ACID PHOSPHATASE AND SUCCINATE AND DEHYDROOROTATE DEHYDROGENASE ACTIVITY DURING INTERACTION BETWEEN ALLOGENEIC LYMPHOCYTES AND TARGET CELLS IN TISSUE CULTURE

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Investigation of acid phosphatase activity during interaction between lymphocytes and target cells showed that its greatest increase is observed in lymphocytes in 1-h and sometimes 3-h cultures, whereas in the cells it was observed after 3-6 h. Initial acid phosphatase activity was higher in immune lymphocytes, but later, on their addition to a culture of L-cells, no significant difference was found between the change in enzyme activity in immune and normal lymphocytes. Acid phosphatase activity in L-cells was lower on the addition of normal than of immune lymphocytes. Activity of the dehydrogenases in the lymphocytes increased until 3 h of incubation and the increase was greater in immune than in normal lymphocytes. Activity of succinate and dehydroorotate dehydrogenases in the L-cells changed at virtually the same times as in the lymphocytes. Increased activity of oxidoreductases and acid phosphatase in the first few hours of contact is evidence of the rapid activation of effector lymphocytes.

KEY WORDS: immune lymphocytes; target cells; acid phosphatase; dehydrogenases.

Contact in tissue cultures between immune lymphocytes and target cells leads to destruction and death of the latter [14]. Different opinions are held regarding the mechanisms lying at the basis of this reaction [1, 3-6, 10]. Death of target cells must be preceded by definite changes in their metabolism and also in the metabolism of the lymphocytes and by activation of lysosomal enzymes.

The investigation described below was carried out to study these problems.

EXPERIMENTAL METHOD

The system of Rosenau and Moon [14] was used. A transplantable line of L-fibroblasts, obtained by Earle from mice of inbred line C3H (f) was used as the target cells [8]. Immune lymphocytes were obtained from regional lymph glands of mice of inbred line BALB/c 8-15 days after immunization with a single dose of L-cells.

Experiments to study the cytotoxic action of immune lymphocytes on L-cells were carried out by the method described previously [4]. Immune and normal lymphocytes of BALB/c mice were washed 3 times, suspended in medium No. 199, and added to washed cultures of L-cells in a concentration $4 \cdot 10^6$/ml. The cells were fixed after 1, 2, 3, 6, 9, and 24 h with a mixture of alcohol and formalin in the ratio of 1 : 1 for determination of acid phosphatase and with cold acetone for 30 sec for determination of the dehydrogenases. Acid phosphatase (3.1.3.2) was determined by the method of Goldberg and Barka [9], succinate dehydrogenase (1.3.99.1) activity by the method of Quaglino and Hayhoe [13], and dehydroorotate dehydrogenase activity

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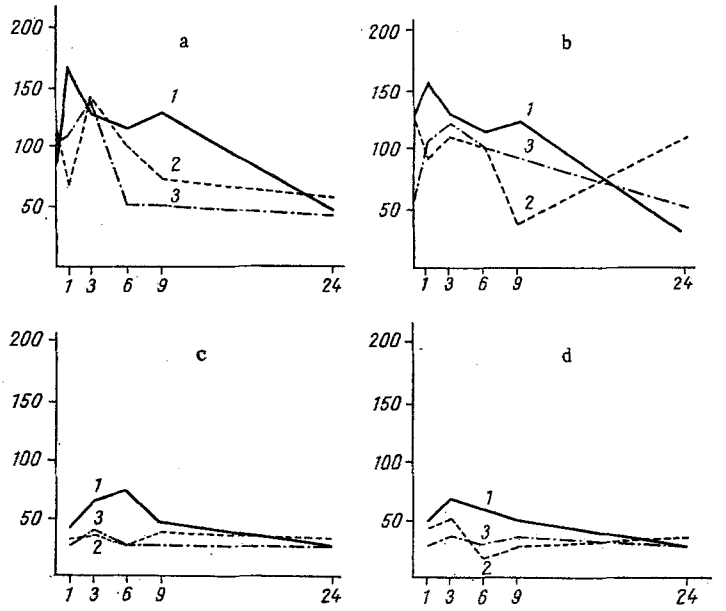

Fig. 1. Activity of acid phosphatase (1) and succinate (2) and dehydroorotate (3) dehydrogenases in immune lymphocytes (a), normal lymphocytes on addition of target cells to the culture (b), and in L-cells during interaction with immune (c) or normal lymphocytes (d). Ordinate, activity of enzymes (in conventional units); abscissa, time after addition of lymphocytes (in h).

by Nartsissov's method [2]. Altogether 100 lymphocytes and 25 L-cells were counted in the preparations and divided into groups depending on the intensity of the reaction (0 – absence, 1 – low, 2 – moderate, 3 – high enzyme activity). The results were expressed by a conventional index as proposed by Karlow [12].

