DETECTION OF CELL-ASSOCIATED ANTIGEN IN A BLAST CRISIS OF CHRONIC MYELOID LEUKEMIA BY THE CYTOTOXIC TEST WITH XENOGENEIC ANTIBODIES

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An antimyeloblastic immune serum (AMS) was prepared by immunizing horses with white blood cells (WBC) from a patient with chronic myeloid leukemia (CML) in the blast crisis stage. The serum was exhausted in relation to antibodies against red and white blood cells of healthy donors. The AMS continued to have a cytotoxic action on blast cells from the blood of 20 of the 42 patients with CML in the blast crisis stage. The AMS did not react with WBC from the blood of patients with CML in the chronic phase or from patients with other forms of leukemia. Morphological observations indicate that the above-mentioned immune serum can be used to identify antigen associated with myeloblasts from the blood of patients with CML in the blast crisis stage.
KEY WORDS: chronic myeloid leukemia; blast crisis; myeloblasts; antimyeloblastic serum; cytotoxic reaction.

The question of the presence of surface antigens associated with leukemic cells remains a matter of dispute and active discussion [1-6]. In the investigation described below an attempt was made to detect such an antigen by means of the cytotoxic test.

In a previous investigation rabbits and goats were immunized with white blood cells (WBC) from a patient with chronic myeloid leukemia (CML) in the chronic phase. The xenogeneic sera thus obtained, exhausted against hemagglutinins and antigens of normal WBC, were found to have a cytotoxic action on peripheral blood cells of patients with CML and did not react with normal thymus and bone marrow cells and WBC [1]. The fact that the serum reacted better with WBC of patients in the blast crisis stage led to the decision to obtain a serum against these particular cells.

EXPERIMENTAL METHOD

Preparation of Antimyeloblastic Serum (AMS). Horses were immunized by four injections of a suspension of WBC from patients with CML in the blast crisis stage in a dose of $1 \cdot 10^{10}$-$1.7 \cdot 10^{11}$ cells per injection. The intervals between the first three injections were 7 days and between the third and fourth injections 14 days. Blood was taken from the horses 7 days after the end of immunization.

The serum was inactivated at 56°C for 30 min and absorbed initially by normal red blood cells (RBC) in a dose of $5 \cdot 10^9$ cells/ml to remove hemagglutinins and hemolysins, and then by a pool of normal WBC from the

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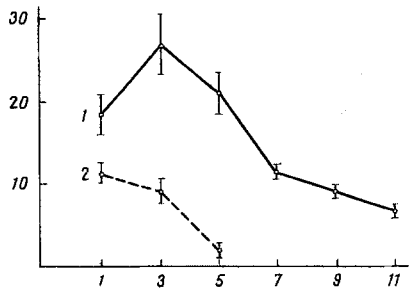

Fig. 1. Cytotoxic action of exhausted AMS on WBC of patients with CML in blast crisis stage and on leukocytes of normal donors (result of 31 determinations): 1) WBC of patients with CML in blast crisis stage; 2) normal WBC. Abscissa, $\log_2$ of dilution; ordinate, cytotoxic index.

peripheral blood of 200 healthy blood donors of groups I-IV in doses of between $4 \cdot 10^7$ and $3.5 \cdot 10^6$ cells/ml in order to remove antibodies against normal WBC antigens.

Performance of the Cytotoxic Test (CTT). In increasing dilutions (1/2-1/4096) AMS was poured into agglutination wells in a volume of 0.15 ml, and 0.05 ml of complement (normal rabbit serum, kept at −20°C) was added to each well. Complement was used in dilutions of $\frac{1}{2}$ or $\frac{1}{4}$, causing death of not more than 6% of the test WBC in a preliminary titration experiment. To the mixture of AMS and complement 0.05 ml of WBC in a concentration of $1 \cdot 10^7$ cells/ml was added. The mixture of AMS, complement, and cells was incubated at 37°C for 30 min, after which 0.25 ml of 0.25% trypan blue was added to each well and the number of dead (stained) and living (unstained) WBC was counted. The total number of WBC counted in the preparation was 100%.

Order of Testing of AMS with Various Cells. The AMS, absorbed with normal RBC and WBC, was tested for completeness of absorption in the CTT with normal WBC. Sera completely exhausted in relation to WBC were tested in the CTT with bone marrow cells of noncancer patients, obtained from the ribs or sternum of persons undergoing operations for pulmonary tuberculosis and cardiovascular diseases, with embryonic liver cells from a 24- to 26-week-old fetus obtained at therapeutic abortion, and with WBC from healthy donors. The exhausted AMS also was tested in the CTT with WBC from the peripheral blood of patients with CML in the blast crisis stage, which was the main part of the investigation.

