COMPARATIVE STUDY OF ULTRASTRUCTURE OF IMMUNE AND NORMAL LYMPHOCYTES TREATED WITH PHYTOHEMAGGLUTININ

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Immune lymphocytes adsorbed on the surface of target cells during the first 3 h of combined incubation are characterized by the presence of an electron-optically dense matrix and by abundance of mitochondria and lipids; the small lymphocytes contain freely scattered ribosomes, which in medium lymphocytes are organized into polysomes and form separate cisterns of the granular endoplasmic reticulum in large lymphocytes, evidence of active protein synthesis by these cells. Cells of the plasma type also were found. Cells treated with phytohemagglutinin for 1 h consisted of a uniform population of small lymphocytes of identical size, with pale cytoplasm containing free ribosomes and single mitochondria. The proportion of medium lymphocytes and blast cells increased with an increase in the period of incubation. These were cells with pale cytoplasm and with freely scattered polysomes, in which a developed granular endoplasmic reticulum was never found. The presence of two types of cells whose ultrastructure reflects their functional characteristics is discussed.

KEY WORDS: *normal and immune lymphocytes; ultrastructure; phytohemagglutinin; target cells.*

The cytological effect of immune lymphocytes on a culture of target cells and blast transformation of lymphocytes under the influence of appropriate sensitizing agents (or mitogens) are model systems of cellular immunity. Blast transformation of lymphocytes reflects the ability of these cells to reproduce themselves, whereas the cytotoxic effect on target cells is an expression of the cytotoxic function of the effector lymphocytes. Existing morphological descriptions of lymphocytes fail to take into account the functional characteristics of these cells [1]. The writers showed previously that the proportion of medium and large pyroninophilic cells increases during the first hours of contact between immune lymphocytes and target cells [2, 3], whereas in the presence of phytohemagglutinin (PHA) the number of blast cells increased during 48-72 h of incubation.

In the investigation described below the ultrastructure of these two types of cells was studied.

EXPERIMENTAL METHOD

Experiments were carried out on inbred BALB/c mice aged 8-16 weeks. Transplant-

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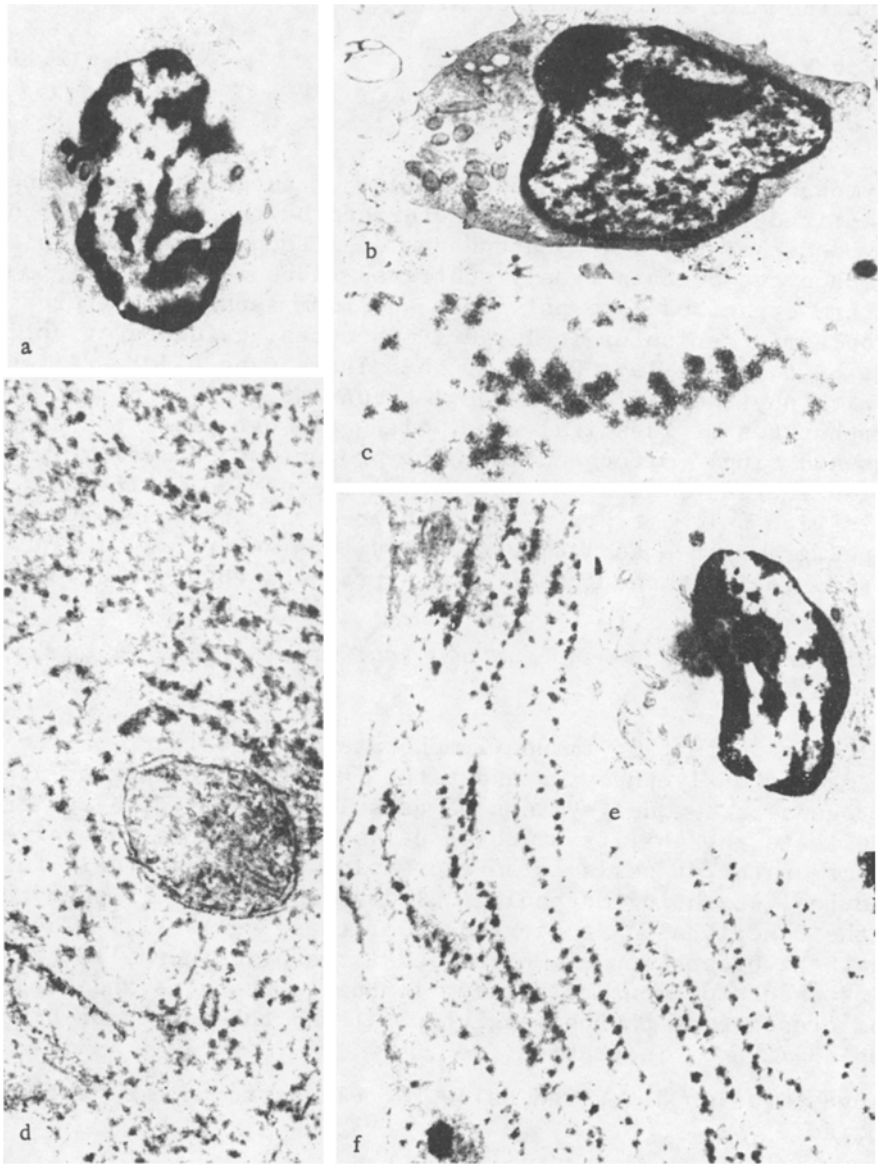

