SELECTIVE ADSORPTION OF IMMUNE DNA-SYNTHESIZING LYMPHOCYTES ON CORRESPONDING TARGET CELLS

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UDC 612.112.94.017.1

Lymphocytes of inbred lines of mice immunized with an allogeneic tumor were labeled with thymidine-H^3 in vitro and in vivo, washed, and incubated with target cells in the presence of "cold" thymidine. Rapid and specific adsorption of the fraction of medium and small lymphocytes, synthesizing DNA, on the corresponding target cells was demonstrated by autoradiography. After preliminary incubation of unlabeled immune lymphocytes with target cells for 2 h, followed by the addition of thymidine-H^3 to the culture medium at various time intervals, the percentage of labeled small and medium lymphocytes adsorbed on the target cells fell, to approach its initial level after contact for 8 h. It is postulated that the small and medium lymphocytes synthesizing DNA are "killers" that are not transformed into blast cells on contact with target cells.

KEY WORDS: immune lymphocytes; target cells; DNA synthesis; selective adsorption.

Ignorance of the pathways of differentiation of effector lymphocytes from their precursors in the course of the reaction to an allograft is due to lack of study of the characteristic features of "killer" T-cells, in order to enable them to be distinguished from the remaining lymphocyte population. Immune small lymphocytes of rats, detectable by accelerated blast transformation in a mixed unidirectional culture, are generated during the first 4 h after transplantation, as is shown by their incorporation of thymidine-H^3 injected in vivo [11]. Among immune lymphocytes adsorbed on the corresponding target cells (TC) there is an immunologically specific increase in the proportion of cells synthesizing DNA compared with the initial population of immune lymphocytes [2].

In order to identify the effector fraction of lymphocytes, a further study was made of the adsorption of lymphocytes labeled with thymidine-H^3 on TC and of the changes in proportion of such lymphocytes in the course of their contact with TC.

EXPERIMENTAL METHOD

Mice of inbred lines CC57BR and B10.D2 age 8-16 weeks, reared in the nursery of the N. F. Gamaleya Institute of Epidemiology and Microbiology, were used. Peritoneal macrophages obtained by means of an irritant and seeded 2 days before the experiment in Leighton's tubes on coverslips in a concentration of $2.5 \cdot 10^5$/ml were used as the TC.

Immune lymphocytes were obtained from the regional lymph glands of CC57BR mice 8 days after immunization with a single dose of cells from a trypsinized MCh26 sarcoma [1], induced in B10.D2 mice with methylcholanthrene and maintained by regular subculture. After being washed 3 times the lymphocytes were suspended in medium No. 199 in a concentration of $20 \cdot 10^6$ cells/ml and incubated with thymidine-H^3 (1 μCi/ml) for 2 h at 37° C in a waterbath with periodic shaking. For incorporation of the label in vivo, thymidine-

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TABLE 1. Change in Proportions of Morphological Groups of CC57BR Anti-B10.D2 Lymphocytes, Previously Labeled with Thymidine-H^3, during Incubation with Target Cells

Incorporation of thymidine-H^3	Lymphocytes	Proportion of labeled lymphocytes† (in %)									
		after incubation with target cells*		After incubation with target cells							
				B10·D2				CC57BR			
				1 h		9 h		1 h		9 h	
In vitro ‡	Small	8/701†	1,14	65/1230†	5,3	115/1433†	8,0	39/1678†	2,3	81/1350†	6,0
	Medium	70/257	29,1	269/484	55,8	503/772	65,5	108/510	21,2	68/245	27,8
	Large	40/46	87,0	76/86	87,7	88/95	93,1	11/12	91,7	3/5	60,0
	Total	124/1000	12,4	410/1800	23,1	76/2300	29,7	158/2200	7,1	152/1600	9,2
In vivo	Small	8/737	1,1	—		174/1664	10,5	—		104/1763	6,0
	Medium	101/221	45,7	—		393/598	65,8	—		146/419	34,0
	Large	40/46	87,0	—		126/138	92,1	—		11/19	57,8
	Total	149/1000	14,9	—		693/2400	28,9	—		261/2200	11,9

* Films of lymphocyte suspensions treated with thymidine-H^3 and washed.
† Ratio between number of labeled and total number of cells of that particular morphological group.
‡ Incubation for 2 h at 37° C.

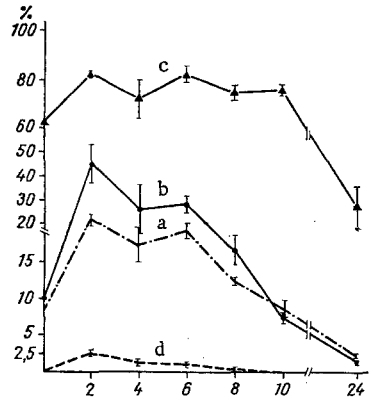

Fig. 1. Proportion of CC57BR anti-B10;D2 lymphocytes adsorbed on B10.D2 target cells for 2 h and labeled with thymidine-H^3, added to medium 2 h before fixation of preparation. Abscissa, time of incubation of lymphocytes with target cells (in h); ordinate, fraction of labeled cells among small (a), medium (b), large (c), and all (d) lymphocytes. Vertical lines indicate twice the standard deviation.

