MORPHOLOGICAL CHANGES IN NORMAL LYMPHOCYTES TREATED WITH PHYTOHEMAGGLUTININ AND METHOTREXATE FOLLOWING THEIR INTERACTION WITH TARGET CELLS

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Interaction between normal allogeneic lymphocytes and target cells was studied in the presence of phytohemagglutinin (PHA) and methotrexate. In the presence of PHA increased adsorption of lymphocytes on the surface of the target cells was observed during the first few hours of interaction. Blast cells appeared 48 h after the beginning of contact and their number reached 56% after 120 h. Meanwhile the number of small lymphocytes fell while the number of medium-sized lymphocytes rose. In the presence of methotrexate the degree of adsorption of lymphocytes on the target cells was indistinguishable from normal; however, if added to a culture of immune lymphocytes, large lymphocytes began to appear during the first few hours of interaction.

Interaction between immune lymphocytes and target cells is a model suitable for studying the mechanisms of transplantation immunity in vitro [19]. The cytotoxic action of nonimmune lymphocytes under the influence of various activators has been demonstrated [5, 8-13, 15, 16, 20]. The substance methotrexate [2], an effective immunodepressant and folic acid antimetabolite, has a similar action. Earlier [1], in a morphological study of interaction between immune lymphocytes and target cells, large lymphocytes appeared during the first few hours of contact between the cells.

This paper describes a morphological study of lymphocytes activated by phytohemagglutinin (PHA) and methotrexate during their interaction with allogeneic target cells.

EXPERIMENTAL METHOD

Inbred BALB/c and C57BL/6j mice aged 8-16 weeks were used.

The target cells were embryonic fibroblasts from C57BL/6j mice grown in tubes and flasks with coverslips as described previously [3]. The immune lymphocytes were obtained from the regional lymph glands of BALB/c mice 8 days after immunization with a single injection of spleen cells of C57BL/6j mice. Normal and immune lymphocytes of BALB/c mice were washed three times, suspended in medium No. 199, and injected into washed cultures of target cells in a concentration of $4 \cdot 10^6$ cells/ml as described earlier [4].

PHA (Difco M) was used in a concentration of 50 μg/ml. Methotrexate (Lederle), batch No. 44-419, 149-211, 0.54-291, was used in a concentration of 0.01 μg/ml.

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TABLE 1. Mean Number of BALB/c Lymphocytes (normal) Immunized with C57BL Cells, Treated with Methotrexate and PHA Adsorbed on the Surface of One C57BL Fibroblast

Lymphocytes	Hours after addition of lymphocytes to culture of target cells						
	1	3	6	9	24	48	96
Normal	1	1,1	1,9	3,3	0,8	0,7	—
Immune	4,3	2,7	2,8	1	1,9	1,2	1,8
Normal + methotrexate	2,2	0,7	1,7	4,7	1,9	1,5	—
Normal + PHA	5,4	9,6	8,2	7,7	3	1,7	0,6

Four series of experiments were carried out in which the target cells were treated with: immune lymphocytes, normal lymphocytes, normal lymphocytes and PHA, and normal lymphocytes and methotrexate. Before addition to the culture of target cells films were made from suspensions of immune and normal lymphocytes and also of lymphocytes incubated with PHA or methotrexate for 1 h at 37°C.

The cells were washed with medium No. 199 and fixed by Carnoy's method 1, 3, 6, 9, 24, 48, 72, and 120 h after addition of the lymphocytes (3 flasks at each time) and stained with azure–eosin or with methyl green–pyronine by Kurnick's method [14].

To study the activation of DNA synthesis in the lymphocytes thymidine-H^3 (specific activity 4 Ci/ml) was added to the culture medium simultaneously with the lymphocytes in a concentration of 0.5 mCi/ml. After fixation the preparations were treated with 4% $HClO_4$ solution for 20 min at 4°C, washed 3 times with distilled water, and coated with type M emulsion. Exposure lasted 7 days.

