POSSIBLE DETECTION OF A HUMAN EMBRYONIC LEUKEMIC ANTIGEN

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The possibility of detecting an embryonic leukemia antigen on blast cells of patients with acute leukemia was studied by means of a cytotoxic test with sera and 7S- and 19S-serum immunoglobulins of placental blood. The presence of an antigen, detectable by antibodies of the placental blood of parturient women but absent on leukocytes of healthy donors, was demonstrated on blast cells of patients with acute leukemia.

KEY WORDS: leukemia; embryo-leukemic antigen; placental sera; antileukemic antibodies.

The appearance of antigens characteristic of embryos in malignant neoplasms has been described in the case of several human and animal tumors. Usually the appearance of "embryonic" antigens can be explained by specific derepression of genes essential for embryonic development, and by the preservation of a few "embryonic" or stem cells in adult tissues, or by a general disturbance of normal genetic control. Evidence of the possible existence of an embryonic leukemic antigen (ELA) has been published. For instance, immunodiffusion tests have shown that in certain forms of leukemia the tissues and blood hemocytoblasts may contain a large quantity of an antigen which is regularly found in the human fetal and neonatal thymus, but is detected in only very small concentrations in the lymphoid organs and mucous membrane of the gastrointestinal tract of the adult [2]. The use of a cytotoxic test [3] has shown that the surface of the lymphocytes of patients with chronic lymphatic leukemia is characterized by antigenic properties that are not found under the same conditions on normal lymphocytes but which have something in common with embryonic tissues.

The existence of an ELA is confirmed by the following facts: 1) sera against carcinoembryonic antigen react selectively with leukemic cells; 2) antileukemic serum is cytotoxic for the leukocytes of about 30% of newborn infants; 3) antigens isolated from membranes of leukemic cells have common properties with antigens of embryonic tissues [5].

The object of this investigation was to study whether ELA can be detected by a cytotoxic test on blast cells of patients with acute leukemia with the aid of antileukemic antibodies from placental blood.

EXPERIMENTAL METHOD

Blast cells from eight children aged from 5 to 12 years with acute lymphoblastic leukemia and leukocytes of 10 healthy blood donors (control group) aged from 7 to 12 years were obtained from heparinized blood. The red cells were lysed with 0.83% ammonium chloride solution. Before use the cells were washed three or four times in Hanks's solution, pH 7.2. Unheated whole placental sera, obtained from umbilical cord blood, were used to detect ELA. 19S- and 7S-globulin fractions were obtained by fractionation of the placental sera on a Sephadex G-200 column [6]. The protein concentration in the 19S fraction varied from 210 to 980 μg/ml and in the 7S fraction from 870 to 1500 μg/ml. Antisera against human γ-globulins (anti-19S and anti-7S) from A. G. Behringwerke (West Germany) and guinea pig complement were used in the cytotoxic test.

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TABLE 1. Results of CTT with Leukemic Cells (cytotoxic index)

Patient	No. of placental serum	Whole serum (WS)	WS + anti-7S	7S	19S	Anti-7S	Anti-19S	7S + anti-7S	19S + anti-19S
A.	12			—					
V.	13	—	—	—	—	—	—	—	—
T.	14			—					
Shch.	15	—	—	—	0,44	0,49	0,26	—	0,39
Ya.	16	—	—	—	—	—	—	0,24	—
P.	17	—	—	0,22	—	0,55	0,66		
P.	18	—	—	0,22	0,51	0,63	0,66	0,86	0,83
P.	19	—	0,34	—	—	0,27	0,35	—	0,39
P.	20	—	—	—	0,44	0,49	0,26	—	0,39
M.	21	—	—	—	0,66	0,42	0,25	—	—
M.	22	—	—	0,28	—	—	—	—	—
Z.	23	Lysis	—						
Z.	24	"							

Legend. —) Cytotoxic index under 0.20.

