ANTIGENIC STRUCTURE OF PHYTOHEMAGGLUTININ-STIMULATED MOUSE LYMPHOCYTES STUDIED WITH THE HELP OF SPECIFIC ANTISERUM

V. G. Nesterenko and L. V. Koval'chuk

UDC 612.112.94.017.1+615.365.018.53.012.6

Experiments with antiserum against intact and phytohemagglutinin (PHA)-transformed lymphocytes showed that a new antigenic determinant (or determinats), not present on the surface of intact lymphocytes or those stimulated briefly (2 h) by PHA, appears on the surface of mouse lymphocytes subjected to prolonged (68 h) PHA stimulation. Meanwhile on the surface of lymphocytes stimulated for a long time by PHA there is a decrease in the number (or density) of antigenic determinants present on the surface of intact lymphocytes.

KEY WORDS: phytohemagglutinin; lymphocyte; antigenic determinant.

There is evidence of a change in the antigenic composition of cells during division [6]. Lymphocytes activated by phytohemagglutinin (PHA) have been shown to stimulate intact syngeneic lymphocytes, and the phenomenon is not connected with the liberation of a blastogenic factor [7].

The object of this investigation was to study the antigenic composition of mouse lymphocytes stimulated with PHA with the aid of antilymphocytic sera. In particular, an attempt was made to obtain a specific serum directed only against PHA-stimulated lymphocytes.

EXPERIMENTAL METHOD

Lymph nodes (cervical, axillary, inguinal) cells of male and female CBA and (CBA × C57BL/6) F_1 mice weighing 20-22 g were cultivated in vitro in the presence of PHA for either 2 or 68 h at 37°C. The method of obtaining the cells, of cultivation, and of morphological assessment of blast transformation of the lymphocytes was described previously [2]. A minor modification consisted of replacement of the human serum by 20% embryonic calf serum. To study the antigenic characteristics of the lymphocytes two types of sera were used: antilymphocytic (ALS) and antiserum against PHA-transformed lymphocytes (ATLS). The ALS were obtained at the Moscow Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR, by immunization of rabbits with two doses of living lymph node cells followed by absorption of the serum with mouse erythrocytes and blood serum [1]. The principle of obtaining the ATLS was that the animals were immunized with materials containing a large number of blast cells and activated lymphocytes, and the serum thus obtained was then absorbed with intact, unstimulated lymphocytes. To obtain the ATLS, chinchilla rabbits were immunized three times intravenously (with intervals of 20 days between immunization) with twice-washed lymph node cells from male CBA mice, cultivated for 68 h with PHA (10^8 living cells per immunization). The rabbits were exsanguinated 7 days after the last immunization. The resulting serum was inactivated at 56°C for 30 min and treated by mass multistage absorption with mouse erythrocytes, liver homogenate, and serum, and also with intact spleen, thymus, and lymph node cells of noninbred mice and of (CBA × C57BL/6) F_1 hybrid mice (to remove antibodies against intact lymphocytes from the original serum). The absorption was continued until all activity of the sera in the hemagglutination test with mouse erythrocytes, in the precipitation test in gel with the serum of intact mice, and in the cytotoxic test with intact lymphocytes had completely disappeared. Three batches of ATLS were obtained. The globulin fraction was isolated from the ALS and ATLS by precipitation with ammonium sulfate at 40% saturation. Activity of the antisera against various intact hematopoietic cells

Department of Immunology, Medico-Biological Faculty, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 7, pp. 836-838, July, 1976. Original article submitted September 2, 1975.

TABLE 1. Cell Composition of Cultivated Lymph Node Cells (in %)

Time of cultivation	Small lymphocytes	Activated lymphocytes	Blasts	Cells transformed into macrophages	Macrophages	Mitoses	Undifferentiated
—	96,7±0,9	0,8±0,2	0,5±0,3	0,2±0,1	0,1±0,1	0	1,7±0,3
2*	92,9±0,8	1,3±0,2	0,8±0,1	1,5±0,3	2,0±0,3	0	1,5±0,3
2	93,0±1,1	1,1±0,1	0,6±0,2	1,8±0,2	1,9±0,1	0	1,8±0,4
68*	19,9±3,2	32,1±4,9	30±3,8	4,2±1,9	6,9±1,7	3,1±0,4	3,8±1,2
68	83±1,4	3,3±0,4	1,8±0,3	3,9±0,7	6,1±1,2	0	2,2±0,5

Cultivation with PHA.

TABLE 2. Cytotoxic Activity of Antisera

Antiserum	Material used		Intact lymphocytes		Lymphocytes cultivated with PHA			
					2 h		68 h	
	to immunize donors	to absorb serum	CTI	CTT	CTI*	CTT	CTI*	CTT
ALS†	lymph node lymphocytes	erythrocytes, blood serum	100	1:4096	100	1:2048	100	1:128
ATLS, batch 1	lymph node cells cultivated for 68 h in presence of PHA	erythrocytes, blood serum, liver homogenate, intact spleen, thymus, and lymph node cells	0	0	0	0	38	1:4
ATLS, batch 2			x	0	0	0	45	1:8
ATLS, batch 3†			0	0	0	0	74	1:32

*Dilution of antiserum 1:4.

