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

S. N. Sura, I. Yu. Chernyakhovskaya,
Z. G. Kadagidze, B. B. Fuks,
and G. Ya. Svet-Moldavskii

UDC 612.014.2-085.23

The mechanism of action of immune lymphocytes in immunologic reactions of the delayed type has not been explained. G. Ya. Svet-Moldavskii has suggested that the harmful action of immune lymphocytes is due to the transfer of nucleic acids or nucleoproteins from them into target cells [1, 2]. It has later been shown that the nuclei of immune lymphocytes act in the same way as the lymphocytes themselves [3].

In the present investigation the interaction between normal and immune lymphocytes of line BALB/c mice and L-cells was studied histochemically and autoradiographically.

EXPERIMENTAL METHOD

Experiments were carried out by the system of Rosenau and Moon [8]. Transplantable L-cells of immune BALB/c mice were used. The method was described previously [3]. Penicillin flasks with cover slips were seeded at the rate of 100,000 L-cells per milliliter of medium No. 199 and 10% bovine serum, inactivated at 56°. After incubation at 37° for 18 h the medium was poured off and a suspension of spleen cells in medium No.199, its concentration adjusted to 4 million cells/ml, was added to the culture. Seven experiments were carried out.

The preparations were fixed 1, 2, 3, 6, 9, 12, and 24 h after addition of the lymphocytes to the L-cells in Carnoy's fluid or methyl alcohol for 7-10 min. DNA was detected histochemically by the Feulgen method and staining with acridine orange (0.012% solution of acridine orange in 0.1N acetate buffer, pH 4.5 for 5 min) after preliminary hydrolysis of the preparations (5 min in 1N HCl solution at 60° [4]). RNA was detected by staining the preparations by Brachet's method with methyl-green pyronine with a ribonuclease control. Both DNA and RNA were detected by staining with toluidine blue and ammonium molybdate with deamination by Van Slyke's method [4, 7].

For control purposes desoxyribonuclease (Research Institute of Organic Chemistry, Siberian Division, Academy of Sciences of the USSR; activity 10,000 activity units/g by Kunitz's method) in 0.005M $MgSO_4$ solution and ribonuclease in vitro in a concentration of 200 μg/ml were used. Incubation continued for 2 h at 37°.

Immune and normal lymphocytes were incubated with uridine-H^3 (specific activity 2.7 Ci/mole in a concentration of 5 μCi/ml) or with alanine-H^3 (specific activity 310 mCi/g) in a concentration of 30 μCi/ml for 1 h in medium No. 199, after which the lymphocytes were washed three times and added to the culture of L-cells. To remove precursors, the preparations were treated in 5% trichloroacetic acid for 15 min at 4°, washed with distilled water, and covered with type M (Motion Picture Research Institute) emulsion. The exposure was for 15 days. After development, the preparations were stained with methyl-green pyronine.

Actinomycin D was added to the culture of L-cells in a concentration of 1.5 μg/ml in medium No. 199 for 20 min, after which the culture was washed twice and the labeled immune and normal lymphocytes were added.

Laboratory of Virology, Institute of Experimental and Clinical Oncology, and Laboratory of Histochemistry, Institute of Human Morphology, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR, E. M. Tareev). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 11, pp. 103-107, November, 1967. Original article submitted February 15, 1967.

