MORPHOLOGICAL AND AUTORADIOGRAPHIC CHANGES IN IMMUNE LYMPHOCYTES ON REACTING WITH ALLOGENEIC TARGET CELLS

B. D. Brondz, S. N. Sura, and A. E. Snegireva

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The morphological composition of the population of cells in immune lymph glands in contact with the corresponding allogeneic target cells changes during incubation for 6-10 h: the proportion of small lymphocytes decreases while that of medium-sized lymphocytes and blast cells rises; these changes are accompanied by an increase in the proportion of lymphocytes labeled with thymidine-H^3 and in the intensity of incorporation of uridine-H^3. These changes are absent or are much less marked in normal adsorbed lymphocytes and in immune lymphocytes not adsorbed on the corresponding target cells or adsorbed on third-party target cells.

The cytotoxic effect (CE) of immune lymphocytes on allogeneic target cells is not the result of passive contact, but of the active function of the lymphocytes, for which respiration [14], functional activity of the lymphocyte membranes [8], and the synthesis [3, 9] and intact structure of the cell proteins [4] are necessary. However, the behavior and fate of the immunolymphocytes during their interaction with target cells have not been studied. The difficulty in the way of such an investigation is the small proportion (not more than 2%) of active lymphocytes in the population [16]. Recently the "peripolesis" of lymphocytes [8], the penetration of their nucleic acids into the cytoplasm of target cells [5], phagocytosis of fragments of dead lymphocytes by target cells [6], and an increase in the proportion of plasma cells during contact with target cells [15] have been described. The connection between these phenomena and the mechanism of CE remains problematical.

The object of the present investigation was to study morphological changes in normal and immune lymphocytes in the course of their interaction with corresponding and with "third-party" target cells.

EXPERIMENTAL METHOD

Experiments were carried out on mice of inbred lines C57BL/10, B10.D2, and A aged 8-16 weeks.

Macrophages of the peritoneal exudate, obtained by injection of an irritant and seeded two days before the experiment in flat-sided test tubes on cover slips in concentrations of $2 \cdot 10^5$/ml, were used as target cells.

Immune lymphocytes were obtained from the regional lymph glands of B10.D2 mice 8 days after a single immunization with cells of an allogeneic sarcoma MKh11, induced with methylcholanthrene and subcultured in C57BL/10 mice.

Laboratory of Immunochemistry of Cancer, Department of Virology and Immunology of Tumors, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. Laboratory of Virology, Institute of Experimental and Clinical Oncology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 1, pp. 70-74, January, 1972. Original article submitted April 16, 1971.

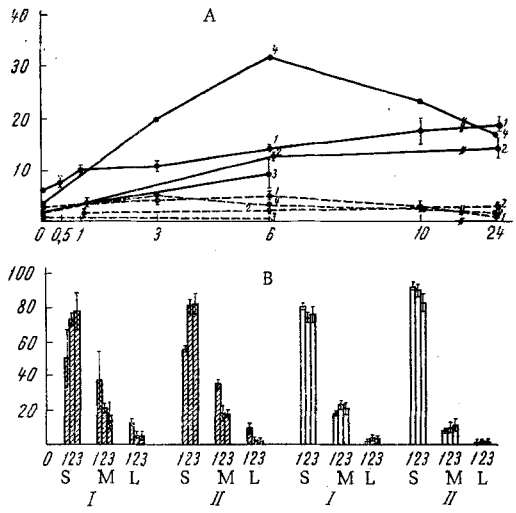

Fig. 1. Changes in proportions of different groups of B10.D2 and B10.D2 anti-C57BL lymphocytes during interaction with target cells. A) C57BL target cells. Continuous line represents immune lymphocytes, broken line normal lymphocytes. 1, 2, 3, 4) Serial Nos. of experiments. Vertical lines show standard deviations. Abscissa, incubation time (in h); ordinate, proportion of blast cells (in %); B) Target cells: C57BL (1), A (2), B10.D2 (3). Incubation for 6 h. Shaded columns represent immune, unshaded columns normal lymphocytes. S – small, M – medium, L – large lymphocytes. I and II) Serial Nos. of experiments. Vertical lines show confidence limits. Ordinate, proportion of lymphocytes (in %).

The experiments to study CE of immune lymphocytes on allogeneic target cells were carried out as described previously [1, 2]. Normal and immune lymphocytes of B10.D2 mice were washed three times, suspended in medium 199, and injected into washed cultures of target cells in concentrations of $5 \cdot 10^6$–$10 \cdot 10^6$/ml. From 30 min to 24 h later the cover slips (3 at each time) and films of suspension of lymphocytes were washed three times with medium 199, fixed for 20 min with methanol, and stained with azure-eosin. The preparations were examined under a magnification of 900×, and in each film 600–1000 "living" lymphocytes (i.e., with normal structure) and the corresponding number of target cells were counted. The lymphocytes were subdivided into small, medium, and large, the large lymphocytes and blast cells being combined into one group.

