DETECTION OF ISOIMMUNE ANTIBODIES AGAINST A POPULATION OF HUMAN B-LYMPHOCYTES

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The rosette-forming power of human lymphocytes was studied before and after their sensitization by isoimmune sera against histocompatibility antigens. Anti-HLA-sera inhibited the ability of T- and B-lymphocytes to form rosettes. Sera inhibiting the rosette-forming ability of a population mainly of B-lymphocytes were found. These sera also had more marked ability to react with a population of B-lymphocytes in the prolonged lymphocytotoxic test.

KEY WORDS: HLA-antibodies; T- and B-lymphocytes; Ia (HLA-DR) antigens.

Antigenic differences between human T- and B-lymphocytes have been described in the literature in recent years [2, 8, 9]. So far, however, no precise information has been given on the formation of isoimmune antibodies against antigens of individual populations of human lymphocytes. No reliable methods of detecting such antilymphocytic antibodies likewise yet exist.

Definite prospects for the discovery of such antibodies may be provided by differences between the functional properties of the T- and B-lymphocytes and, in particular, their ability to form rosettes with heterologous erythrocytes.

In this investigation described below the possibility of detecting isoimmune antibodies against individual populations of lymphocytes with the aid of the inhibition of rosette formation test was studied.

EXPERIMENTAL METHOD

The rosette-forming ability of lymphocytes of healthy blood donors was studied before and after sensitization of the cells with the test serum.

Lymphocytes were obtained from defibrinated blood by centrifugation at 200g for 20 min on a gradient with specific gravity 1.077 [4, 6]. Lymphocytes were sensitized at 37°C for 1 h with the sera of multiparous women, of patients who had received frequent blood transfusions, or volunteers artificially immunized with leukocytes.

The rosette-forming ability of the T-lymphocytes was assessed on the basis of spontaneous rosettes formed with native sheep's erythrocytes [1, 3]. The ability of B-lymphocytes to form rosettes was studied with sheep's erythrocytes loaded with heteroimmune antisheep antibodies and complement. For this purpose, a hemolytic rabbit serum was used in a dilution of 1:150. Group O (I) human serum, diluted 1:6, was used as complement. Sensitization of the erythrocytes with serum and complement was carried out for 1 h with each component at 37°C. A suspension of lymphocytes in a concentration of 1000-2000 cells/mm^3 and a suspension of erythrocytes in a concentration of 70,000-80,000 cells/mm^3, made up in Hanks' solution, were used for the rosette-formation test.

To 0.1 ml of the suspension of erythrocytes 0.05 ml of the lymphocyte suspension was added. To stimulate spontaneous rosette formation, 0.025 ml of group AB (IV) human serum, adsorbed with sheep's erythrocytes, was added to the suspension of lymphocytes and erythrocytes. The tubes were centrifuged for 5 min at 70g and kept for 30 min in a refrigerator at 4°C, after which the residue was carefully broken up with a pipet and the number of rosettes determined by counting them in a Goryaev's chamber.

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TABLE 1. Inhibition of Rosette-Forming Ability of Human Lymphocytes by Isoimmune Sera

Group of sera	No. of serum	Donor of lymphocytes	Rosette-forming ability of lymphocytes					
			T-population			B-population		
			before sensitization, %	after sensitization with serum, %	index of rosette formation inhibition (in fractions of a unit, + or -)	before sensitization, %	after sensitization with serum, %	index of rosette formation inhibition (in fractions of a unit, + or -)
I	922	N-va	51	38	0,25 (—)	40	34	0,15 (—)
	710	N-va	51	39	0,23 (—)	40	34	0,15 (—)
	999	Kh-na	58	40	0,31 (—)	34	32	0,05 (—)
	1002	S-na	48	44	0,08 (—)	39	34	0,12 (—)
	710	N-na	60	45	0,25 (—)	41	30	0,26 (—)
II	M-v	K-ya	53	23	0,56 (+)	19	8	0,57 (+)
	T-v	S-v	40	23	0,42 (+)	22	10	0,54 (+)
	R-g	M.V-va	30	15	0,50 (+)	20	10	0,50 (+)
	R-g	M.Ya-ko	45	21	0,53 (+)	15	7	0,53 (+)
	K-i	M-na	50	25	0,50 (+)	15	9	0,40 (+)
	K-i	V-na	45	15	0,66 (+)	19	8	0,55 (+)
	K-Ya	M-v	53	23	0,57 (+)	19	8	0,68 (+)
	1014	Kh-na	58	15	0,74 (+)	18	14	0,58 (+)
	1014	S-na	48	7	0,85 (+)	39	17	0,56 (+)
	964	N-va	51	26	0,49 (+)	40	16	0,60 (+)
	802	N-na	60	39	0,35 (+)	40	22	0,46 (+)
	1108	V-va	45	18	0,60 (+)	25	14	0,44 (+)
III	1171	Sh-r	35	33	0,06 (—)	23,5	11	0,52 (+)
	943	Sh-r	33	29	0,12 (—)	24	15	0,37 (+)
	1107	N-na	60	43	0,30 (—)	41	22	0,46 (+)