For the investigation the direct and indirect complement-dependent cytotoxic test (CTT) of Gorer and O'Gorman were used in Lezhneva's modification [1,4]. The direct CTT was carried out as follows. Target cells isolated from heparinized blood were added to serological tubes at the rate of 400,000 cells per tube. The cells were incubated with the test fractions (0.1 ml per tube) at 37°C for 30 min. In the control the target cells were incubated under the same conditions but with Hanks's solution. Guinea pig complement (0.1 ml) was then added to all the tubes. Incubation with complement continued for 40 min at 37°C. The living and dead cells were counted with the aid of Trypan Blue in a Goryaev chamber. The cytotoxic index was calculated by the formula:

$$\frac{\%\text{ of dead cells in experiment} - \%\text{ of dead cells in control}}{\%\text{ of living cells in control}}$$

The results were considered to be positive if the cytotoxic index was higher than 0.20. For the indirect CTT, after incubation of the target cells with immunoglobulin fractions the cells were washed in 2 ml of Hank's solution and 0.1 ml antiserum was added to each tube. The tubes were incubated at 37°C for 30 min, after which (just as in the direct CTT) they were incubated with complement under the same conditions, the number of living and dead cells was counted, and the cytotoxic index was calculated.

EXPERIMENTAL RESULTS

Altogether 24 placental sera and their 19S and 7S fractions were studied by the cytotoxic test. Thirteen placental sera were tested on leukemic cells from eight patients and 11 placental sera and their 19S and 7S fractions were tested on leukocytes of 10 healthy blood donors (control).

In the control no human ELA could be detected on cells from the healthy donors. In every case the cytotoxic tests were negative. Leukocytes of one donor were tested with two sera and each sample of leukocytes from nine donors was tested with one placental serum.

As Table 1 shows, leukemic cells of five patients were tested once in the CTT. In three of them (A., V., and T.) the results were negative (it will be noted, however, that in two cases the investigation was carried out only with the 7S-fraction of placental sera). In two patients tested once only, positive results were obtained: in patient Shch. (serum No. 15) in the direct CTT with the 19S protein fraction and in patient Ya. (serum No. 16) in the indirect CTT with 7S + anti-7S (positive results of the indirect CTT were considered only if the results with the corresponding direct CTT were negative). In the latter case the placental serum could be presumed to contain noncytotoxic antibodies specific for ELA.

Leukemic cells of three patients (P., M., and Z.) were tested repeatedly with different placental sera (Nos. 17-24). In every case but one (patient P., serum No. 19), in which positive results were obtained only with the antisera, the results were positive in both variants of the CTT with the immunoglobulin fractions. It is interesting that different fractions of different sera gave a positive reaction with leukocytes from the same patient.

For instance, the 7S fraction was cytotoxic with cells of patient P. in serum No. 17, the 19S-fraction was cytotoxic in serum No. 20, and the 7S and 19S fractions in serum No. 18. When serum No. 21 was tested with the cells of patient M. antibodies belonging to the 19S immunoglobulin class were found, while the test with serum No. 22 revealed class 7S antibodies. Only whole placental sera (Nos. 23 and 24) were tested with the cells of patient Z. In both cases subtotal lysis of the leukemic cells was observed.

Besides the CTT in the above-mentioned modifications, numerous previous investigations undertaken by workers in the writers' laboratory showed that sera and their 19S and 7S fractions from healthy donors do not contain antibodies against leukemic antigens.

The results of these investigations suggest that a so-called embryoleukemic antigen is present on the surface of blast cells of patients with acute lymphoblastic leukemia; the antigen is detectable by the cytotoxic test with complement-dependent antibodies contained in placental blood. The specificity of this phenomenon is confirmed by the following considerations: 1) significant cytotoxicity is found only when placental sera and their 19S and 7S fractions and leukemic target cells are used (in both the direct and the indirect CTT); 2) the reactions were negative with a combination of cells from healthy donors and placental sera and their 19S and 7S fractions; 3) reactions also were negative with a combination of leukemic cells and sera and their 19S and 7S fractions from healthy blood donors.

Antibodies against an embryonic antigen can evidently accumulate in the serum of pregnant women during development of the fetus; in some women antibodies of the 19S-globulin type are evidently formed whereas in others the antibodies are of the 7S-globulin type; in some pregnant women both 19S and 7S antibodies are formed simultaneously; in one parturient woman no antibodies could be detected by the direct cytotoxic tests and they were revealed only by the indirect CTT.

These results correlate with data obtained by other workers mentioned above and indicating the possible existence of an embryonic-leukemic antigen.

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