ELIMINATION OF LARGE PYRONINOPHILIC CELLS BY THE ACTION OF PHYTOHEMAGGLUTININ

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UDC 612.112.12-06:612.111.44

Disappearance of medium-sized and large pyroninophilic lymphocytes, adsorbed on the surface of target cells, was shown to take place after the addition of phytohemagglutinin (PHA). After incubation for 45 min in the presence of PHA no cisterns of the granular endoplasmic reticulum could be found in the lymphocytes and the mitochondria were fewer in number. Cells labeled with thymidine-H^3 had practically completely disappeared. A population of small lymphocytes with smooth outlines and pale cytoplasm, poor in organelles, and with ribosomes scattered freely in them appeared. After incubation for 24-48 h they were transformed into blast cells, larger cells with a pale nucleus and pale cytoplasm, in which no cisterns of the granular endoplasmic reticulum could be found.

KEY WORDS: phytohemagglutinin; immune lymphocytes; large pyroninophilic lymphocytes; target cells.

Phytohemagglutinin (PHA) is a powerful mitogen which induces blast transformation of lymphocytes in vitro [13] and induces a cytotoxic action of normal lymphocytes in a culture of target cells [10, 11]. The writers showed previously that during the first hours of incubation of normal and also of immune lymphocytes with PHA, only a very few medium-sized and large pyroninophilic cells can be found in the cytotoxic test [1]. Electron-microscopic investigation revealed an electron-optically dense matrix, elements of a reticulum, and cells resembling plasma cells in their structure in the population of immune lymphocytes and a qualitatively different morphological picture in cells incubated with PHA [2].

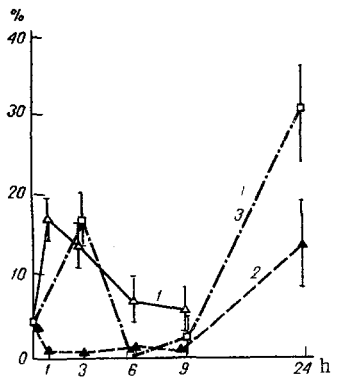

Fig. 1. Elimination of large pyroninophilic cells by the action of PHA: 1) immune lymphocytes; 2) immune lymphocytes + PHA; 3) immune lymphocytes + PHA 3 h after beginning of contact with culture of L-cells. Abscissa, time after addition of lymphocytes to culture of L-cells (in h); ordinate, number of cells (in %).

In this investigation the action of PHA during the first 15-90 min of incubation on the structure and ultrastructure of immune lymphocytes in a culture of target cells was studied.

EXPERIMENTAL METHOD

Experiments were carried out with the system of Rosenau and Moon [16]. Inbred BALB/c mice aged 8-16 weeks were used as the source of lymphocytes, and transplantable L-cells grown in tubes and flasks with cover slips by the method described previously [3] were used as the target cells.

Laboratory of Systemic Blood Diseases, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR. Department of Electron Microscopy, N. E. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 9, pp. 107-111, September, 1975. Original article submitted June 27, 1974.