EXPERIMENTAL RESULTS

Acid phosphatase activity in the lymphocytes varied in different experiments. From 20 to 80% of cells with low, high, and moderate enzyme activity were found in the films. As a rule no clearly defined granules could be observed in the lymphocytes but the reaction product was distributed diffusely in most cells. This character of arrangement of the reaction product was also found in the cells at different times of incubation. No differences could be found in the change in acid phosphatase activity in lymphocytes isolated from immune and normal animals. The greatest increase in enzyme activity was found in 1-h cultures (Fig. 1a, b), but in some experiments this maximum was shifted to 3 h. At these same times acid phosphatase activity was found in nearly all cells and, in addition, the number of lymphocytes with moderate and high enzyme activity was increased. Later the activity of the enzyme fell gradually.

Incubation of lymphocytes without L-cells also was accompanied by increased activity of the enzyme, but the increase was less marked than the changes in cultures of lymphocytes with L-cells. No differences could be found in the dynamics of acid phosphatase activity between immune and nonimmune lymphocytes incubated without L-cells.

During determination of acid phosphatase activity in the L-cells, just as in the lymphocytes, the intensity of deposition of the reaction product varied. The dynamics of changes in enzyme activity in the L-cells resembled the course of the increase in acid phosphatase activity in the lymphocytes, but the maxima of activity in the target cells were displaced in time. In the earlier periods acid phosphatase activity in these objects was low or moderately high and the reaction product in the L-cells was distributed either as granules or diffusely. By 3-6 h the enzyme activity was sharply increased; no clearly defined granules could be observed and the distribution of the reaction product gave the impression of lysis of the structures (Fig. 1c). At these stages it was particularly difficult to differentiate acid phosphatase activity in the lymphocytes, for nearly all the lymphocytes were in close contact with L-cells. In the original state activity of the oxidoreductases as a rule was low. Either a low-density diffuse deposition of the enzyme or clearly defined, deeply stained single granules were observed. In 1-h cultures the activity of these enzymes

remained low, and a particularly sharp increase was found after 3 h of incubation. In individual cultures this increase in dehydrogenase activity was not seen until after exposure for 6 h, and later the activity of the oxidative enzymes fell (Fig. 1b). Activity of the oxidoreductases in the L-cells changed at virtually the same times as in the lymphocytes. Deposition of the reaction product in the L-cells in the early stages of incubation, both diffuse and as discrete granules, increased. Individual cells with very high enzyme activity could be observed in these preparations. No discrete granules could be found in such objects and deposition of the enzymes was confluent in character (Fig. 1c).

Analysis of the results showed the following patterns: an increase in acid phosphatase activity during the first hour of incubation in the lymphocytes and during the next 3-6 h in the target cells. This increase in activity in the L-cells may be connected with activation of a latent enzyme which is liberated during destruction of the subcellular structures, or with active synthesis of the enzyme de novo. A previous investigation [5] showed that 3 h after addition of lymphocytes to a culture of target cells fragments of destroyed nuclei of lymphocytes, as well as whole lymphocytes and L-cells vacuolated in their cytoplasm, appeared in the cytoplasm of the target cells, in agreement with observations of other workers [7, 11]. Lymphocytes are adsorbed on the surface of target cells during the first hours of contact. Evidently the transfer of an activator or lymphotoxin can take place at these times [6]. The entry of foreign material evidently leads to destruction of the lysosomes and massive liberation of enzyme. Evidence of this is given by the diffuse distribution of the reaction product and the absence of discrete granules observed in the cytoplasm of the L-cells, suggesting lysis of subcellular structures. By 24 h the acid phosphatase activity in the L-cells was reduced by half, but its total disappearance was not observed. In later stages of incubation, some synthesis of this enzyme de novo possibly takes place.

Activity of oxidoreductases in the L-cells was practically identical after the addition of either immune or normal lymphocytes. It increased toward 3 h of incubation, but by 6 h the activity of the oxidoreductases was below its original level. The decrease in activity of succinate dehydrogenase and dehydroorotate dehydrogenase indicate depression of general metabolism in the target cells.

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