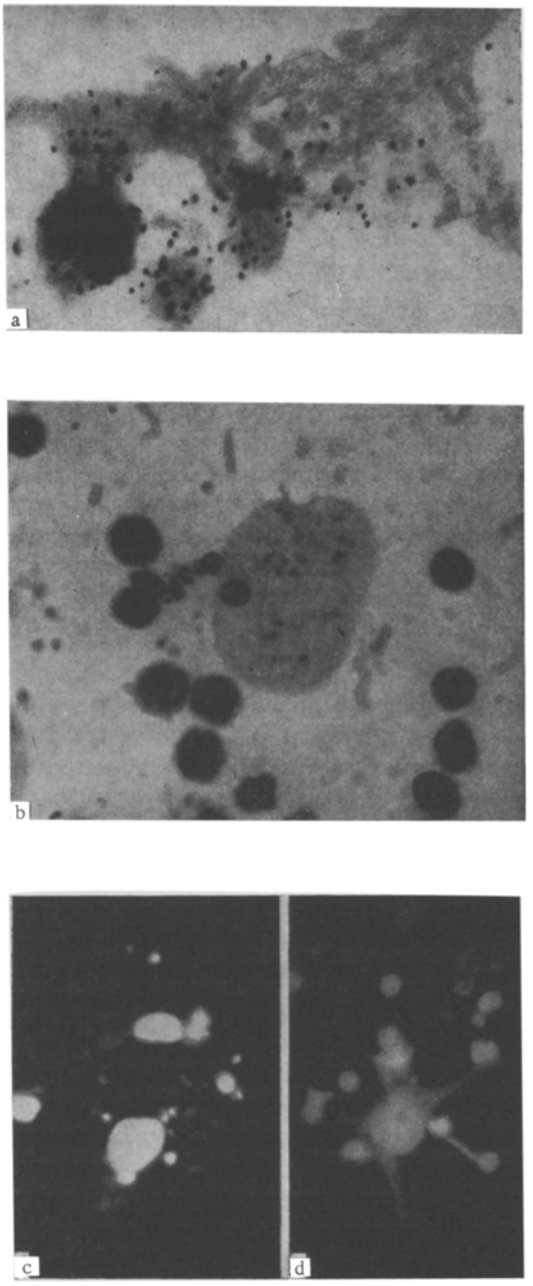

Fig. 1. Formation of cytoplasmic bridges between lymphocytes and target cells and transfer of DNA and RNA along them. a) Transfer of RNA from lymphocyte into target cell along cytoplasmic bridge. Methyl green–pyronine, magnification 10 × 90; b) nuclear band running from lymphocyte to nucleus of L-cell. Feulgen, magnification 10 × 90; c) droplets of DNA-containing material in cytoplasm of α-cell. Acridine orange after preliminary hydrolysis, magnification 10 × 90; d) DNA-containing bridge between two lymphocytes. Acridine orange after preliminary hydrolysis, magnification 10 × 90.

EXPERIMENTAL RESULTS

From 1-2 h after addition of the lymphocytes to the culture, cytoplasmic bridges were found between them and the L-cells. They could be seen during phase-contrast microscopy of unstained preparations, in films stained with hematoxylin-eosin, and also by all the histochemical methods described above. The length of the bridge was approximately equal to the diameter of the lymphocyte. Autoradiographic analysis

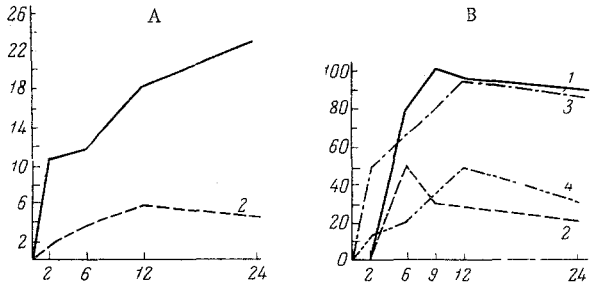

Fig. 2. Dynamics of transfer of nucleic acids into target cells after addition of immune and normal lymphocytes. A) Mean number of granules of label over cytoplasm of target cells after addition of immune and normal lymphocytes. B) Number of L-cells (in percent) in whose cytoplasm DNA or labeled RNA was found. A: abscissa—hours after addition of immune and normal lymphocytes to L-cells; ordinate—number of granules of label; 1) mean number of granules of label above one L-cell after addition of immune lymphocytes; 2) mean number of granules of label above one L-cell after addition of normal lymphocytes. B: abscissa—percentage of cells; ordinate—the same as in A; 1) percentage of L-cells in whose cytoplasm DNA was found after addition of immune lymphocyates; 2) percentage of cells in whose cytoplasm DNA was found after addition of normal lymphocytes; 3) percentage of cells over which DNA was found after addition of normal lymphocytes; 3) percentage of cells over which granules of label were found after addition of immune lymphocytes; 4) percentage of cells over which granules of label were found after addition of normal lymphocytes.

and staining the films by Brachet's method with a ribonuclease control demonstrated the presence of RNA-positive material in the region of the bridges. In two experiments, immune and normal lymphocytes labeled beforehand with uridine-H^3 were added to the L-cells. The mean number of granules over the immune lymphocytes 2 h after addition to the L-cells was 10.4 per cell, compared with three per cell above the normal lymphocytes. Label could be found in the L-cells soon (1-2 h) after addition of the lymphocytes (Fig. 1, a).

After two hours mainly those cells to which lymphocytes were attached were labeled. By this time label was found mainly in the cytoplasm, but after 6 h granules could also be seen both above the nucleus and above the cytoplasm. Sometimes cytoplasmic bridges running from a labeled lymphocyte to a target cell not yet containing label were themselves labeled (Fig. 2, A). When normal lymphocytes were added to the culture the L-cells also were labeled, but much less intensively.

The possibility of reutilization of labeled precursors entering the medium from disintegrated lymphocytes was investigated in experiments with actinomycin D (1 μg/ml). The results of these experiments showed that blocking synthesis of ribosomal RNA had no effect on appearance of the label in the cytoplasm of the L-cells.