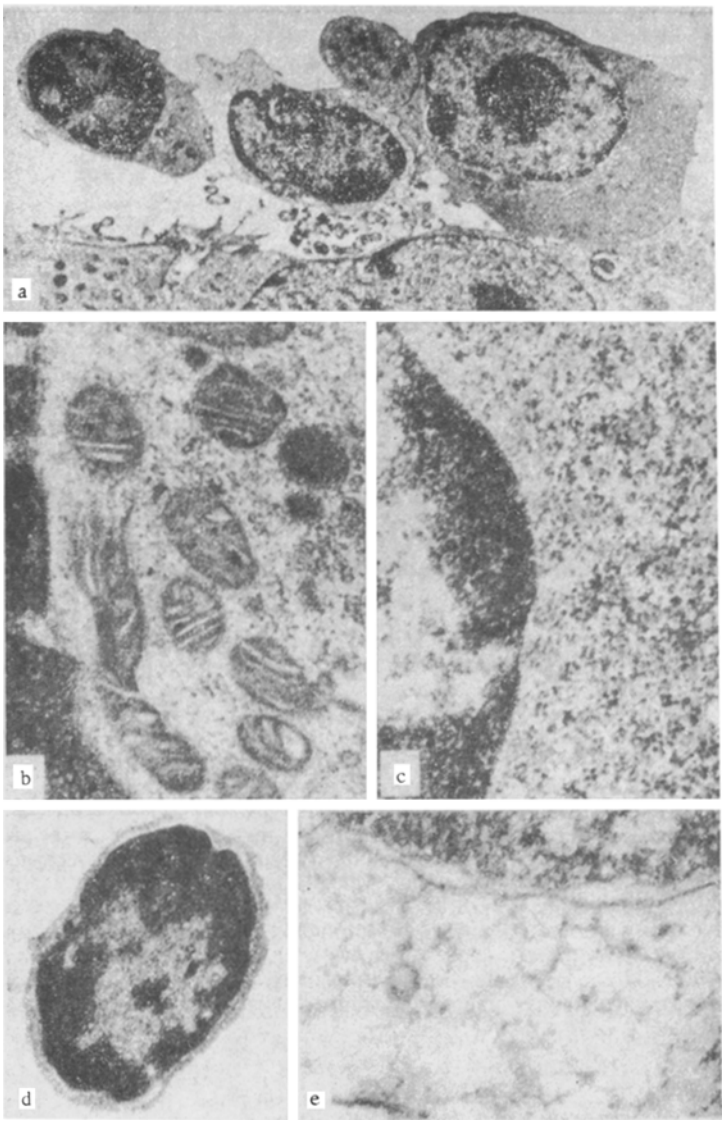

Fig. 2. Ultrastructure of immune lymphocytes 15-90 min and 48 h after incubation with PHA: a) lymphocytes after incubation for 15 min with PHA (2500 ×); b, c) areas of cytoplasm of lymphocytes after incubation for 15 min with PHA (30,000 ×); d) lymphocytes after incubation for 90 min with PHA (3500 ×); e) area of cytoplasm of lymphocytes after incubation for 48 h with PHA (100,000 ×).

Immune lymphocytes were obtained from the regional lymph glands of BALB/c mice 8 days after a single immunization with L-cells ($11 \cdot 10^6$–$20 \cdot 10^6$ cells per mouse). Normal and immune lymphocytes of BALB/c mice were obtained by means of a Potter's homogenizer, washed 3 times, suspended in medium No. 199, and added to washed cultures of L-cells in a concentration of $4 \cdot 10^6$ cells/ml. PHA (Difco) was used in a concentration of 50 μg/ml. Altogether 5 series of experiments were carried out, with immune lymphocytes, normal lymphocytes, immune lymphocytes and PHA, and normal lymphocytes and PHA added to the culture of target cells. In one series of experiments, 3 h after the beginning of incubation the immune lymphocytes were removed, and PHA was added to fresh medium not containing cells. Films were made at the same time from suspensions of normal and immune lymphocytes and also of lymphocytes which had been incubated with PHA for 1.5 h.

The cells were washed with medium No. 199 and fixed by Carnoy's method 1, 3, 6, 9, 24, and 48 h after the addition of lymphocytes to the culture of L-cells (3 flasks at each time) and stained with methyl green-pyronine. In order to tag the large pyroninophilic cells (LPC), thymidine-H^3 (specific activity 11 Ci/mmole)

was added in a concentration of 3 μCi/ml to the suspensions of lymphocytes and also to the cultures in the experiments in which the immune lymphocytes were removed, 3 h before the addition of PHA.

These preparations were washed 3 times with medium No. 199 heated to 37°C, fixed by Carnoy's method, treated with a 4% solution of $HClO_4$ for 20 min at 4°C, washed 3 times with distilled water at the same temperature, and coated with type M (NIKFI) emulsion. The duration of exposure was 4 days.

