DISCOVERY OF B CELL ANTIGEN ON BLAST CELLS OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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The presence of a common antigen on B lymphocytes of healthy blood donors and on myeloblasts of patients with chronic myeloid leukemia (CML) in the blast crisis (BC) stage was established with the aid of an antimyeloblast serum by the indirect surface immunofluorescence test. In the cytotoxic test this antigen was found on the blast cells of 27 patients with CML in the BC stage and in three of 11 patients with acute lymphatic leukemia, in one of eight patients with chronic lymphatic leukemia, and in both of two patients with undifferentiated leukemia. No antigen was found on the peripheral blood cells of healthy donors by this test.

KEY WORDS: chronic myeloid leukemia; antimyeloblast serum; B cell antigen.

The writers previously obtained a xenogeneic antimyeloblast serum (AMS) reacting specifically with the blast cells of 50% of patients with chronic myeloid leukemia (CML) in the blast crisis (BC) stage [1, 2].

The object of this investigation was to study the nature of this antigen.

EXPERIMENTAL METHOD

The scheme of immunization of a horse with peripheral blood leukocytes from a patient (male) with CML in the BC stage, absorption of the antiserum, and the procedure of the cytotoxic test (CTT) were described previously [3]. T lymphocytes, forming stable E rosettes with sheep's red blood cells treated with neuraminidase, were separated from B lymphocytes by centrifugation in a Ficoll-Hipac density gradient. The purity of the cell population was 95 and 40% for T and B lymphocytes respectively.

Titration of the B lymphocytes was carried out by determination of surface immunoglobulins by the direct immunofluorescence test (IFT), using standard rabbit serum against human globulins, labeled with FITC. The AMS, absorbed in doses of $5 \cdot 10^8$ and $5 \cdot 10^9$ cells/ml, were tested in the indirect surface immunofluorescence test.

TABLE 1. Reactivity of AMS in CTT with Cells from Healthy Blood Donors

Cells	Results of test*
Peripheral blood leukocytes	0/105
Granulocytes	0/13
Lymphocytes	0/13
T lymphocytes	0/13
B lymphocytes	0/13
Bone marrow cells	0/4
Fetal liver cells	0/3

*Here and in Table 2, numerator gives number of positive results, denominator total number of samples tested.

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TABLE 2. Reactivity of AMS in CTT with Peripheral Blood Cells of Patients with Various Forms of Leukemia

Form of leukemia	Results of test
Chronic myeloid leukemia in blast crisis stage	27/57
Chronic myeloid leukemia in chronic phase	0/16
Acute myeloid leukemia	0/13
Chronic lymphatic leukemia	1/8
Acute lymphatic leukemia	3/11
Undifferentiated leukemia	2/2
Acute erythroid leukemia	0/1

Leukemic cells were incubated with papain solution, used in concentrations of 0.18, 0.19, and 0.045%, previously mixed with an equal volume of 0.01% glutathione solution. Incubation was carried out at 37°C for 90 min in an atmosphere of 5% CO_2. The cells were tested 18 h later in the CTT.

EXPERIMENTAL RESULTS

Titration of the AMS in the CTT showed that the serum, absorbed by peripheral blood cells from 250 healthy blood donors in a dose of 40 million cells/ml, did not react with the peripheral blood cells of 105 healthy donors or with the lymphocyte and granulocyte fractions. This serum also did not react with adult human bone marrow cells or with the liver cells of a 24-week human fetus (Table 1). The AMS had a cytotoxic action on peripheral blood cells of 27 or 57 patients with CML in the BC stage, three of 11 patients with acute lymphatic leukemia (ALL), one of eight patients with chronic lymphatic leukemia (CLL), and both of two patients with acute undifferentiated leukemia (Table 2). The titer of the serum against cells from patients with CML in the BC stage was 1:32-1:128; the maximal cytotoxic index correlated clearly with the percentage of blast cells in the peripheral blood. Blast cells from patients with CML in the BC stage that did not react in the CTT did not acquire sensitivity to the action of AMS even after their treatment with papain, i.e., the areactivity of these cells was not due to masking of the surface antigens with sialic acid or blocking antibodies.

