ERYTHROBLAST ANTIGEN IN MOUSE VIRUS LEUKEMIAS

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An antigen differing from the type-specific and group-specific antigens in electrophoretic mobility and immunochemical specificity was found in mice with Rauscher virus leukemia. This antigen was presented in the serum and spleen of various lines of healthy mice. It was not found by the immunofluorescence method in red cells or lymphocytes but was regularly detected on the surface of erythroblasts and was therefore called AG-EB. AG-EB is not identical with embryonic hemoglobin. It is evidently a specific marker of a particular stage in the differentiation of cells of the erythroid series.

In virus leukemias of mice group-specific antigens (GSA) [10] and type-specific antigens (TSA) [11] are constantly found. In some mouse leukemias antigens of a different specificity have also been described and, in particular, an antigen common to leukemic cells and the thymus [8] and to leukemic cells and the mammary gland [12].

This paper describes the immunochemical characteristics of yet another antigen found in the course of study of Rauscher leukemia that proved to be a specific antigen of mouse erythroblasts (AG-EB).

EXPERIMENTAL METHOD

Mice of lines C57BL/He, C57BL/10Sn, C3H/Sn, BALB/c, and B10/D2 were obtained from the laboratory of inbred animals of the nursery of the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR; (C57BL × CBA)F_1 hybrids were obtained from the Central Institute of Blood Transfusion, Ministry of Health of the USSR.

Colonies of hematopoietic tissue were obtained by the method of Till and McCulloch [13] in (C57BL × CBA)F_1 mice irradiated with a dose of 800 rad.

Rauscher leukemia was transplanted in BALB/c mice by intraperitoneal injection of a suspension of leukemic cells. Mice with Friend, Moloney, Stepin-Zil'ber, and Mazurenko leukemias were obtained from the Laboratory of Etiology of Leukemias, IÉKO.

Rauscher virus (RV) was isolated from the plasma of mice with a developed leukemia by ultracentrifugation in a sucrose density gradient [3]. Antiserum against RV was obtained by inoculating rabbits in the popliteal lymph glands [3].

A test system for GSA consisting of monospecific antiserum against GSA, kindly provided by A. I. Gusev, and a splenic extract from mice with Moloney leukemia, taken in the equivalent dilution, was used.

Saline extracts were prepared by grinding the tissues in a mortar in Ringer's solution in the ratio of 1:3 [3]. The precipitation test in agar was carried out by the method of Gusev and Tsvetkov [2]. Analytical immunoelectrophoresis was carried out in 1% agarose, in veronal-medinal buffer, pH 8.6, $\mu = 0.025$ [1].

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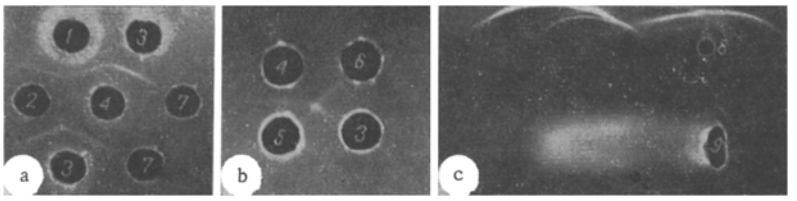

Fig. 1. Immunochemical characteristics of AG-EB: a) reaction between electrophoretic fraction of RLS and test system for AG-EB and antibodies against AG-EB; b) comparison of AG-EB with GSA; c) immunoelectrophoretic characteristics of AG-EB. 1) antibodies against AG-EB; 2) electrophoretic fraction of RLS containing AG-EB; test system for AG-EB; 3) antiserum against Rauscher virus exhausted by NMS; and 4) plasma of BALB/c mice with Rauscher leukemia, concentrated twofold; test system for GSA; 5) antiserum against GSA; 6) splenic extract from mice with Moloney leukemia; 7) physiological saline; 8) normal mouse serum; 9) splenic extract of mice with Rauscher leukemia. In c: top gutter contains antiserum against NMS, bottom gutter contains monospecific antiserum against AG-EB.

TABLE 1. Distribution of AG-EB in McCulloch's Foci in the Spleen of Irradiated Mice and in a Suspension of Mouse Bone Marrow

No. of specimen	Morphological characteristics of specimen	Cells of erythroid series		Cells of myeloid series	
		% fluorescent	% not fluorescent	% fluorescent	% not fluorescent
1	Erythroid colony	85	15	—	—
2+3	Mixed colony: erythroid and myeloid cells	64	9	0	27
4	Mixed colony with preponderance of of cells of the myeloid series	30	8	0	62
5	Mixed colony: cells of the erythroid and myeloid series 1:1	48	2	0	50
6	Suspension of bone marrow from BALB/c mice	25	6	0	69

The test antigen was isolated from splenic extract of mice with Rauscher leukemia (RLS) by preparative electrophoresis in 1% agarose in the same buffer [1]. The RLS was homogenized in a mechanical mincer (Ultra-Turrax, Janke & Kunkel KG) in Ringer's solution in the ratio of 1:3. The extract was clarified by centrifugation at 20,000 g for 30 min, concentrated with polyethylene glycol (molecular weight 40,000) 3-4 times, and dialyzed against veronal-medinal buffer, pH 8.6. Fractions obtained by preparative electrophoresis were concentrated with Sephadex G-50 medium or by polyethylene glycol by 5-6 times. The presence of AG-EB and GSA in the resulting fractions was determined by the precipitation test in agar with the appropriate test systems.