† Globulin fraction was used.

and similar cells cultivated with PHA was determined by the cytotoxic test [5]. The antiserum was characterized by a cytotoxic index (CTI) and cytotoxic titer (CTT). $CTI=[(a-b)/(100-b)]\cdot 100\%$ [5], where a is the percentage of killed cells in the experimental series and b the percentage of killed cells in the control; CTP is the maximal dilution of antiserum causing death of twice as many cells as die during incubation with complement [4]. The results were subjected to statistical analysis by Lord's method and confidence limits were determined with a probability of 0.95 [3].

EXPERIMENTAL RESULTS

The cytological composition of the cell suspensions (lymph nodes) used for immunization of the rabbits and as test objects in the cytotoxic test is given in Table 1. Clearly the cell composition varied in the different groups.

For instance, the number of stimulated cells exceeded 60% in the case of combined cultivation with PHA for 68 h (only 5% of stimulated cells after cultivation for 68 h without PHA). The cell composition of the intact suspension was virtually the same as that of the suspension cultivated for 2 h (with or without PHA).

Data for the cytotoxic action of antisera on the various cell suspensions are given in Table 2 and Fig. 1. The ALS had a broad spectrum of cytotoxic action and, in optimal dilutions, it killed all the cells, whether intact or cultivated (CTI=100%). Under these circumstances the intact lymphocytes were more sensitive to the action of ALS than cells cultivated for 68 h. In a dilution of 1:256-1:512, for instance, 90% of the intact but only 25% of the cultivated cells died. The ALS titer in the test with intact lymphocytes was 1:4096 and in the test with cultivated lymphocytes 1:128.

ATLS had a cytotoxic action only against cells cultivated for 68 h in the presence of PHA, among which transformed lymphocytes accounted for over 60%. In the test with these cells, the cytotoxic index for individual batches of ATLS varied from 38 to 74% and the titer from 1:4 to 1:32. The ATLS had no toxicity against intact lymph node cells from male and female CBA and (CBA × C57BL/6) F_1 mice (CTI and CTT both 0) and also against bone marrow, thymus, and spleen cells.

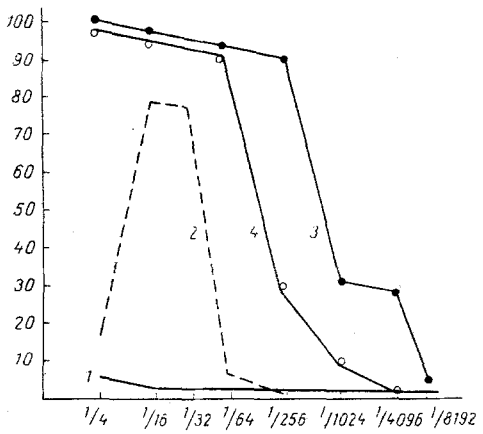

Fig. 1. Titration of antisera with intact lymph node cells from CBA mice and with similar cells cultivated for 68 h with PHA. Abscissa, dilution of antiserum; ordinate, cytotoxic index (in %). 1) ATLS, intact cells; 2) ATLS, PHA-stimulated cells; 3) ALS, intact cells; 4) ALS, PHA-stimulated cells. Globulin fractions of antisera used (protein concentration in ATLS 57.5 mg/ml, in ALS 47.5 mg/ml).

Lymphocytes incubated for 2 h in the presence of PHA were similar in their antigenic composition to intact, uncultivated lymphocytes in these tests. They showed high sensitivity to the cytotoxic action of ALS but were insensitive to the action of ATLS.

It can be concluded from these results that there was a change in the antigenic structure of the stimulated lymphocytes. For instance, a new antigenic determinant (or determinants) absent from the surface of intact lymphocytes appeared on the surface of mouse lymphocytes during prolonged stimulation by PHA (68 h). The appearance of this antigenic determinant made the cells sensitive to the action of ATLS. Meanwhile, the number (or density) of antigenic determinants present on the intact lymphocytes was much smaller on lymphocytes stimulated for a long time by PHA, so that the cultivated lymphocytes were less sensitive to the action of ALS. These changes were equally well marked on lymphocytes of male and female CBA and (CBA × C57BL/6)F_1 mice and they appeared only during prolonged stimulation of the lymphocytes with PHA. The nature and importance of changes in the antigenic composition of lymphocytes during stimulation are not yet known.

LITERATURE CITED

1. N. A. Kraskina, O. V. Kotel'nikova, A. P. Alliluev, et al., in: Transplantation of Organs and Tissues [in Russian], Gor'kii (1970), pp. 113-114.
2. V. G. Nesterenko and L. V. Koval'chuk, in: Immunodepression During Allografting [in Russian], No. 3, Tashkent (1975), pp. 62-63.
3. G. W. Snedecor, Statistical Methods Applied to Sesearch in Biology and Medicine [Russian translation], Moscow (1961).
4. M. Carraz and J. Traeger, Rev. Inst. Pasteur Lyon, 1, 17 (1967).
5. M. Schlesinger and I. Yron, J. Immunol., 104, 798 (1970).
6. D. B. Thomas and B. Phillips, J. Exp. Med., 138, 64 (1973).
7. M. E. Weksler, J. Exp. Med., 137, 799 (1973).