After staining by Feulgen's method, with toluidine blue and ammonium molybdate, and also with acridine orange, transfer of DNA from lymphocytes into target cells could be seen (Fig. 1, b). These methods revealed that dense, convoluted, DNA-containing bands or spherical structures extended from the nuclei of some lymphocytes toward the cytoplasm or nuclei of the L-cells. Their number reached a maximum in the early stages of contact between lymphocytes and L-cells, while numerous tiny granules of DNA-containing material appeared in the cytoplasm of the L-cells after 6, 9, and 12 h (Fig. 1, c). Curves showing the number of L-cells in whose cytoplasm DNA granules were found are illustrated in Fig. 2, B. The number of bridges in cultures with immune lymphocytes was twice their number in cultures with normal lymphocytes and reached its maximum by 2-3 h; DNA granules began to appear in the cytoplasm of the L-cells at the same time. By 9 h, DNA could be seen in the cytoplasm of nearly all L-cells, and at this time cells began to appear in whose cytoplasm the tiny DNA droplets, grouped around the periphery, sometimes formed a bright red fringe. After 24 h the number of L-cells whose cytoplasm contained DNA showed a slight decrease. The number of L-cells whose cytoplasm contained DNA granules was only half as great in the cultures with normal lymphocytes.

Bridges containing DNA were also observed between pairs of lymphocytes sometimes located far apart (Fig. 1, d).

Autoradiographically, the transfer of protein could also be observed from lymphocytes into L-cells. In these experiments, the mean number of granules per immune lymphocyte was 3.6, compared with 0.2 per normal lymphocyte. After introduction of immune lymphocytes labeled beforehand with alanine-H^3 into the culture, granules appeared above bridges between lymphocytes and L-cells: only 5% of L-cells were labeled and the mean number of granules above one labeled L-cell was 18.

Cytoplasmic bridges between macrophages and histiocytes were first described by Aronson. Schoenberg [9] observed areas with disappearance of cytoplasmic membranes between cells of the lymphoid series in a lymph gland by means of the electron microscope.

In the present experiments, cytoplasmic bridges regularly appeared in the early stages after addition of lymphocytes to the culture of L-cells. Continuous migration of cytoplasm of the lymphocyte into the cytoplasm of the L-cell and absence of the boundary visible in the optical microscope suggest that this phenomenon is not accidental.

The problem of transfer of RNA from lymphocytes into target cells is complicated by the possibility of label reutilization. However, the label appeared mainly in the region of the cytoplasmic bridges, above the cytoplasm of the L-cells to which, as a rule, lymphocytes had already adhered in the early periods of the experiment. The intensity of label in the L-cells increased from 2 h until 24 h, not by an increase in the number of granules in the target cells, but by an increase in the number of labeled L-cells.

The discovery of numerous granules of DNA-containing material in the cytoplasm of the L-cells was preceded by the appearance of nuclear bridges running from the lymphocytes to the target cells. Nine hours after addition of immune lymphocytes, droplets of DNA-containing material were observed in the cytoplasm of 100% of cells. After contact with normal lymphocytes DNA was observed in the cytoplasm of 50% of L-cells. Later, after 24 h, DNA disappeared from the cytoplasm of the L-cells. No changes were found in the nuclei of the L-cells visible in the ordinary microscope.

The possibility of migration of genetically active material from cell to cell has been proved by somatic hybridization experiments [6]. The pictures observed indirectly confirm views according to which interaction between lymphocyte and target cell is lethal hybridization of the cells.

LITERATURE CITED

1. G. Ya. Svet-Moldavskii, S. N. Zinzar, and D. A. Egorkina, In the book: Proceedings of the Second Congress of Hygienists, Microbiologists, Epidemiologists, and Infectious Diseases Specialists of the Latvian SSR [in Russian], 149, Riga (1963).
2. G. Ya. Svet-Moldavskii et al., Nature, 202, 303 (1964).
3. G. Ya. Svet-Moldavskii and I. Yu. Chernyakhovskaya, Nature, 204, 799 (1964).
4. B. B. Fuks, S. N. Sura, V. V. Brzhevskii, et al., Tsitologiya, No. 4, 433 (1967).
5. M. Aronson, J. Exp. Med., 118, 1083 (1964).
6. B. Ephrussi and S. Sorieul, C. R. Acad. Sci., Paris , 254, 181 (1962).
7. R. Love and G. Rabotti, J. Histochem. Cytochem., 11, 187 (1963).
8. W. Rosenau and H. D. Moon, J. Nat. Cancer Inst., 27, 471 (1961).
9. Schoenberg, Lancet., 1, 7394, (1965).