Morphological Criteria of the Cytotoxic Action of AMS. For morphological identification of cells sensitive to the action of the serum, the number of separate populations of WBC was determined in a mixture of cells, serum, and complement taken in the ratio of 1 : 3 : 1 by volume, before and after incubation at 37°C for 30-60 min. Films stained with azure−eosin by Romanovsky's method were obtained from the mixture before and after the end of incubation and 200 WBC were counted in each film. The cytotoxic action of the serum on the various populations of WBC was determined from the difference in their number in the initial (before incubation) and final (after the end of incubation) preparations.

EXPERIMENTAL RESULTS

The AMS absorbed by WBC from the peripheral blood of healthy donors in a dose of $1.5 \cdot 10^8$-$2.5 \cdot 10^8$ cells/ml was completely exhausted in relation to normal WBC from the blood of the 65 donors tested. This AMS also did not react when tested with normal bone marrow and embryonic liver cells. Meanwhile, this serum had a cytotoxic action on the peripheral blood cells of 20 of the 42 (47.6%) patients with CML in the blast crisis stage (Figs. 1 and 2). A morphological study of the preparations obtained from a mixture of cells sensitive to the action of the cells, complement, and serum showed that the serum acted in a strictly definite direction on peripheral blood myeloblasts; on incubation of the mixture for 30 min a considerable decrease in the number of myeloblasts was observed in the preparations, whereas the number of the other cells (promyelocytes, myelocytes, juvenile cells, and so on) was virtually unchanged. The degree of the decrease in the number of myeloblasts correlated with the percentage of killed cells in the CTT.

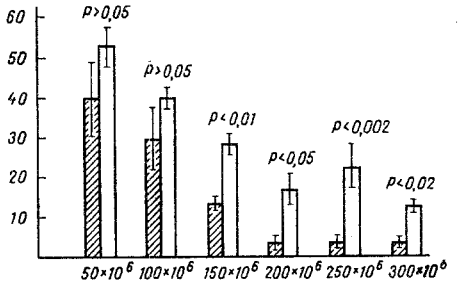

Fig. 2

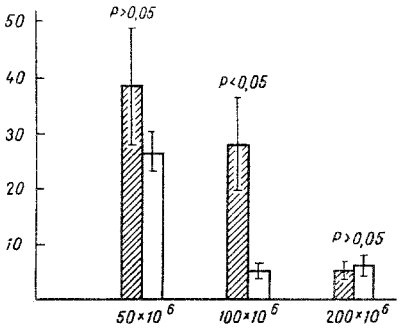

Fig. 3

Fig. 2. Cytotoxic action of AMS on WBC of patients with CML in blast crisis stage. Shaded columns indicate normal WBC; unshaded columns WBC of patients with CML in blast crisis stage. Abscissa, number of normal WBC taken for absorption of serum; ordinate, cytotoxic index.

Fig. 3. Cytotoxic action of AMS on WBC of patients with acute myeloid leukemia. Shaded columns indicate normal WBC; unshaded columns WBC of patients with acute myeoloid leukemia. Abscissa, number of normal WBC taken for absorption of serum; ordinate, cytotoxic index.

The myeloblastic sera mentioned above also were studied for the presence of antibodies against WBC from the peripheral blood of 12 patients with CML in the chronic phase and also of patients with other forms of leukemia (six patients with acute myeloid leukemia, five with acute lymphatic leukemia, seven with chronic lymphatic leukemia). In this case the sera did not react in the CTT against WBC of patients with the above forms of leukemia. In the course of the work it was noted that sera not completely absorbed with respect to normal antigens reacted to a much lesser degree with peripheral blood WBC of patients with acute myeloid and lymphatic leukemias than with WBC of healthy donors, possibly an indication of antigenic simplification of the patients' WBC (Fig. 3).

The results described above are evidence that an antigen could be detected by means of a specific AMS in the WBC of patients with CML in the blast crisis stage. This antigen, associated with leukemic cells, was possibly an antigen not present in normal WBC or present in them in minimal amount, not detectable by the cytotoxic test used. The suggestion cannot be completely ruled out that the "leukemic" antigen discovered may belong to the system of HLA antigens, forming a specific association on the surface of WBC of patients with CML that differs from the association of these antigens on WBC of healthy donors. However, this suggestion is not confirmed by the results showing that this particular antigen disappeared during a short remission and then was again detected in the CTT during a fresh relapse (blast crisis) in the same patient. The immune xenogeneic serum likewise did not react with leukemic cells of patients with acute myeloid leukemia or chronic and acute lymphatic leukemia in the stage of relapse and remission; this evidently indicated the absence of common antigens between leukemic cells of patients with these forms of leukemia and patients with CML in the blast crisis stage.

The absence of cross reactivity between WBC of patients with CML in the blast crisis stage and patients with acute myeloid leukemia may also be of practical importance, for attempts have been made to immunize patients with CML with WBC from patients with acute myeloid leukemia in order to prevent the blast crisis state [7].

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