Fig. 1. Ultrastructure of immune lymphocytes 3 h after contact with L-cells: a) medium lymphocyte, 3500×; b) large lymphocyte, 3500×; c) fragment of preceding photograph, linear polysome, 200,000×; d) area of cytoplasm of large lymphocyte, cisterns of granular endoplasmic reticulum, 100,000×; e) plasma cells; f) fragment of previous photograph, ergastoplasm, 40,000×.

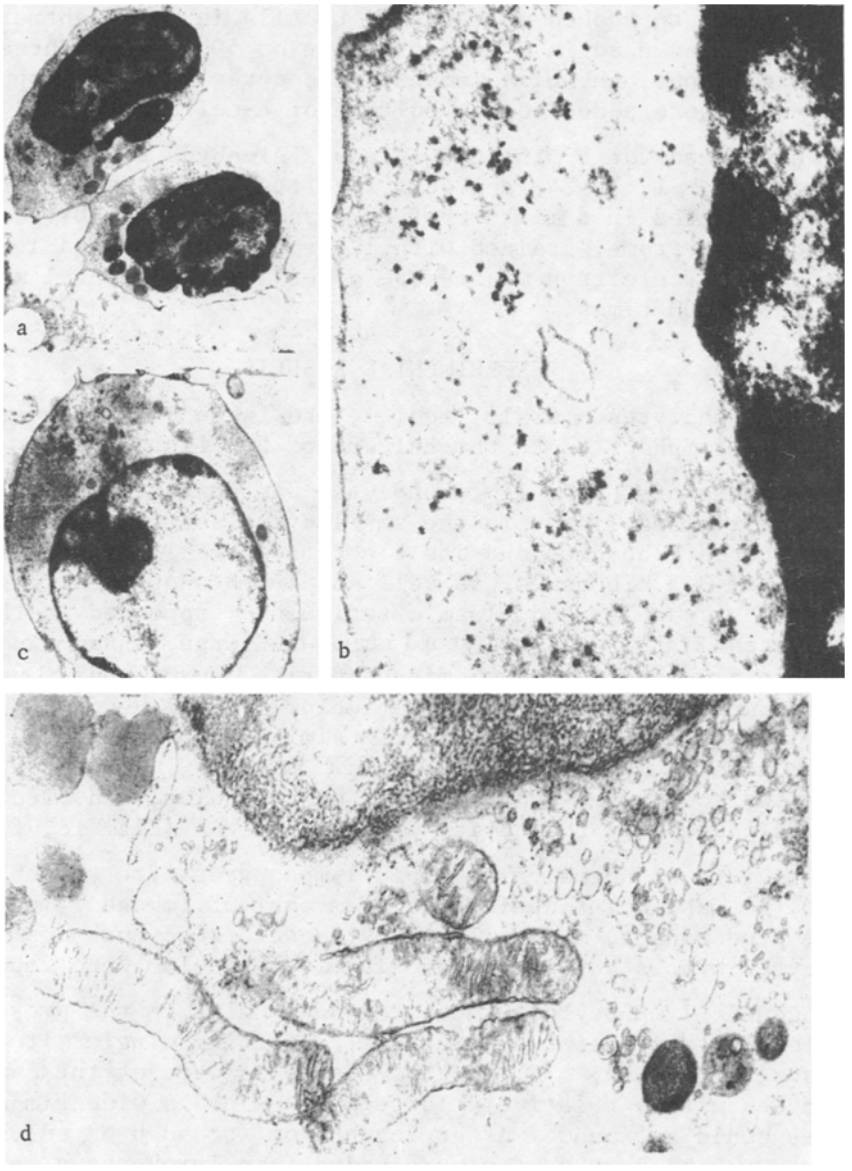

Fig. 2. Ultrastructure of normal lymphocytes incubated with PHA 3 and 48 h after contact with L-cells: a) medium lymphocytes 3 h after contact with target cells, 2500×; b) fragment of previous photograph, single ribosomes and polysomes can be seen in cytoplasm of lymphocyte, 40,000×; c) blast cell, 48 h after contact of lymphocytes with target cells, 2500×; d) area of blast cell, numerous vacuoles, collections of lipids, and mitochondria can be seen in the cytoplasm, 17,000×.

able L-cells, grown in tubes and flasks with coverslips [4], were used as target cells.

Immune lymphocytes were obtained from the regional lymph glands of BALB/c mice 8 days after a single immunization with L-cells.

Normal and immune BALB/c lymphocytes were washed three times, suspended in medium No. 199, and added to washed cultures of L-cells in a concentration of 4×10^6/ml; PHA (PGAM, Difco) was used in a concentration of 50 µg/ml. Three series of experiments were carried out, in which immune lymphocytes, normal lymphocytes, and normal lymphocytes + PHA were added to the culture of L-cells.