In each preparation from 300 to 1000 cells were counted depending on the number of "living" lymphocytes, which must not be below 200. The mean number of lymphocytes adsorbed on one target cell was determined in 100 target cells. Large lymphocytes and blast cells were combined into one group described as large lymphocytes.

EXPERIMENTAL RESULTS

The numbers of large lymphocytes in the original suspensions was 1.4% among normal lymphocytes, 1% among lymphocytes treated for 1 h with PHA, 1.6% among lymphocytes treated with methotrexate, and 3.6% among immune lymphocytes. During the first few hours of contact the lymphocytes were adsorbed on the surface of the target cells. On the average 9.6 lymphocytes treated with PHA, 0.7 lymphocyte treated with methotrexate, 2.7 immune lymphocytes, and 1.1 normal lymphocytes were adsorbed on one target cell 3 h after combined incubation (Table 1).

The number of large, medium-sized, and small lymphocytes was counted among the "living" lymphocytes only. A high percentage of small lymphocytes was observed in the group of cells treated with PHA in the first 9 h of contact (86-97). The number fell after 24 h to 59-75% and after 48 h to 31-35%. The percentage of medium-sized lymphocytes increased from 1.5-13.4 in the first 9 h of contact to 19-38% after 24 h; after 48 h their number was 42-46.5%, and by 96 h it had fallen to 28-33%. The number of large lymphocytes began to increase after 48 h of contact (18-27%) and reached 36-56% after incubation for 3-4 days (Fig. 1, I and Fig. 2b).

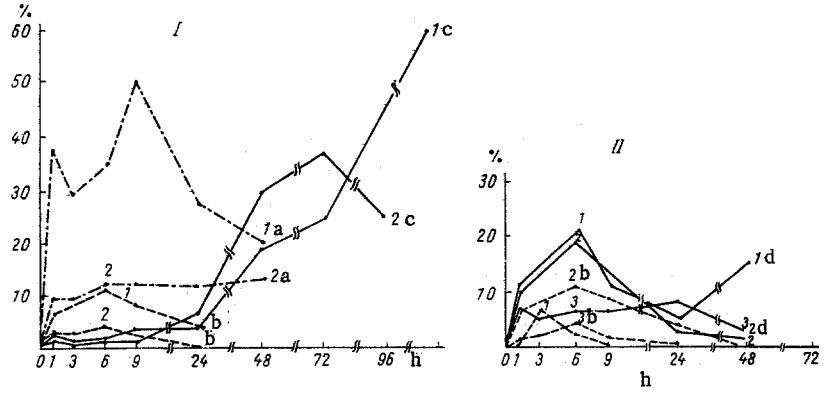

Fig. 1. Proportion of large lymphocytes among lymphocytes of BALB/c mice: a) immunized with C57BL cells; b) normal; and c) treated with PHA (I) and methotrexate (II). Abscissa, time (in h); 1, 2, 3) No. of experiment; ordinate, proportion of cells.

