ONCOLOGY

THE ANTIGENIC PROPERTIES OF THE RED CELLS IN LEUKEMIA *

COMMUNICATION I. DIFFERENTIATION OF THE ANTIGENIC PROPERTIES OF NORMAL AND LEUKEMIC RED CELLS BY THE SPECIFIC DELAY OF PRECIPITATION TEST IN AGAR USING ANTISERUM AGAINST LEUKEMIC SPLENIC TISSUE

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We showed in previous research that tissues with leukemic infiltration differ in their antigenic properties from the corresponding normal tissues. Antigenic differences between leukemic and normal tissues could be found both by the anaphylaxis with desensitization test [2, 3] and by the specific delay of precipitation test in agar [4, 5].

Proliferating leukemic cells may be found not only in the tissues but also in the blood stream of patients with leukemia. Products of destruction of these cells may also enter the blood stream.

There are reports in the literature of patients with leukemia developing an autoimune hemolytic anemia [7, 8, 10 and others]. The factor responsible for modifying the antigenic properties of the red cells and causing autoimmunization is still unknown [6].

Assuming that the antigenic properties of the red cells are altered by adsorption of products of destruction of leukemic cells with a definite antigenic specificity, we compared the antigenic properties of normal and leukemic red cells, using antiserum to leukemic splenic tissue for their differentiation. The antigenic properties of the red cells were studied by the Ouchterlony specific precipitation in agar test [7] and by the specific delay of precipitation test.

EXPERIMENTAL METHOD

The precipitation test was carried out at room temperature in Petri dishes with a diameter of 96 mm. The dishes were filled with 1% transparent agar containing 0.8% NaCl and a preservative – methiolate (1:10,000). The volume of agar poured into the dishes was 24 ml (7 ml for the bottom layer and 17 ml for the top). The antigens and antiserum were poured into holes made in the top layer of agar. The results were read on the next day and again on the 2nd-4th day.

The antisera used in the test were obtained by immunization of rabbits with antigens from the splenic tissue of persons dying from leukemia or from trauma. The immunizing antigens consisted of a saline extract of the tissues of the whole spleen, of the hyaloplasm fraction and a nuclear fraction. The hyaloplasm fraction was obtained by removal of the nuclei and the cytoplasmic granules from the homogenate of splenic tissue by centrifugation for 1 hour at 20,000 rpm, and the nuclear fraction by the method of Schneider and Petermann [10]. The rabbits were immunized with an additive as suggested by Freund. 4 cycles of immunization were carried out. Each cycle consisted of 4 injections at 5-day intervals: 1st injection – 33 mg of antigen protein with addi-

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tive (lanolin + vaseline with killed BCG) by Freund's method, the 2nd, 3rd and 4th injections without additive. The interval between the cycles was 6 weeks. Blood was taken in rising doses of antigen – 66, 99 and 132 mg – on the 7th, 14th and 21st day after the conclusion of the immunization cycle.

Antigens for the test were prepared from the red cells of patients with leukemia and from healthy donors of the same blood group (by the ABO and Rh systems).*

Differentiation of the Antigenic Properties of the Red Cells of Patients with Leukemia and Healthy Donors of the Same Blood Group by the Specific Delay of Precipitation Test in Agar

Details of tests	Patients with				Total	Healthy donors
	chronic lymphatic leukemia	chronic myeloid leukemia	acute leukemia (hemocytoblastosis)	aleukemia leukemia		
Total number of tests	91	132	36	34	293	167
Number of tests giving a positive result	79	124	35	24	262	0
Percentage of positive tests	87	94	97	70	89	0

The red cells were carefully separated from the leucocytes by preparation of a blood clot (the clot was washed three times with physiological saline and then the pale, upper part was cut away with scissors). The red cells were then suspended 1:5 in physiological saline at pH = 7.2, and homogenized in a Waring-type blender in the cold for 10 minutes. The homogenate was clarified by centrifugation at 10,000 rpm for 60 minutes. The precipitate was discarded. The supernatant fluid was preserved with merthiolate and was used as antigen. The protein content of each antigen in mg was determined. The antigens were stored in the cold. Before the experiment the antigens were diluted so that 1 ml of each antigen contained the same quantity of protein. Red cells of healthy donors of the same group were pooled before preparation of antigen.

In the performance of the specific delay of precipitation test in agar, an excess of antigen from healthy donors' red cells was injected in the first place.

In these conditions the antiserum gave no precipitation lines during the test with healthy donors' red cells. If the dose of antigen introduced into the agar was too small, only a part of the antigenic spectrum of the healthy donors' red cells was suppressed.

Equal doses of all the test antigens were used in the reactions.

EXPERIMENTAL RESULTS

During the performance of the specific precipitation in agar test with antiserum to leukemic splenic tissue and with antigens from the red cells of patients with leukemia and from healthy donors, it was found that the former gave a more complex precipitation spectrum than the latter.

This was shown particularly clearly by the specific delay of precipitation test in agar (Figs. 1-3).

