DIFFERENCE IN THE ABILITY OF HLA SERUM TO REACT WITH T AND B LYMPHOCYTE POPULATIONS

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UDC 612.112.94.017.1

The ability of HLA sera to react with T and B lymphocytes of human blood was studied. The lymphocytes were separated by removal of one of the cell populations. The method of rosette formation was used, followed by centrifugation in a density gradient and adsorption of B lymphocytes on synthetic fiber. After removal of the B cells the cytotoxic activity of the HLA sera was reduced. Removal of T lymphocytes did not affect the result of the lymphocytotoxic test. It is postulated that B lymphocytes contain more determinants of HLA antigens than T lymphocytes.

KEY WORDS: HLA antigens; T and B lymphocytes.

The lymphocytes of animals and man constitute a heterogeneous population [1, 4, 7]. The T and B subpopulations of lymphocytes differ in several characteristic immunological features: ability to undergo blast transformation [12, 20] and rosette formation [13, 15], and the presence of immunoglobulins on their surface [5, 6, 11, 17].

The object of this investigation was to study the ability of human T and B lymphocytes to react with anti-HLA sera.

EXPERIMENTAL METHOD

Lymphocytes of healthy blood donors were studied. The main method used for the investigation was the lymphocytotoxic test with anti-HLA test sera [15, 19]. Lymphocytes were obtained from defibrinated blood or from blood taken into heparin by allowing it to stand with high-molecular-weight dextran and centrifuging the suspension of cells of the supernatant fluid in a density gradient consisting of a mixture of 10 parts of a 33.9% solution of verografin (amidotrizoate) with 24 parts of a 9% solution of ficoll, made up in distilled water [10, 14].

Subsequent fractionation of the cells was carried out by the removal of one of the subpopulations of lymphocytes. The T lymphocytes were removed by rosette formation with sheep's red cells followed by centrifugation in a density gradient solution at 230g [13]. The same principle of gradient centrifugation was used to remove the B lymphocytes, which formed rosettes with allogeneic Rh^+ red cells sensitized with incomplete Rhesus antibodies [2,3,9].*

The method of removal of B lymphocytes from a suspension of leukocytes based on their ability to be adsorbed on synthetic fiber [13] also was used. For this purpose, 10 ml of the cell suspension was incubated with 1 g polyacrylonitrile fiber for 45 min at 37°C. By the use of these methods, the number of lymphocytes of the T and B populations could be reduced separately by seven to ten times.

The number of lymphocytes of each population was estimated from the number of rosettes with sheep and allogeneic red cells, counted per 1000 lymphocytes.

*The writers are grateful to S. I. Donskov, of the Laboratory of Immunohematology, for helping with the fractionation of the lymphocyte populations.

Laboratory of Immunohematology, Central Institute of Hematology and Blood Transfusion, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Kosyakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 6, pp. 698-699, June, 1976. Original article submitted May 20, 1975.

TABLE 1. Cytotoxic Activity (in % of dead cells) of Anti-HLA Sera with Respect to Lymphocytes Deprived of Their T or B Subpopulations

Serum	Dilutions of serum	Specificity	Donor of lymphocytes	Suspension of lymphocytes			Methods used to separate T and B lymphocytes
				initial	deprived of T lymphocytes	deprived of B lymphocytes	
M-v	1:2	HLA1+	V-va	38	56	20	Rosette formation followed by centrifugation in density gradient
	1:4	HL-A9		22	53	17	
	1:8			16	53	20	
	1:16			6	50	20	
	—			3	4	2	
K-v S	1:2			51	58	11	
	1:4	HL-A1+		34	50	11	
	1:8	HL-A11	V-v	25	34	11	Ditto
	1:16			11	35	14	
	—			2	8	2	
Ts-m	1:2	HL-A1+		89	76	70	
	1:4	HL-A3+		87	62	18	
	1:8	HL-A11	Pe-v	50	49	16	"
	1:16			39	32	14	
	—			3	3	4	
T-v	1:2			31	33	12	
	1:4	HL-A1 +		30	32	16	
	1:8	HL-A9	M-va	29	29	12	
	1:16			20	15	2	
	—			6	4	2	
Ku-v	1:2			71	68	50	
	1:4			68	57	39	
	1:8	W 25	K-ya	60	48	29	"
	1:16			56	44	30	
	—			15	11	4	
Ts-n	1:2			50	49	20	
	1:4	HL-A9+		32	49	13	
	1:8	HL-A17	G-va	21	29	11	"
	1:16			19	32	15	
	—			5	5	5	
R-g. M	whole		B-v	60		10	Adsorption of B lymphocytes on synthetic fiber
	1:2	HL-A2		50		<10	
	1:4			35		<10	
K-v	whole	HL-A8+		50		10	Ditto
	1:2	HL-A14	T-n	35		10	Ditto
	1:4			30		<10	
Sh-v	whole			50		25	
	1:2			25		25	"
	1:4	W26	S-v	25		10	
R-g I	whole	HL-A1+	P-v	50		10	"
	1:2	HL-A3+ W15 W18		60		10	

EXPERIMENTAL RESULTS

Tests of samples of lymphocytes from 28 blood samples with 12 HLA sera gave consistent results. Removal of lymphocytes of the B population by gradient centrifugation of the rosettes and also by adsorption on synthetic fiber led in every case to a well-marked lowering of the results of lymphocytotoxic test (Table 1). The clearest results were obtained by the use of the sera in dilutions of between 1:2 and 1:16. Meanwhile removal of lymphocytes of the T subpopulation did not lead to any appreciable decrease in the level of the lymphocytotoxic test compared with the original suspension from the same donor.

The tests indicate that the positive result of the lymphocytotoxic test with anti-HLA sera was due principally to the reaction of the antibodies with lymphocytes of the B subpopulation. It can be postulated that the B lymphocytes contain more determinants of HLA antigens or that anti-HLA sera also contain tissue-specific antibodies against B lymphocytes.

These results are in agreement with those obtained by other workers [18] who, in experiments on guinea pigs, also found that B lymphocytes contain more transplantation antigens than T lymphocytes.

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ROLE OF DIFFERENT TYPES OF CELLS IN THE EFFECT OF STIMULATION OF IMMUNOGLOBULIN SYNTHESIS IN MIXED CULTURES

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UDC 612.017.1.014.2-085.23

The synthesis of antibodies and nonspecific immunoglobulins in mono- and mixed cultures of immune lymph nodes and intact bone marrow after removal of adherent cells or T cells was studied by a radioisotope method using immunosorbents. Treatment of the lymph node cell population with anti-θ serum depressed antibody synthesis to 30%, whereas removal of the adherent cells depressed it to 70%. In mixed cultures of immune lymph node cells, after removal of adherent cells, and intact bone marrow cells no effect of stimulation of immunoglobulin synthesis was observed, but treatment of immune lymph node cells with anti-θ serum doubled this effect. The possible mechanisms of the effect of stimulation of immunoglobulin synthesis in mixed cultures are discussed and it is concluded that cooperation between individual types of cells is essential in the productive phase of the immune response.

KEY WORDS: immune response; intercellular interaction; T cells; A cells.

Previous investigations have shown that the intensity of antibody synthesis is two to three times greater in a mixed culture of immune lymph node cells and intact bone marrow cells than in a monoculture [3, 4, 11, 12]. This effect has been shown to be connected with the appearance of an additional number of antibody-forming cells in the population of the immune lymph nodes through the participation of a humoral factor secreted by intact bone marrow cells [2, 5, 9]. The view has been substantiated that, besides cellular cooperation observed

Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 6, pp. 700-702, June, 1976. Original article submitted September 27, 1975.