H^3 was injected intraperitoneally into the mice in a dose of 1 μCi/g body weight, 4 times at intervals of 3 h, and the lymphocytes were removed 30 min after the last injection. Cells treated with thymidine-H^3 were washed 3 times, suspended in medium with "cold" thymidine (50 μg/ml), and added in a concentration of $5 \cdot 10^6$ ml to washed cultures of B10.D2 (experimental) and CC57BR (control) cultures of TC, three parallel cultures for each sample of lymphocytes. The preparations were washed after incubation for various times, fixed with methanol, treated for autoradiography, and exposed as described previously [2]. Preparations and films were stained by Giemsa's method and from 600 to 1000 lymphocytes were counted in each. Besides morphological criteria, to differentiate the lymphocytes the diameter of the nuclei was measured: under 7 μ – small lymphocytes; 7-10 μ – medium; over 10 μ – large lymphocytes.

EXPERIMENTAL RESULTS

In the initial suspensions 12-15% of lymph-gland cells were labeled with thymidine-H^3, including 87% of large, 29% (in vitro) or 46% (in vivo) of medium, and about 1% of small lymphocytes; treatment with thymidine-H^3 in vitro and in vivo gave similar results (Table 1). After incubation for 1 h with TC the percentage of labeled cells in contact with the corresponding TC (B10.D2) was doubled, on account of labeled small lymphocytes (a proportion of which was increased five-fold) and medium lymphocytes (doubled). This effect was not present after incubation of the same immune lymphocytes for 1 h with syngeneic TC (CC57BR). After incubation for 9 h the proportion of labeled small and medium lymphocytes was even greater in the specific system, but less so after contact with syngeneic TC. Conversely, the proportion of labeled large lymphocytes (87-93%) showed no significant change in the course of incubation, but fell to 58-60% during contact for 9 h with syngeneic TC (Table 1). The fraction of small and medium lymphocytes, synthesizing DNA, was thus selectively adsorbed on the corresponding TC during the first hour of contact.

To study the subsequent fate of these cells, after the incubation of unlabeled immune lymphocytes with B10.D2 TC, the medium together with nonadherent lymphocytes was removed after incubation for 2 h, the cultures were washed, and at various times of incubation of the lymphocytes with TC (0, 2, 4, 6, 8, and 22 h) thymidine-H^3 (1 Ci/ml) was added to the medium, and 2 h later the cultures were washed 3 times and treated as described above. The ability of immune lymphocytes, adsorbed on TC for 2 h, to synthesize DNA during the same time interval (2 h), chosen at different periods of incubation (from 0 to 24 h) with TC, was thereby studied (Fig. 1).

In the initial suspension during incubation for 2 h without TC 8.2% of cells were labeled, including a fraction of a percent of small lymphocytes, 10.6% of medium, and 61.1% of large lymphocytes. After incubation for 2 h with TC the proportion of labeled lymphocytes adsorbed on TC was 2.5 times greater than initially, on account of medium (an increase of 4.5 times) and small lymphocytes (an increase of 10 times). At later periods of incubation with TC the percentage of labeled medium and small lymphocytes fell, so that after 8 h these values were indistinguishable from initially. The proportion of labeled large lymphocytes was reduced (to 25%) only in the late stages of incubation with thymidine-H^3 (between 22 and 24 h). During further incubation with TC of that fraction of immune lymphocytes that was adsorbed on TC during the first 2 h, no increase in DNA synthesis was observed in the cells of any category; conversely, the fraction of labeled lymphocytes decreased.

Selective accumulation of blast cells during incubation of immune lymphocytes with TC [2] is presumably the result not of blast-transformation, but of selective adsorption of blast forms on TC. Since a fraction of small and medium lymphocytes synthesizing DNA and adsorbed rapidly on the corresponding TC was found in the suspension of immune lymphocytes, the possibility cannot be ruled out that the cytotoxic effect was due to these cells. It does not follow from this that a certain fraction of effector lymphocytes, varying with the period of immunization, may not belong to the blast cells [4, 7, 8]. However, removal of all the blast cells from the suspension by centrifugation in a density gradient does not abolish the cytotoxic

effect of the lymphocytes [3]. Since induction and realization of cellular immunity are the functions of two different subpopulations of T-lymphocytes [9], between which synergic interaction exists [5, 6, 9, 10], it is now an urgent problem to study the need for proliferation of each of them for the production of effector lymphocytes and also the possibility of the change from one into the other in the course of the reaction of graft antigens.

The authors are grateful to Professor A. Ya. Fredenstein and Dr. E. A. Luria for useful advice and to G. N. Vornakova for expert technical help. The work was partly subsidized by the World Health Organization.

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