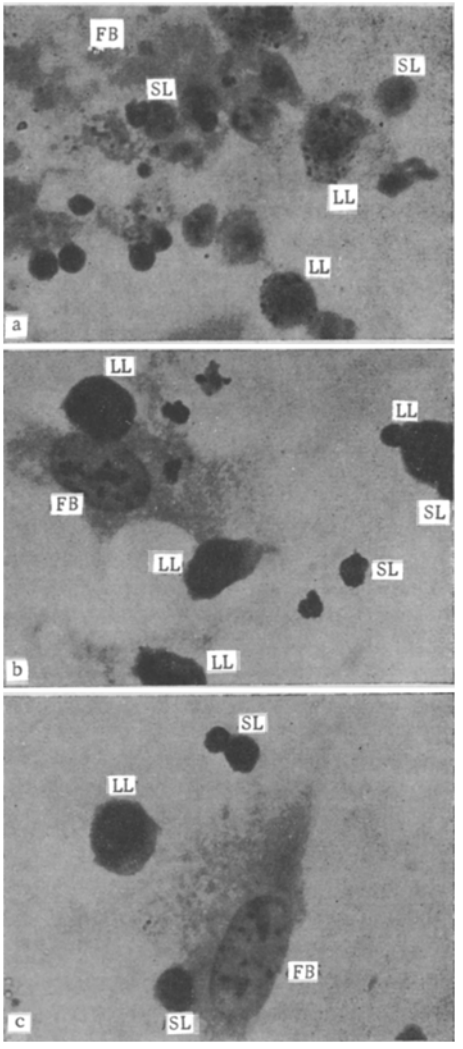

Fig. 2. Morphological and autoradiographic pictures of BALB/c lymphocytes treated with PHA (a and b) and with methotrexate (c), added to the C57BL culture: a and b – 48 h, c – 3 h after beginning of contact. LL) Large lymphocyte; SL) small lymphocyte; FB) fibroblast; 900×.

The number of labeled cells increased toward 24-48 h of incubation and after 72-96 h nearly all the large lymphocytes were labeled (Fig. 2a). Whereas in the experiment with PHA from 1 to 3% of cells were labeled in the first few hours, on the addition of immune lymphocytes to the system from 10 to 12% of labeled lymphocytes were found on the target cells.

Among lymphocytes treated with methotrexate large lymphocytes began to appear during the first few hours of contact (Fig. 1, II and Fig. 2c). Their number after 6 h reached 19-21% and it fell toward the end of the day. The number of medium-sized lymphocytes after incubation for 6 h was 55%, and this also fell toward the end of the day. The number of small lymphocytes gradually increased, and after 48 h it was 70-90%. In the original suspensions the lymphocytes treated with methotrexate did not incorporate thymidine-H^3. During the first few hours of contact with the target cells the number of labeled lymphocytes increased to correspond to the number of large lymphocytes adsorbed on the surface of the target cells and it fell toward the end of the day.

On incubation of the immune lymphocytes with the target cells a marked increase was found in the number of large lymphocytes during the first few hours of contact: 22-36.3% after incubation for 1 h (Fig. 1, I). The number of medium-sized lymphocytes increased toward 24 h of incubation and then fell. The percentage of small lymphocytes fell toward the end of the first day, then gradually increased to 95 after incubation for 96 h. On the addition of normal lymphocytes the percentage of large lymphocytes was low and they could be observed only during the first few hours of contact. In the different experiments the percentage of large lymphocytes 2 h after the beginning of incubation varied from 6 to 0.6. The number of medium-sized lymphocytes at the various times ranged from 20 to 4% (after incubation for 72 h); the percentage of small lymphocytes was not below 70, and 72 h after the beginning of contact it was 96. In the presence of PHA the normal mouse lymphocytes adsorbed on the surface of the target cells were thus converted after 72-120 h into blast cells. The cytotoxic action of lymphocytes on target cells in the presence of PHA has been studied in detail [10-12, 17, 18]. Electron-microscopic investigations of transverse sections through the cells of a monolayer [6, 7] demonstrated intensive adsorption of lymphocytes on target cells and the development of close contact over large areas of the surface of those cells. In the present experiments adsorption of lymphocytes on the surface of target cells was observed during the first few hours of contact; destruction of the monolayer of target cells was observed after 24-48 h and the formation of blast cells after 72-120 h.

The experiments with immune lymphocytes showed that most large lymphocytes are found during the first few hours of contact.

Morphological changes in normal lymphocytes in the presence of methotrexate were similar to the changes in the population of immune lymphocytes, although the original suspension of lymphocytes treated with methotrexate contained only 1.6% of large lymphocytes. The mechanism of action of methotrexate in this system is not clear.

The process of interaction of immune lymphocytes and normal lymphocytes after treatment with PHA or methotrexate with target cells is thus not identical.

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