To study synthesis of RNA or DNA in the lymphocytes, uridine-H^3 (Radiochemical Center, Amersham, England; 460 mCi/mmole) in a concentration of 3.5 μCi/ml or thymidine-H^3 (from the same firm, 2.7 Ci/mmole) respectively was added to the culture medium. The thymidine-H^3 concentration fell from 6 to 1 μCi/ml as the incubation period lengthened from 1 to 24 h. A 1:1 mixture of Eagle's solution and 0.5% lactalbumin hydrolyzate solution was used as culture medium in the experiments with uridine-H^3, and medium 199 in the experiments with thymidine-H^3. Uridine-H^3 was added 1 h before fixation of the specimens, and thymidine-H^3 was added at the same time as the lymphocytes. At different times after the beginning of incubation the preparations were washed as described above, fixed in Carnoy's fluid, treated with 4% $HClO_4$ solution for 20 min at 4°C, washed three times with distilled water, and coated with type M (NIKFI) emulsion. After exposure for 7 days and staining with methyl green-pyronine, at least 200 "living" lymphocytes (i.e., lymphocytes with a green nucleus and pink cytoplasm) were counted in each preparation, the total number being made up of 80–150 small and 40–60 medium lymphocytes and 15–25 blast cells.

EXPERIMENTAL RESULTS

The proportion of blast cells in the original suspensions varied from 2 to 6% among the immune lymphocytes and 0.9–3% among the normal lymphocytes in different experiments.

In the first few hours after the beginning of incubation immune and normal lymphocytes were intensively and equally adsorbed on the target cells; after 10 h the difference was appreciable; and after 24 h it was highly significant: the number of adsorbed normal lymphocytes was considerably reduced. In the course of incubation with the corresponding target cells significant changes took place in the population of immune lymphocytes adsorbed on the cover slip: the proportion of small lymphocytes fell gradually from 53–72% after 1 h of incubation to 24–38% after 24 h; the proportion of medium lymphocytes increased from 20–37% after 1 h to 48–62% after 24 h; and the proportion of blast cells increased to 9–13% after 6 h (in one experiment to 31%) and reached 14–21% 24 h after the beginning of incubation. Conversely, in the population of normal lymphocytes no significant changes took place on incubation with target cells (Fig. 1A). The mean number of blast cells adsorbed on one target cell increased during incubation of the immune lymphocytes from 1 to 10 h from 0.3 to 0.6 respectively. This index in normal lymphocytes did not exceed 0.14.

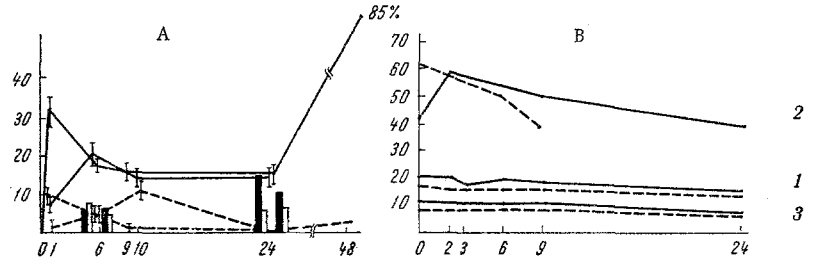

Fig. 2. Incorporation of thymidine-H^3 (a) and uridine-H^3 (b) into B10.D2 and B10.D2 anti-C57BL lymphocytes during interaction with C57BL target cells (curves) and A target cells (columns). Abscissa, time of incubation (in h); ordinate: A) proportion of labeled lymphocytes (in %), B) mean label per labeled lymphocyte. Continuous line and shaded columns show immune lymphocytes, broken line and unshaded columns normal lymphocytes. 1) Large; 2) medium; 3) small lymphocytes. Vertical lines indicate standard deviation.

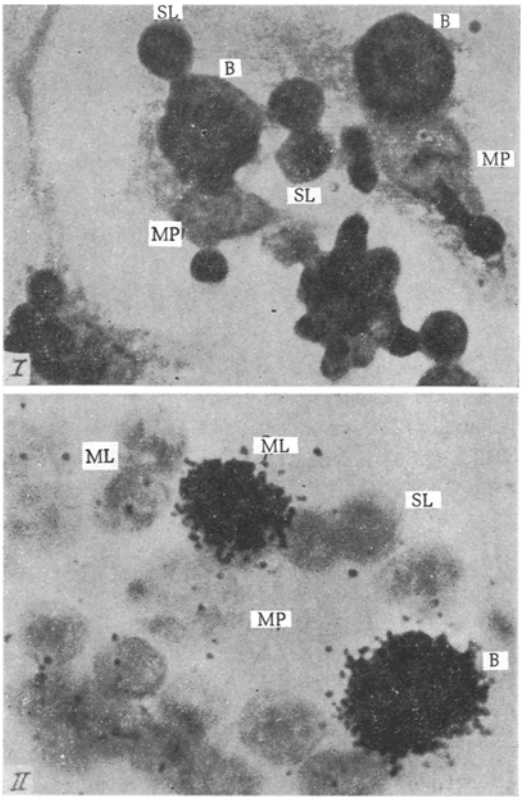

Fig. 3. Morphological (I) and autoradiographic (II) pictures of B10.D2 anti-C57BL lymphocytes incubated with C57BL macrophages for 3 h. MP) macrophages; B) blast cells; SL) small lymphocytes; ML) medium lymphocytes; stains: I) azure-eosin; II) thymidine-H^3; methyl green-pyronine, 900×.

