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ANTIBODIES AGAINST AN INTERSPECIFIC ERYTHROKARYOCYTE ANTIGEN IN PATIENTS WITH PARTIAL RED CELL APLASIA OF THE BONE MARROW

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KEY WORDS: partial red cell aplasia of bone marrow; erythrokaryocytes; interspecific antigen.

Partial or "pure" red cell aplasia (PRCA) of bone marrow is a disease characterized by selective damage to the erythroid branch of hematopoiesis with a reduction in the number of bone-marrow erythrokaryocytes which may amount to total disappearance, reticulocytopenia, and severe normochromic anemia. Antibodies with a selective cytotoxic action on nucleated red cells in the bone marrow have been found in the plasma of some patients with PRCA [7, 8].

A previous investigation showed that an antigen immunologically similar to mouse erythroblast antigen (EB-AG) described perviously [2], is present on the surface of erythrokaryocytes in adult human bone marrow and embryonic human liver.

In the present investigation an attempt was made to identify the antigenic specificity of antibodies from patients with PRCA by testing the patients' sera in the *in vitro* cytotoxicity test (CTT) and the indirect immunofluorescence test (IFT) against target cells of different types containing surface interspecific mammalian erythrokaryocytic antigen, by the use of absorption tests, and by blocking the IFT by serum against mouse EB-AG.

EXPERIMENTAL METHOD

Sera from 19 patients with PRCA and from 14 patients with autoimmune hemolytic anemia (AIHA), with incomplete thermoagglutinins against mature peripheral erythrocyte antigen (control), and pools of 10-20 healthy donors' sera were inactivated by heating to 56°C for 30 min. Before the test with human cells the sera were exhausted twice with an equal volume of peripheral erythrocytes and, in some cases, by a freeze-dried preparation of human amniotic fluid. In tests with mouse cells the sera were first absorbed with equal volumes of erythrocytes (twice) and thymocytes of BALB/c mice. Spleen cells of mice with Rauscher leukemia, erythrokaryocytes from mouse and human embryonic liver, normoblasts from bone marrow of patients with microspherocytosis and from the spleen of a patient with H hemoglobinopathy, with a high content of erythrokaryocytes, and also bone marrow cells from a patient with acute erytheoleukemia, were used as target cells for the IFT and CTT. All suspensions contained about 80% of erythrokaryocytes. The methods of preparation of the cell suspensions, of conducting the IFT, and of obtaining sera and monospecific antibodies against EB-AG were described previously [3]. The CTT was performed by a modified method of Gorer and O'Gorman, using the indirect variant with additional treatment of the cells with serum against human IgG [5]. IFT blockade was carried out by incubating the cells with monospecific antibodies

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TABLE 1. IFT (% of fluorescent cells) on Sera from Patients with PRCA and Antibodies against EB-AG with Human Erythrokaryocytes and Cells from Mice with Rauscher Leukemia (M ± m)

Sera tested	Target cells									
	Rauscher leukemia cells		thymocytes of intact mice		blast cells of human erythromyelosis		erythrokaryocytes of human bone marrow and spleen		human fetal thymocytes	
	n	*M±m*	*n*	*M±m*	*n*	*M±m*	*n*	*M±m*	*n*	*M±m*
Healthy donors (pooled sera)	5	6,6±0,8	5	5,8±1,4	1	5	5	6,6±2,2	5	6,7±1,7
Sera of patients with AIHA	14	8,6±0,8	14	8,1±0,9	3	7,3±2,8	6	7,2±1,3		—
Sera of patients with PRCA:										
Group I	7	7,4±1,7	7	5,0±0,7	4	8,0±2,4	4	12,0±4,4	4	9,0±2,9
Group II	9	51,1±5,8	9	6,3±1,2	4	55,0±7,3	6	66,9±7,4	6	7,3±1,2
Antibodies against EB-AG		80		5		65		62		2

TABLE 2. CTT on Sera from Patients with PRCA and Antibodies against EB-AG with Human Erythrokaryocytes and Cells from Mice with Rauscher Leukemia (M ± m)

Sera tested	Target cells					
	Rauscher leukemia cells			erythrokaryocytes of human embryonic liver		
	% of dead cells		cytotoxicity index	% of dead cells		cytotoxicity index
	n	*M±m*	*M±m*	*n*	*M±m*	*M±m*
Healthy donors (pooled sera)	3	12,0±1,2	—	4	11,3±1,7	—
Sera of patients with PRCA	6	34,7±2,1	0,26±0,02	5	40,0±2,6	0,32±0,33
Sera of patients with AIHA	3	15,7±1,5	0,04±0,01	4	15,5±1,4	0,04±0,01
Intact rabbit serum	3	12,7±3,5	—	3	15,0±3,2	—
Anti-EB-AG serum	3	72,7±4,0	0,68±0,03	3	53,0±2,3	0,45±0,01

TABLE 3. Investigation of Immunospecificity of Serum Antibodies of Patient with PRCA (d-v) in CTT and IFT by Absorption Test