The cells were fixed for 5 min with 1% glutaraldehyde solution in phosphate buffer after incubation for 1, 3, 24, and 48 h, and then fixed by Dalton's method [8]. The specimens were mounted in a mixture of Epon and Araldite. Ultrathin sections were cut on the LKB-4880 ultratome, stained with 1% uranyl acetate and lead citrate, and examined in the JEM-100V electron microscope under an instrumental magnification of 5,000, 30,000, and 50,000 times.

EXPERIMENTAL RESULTS

All forms of lymphocytes — small, medium, and large — were observed 3 h after addition of the immune lymphocytes to the culture of L-cells.

The ultrastructure of the small lymphocytes was characterized by the presence of individual mitochondria and lipids in the small cytoplasm, whereas the hyaloplasm contained freely scattered ribosomes and its electron-optical density was high. Polysomes, organized from 3-5 ribosomes, as well as elements of the ergastoplasm — separate cisterns of the granular endoplasmic reticulum — appeared in the cytoplasm of the medium lymphocytes (Fig. 1a). The cytoplasm of the large lymphocytes had an electron-optically dense matrix and contained many mitochondria, lipids, and polysomes. (Fig. 1b, c). As a rule the granular endoplasmic reticulum was well developed in the large lymphocytes (Fig. 1d). In some cells morphologically similar to plasma cells, cisterns of the granular reticulum were arranged in parallel rows (Fig. 1e, f), linear polysomes were localized on the surface of the membranes of the reticulum, and electron-optically dense material was present in the cavity of the cisterns (Fig. 1d, f).

The ultrastructure of normal allogeneic lymphocytes after incubation for 3 h with a culture of L-cells was indistinguishable from that of immune lymphocytes, but small lymphocytes were predominant, medium lymphocytes were seen much less frequently, and no large lymphocytes with a developed endoplasmic reticulum were found.

Normal allogeneic lymphocytes, incubated on L-cells in the presence of PHA, had an electron-optically translucent hyaloplasm, containing single mitochondria, ribosomes, and polysomes (Fig. 2a, b). Most of the cells were of the same size: small (medium) lymphocytes with a relatively large nucleus and a wide rim of cytoplasm; no large lymphocytes could be found. After incubation for 48 h on cultures of L-cells to which immune or normal lymphocytes were added, the lymphocytes were nearly all destroyed. During incubation of normal allogeneic lymphocytes in the presence of PHA, medium and large lymphocytes were observed; their cytoplasm was characterized by low electron-optical density and by the presence of numerous pinocytotic vacuoles and collections of lipids; the endoplasmic reticulum consisted mainly of smooth-outlined vacuoles, whereas the granular endoplasmic reticulum consisted of spherical vacuoles, with polysomes situated on their surface (Fig. 2c, d).

Large pyroninophilic cells and precursors of plasma cells have been described during rejection of a graft and also at the site of application of the sensitizing agent during hypersensitivity of delayed type (HDT) in vivo [10, 12]. Large pyroninophilic cells were found by Chertkov et al. after injecting cells of allogeneic bone marrow into monkeys previously irradiated in a dose of 2000 rad [5]. The present writers previously found an increase in the proportion of medium and large immune lymphocytes 1-3 h after the beginning of their incubation in a culture of target cells

[2, 3]. Large pyroninophilic cells with thin, spongy cytoplasm, containing many polysomes and not deprived of its endoplasmic reticulum have been found on xenogeneic immunization in vitro; they were described by Ginsburg et al. [9]. In the opinion of these workers, these are effector cells, i.e., cells responsible for destruction of the targets in vitro. Activation of immune lymphocytes during contact with antigen, responsible for their effector function during cellular immunity and HDT, is not accompanied by cell division [6, 11]. The ability of immune lymphocytes to undergo blast transformation and to induce a reaction of HDT in vitro on contact with antigen has also been found not always to correlate [7, 12].

The experiments thus showed that immune lymphocytes, and also normal allogeneic lymphocytes, during the first few hours of contact with cellular antigen constitute a heterogeneous population of cells ranging from small lymphocytes with a narrow rim of cytoplasm to large lymphocytes with a developed endoplasmic reticulum. These cells are characterized by the presence of an electron-optically dense matrix, lipids, numerous mitochondria, and ribosomes, which are freely scattered in the cytoplasm of the small lymphocytes, but organized into polysomes in the medium and large lymphocytes, in which areas of ergastoplasm also are found. In some cells cisterns of the granular endoplasmic reticulum are arranged in parallel rows. These cells in structure resemble plasma cells, and their role in the cytotoxic effect remains uncertain. Conversely, lymphocytes treated with PHA during the first few hours of contact are of approximately equal size, with a pale matrix, they have neither mitochondria nor polysomes, and no endoplasmic reticulum can be seen in their cytoplasm. After incubation for 48 h these cells increase in size considerably, but their ultrastructure differs sharply from that of the large lymphocytes observable during the first hours of contact: they have a pale cytoplasm in which polysomes are freely scattered, but no developed granular endoplasmic reticulum.

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