The changes in the relative proportions of the cells described above were absent in the same experiments in films of immune lymphocytes not adsorbed on C57BL target cells, but "free-floating" in the culture medium. These changes likewise did not occur during incubation of immune lymphocytes with syngeneic target cells of B10.D2 mice or with "third-party" allogeneic target cells of line A mice (Fig.1B), on which the B10.D2 anti-C57BL lymphocytes have no cytotoxic effect [2].

The proportion of immune lymphocytes labeled with thymidine-H^3 increased to 20-30% 1-6 h after the beginning of incubation with C57BL target cells and it fell to 13-14% after 24 h. The proportion of labeled normal lymphocytes incubated with the same target cells and of immune lymphocytes incubated with A target cells did not exceed 10%, although in one case it rose to 11-13% after 24 h (Fig. 2A).

On the addition of uridine-H^3 to the medium label was found in 95-98% of living lymphocytes. During the first 6 h of incubation the mean label per labeled lymphocyte was much higher in the immune lymphocytes incubated with the corresponding target cells than in normal or immune lymphocytes incubated with "third-party" target cells. Determination of the mean label (in the experiment with uridine) and the proportion of labeled cells (in the experiments with thymidine) in groups of small, medium, and large lymphocytes separately showed no significant differences between the immune and normal lymphocytes incubated with target cells (Fig. 2b). This means that the difference in the dynamics of DNA and RNA synthesis between the immune and normal lymphocytes on contact with target cells was due, not to changes in synthesis within individual morphological groups of lymphocytes, but to the quantitative changes between the populations themselves as described above.

The morphological and autoradiographic pictures are illustrated in Fig. 3.

During the first 6-10 h of contact between immune lymphocytes and target cells the morphological composition of the population thus changed: the proportion of small lymphocytes was reduced while the proportions of medium lymphocytes and blast cells increased, and these changes were accompanied by an increase in the synthesis of DNA and RNA in the population of lymphocytes as a whole. These changes were immunologically specific and were detected only in the presence of CE. They were absent or were much less conspicuous in immune lymphocytes not absorbed on the corresponding target cells or adsorbed on syngeneic or "third-party" allogeneic target cells. It must be supposed that the increase in the proportion of "activated" lymphocytes is essential for CE, which may be connected with the synthesis of protein, destroying the target cells, in those lymphocytes [11]. This hypothesis is in agreement with results indicating the absence of CE after destruction of blast cells by L-asparaginase [7] in experiments in which rat lymphocytes acted in vitro on mouse target cells.

The increase in the proportion of medium lymphocytes and blast cells during contact with target cells could be due either to selective absorption of these cells from the original suspension or to blast-transformation of small lymphocytes responsible for the CE [13, 16]. This problem requires further study.

Since activation of DNA synthesis in immune lymphocytes does not occur even under optimal conditions before 48 h of incubation with the corresponding soluble antigen [10], membrane antigens of living target cells can be regarded as a more powerful inducer of blast-transformation than soluble or corpuscular antigens isolated from living cells [12].

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LITERATURE CITED

1. B. D. Brondz, Folia Biol. (Prague), 10, 164 (1964).
2. B. D. Brondz, Folia Biol. (Prague), 14, 115 (1968).
3. B. D. Brondz and E. V. Sidorova, Biokhimiya, 34, 1168 (1969).
4. B. D. Brondz, A. E. Snegireva, and Yu. A. Rassulin, Probl. Gematol., No. 4, 11 (1971).
5. S. N. Sura, I. Yu. Chernyakhovskaya, Z. G. Kadagidze, et al., Exp. Cell Res., 48, 656 (1967).
6. S. N. Sura, A. F. Bykhovskii, Z. G. Kadagidze, et al., Byull. Éksperim. Biol. i Med., No. 9, 98 (1960).
7. M. C. Berenbaum, H. Ginsburg, and D. M. Gilbert, Nature, 227, 1147 (1970).
8. P. Biberfeld, G. Holm, and P. Perlmann, Exp. Cell Res., 52, 672 (1968).
9. K. T. Brunner, J. Mauel, J. C. Cerottini, et al., Immunology, 14, 181 (1968).
10. D. G. Chalmers, E. H. Cooper, C. Evans, et al., Internat. Arch. Allergy, 32, 117 (1967).
11. D. C. Dumonde, L. A. Wolstencroft, G. S. Panayi, et al., Nature, 224, 38 (1969).
12. D. A. Hardy and N. R. Ling, Nature, 221, 545 (1969).
13. D. S. Janovsky, W. Rosenau, and H. D. Moon, Proc. Soc. Exp. Biol. (New York), 115, 77 (1964).
14. D. Perlmann, H. Perlmann, J. Wasserman, et al., Internat. Arch. Allergy, 38, 204 (1970).
15. L. Weiss, J. Immunol., 101, 1346 (1968).
16. D. B. Wilson, J. Exp. Med., 122, 143 (1965).