By 9 h DNA could be seen in the cytoplasm of 100 per cent of L cells, and at the same time cells appeared in the cytoplasm of which DNA-containing particles were localized in the perphery, sometimes forming a bright red fringe. In 24 h the number of L cells showing DNA granules in the cytoplasm was twice as low in cultures with normal lymphocytes. Fig. 7 shows the dynamics of the formation of DNA-containing bridges between L cells and immune or normal lymphocytes. It is well correlated with the appearance of DNA-containing particles in the cytoplasm of L-cells (Fig. 1). Further studies will reveal whether or not all DNA-containing granules transfer to the cytoplasm of L-cells via the bridges. It is not excluded that some fragments of lymphocyte nuclei appears in the L-cells due to pinocytosis. DNA-containing bridges were also observed between pairs of lymphocytes located sometimes at a considerable distance from each other (Fig. 6). In the 3–6 h cultures their quantity is up to 4 per cent.

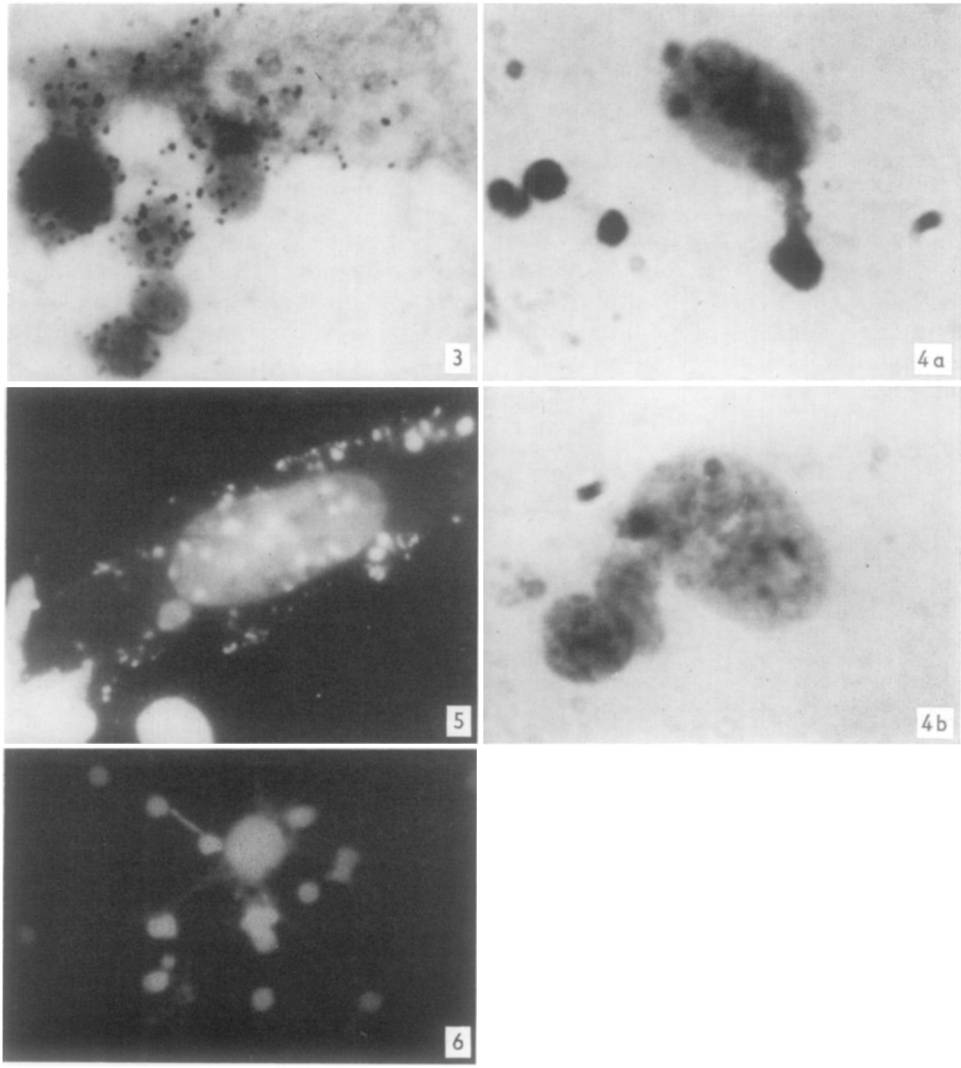

Fig. 3.—L-cell connected by cytoplasmic bridge with heavily labelled lymphocyte at 2 h after addition of lymphocytes to L-cell culture. Granules of the label are seen in the area of the cytoplasmic bridge and partially in the cytoplasm of L-cell. Stain: methyl-green-pyronine. 10 × 90.

Fig. 4 *a* and *b*.—A bridge containing DNA stretching from lymphocyte to the nucleus of L-cell (3 h after addition of lymphocytes to L-cells). Feulgen stain. 10 × 90.

Fig. 5.—L-cell in the cytoplasm of which numerous DNA granules are found (9 h after addition of lymphocytes to L-cells). Stain: acridine orange. 10 × 90.

Fig. 6.—A bridge containing DNA between two lymphocytes (9 h after addition of lymphocytes to L-cells). Stain: acridine orange. 10 × 90.

When immune lymphocytes previously labelled with ^{3}H-alanine were added to the culture, appearance of granules above bridges between lymphocytes and L cells was observed. Two h after addition of lymphocytes only 5 per cent of L cells were labelled, and the average number of granules above one labelled L cell was 18.

The bridge formation and transfer of lymphocyte DNA-containing granules in the cytoplasm of 100 per cent of L cells very likely supports the suggestion by Svet-Moldavsky that the effect of lymphocytes in transplantation immunity and other delayed type responses is connected with the transmission of the informative structures between the lymphocyte and target cell [2, 6–10]. It is possible that the DNA-containing lymphocyte-lymphocyte bridges have something to do with the immunological memory. The study of function of DNA-containing particles of lymphocytes in the cytoplasm of L-cells will show whether the above evidence is a particular kind of cell hybridization.

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POSSIBLE PRESENCE OF DNA IN INTERCELLULAR BRIDGES

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The occurrence of conjugation among procaryotes has been known for a long time [11, 12]. During this process a small bridge is formed between the conjugating cells, which establishes direct cytoplasmic contact [1].

Structures superficially similar to these bridges have been observed connecting mammalian cells [2, 3, 13]. Under certain circumstances cells make use of bridges or processes presumably for the transfer of material, for example, the so-called dendritic macrophage [20] and the lymphocyte with its uropode [15]. Furthermore, with electron microscopy it has been established beyond doubt that cytoplasmic continuity can exist between "macrophage" and lymphocyte in the lymph node [19] and

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