Target cells	Variants of additional absorption of serum from patient with PRCA	CTT			IFT		
		% of dead cells	cytotoxicity index	% neutralization	% of fluorescent cells	cytotoxicity index	% neutralization
Erythrokaryocytes of liver from 6-12-week human embryo	—	52	0,43	—	65	0,61	—
	Erythrokaryocytes of human embryonic liver	18	0,03	90	15	0,05	90
	Rauscher leukemia cells	14	—	100	18	0,09	85
	Adult human liver cells	50	0,41	5	62	0,58	5
Erythrokaryocytes from mouse embryonic liver	—	49	0,43	—	55	0,47	—
	Erythrokaryocytes of human embryonic liver	10	0,06	100	16	0,01	98
	Adult human liver cells	18	0,08	81	12	0,00	100
Spleen cells from mice with Rauscher leukemia	—	48	0,42	—	60	0,55	—
	Erythrokaryocytes of human embryonic liver	21	0,12	71	25	0,15	73
	Adult human liver cells	17	0,07	82	18	0,06	87

TABLE 4. Test of Blocking Immunofluorescence of Sera from Patients with PRCA with Rauscher Leukemia Cells by Serum against EB-AG

Sera studied	% of fluorescent cells after blockade		Change in intensity of fluorescence	% blockade of IFT	% enhancement of IFT
	by intact rabbit serum	by anti-EB-AG serum			
Healthy donors' serum	6	8	No change	—	—
	10	10	Same	—	—
	10	10	"	—	—
Serum from patients with PRCA					
D-v	68	30	Decrease	61	—
Ch-a	62	95	Increase	—	39
S-a	21	80	Sharp increase	—	84

against EB-AG or with healthy rabbit serum, followed by treatment consecutively with second order sera (from the patient with PRCA or healthy donors) and with serum against human IgG labeled with fluorescein isothiocyanate. The formula for calculating the efficiency of blocking and percentage of neutralization in the absorption test was given previously [6].

EXPERIMENTAL RESULTS

In nine of 16 sera from patients with PRCA studied in the IFT antibodies reacting specifically with the membrane of Rauscher leukemia cells and with human erythrokaryocytes of various originwere discovered (Table 1). The same coincidence of immunologic activity of the sera was revealed by the CTT (Table 2): Six of the seven sera tested from patients with PRCA were cytotoxic for mouse leukemia cells and for embryonic human erythrokaryocytes.

Antigenic specificity of the serum antibodies of one patient with PRCA (patient D-v) was investigated by the absorption test in the CTT and IFT, using three types of target cells. Activity of the sera atainst all cells was found to be neutralized by preliminary exhaustion of the serum both by human erythrokaryocytes and by mouse leukemia cells (Table 3).

Antibodies of the patient with PRCA thus reacted with an interspecific antigen common to the membrane of preerythrocytic mammalian nucleated cells and cells of Rauscher virus erythroleukemia. To identify this antigen with mouse EB-AG, Rauscher leukemia cells were treated beforehand with antibodies against EB-AG, after which the IFT was carried out with sera from three patients with PRCA and from healthy donors. The result of this treatment in experiments with the sera of two patients was a paradoxical increase in the percentage of fluorescent cells and an increase in the intensity of fluorescence (Table 4). This phenomenon canbe explained by the presence of antigen–antibody complexes in the sera studied. With target cells covered with antibodies against EB-AG these complexes are specifically bound through the antigenic component, and it is this which causes enhancement of the reaction. The immune reaction of the serum from patient D-v with Rauscher leukemia cells, which was blocked by anti-EB-AG serum, was evidently due to antibodies against this antigen not present in complex form. Serum against EB-AG also blocked the reaction of patient D-v's serum with mouse and human embryonic erythrokaryocytes.

The results show that the development of PRCA, at least in some patients, is associated with the appearance of antibodies against an interspecific antigen of mammalian erythrokaryocytes (IAME), which may be similar to EB-AG. The elucidation of this fact would greatly improve the prospects for the diagnosis and study of the pathogenesis of the disease and also, possibly, its immunotherapy in conjunction with cytostatic agents. The autoimmune response to IAME is not the only factor in the mechanism of the pathogenesis of PRCA. In some cases antibodies against erythropoietin have been found in patients' serum [8]. Previously, by means of the aggregate-hemagglutination test [4], the obligatory presence of class A and,

less frequently, class G, immunoglobulins possessing the properties of antibodies, but not damaging mature erythrocytes [1], was demonstrated on the surface of the peripheral erythrocytes of patients with PRCA. The role of these antibodies, directed against the Pr antigen of the erythrocyte membrane, in the pathogenesis of PRCA is not yet clear.

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REACTION OF SERA OF MYASTHENIA PATIENTS WITH THE SURFACE ANTIGENIC STRUCTURE OF THYMUS LYMPHOCYTES OF HEALTHY SUBJECTS AND PATIENTS WITH MYASTHENIA GRAVIS

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The blood of patients with myasthenia gravis contains antibodies against certain heteroorganic antigens of epithelial tissue of the human thymus. It has been shown by the immunofluorescence test, for instance, that the blood of patients with myasthenia contains antibodies against antigens of myoid cells common with antigens of skeletal muscle and myocardium [1, 5], and also against an antigen of the epithelial reticulum of the human thymus, common with the epithelium of the skin [2]. Deposition of immune complexes containing immunoglobulins of the M, A, and G classes has been found in sections of the thymus from patients with myasthenia by the direct immunofluorescence method [3]. Because of the distribution of the immune complexes it has been suggested that they contain antibodies against thymus tissue antigens and, primarily, against antigens of its lymphoid cells.

The object of the present investigation was thus to demonstrate antibodies against antigens of human thymus lymphoid cells in the spleen of patients with myasthenia and also to compare the reactions of these sera with lymphocytes from healthy human lymphoid organs and from the thymus of patients with myasthenia in order to discover any possible antigenic changes in the thymus lymphocytes in this disease.

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