Monospecific antibodies against AG-EB were obtained by decomposing the corresponding precipitate in acid medium [5].

The presence of AG-EB in sections and cell suspensions from various mouse organs was detected by the indirect fluorescent antibody method [14]. Cell suspensions for immunofluorescence were obtained and treated by Dorfman's method, using a Formvar film [9]. The preparations were dried, mounted in 50% glycerol, and examined in the ML-2 luminescence microscope. To estimate the results 200 cells were counted and the percentage of luminescent cells determined. Sections from the various mouse organs, 3 μ in thickness, were cut in a cryostat (-20°C) from pieces of tissue frozen to -70°C. The sections were fixed in acetone for 10 min, then treated with antibodies against AG-EB and with donkey antibodies against rabbit γ globulin labeled with fluorescein isothiocyanate [6].

Sections, squash preparations, and films from cell suspensions for morphological analysis were stained by the Romanowski–Giemsa method (exposure with dye 30 min for sections, 5 min for squash preparations and films).

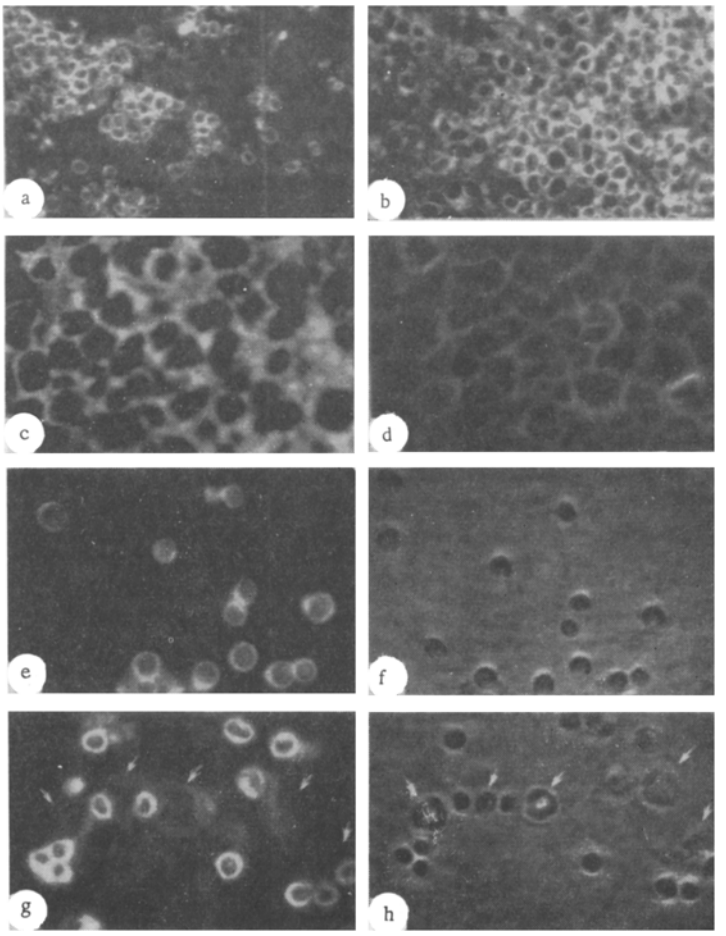

Fig. 2. Localization of AG-EB in sections of the spleen of BALB/c mice, healthy and affected with Rauscher leukemia (a-d), and in cell suspensions from colonies formed in irradiated mice (e-h). a) Section through spleen of a healthy mouse; b, c, d) sections through spleen of mouse with Rauscher leukemia; e, f) cell suspension from erythroid colony; g, h) cell suspension from mixed colony; a, b, c, e, f, g, h) incubation with antibodies against AG-EB; d) incubation with antibodies against AG-EB, neutralized by electrophoretic fractions containing AG-EB; f, h) similar fields of vision to e, g, examined under phase contrast. Arrows indicate cells of myeloid series in which no AG-EB were found. Magnification a, b) 120 ×; c-h) 270 ×.

EXPERIMENTAL RESULTS

Antiserum against RV contained antibodies against several components of normal mouse serum (NMS), against GSA, and against another antigen subsequently named AG-EB. Antibodies against GSA were present in two of six rabbits and antibodies against AG-EB in all six. Antisera not containing antibodies against GSA were used.

