ANTIGENIC PROPERTIES OF THE RED CELLS IN LEUKEMIA

COMMUNICATION III. SPECIFIC INHIBITION OF PRECIPITATION REACTION IN AGAR ON A GLASS SLIDE USING RED CELLS FROM LEUKEMIA PATIENTS

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By means of Ouchterlony's specific inhibition of precipitation reaction in agar [4, 5], it has been possible to detect a difference in the antigenic properties of the red cells of healthy donors and of patients with acute leukemia, chronic lymphatic and myeloid leukemias and reticulosis [1-3].

The possibility of differentiation of the red cells from patients with leukemia and those from healthy donors has opened prospects for the use of this method as an accessory diagnostic test.

In order to simplify the technique and to obtain more rapid results, we decided to use the precipitation reaction in agar on a glass slide.

EXPERIMENTAL METHOD

The test was performed on ordinary glass slides, thoroughly freed from fat, with ground ends on which the number of the experiment was marked.

For the test we used 1% transparent agar, which was first freed from contaminants by precipitation with calcium chloride, filtration and washing for 72 hours in tap water.

One drop of molten sterile agar, with merthiolate preservative (1:10,000) was placed on the edge of a warm glass slide. A thin film was made, like a blood film. After the film had dried, a second layer of agar was applied, 1-2 ml in volume.

Next, by the use of a special metal drill, connected by a rubber tube to a glass end-piece, shallow holes of equal diameter were drilled in the coagulated agar. The drilling was done carefully in order to avoid damaging the lower layer of agar and the side walls of the holes. The agar fragments produced during drilling were removed by suction through the rubber tube.

The arrangement of the holes on the slide may be seen in Fig. 1.

The test was carried out in two stages: the first: into the central hole was poured the antigen from the healthy donors' red cells in a titrated dose; the second: into the same hole, after 4-13 hours (after complete diffusion of the antigen), was poured 0.01 ml of antiserum obtained after immunization of rabbits with red cells

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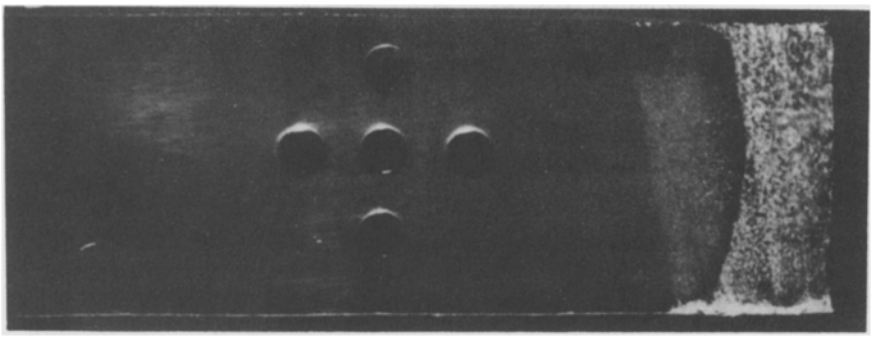

Fig. 1. Glass slide with holes drilled in the layer of agar.

from patients with leukemia, and into the peripheral holes were poured antigens from red cells of healthy donors and patients, also in volume of 0.01 ml.

All the antigen preparations were preliminarily standardized for protein content per ml. The dose of each preparation in an experiment did not usually exceed 0.1 mg of protein.

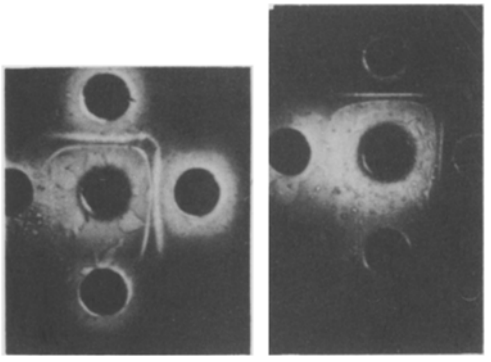

Fig. 2. Specific inhibition of precipitation reaction in agar with antiserum to red cells from patients with leukemia. In the agar are antigens from the red cells of healthy donors. In the holes: in the center — antiserum to red cells from patients with leukemia; in the top and right: antigens from red cells of a patient with chronic myeloid leukemia; antigens from the red cells of a patient with acute leukemia; in the bottom and left: antigens from the red cells of healthy donors of the same blood group.

Each antigen was tested on not less than 4 glass slides. The patients' red cells were compared with the healthy donors' red cells of the same blood group by the ABO and Rh systems. The slides were left at room temperature in a humid chamber. The results were read after 4-18 hours.

In all,34 patients with leukemia were tested (8 with acute leukemia, 6 with chronic lymphatic leukemia, 12 with chronic myeloid leukemia, 3 with aleukemic leukemia and 5 with reticulosis) as well as 36 healthy donors. Each specimen of blood was submitted to not less than 4 tests.

EXPERIMENTAL RESULTS

The antigenic spectrum of the red cells from both normal and leukemic patients was complex, and therefore in the differentiation of the antigenic properties of patients and healthy donors we recommend the use of the specific inhibition of precipitation reaction in agar. This reaction differs from the ordinary precipitation reaction agar, introduced by Ouchterlony [5], by the fact that antigen from the red cells of normal donors is preliminarily introduced into the agar.

With a properly selected dose, this permits total suppression of formation of precipitation lines in the agar during the reaction between the antiserum and the red cells of healthy donors. If under these circumstances the antiserum preserves its power to give precipitation lines with the red cells of patients with leukemia, the result is regarded as positive (Fig. 2, a and b).

In the table are shown the results of the specific inhibition of precipitation reaction on a glass slide.

As can be seen from the results in the table, in 119 of 138 tests (i.e., 86%) the red cells of the patients were clearly distinguished in their antigenic properties from the red cells of the healthy donors. The method of specific inhibition of precipitation on a glass slide may thus be used to differentiate the red cells of patients with leukemia and of healthy donors of the same blood group (by the ABO and Rh systems). The method is simple, requires small quantities of ingredients and gives quick results.

Specific Inhibition of Precipitation Reaction in Agar on a Glass Slide, with Antigens from the Red Cells of Patients with Leukemia and Healthy Donors

Tests	Patients with a diagnosis of						Healthy donors
	acute leukemia	chronic lymphatic leukemia	chronic myeloid leukemia	aleukemic leukemia	reticulosis	total	
Total number of tests	33	24	48	12	21	138	130
Number of positive tests	27	21	42	12	17	119	0
Percentage of positive tests	82	90	87	100	81	86	0

The character of the antigenic differences which we found is being studied. *

SUMMARY

The authors used the reaction of specific inhibition in agar on a slide by employing the antiserum for the red cells with high precipitating titer of patients suffering from leukemia. In 86% of the cases it was possible to reveal a difference in the antigenic properties of red cells obtained from the patients suffering from leukemia (acute leukemia, chronic myelo- and lympholeukemia, reticulosis and aleukemic leukemia) and in those of healthy donors with the same blood group (according to the ABO and Rh systems).

The method of specific inhibition of precipitation in agar on a slide enables one to quickly differentiate by their antigenic properties the red cells of healthy donors and those of the patients suffering from leukemia.

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** See English translation.