IDENTIFICATION OF SOME FORMS OF HUMAN LEUKEMIAS BY MEANS OF POLYCLONAL ANTIBODIES TO AN ERYTHROBLAST ANTIGEN

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The classification of leukemias is based on cytochemical and morphological characteristics of the tumor cells. Recently, however, various immunologic markers, including differential surface antigens of hematopoietic cells, have been used for the more exact diagnosis of leukemias. These markers include a broad spectrum of polyclonal and monoclonal antibodies [7]. Meanwhile markers of erythroid cells (with the exception of spectrin and glycophorin, found in large quantities in mature erythrocytes) have virtually not been studied.

The writers previously described an interspecific erythroblast antigen (EB-AG), a specific differential marker of hematopoietic cells of the erythroid series [2, 3, 8].

The aim of this investigation was to study the possibility of using polyclonal antibodies (PAB) against EB-AG as a diagnostic test to identify human leukemias, particularly of the acute kind.

EXPERIMENTAL METHOD

Serum against EB-AG was obtained by immunizing rabbits by injecting a precipitate into the popliteal lymph nodes. The precipitate was formed with antigens to EB-AG and serum of mice with phenylhydrazine-induced anemia, containing EB-AG. The resulting antiserum was exhausted with lyophilized mouse serum and incubated with cells of murine suspension erythroid culture K-2 [4] for30 min at 37°C and overnight at 4°C. The PAB with K-2 cells were eluted with HCl-glycine buffer (pH 2.8), neutralized with $NaHCO_3$ to pH 7.2, and dialyzed against buffered physiological saline. The PAB gave a reaction of complete identity with the test system for EB-AG in the agar diffusion test (Fig. 1b), conducted by the modification in [1].

Electrophoresis of the lysates (1% Triton X-100, from Serva, West Germany) of K-2 mouse cells and human embryonic liver cells in a 7-22% polyacrylamide gel gradient, in the presence of sodium dodecylsulfate, was carried out by the method described in [5]. Protein was transferred to a nitrocellulose filter (Millipore) by passive diffusion overnight at 25°C. For development, the method described in [10] was used, with slight modifications. As the substrate for peroxidase, 4-chloronaphthol (from Sigma, USA) was used.

Exhausted antiserum against EB-AG revealed a protein with mol. wt. of 69 kilodaltons (kD) in lysates of K-2 cells and human embryonic liver cells (Fig. 2). Electrophoresis followed by staining of preparations of thoroughly washed precipitation bands from the test system (Fig. 1b) in the immunoblotting test revealed the same protein with mol. wt. of 69 kD (Fig. 1c, d). Electrophoresis of proteins of the precipitate in the highest possible dilution for electrophoresis is illustrated in Fig. 1a.

Cell suspensions from the peripheral blood and bone marrow of patients with different forms of leukemias, of human embryonic liver cells, of normal human thymus and kidney cells and erythrocytes, and also suspension cultures of human erythroid (K562) and myeloid (HL-60) cells [6, 9], generously provided by Dr. Mach, were used. Nucleated cells were isolated in a one-step Ficoll–Hypaque gradient. These cells were then washed off in 0.5% lactalbumin hydrolysate by centrifugation five times at 1000 rpm for 5 min.