The results of this test are summarized in the table. As may be seen from the table, during the examination of the blood of leukemia patients positive results were obtained in 262 of the 293 tests. The highest percentage of positive results was obtained in the tests on patients with acute leukemia (97), the lowest – but still considerable (70) – in the tests with aleukemic leukemia. The possibility of differentiation between the red cells of patients with aleukemic leukemia and of healthy donors by means of their antigenic properties is undoubtedly of great interest.

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The number of positive tests was related to the precipitation titer of the antiserum used in the reaction and to the spectrum of the antibodies which it contained. In our experiments the greatest activity was shown by the antiserum to tissue of the whole spleen, but the other two leukemic antisera also gave good results. The antserum obtained by immunization of rabbits with the nuclear fraction of leukemic splenic tissue, for instance, gave clearly positive results in the specific delay of precipitation test with antigens from red cells of patients with leukemia, whereas antiserum to the nuclear fraction of normal splenic tissue did not react in the delaying test with either the red cells of healthy donors or the red cells of patients with leukemia.

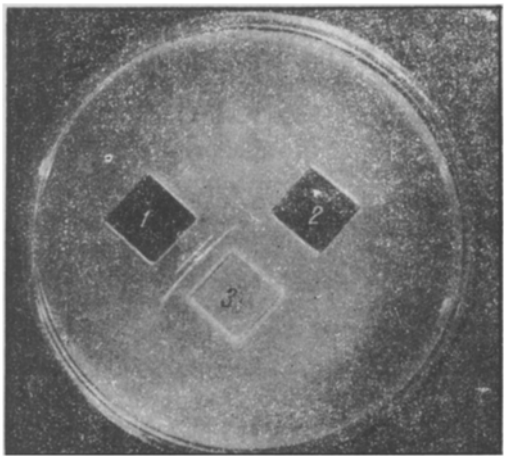

Fig. 1. Specific delay of precipitation test in agar with antiserum to leukemic splenic tissue and antigens from red cells of patients and healthy donors of the same blood group. The agar contained antigens from healthy donors' red cells: hole 1) red cells from patients with chronic lymphatic leukemia; hole 2) healthy donors' red cells; hole 3) antiserum to tissue from a leukemic spleen.

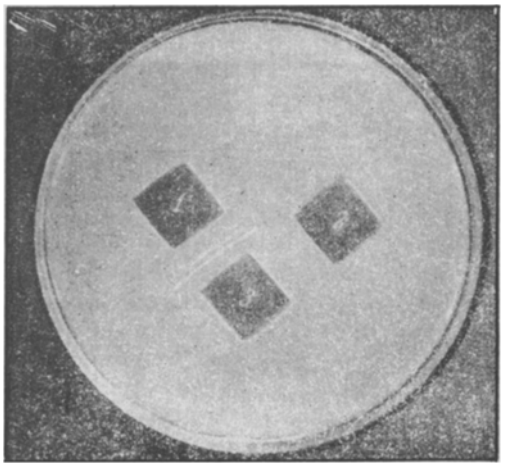

Fig. 2. The same as in Fig. 1. In hole 1) red cells from a patient with chronic lymphatic leukemia; hole 2) healthy donors's red cells; hole 3) antiserum to tissue from a leukemic spleen.

During the performance of the specific delay of precipitation test with antigens from patients with lymphogranulomatosis and with antiserum to tissue from the whole leukemic spleen, no positive results were obtained in any of the 12 tests. The red cells of a patient with the clinical diagnosis of "hypoplastic anemia", on the other hand, gave a clearly marked positive reaction (Fig. 3).

The investigations which we carried out showed that it is possible to differentiate between the red cells of patients with leukemia and those of healthy donors by means of their antigenic properties.

Fig. 3. The same as in Figs. 1 and 2. In hole 1) red cells from a patient with chronic lymphatic leukemia; hole 2) red cells from a patient with hypoplastic anemia; hole 3) red cells from a patient with an exacerbation of chronic myeloid leukemia, hole 4) healthy donors' red cells; the central hole contained antiserum to tissue from a leukemic spleen.

As a result of these investigations, many new problems arise: to what is due the change in the antigenic properties of the red cells in leukemia, what is the character of the changes, do the red cells of patients with leukemia acquire the power of causing the production of antibodies which will react specifically with the altered red cells, and are these changes peculiar to leukemia or do they occur in other neoplastic disease [1]. In subsequent research we shall endeavor to shed light on these problems.

SUMMARY

By their antigenic properties the erythrocytes of leukemic patients differ from those of healthy donors of the same blood group (by the ABO and Rh systems). These differences are revealed in the reaction of specific inhibition of precipitation in agar with the antiserum obtained by immunization of rabbits with the leukemic splenic tissue.

By inhibiting precipitation through introduction of surplus erythrocytes of healthy donors into agar, the antiserum would not reveal their full range (incomplete inhibition) or, in reacting with them, would not form any lines of precipitation at all. It was against this background that the lines of precipitation formed in reaction of the antiserum with the erythrocytic antigens of leukemic patients were especially pronounced.

In 262 tests out of 293 (in 89% of all cases) the erythrocytes of patients suffering from various forms of leukemia could be differentiated from those of healthy donors by their antigenic properties.

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