The number of rosettes was expressed as a percentage, after which the index of inhibition of rosette formation was calculated by the equation:

$$IIR = 1 - \frac{A}{K},$$

where IIR is the index of inhibition of rosette formation, A the number of rosettes after sensitization of the lymphocytes with the test serum; K the number of rosettes before sensitization of the lymphocytes with the serum (control).

The result was considered to be positive (+) if the index of inhibition of rosette formation was 0.35 or higher.

Besides studying inhibition of the rosette-forming ability of the lymphocytes by means of sera, they were also investigated in the microlymphocytotoxic test. The variant with prolonged incubation for up to 2 h with complement was used. A suspension of lymphocytes prepared in the usual way and also a suspension enriched with B-lymphocytes were used in the test [2, 5].

In some experiments the sera were absorbed with platelets to remove antibodies against HLA-antigens. After addition of 1×10^9-2×10^9 platelets to 0.1 ml of serum the suspension was incubated at 37°C for 1 h.

EXPERIMENTAL RESULTS

Altogether 52 sera were tested, each of them with lymphocytes from 3 to 12 donors.

The results of these investigations showed that the sera can be divided into three groups depending on their action. Group I contained 40 sera without any marked ability to inhibit rosette formation. The index of inhibition of rosette formation for these sera varied from 0.05 to 0.30 (Table 1).

Group II included nine sera (M-v, T-v, R-g, K-i, and K-ya, 1014, 964, 802, 1108). They inhibited the rosette-forming ability of both (T- and B-) lymphocyte populations. Investigation of these sera by the microlymphocytotoxic test showed that they contained powerful isoimmune antibodies. The results of the lymphocytotoxic test corresponded to 4+, or 76-100% of dead cells. Two of these sera contained anti-HLA-A1 antibodies, and another two contained HLA-A2.

Group III included three sera (1171, 943, 1107) with marked ability to inhibit rosette formation by B-lymphocytes. The index of inhibition of rosette formation for B-lymphocytes was 0.52, 0.37, and 0.46, and for T-lymphocytes 0.06, 0.12, and 0.3 respectively. Sera 1171, 943, and 1107 also contained lymphocytotoxic

antibodies, and gave a stronger reaction in relation to some donors with a lymphocyte suspension enriched with the B-population. In one case a serum (K-v) containing HLA-A1 antibodies and, on the basis of its rosette-inhibiting activity, belonging to group II, was absorbed by the thrombocytes of a donor who reacted with it in the lymphocytotoxic test. After absorption it lost its ability to inhibit rosette formation by T-lymphocytes. It still remained capable of inhibiting rosette formation by B-lymphocytes.

The investigations thus showed that HLA-antibodies can inhibit rosette formation by T- and B-lymphocytes of a donor containing the corresponding histocompatibility antigen. Several sera were able to inhibit rosette formation mainly by B-lymphocytes. This evidently indicates that they contain antibodies against the Ia (HLA-DR) system of antigens of human B-lymphocytes.

The inhibition of rosette formation test is a promising method for the detection of antibodies against individual lymphocyte populations.

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SYNGENEIC CEREBRAL CORTICAL TISSUE AS A STIMULATOR OF IMMUNOGENESIS IN THYMECTOMIZED MICE

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Experiments on CBA mice showed that homogenate of gray matter (cortex) of syngeneic brain contains components which restore the population of splenic T-cells in thymectomized animals and stimulate the immune response to sheep's red blood cells. Homogenate of white matter has much weaker activity; indeed it could be due to gray matter contaminating the preparation. Homogenate of syngeneic muscle tissue has no biological activity.

KEY WORDS: thymus; brain; cross-reacting antigens; stimulation of immunogenesis.

The θ-antigen of thymocytes in various species of animals is known to be present also in the brain [1, 7, 11]. This antigen is associated chiefly with the gray matter (cortex) of the brain and is virtually absent in the white matter [1, 6]. It has also been shown that extracts of thymus can stimulate the immune response in animals especially after thymectomy, and under these circumstances they functionally replace the receptors of the T-cells [2, 10, 13]. The question arises, do brain antigens have a similar effect?

The object of this investigation was to study the effect of homogenates of the cortex and white matter of syngeneic brain on the primary immune response and on the thymus-dependent lymphocyte population in thymectomized mice.

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