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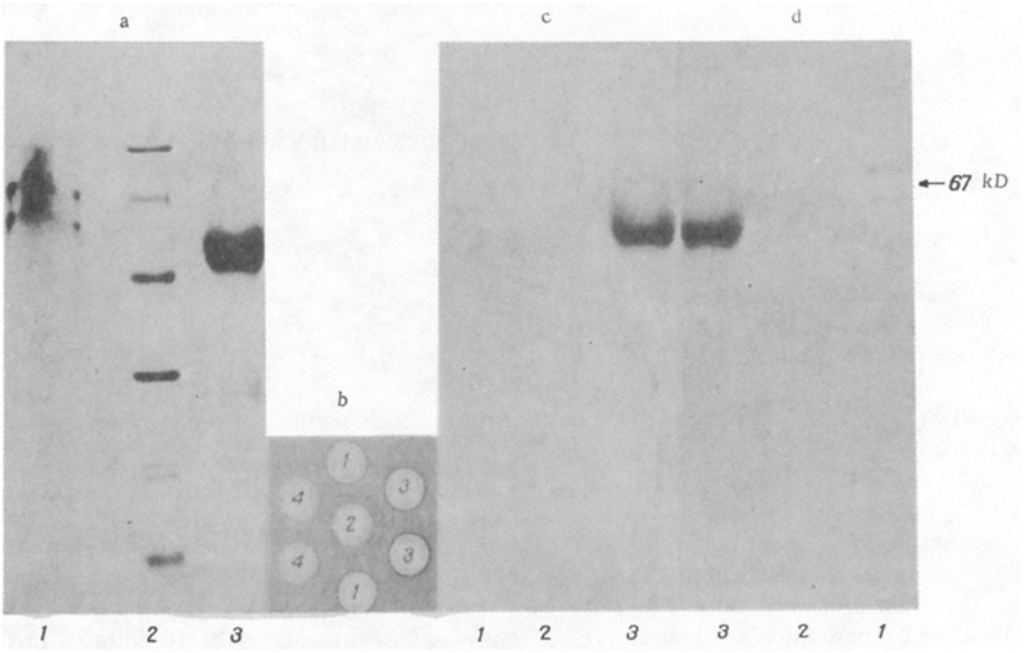

Fig. 1. Immunochemical and electrophoretic analysis of EB-AG. a) Electrophoresis of proteins of eluted bands of precipitate (1), of marker proteins (from Pharmacia, Sweden): phosphorylase B, 94 kilodaltons (kD), bovine serum albumin (94 kD, 67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20 kD), α-lactalbumin (14 kD) (2); preparation of rabbit antibodies (3); b) agar precipitation test with exhausted serum against EB-AG (1), with unpurified EB-AG preparation from spleens of erythroleukemic mice, with physiological saline (4); c) immunoblotting of proteins in band of precipitate. Staining with peroxidase-labeled goat antibodies against rabbit IgG (control): 1) proteins of band of precipitate, 2) marker proteins (the same as in Fig. 1a), 3) a preparation of rabbit antibodies (see 1a), d) the same, stained successively with antiserum against EB-AG and peroxidase-labeled goat antibodies against rabbit IgG: 1-3) the same as in Fig. 1c.

The indirect immunofluorescence test (IIT) was carried out on a monolayer of animal cells fixed to slides with polylysine (0.1 mg/ml). Washed off living cells were treated with PAB against EB-AG and then with serum against rabbit γ-globulins, labeled with fluorescein isothiocyanate (Produced by the N. F. Gamaleya Institute of Epidemy and Microbiology). The preparations were examined in a luminescence microscope (Opton, West Germany). Altogether 200 cells were counted and the percentage of luminescent cells determined.

The diagnosis was based on the results of cytochemical, morphological, and clinical examination of the patients.

EXPERIMENTAL RESULTS

When a suspension of human embryonic (10-12 weeks) liver cells containing about 85% of erythroblasts was treated with PAB against EB-AG more than 80% of EB-AG-positive cells were discovered (Table 1); 90% of cells of the K562 erythroid culture reacted with PAB. High specificity of the PAB was confirmed by absence of fluorescence on nonerythroid human cells (Table 1).

Cells from 43 patients with different forms of leukemias were analyzed in the IIT. Virtually no luminescence was found on the surface of cells from patients with acute and chronic lymphatic and myeloid leukemias, or of patients with acute monocytic leukemia (Table 2). Cells of all patients with erythromyelosis reacted with PAB.