During cytochemical investigation of blast cells from the blood of 32 patients with CML in the BC stage it was found that in 12 cases the reaction of the cells to peroxidase and Sudan was positive (Group 1), but in 20 cases it was negative (Group 2). In the CTT the AMS reacted with blast cells of all patients of group 1 but with the cells of only four of 20 patients (21.7%) of patients in group 2.

Specific fluorescence of the membrane of B lymphocytes from the healthy donors under the influence of AMS was found in the indirect surface IFT. The fluorescence resembled a "cap" or continuous ring. T lymphocytes and granulocytes of healthy donors gave no fluorescence under the influence of AMS. Myeloblasts from patients of group 1 with CML in the BC stage (Sudan- and peroxidase-positive) gave clear fluorescence in the three tested cases under the influence of AMS, whereas fluorescence was not observed in preparations from peripheral blood of the patients of group 2 with CML in the BC stage (Sudan- and peroxidase-negative) which did not react in the CTT. Clear correlation was thus found between the results of the CTT and IFT.

Blast cells of patients with CML in the BC stage, reacting with AMS in the CTT, were characterized histochemically as Sudan-, peroxidase-positive, i.e., as myeloblasts. Meanwhile the blast cells of patients with AML, also characterized as Sudan- and peroxidase-positive, did not react with this antiserum. Of the 20 patients with CML in the BC stage with less well-differentiated blast cells (Sudan- and peroxidase-negative) a positive CTT was observed in only four cases. A common antigen was discovered by the indirect surface IFT on the surface of B lymphocytes of the healthy donors and peripheral blood myeloblasts of patients with CML in the BC stage.

A normal surface B cell antigen (or antigens) thus appeared on myeloblasts of patients with CML in the BC stage. This antigen was not detected on undifferentiated blast cells of patients with CML in the BC stage, on myeloblasts of patients with AML, and on promyelocytes, i.e., it was found only at a certain stage of differentiation of cells of the myeloid series.

It must, however, be emphasized that during the investigation of patients with ALL, in three of 11 cases the presence of a surface antigen testable with the aid of AMS was discovered in the CTT. The reason for this phenomenon is not yet clear, for cell markers were not studied in these patients. According to recently published observations a B cell antigen is present on the membrane of leukemic cells [3-7]. For instance, by using antiserum against B cell P 28,33 (Ia-like) antigen, Janossy et al., [5] found bright fluorescence in the

IFT on cells of patients with CLL, ALL, and a "lymphatic" variant of CML in the BC stage and moderate fluorescence in cases of AML and a "myeloid" variant of CML in the BC stage. This antigen also was present on myeloblasts from the bone marrow of healthy donors and of patients with myeloproliferative diseases. The B cell antigen was found on the surface of 90% of blast cells of patients with AML, but only in 15-20% of blast cells of patients with CML in the BC stage [8]. B-cell P23,30 (Ia-like) antigen was found on the surface of cells from patients with CLL, ALL, and AML [7]. This antigen was absent on blast cells of patients with CML in the BC stage. These workers conclude that this antigen may be an early-differentiating antigen. Their observations also indicate that blast cells of patients with AML are less differentiated than cells of patients with CML in the BC stage.

To counterbalance these investigations, in the present experiments a B cell (Ia-like) antigen was found on the surface of Sudan-, peroxidase-positive myeloblasts from the peripheral blood of patients with CML in the BC stage. This antigen could not be found on undifferentiated blast cells of patients with CML in the BC stage or on myeloblasts of patients with AML and promyelocytes of patients with CML in the chronic phase. It is an interesting fact that two variants of blast crisis could be distinguished in patients with CML by means of AMS. It is to be hoped that the identification of two different types of blast crisis in patients with CML will prove to be of therapeutic and prognostic importance.

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