To obtain monospecific antiserum against AG-EB the antiserum was neutralized with one volume of NMS. The same antiserum and the plasma of BALB/c mice affected with Rauscher leukemia, concentrated twofold, were used as the test system for AG-EB in the precipitation test in agar (Fig. 1a).

To detect antibodies against AG-EB the monospecific antiserum was clarified for 30 min at 13,000 g and the neutralizing dose of RLS extract, clarified at 9,000 g for 20 min, determined beforehand was added. The antibodies eluted from the resulting precipitate were identified with a test system for AG-EB (Fig. 1a). These antibodies were used to locate the AG-EB by the immunofluorescence method (Fig. 2).

TABLE 2. Immunofluorescence Test for Antibodies against AG-EB with Various Cells from BALB/c Mice

Type of cells	% of fluorescent cells
Spleen cells from mice with Rauscher's leukemia	86
Normal spleen cells	9,5
Erythrocytes	3,5
Thymus cells from mice with Rauscher's leukemia	5
Thymus cells from normal mice	2

Comparison of the test system for AG-EB with the test for GSA and in the agar precipitation test demonstrated their complete immunologic difference (Fig. 1b).

After immunoelectrophoresis of the RLS extracts and development with monospecific antiserum against AG-EB, a precipitation arc was found in the zone of the serum β globulins (Fig. 1c), whereas TSA of Rauscher leukemia had the mobility of α_2 globulins [1] and GSA the mobility of γ globulins [7]. During electrophoresis the activity of AG-EB fell sharply and it could be detected and isolated only by the use of highly concentrated RLS extracts. After preparative electrophoresis AG-EB also was discovered in the zone of the β globulins. The electrophoretic fraction containing AG-EB completely neutralized the antibodies used for its detection in the precipitation test in agar and by the immunofluorescence method (Fig. 2d).

The precipitation test in agar with the test system revealed AG-EB in the serum and plasma of BALB/c mice with Rauscher leukemia and also in normal mice, but in much smaller amounts. It was also present in splenic extracts from mice of all the lines studied, but with age the quantity of AG-EB decreased appreciably. In mice with the leukemias studied AG-EB was found only in splenic extracts from animals with Rauscher and Friend leukemias.

To determine which cells contained AG-EB, the indirect immunofluorescence method was used. AG-EB was found in cryostat sections of the spleens of BALB/c mice, both healthy and affected with Rauscher leukemia (Fig. 2a, b, c), but in the sections of the "leukemic" spleens nearly all the cells were luminescent, compared with only individual groups of cells in the normal spleen. Fluorescence was observed at the periphery of the cells but not in the nuclei. Parallel with the sections for immunofluorescence, histological preparations were stained by the Romanowsky–Giemsa method. However, no cells containing AG-EB could be identified in such preparations from the normal spleen.

To investigate this problem McCulloch's foci were obtained in mice in two experiments. In one experiment mice were irradiated and injected with a bone-marrow cell suspension from syngeneic animals. In the other experiment the mice were irradiated only. In both cases clearly visible discrete cell colonies were formed after 8-10 days in the spleen of the mice. Some of the colonies were erythroid and some were mixed, consisting of erythroid and myeloid cells; no lymphoid colonies were formed in the spleen [4]. Suspensions were prepared from individual colonies whose morphological characteristics were first determined in impressions from each colony stained by the Romanowsky–Giemsa method. A monolayer was obtained from the suspension on Formvar film and treated with antibodies against AG-EB. As is clear from Table 1 and Fig. 2e-h, the cells of the myeloid series did not fluoresce. In the erythroid colony there were 85% of fluorescent cells, but in the mixed colonies the percentage of fluorescent cells correlated with the number of cells of the erythroid series. All the fluorescent cells were erythroblasts. These results were confirmed in a suspension of bone marrow, in which only the erythroblasts were fluorescent (Table 1).

Determination of AG-EB in the various cell suspensions showed that erythrocytes, like thymus and spleen cells, contained hardly any of this antigen (Table 2).

AG-EB is not embryonic hemoglobin, for only traces of it could be found by the agar diffusion method, and then only in highly concentrated blood lysates from mouse embryos.

The AG-EB found in Rauscher leukemia thus differed from the TSA of Rauscher leukemia and also from GSA in its immunochemical specificity and electrophoretic mobility.

AG-EB is evidently an antigen that is characteristic of a certain stage in the differentiation of cells of the erythroid series. It is contained on the surface, for it can be detected by the immunofluorescence method in living cells. In this respect it resembles the specific surface antigens of the thymus, lymphocytes, and plasma cells and it can evidently serve as a specific marker for cells of the erythroid series. The sharp increases in the content of AG-EB in Rauscher and Friend leukemias is explained by the fact that both these forms of leukemia are erythroblastoses.

So far as the presence of this antigen in preparations of Rauscher virus used to obtain antiserum is concerned, this can be explained either by their contamination with cell fragments or by the incorporation

of this antigen into the virus membrane during its reproduction. If monospecific antibodies against AG-EB are available, this can be studied by means of an electron-microscopic investigation.

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