The results of the IIT, incidentally, correlated closely with those of cytochemical analysis of the leukemic cells. For instance, 75% of erythroblasts were discovered cytochemically in bone marrow from a patient with erythromyelosis, containing 65% of EB-AG-positive cells. In the course of treatment of this patient

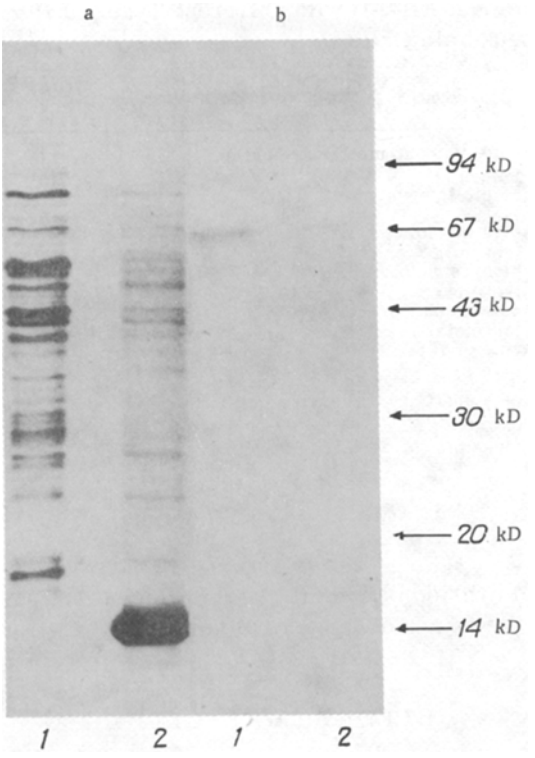

Fig. 2. Electrophoresis (a) and immunoblotting (b) of Triton lysates of K-2 mouse erythroleukemic cells (1) and normal human embryonic (8-12 weeks) liver cells (2). Filters treated successively with PAB and peroxidase-labeled goat antibodies against rabbit IgG.

TABLE 1. Immunofluorescence Reaction of PAB Against EB-AG with Various Human Cell Suspensions

Target cells	% of positive cells
Erythroid culture K562	90
Embryonic liver (10-12 weeks)	80
Fetal thymus (6 months)	2
Peripheral blood erythrocytes	3
Neonatal kidneys	3
Myeloid culture (Hl-60)	0

the number of erythroblasts in the peripheral blood fell to 8.5% as shown by the results of the IIT, and fluorescence was observed in 8% of cells in peripheral blood films.

The group of patients with acute undifferentiated leukemias, developing against the background of a lesion affecting one hematopoietic series (Table 2) is particularly interesting. Three of the six patients investigated developed leukemias against the background of partial red-cell aplasia. In four of six patients EB-AG were detected on the surface of the leukemic cells. The number of blast cells in the films examined was about 30-60%, which corresponded to the number of luminescent cells. Blast cells which could not be placed in any particular hematopoietic series on the basis of morphological and cytochemical criteria thus contained EB-AG on their surface.

These results demonstrate the high specificity of PAB against EB-AG. With their aid it is evidently possible to determine the exact genealogy of cell populations in some cases of acute leukemia which cannot yet be identified either histochemically or morphologically.

TABLE 2. Immunofluorescence Reaction of PAB Against EB-AG with Different Types of Human Leukemic Cells

Diagnosis	No. of patients investigated	No. of positive cases	% of positive cells
Acute lymphatic leukemia	11	0	0
Chronic lymphatic leukemia	4	0	0
Acute myeloid leukemia	4	0	0
Chronic myeloid leukemia	9	1	4
Acute monocytic leukemia	5	0	0
Acute erythromelosis	4	4	55, 65 40, 60
Acute undifferentiated leukemia	6	4	0, 0, 36 40 61, 68

Recently the writers obtained hybridomas secreting monoclonal antibodies against human erythroid cells, and it is proposed to use them for the immunodiagnosis of human erythroleukemias and acute undifferentiated leukemias.

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