ANTIGENIC MARKER OF HUMAN ERYTHROKARYOCYTES SIMILAR TO MOUSE ERYTHROBLAST ANTIGEN

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Identical antigenic determinants were detected by immunofluorescence and the cytotoxic test on the surface of human erythrokaryocytes with the aid of antibodies against a specific antigen of mouse erythroblasts (EBAG) discovered previously during a study of Rauscher leukemia. The antigen is present on the membrane of most nucleated erythroid cells of the liver of human embryos in the early stages of development and in adult human bone marrow, but is not found in fetal thymocytes, neonatal kidney cells, adult human liver, or in peripheral blood erythrocytes. EBAG evidently possesses an interspecific determinant which is common for mammalian nucleated erythroid cells and which may serve as their specific marker.
KEY WORDS: erythroblast antigen; immunofluorescence method; cytotoxic reaction; human erythrokaryocytes.

A surface marker antigen specific for the early stages of differentiation of cells of the erythroid series (erythroblasts) in mice (EBAG) was found for the first time and its immunochemical properties determined during a study of Rauscher erythroleukemia [3].

In the present investigation the possibility of using anti-EBAG antibodies for the detection of similar antigenic determinants on the surface of human erythroid cells was studied by means of the immunofluorescence method and the cytotoxic test in vitro.

EXPERIMENTAL METHOD

Serum against mouse EBAG was obtained by immunizing rabbits by injection of Rauscher virus, isolated by ultracentrifugation in a sucrose density gradient from the plasma of BALB/c mice affected with Rauscher leukemia, into the popliteal lymph nodes [2, 3].

Monospecific antibodies against EBAG were obtained by decomposing the precipitate in acid medium [5]. For this purpose the immune serum was first exhausted with normal mouse serum, clarified by centrifugation for 30 min at 13,000g, and treated with a predetermined dose of splenic extract from leukemic mice. The resulting precipitate was washed and decomposed in acid medium (pH 3.2). Full details of the method of obtaining the antibodies were given previously [3].

Besides monospecific antibodies, an original serum against EBAG also was used. In that case the serum was preincubated with an equal volume of healthy human plasma and then adsorbed twice with an equal volume of peripheral erythrocytes (a mixture from donors of different blood groups) and also with liver powder or freeze-dried human amniotic fluid in the proportion of 80 mg to 1 ml serum. Serum of an intact rabbit was subjected to the same treatment. Reactions with fetal or neonatal thymus cells and with peripheral blood erythrocytes acted as the control of completeness of adsorption.

Cell suspensions of erythrokaryocytes were prepared from two types of material.

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TABLE 1. Immunofluorescence Reaction between Monospecific Anti-EBAG Antibodies and Human Cells

Material tested (target cells)	Percentage of luminescent cells
Embryonic liver erythrokaryocytes	80
Thymus of 6-month fetus	2
Neonatal kidney	3
Adult erythrocytes	0

TABLE 2. Cytotoxicity Test (CTT) and Immunofluorescence Test (IFT) of Anti-EBAG Serum with Human Erythrokaryocytes

Target cells	Serum	Variants of additional adsorption of anti-EBAG serum	CTT			IFT		
			% of dead cells	index of cytotoxicity	% neutralization	% of luminescent cells	index of immunofluoresence	% neutralization
Liver erythrokaryocytes from 6-12-week human embryo	Anti-EBAG	—	53	0,44	—	80	0,79	—
		Adult human liver cells	48	0,38	14	78	0,77	—
		Spleen cells of mice with Rauscher leukemia	18	0,02	94	4	0,01	98
		Erythroid cells of human embryonic liver	19	0,03	92	6	0,03	96
	Serum of intact rabbit (control)	—	16	—	—	3	—	—
Adult human bone marrow	Anti-EBAG	—	—			56	0,55	—
		Thymus cells of mice with Rauscher leukemia	—			52	0,51	7
		Spleen cells of mice with Rauscher leukemia				5	0,03	94
	Serum of intact rabbit	—	—			2	—	—

Fig. 1. Liver of 6-week human embryo: clusters of normoblasts (cells with a dense nucleus) can be seen among hepatocytes (cells with a pale nucleus). Histological section stained with hematoxylin-eosin, 200×.