From 300 to 500 cells were counted in each preparation, depending on the number of "living" lymphocytes, which had to be not less than 200. In the experiments in which PHA was added 3 h after the beginning of incubation of the immune lymphocytes with L-cells the cultures were fixed for electron-microscopic investigation for 5 min in 1% glutaraldehyde solution in phosphate buffer, then by Dalton's method after 15, 30, 45, and 90 min. The preparations were then embedded in a mixture of Epon and Araldite by Dalton's method [8]. Ultrathin sections were cut on the LKB-4880 ultratome and stained with a 1% aqueous solution of uranyl acetate and lead citrate; the preparations were examined in the IEM-100V electron microscope with an instrumental magnification of 5000, 30,000, and 50,000 ×.

EXPERIMENTAL RESULTS

The proportion of a plaque-forming cells (PFCs) in the population of immune lymphocytes incubated with PHA in a culture of L-cells did not exceed 5-6% during the first 9 h of incubation, whereas the proportion of these cells during incubation without PHA after contact for 6-9 h with L-cells reached 20%. Later the proportion of PFC in this group of experiments gradually decreased, whereas in the presence of PHA the number of blast cells increased, to reach 34% after incubation for 48 h (Fig. 1). The content of medium-sized lymphocytes also fell: 9% in the experimental and 18% in the control series (of all cells incubated without PHA). The addition of PHA to the culture of immune lymphocytes 3 h after the beginning of incubation led to a decrease in the number of PFCs from 17 to 1% (Fig. 1) and of medium-sized lymphocytes from 33 to 3%. The content of cells labeled with thymidine-H^3 fell from 10-12 to 1-2%.

A study of the ultrastructure of the immune lymphocytes showed that after incubation for 15-45 min with PHA no cisterns of the granular endoplasmic reticulum could be seen in the cells (Fig. 2a, b, c); after 90 min (Fig. 2d) a qualitatively new and homogeneous population of cells appeared: with pale cytoplasm, containing only free ribosomes and single mitochondria, and not containing any graunular endoplasmic reticulum. Only solitary ribosomes were found in the cytoplasm of the cells 46 h after incubation (Fig. 2e).

According to Rosenau's observations [17], the addition of PHA to the culture medium reduces the intensity of the cytotoxic effect. Instead of immune PFCs, the lymphocytes are transformed into PHA-blasts, with no cytotoxic activity [6]. The addition of concanavalin A to the medium had a similar action [19]. Inhibition of the cytotoxic activity of rat lymphocytes against mouse fibroblasts has also been demonstrated after destruction of PFCs by L-asparaginase [5]. The use of labeled acridine, which binds free phosphate groups of DNA, showed that during the first 7 min of incubation of the lymphocytes with PHA the bond between DNA and histone is weakened and the structure of the chromatin is altered [9, 15, 18]. The action of PHA is connected with derepression of parts of the genome, as confirmed by an increase in the acetylation of nuclear histones [14] and stimulation of the phosphorylation and dephosphorylation of histones and lipoproteins in the lymphocytes [12]. RNA synthesis is activated 15 min after the addition of PHA [14], but during the first 30 min of incubation the cytoplasmic RNA of the lymphocytes is destroyed, after which synthesis of a new RNA begins; during the first few hours of action of PHA this RNA is not ribosomal [7].

In the present experiments disappearance of the medium-sized and large pyroninophilic lymphocytes was demonstrated after the addition of PHA. The number of LPCs was reduced by about 15 times and the number of medium-sized lymphocytes by 10 times after incubation for 3 h with PHA. The number of cells labeled with thymidine-H^3 decreased from 10-12 to 1-2%, indicating destruction of cells synthesizing DNA in the presence of PHA. This conclusion does not, however, extend to the fate of the effector lymphocytes, and no direct proof of their death was obtained. On electron-microscopic examination no cisterns of the granular endoplasmic reticulum could be found in the lymphocytes 15-45 min after incubation of the immune lymphocytes with PHA, and only free ribosomes could be seen in their hyaloplasm, their number decreasing considerably after 24-48 h. The phenomenon of morphological dedifferentiation is also characteristic of other cell organoids, especially mitochondria. Mainly cells with a small amount of cytoplasm and with a dedifferentiated endoplasmic reticulum were seen in the preparations 90 min after incubation with PHA.

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