Fig. 2. Monocytes of liver normoblasts from 6-week human embryo, treated with anti-EBAG serum: bright luminescence of membrane. Luminescence microscopy, 900 ×.

Fig. 3. Monolayer of thymocytes of 6-month human fetus, treated with anti-EBAG serum: luminescence absent. Luminescence microscopy, 900 ×.

1. The liver of embryos obtained on the day of therapeutic abortion from healthy women between the 6th and 12th weeks of pregrancy. The suspension was prepared by frequently washing pieces of liver, dispersed by dissection needles, in a 5% solution of lactalbumin hydrolysate in Hank's solution, pH 7.2. Centrifugation at 1000 rpm for 2-3 min alternated with repeatedly allowing the resuspended sediment to stand in the cold for 5-10 min to remove any contaminating hepatocytes. The number of erythrokaryocytes in films from the resulting suspensions, stained by Pappenheim's method, exceeded 80% of the total number of cells.

2. Bone marrow was obtained by sternal puncture from patients of group O (I) with microspherocytic hemolytic anemia. In the myelogram of these patients erythrokaryocytes accounted on average for 60% of all nucleated cells. Bone marrow was placed in the same medium as embryonic liver, with the addition of heparin, and was repeatedly washed under the same conditions. In films from the resulting suspension erythrocytes accounted for 55-70% of all cells.

The indirect immunofluorescence test was carried out on an artificial monolayer of living cells adherent to glass. The specimens were examined in the ML-2 luminescence microscope, 200 cells were counted, and the percentage of luminescent cells determined. The cytotoxic test in vitro was carried out by a modified method of Gorer and O'Gorman [4], using fresh guinea pig serum as complement.

EXPERIMENTAL RESULTS

The most convenient object for testing the activity of anti-EBAG antibodies against human erythroid cells was found to be embryonic liver in the early periods of development, at the stage of intensive erythropoiesis.

Most cells of the resulting suspensions were erythronormoblasts (Fig. 1). Practically all these cells gave intensive luminescence on treatment with monospecific anti-EBAG antibodies (Fig. 2). The specificity of this reaction was confirmed by the absence of fluorescence on fetal thymocytes (Fig. 3), neonatal kidney cells, and adult human peripheral blood erythrocytes Table 1).

Similar results were obtained by the use of the initial anti-EBAG serum, after removal of antibodies against heteroantigens by adequate adsorption. These sera were highly active in the immunofluorescence test and also, although rather less so, in the complement-dependent cytotoxicity test (Table 2).

The specificity of these reactions was completely confirmed by the results of additional adsorption tests. Adsorption of the serum both by the target cells themselves and by Rauscher mouse leukemia cells completely neutralized both reactions, whereas adsorption by adult human liver cells did not reduce the activity of the serum tested.

The same antigen also was found on the surface of adult bone marrow erythrokaryocytes (Table 2), with a higher content of cells of the erythoid series as a result of compensatory regeneration (hemolytic anemia). In this case also the reaction was neutralized by adsorption of the serum with mouse erythroid leukemia cells. The results as a whole indicate that an antigen with a common determinant with EBAG and, consequently capable of acting as a marker of the cell population, is present on the membrane of human cells belonging to the pre-erythrocytic stages of differentiation.

This fact can be used to solve various problems.

Antigens of adult erythrocytes have now been studied in considerable detail, and as a result many anemias have been shown to be autoimmune in nature and associated with a response to various concrete erythrocytic antigens [1]. Meanwhile practically nothing is known of the antigens of the pre-erythrocytic stages of development of human cells of the erythroid series, and this is a handicap to the study of the pathogenesis of diseases associated with selective damage to the bone-marrow precursors of mature erythrocytes (partial erythrocytic aplasia) or with the depression of the various branches of medullary hematopoiesis (pancytopenia).

The use of the test for EBAG may perhaps make it possible to define also the genealogy of the cell populations in some cases of leukemia that cannot at present be differentiated either by their morphological features or the histochemical data, in much the same way as antigenic markers of mouse lymphoid tissue cells of the Thy, TL, Ly, and PC types can be used for similar purposes experimentally.

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