ANTIGENIC DIFFERENCES BETWEEN NORMAL AND LEUKEMIC TISSUES

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Considerable attention has recently been paid by researchers to the problem of leukemia. This is due, above all, to the rising incidence of the leukemias, the etiology and pathogenesis of which have still, unfortunately, received inadequate study. This explains the attempts that have been made to use immunological methods in the study of the antigenic structure of leukocytes, erythrocytes, and tissues in normal and leukemic subjects. The discovery of specific differences between leukemic cells and tissues and those from normal subjects would bring us nearer to the elucidation of the etiology of this disease, and would also give hope of the development of immunolo-ical methods of treatment of leukemia.

There are few reports in the literature dealing with the discovery and study of a specific antigen in leukemic tissues and cells. Steinberg and Martin [11] found no difference in the antigenic properties of normal and leukemic leukocytes by means of the leukoagglutination reaction. Similar results were obtained by Seligman and co-workers [10], using the method of precipitation in jelly, who found 3 or 4 specific components in both normal and leukemic leukocytes. The use of the method of diffusion in agar by Kemgold and co-workers [9] revealed only quantitative differences in the antigenic composition of normal and leukemic leukocytes. A. A. Rakityanskaya and S. S. Kharamonenko [7] were unable to differentiate between the leukocytes of patients with myeloid leukemia and healthy persons by means of the agglutination reaction.

More promising results were obtained by the study of the antigenic composition of the erythrocytes and tissues of patients dying from leukemia. In 1949 L. A. Zil'ber and V. A. Parnes [1], using the method of anaphylaxis with desensitization, discovered an antigen in the spleen of leukemic patients which was not present in the spleen of healthy persons. Further investigations conducted by V. A. Parnes [3, 6] showed that the specific antigen is present in other organs besides the spleen of leukemic patients: in the liver, kidneys, lymphatic glands, bone marrow, and also the erythrocytes. Parnes associates the specificity of leukemic tissue with at least two antigenic components, one of which is common to all forms of leukemia, while the other is specific for each individual form of the disease [4]. Besides the appearance of new antigens in leukemic tissues, the loss of certain typical antigenic components of normal tissues was also discovered [5].

M. O. Raushenbakh [8] confirmed Parnes' discoveries, and by means of the reaction of anaphylaxis with desensitization, demonstrated that the tissues and urine of patients with acute leukemia contained specific antigens not present in healthy subjects.

In the present research our object was to compare the antigenic properties of leukocytes and tissues from leukemic patients and healthy human subjects.

EXPERIMENTAL METHOD

The material studied consisted of the leukocytes from 25 patients with acute and chronic leukemia, and also tissue from the liver and spleen of 2 patients dying from leukemia. Control tests were made on the leukocytes (8 samples) and erythrocytes (10 samples) from healthy donors*, and tissues from the liver and spleen of 19 healthy persons killed in accidents. Both the experimental and control material was obtained from persons of different blood groups in the ABO system.

* We obtained the material from the leukemic patients and donors from the Central Institute of Hematology and Blood Transfusion through the courtesy of Dr. O. D. Tskhovrebova, to whom we express our gratitude.

The tissue antigens for the immunization of rabbits and for the complement fixation reaction were prepared by grinding the tissue in a mortar with the continuous addition of physiological saline in a volume of 20 ml/g tissue. After centrifugation of the suspension at 3500-4000 rpm the supernatant fluid was removed and used as an antigen. The antigens were prepared from the blood cells by freezing and thawing. The residue of leukocytes washed with physiological saline was frozen 3 or 4 times in a mixture of dry ice with alcohol, and thawed in a water bath at 55-57°. The leukocytes were then transferred to a mortar and thoroughly ground with physiological saline (10 ml of saline to 1 ml of leukocytes). Grinding of the leukocytes in the mortar was also accompanied by freezing and thawing for at least 3 times. The suspension was then centrifuged for about 30 minutes at 3500-4000 rpm. The supernatant fluid was used as antigen. When the erythrocytes were extracted, the stroma washed free from hemoglobin was used. In this case the technique of preparation of the antigen was indistinguishable from that described for preparing antigen from the leukocytes.

TABLE 1. Investigation of Antigens from Leukocytes of Leukemic Patients, Donors' Erythrocytes, and Tissues from a Healthy Human Subject

Serum	Dilution of serum	Antigens from leukocytes of leukemic patients		Antigens from erythrocytes of healthy donors of group			Antigens from tissues of a healthy human	
		M. A, β	N. O, α β	O, α, β	A, β	B, α	spleen (AB, O)	liver (AB, O)
No. 945 to leukocytes of leukemic patient M.	1:40	++++	++++	–	–	–	+	–
	1:80	++++	++++	–	–	–	±	–
	1:160	++++	++++	–	–	–	–	–
	1:320	+++	+++	–	–	–	–	–
No. 28 to leukocytes of leukemic patient N.	1:40	++++	++++	–	–	–	±	–
	1:80	+++	++++	–	–	–	–	–
	1:160	++	+++	–	–	–	–	–
	1:320	+	++	–	–	–	–	–
No. 4 to spleen of a healthy human subject	1:40	–	–	–	–	–	+++	–
	1:80	–	–	–	–	–	+++	–
	1:160	–	–	–	–	–	–	–
No. 1 to liver of a healthy human subject	1:40	–	–	–	–	–	+	++++
	1:80	–	–	–	–	–	–	++++
	1:160	–	–	–	–	–	–	++

Legend: ++++, +++, ++, +, ± denote different degrees of a positive reaction; – denotes a negative reaction.

In order to prepare specific sera, rabbits were immunized with antigens from the leukocytes and spleen of leukocytes and spleen of leukemic patients in accordance with the scheme which we previously used for obtaining antitumor sera [2]. Two groups of rabbits were used for immunization with leukocytes. The rabbits of one group were immunized with the leukocytes of patient M with chronic myeloid leukemia and of blood group A; the rabbits of the second group with leukocytes from patient N, of blood group O. Control organ-specific sera were produced by immunizing rabbits with saline extracts of the liver and spleen of healthy persons killed in road accidents.

The complement fixation reaction was carried out in a volume of 2.5 ml at 37°. The dose of antigen for the test was determined by preliminary titration in the presence of complement.

EXPERIMENTAL RESULTS

The serological study of the sera obtained by immunization with the spleen of the leukemic patient did not reveal any relative increase in production of antibodies to patient's spleen. The sera contained antibodies in high titers not only to patient's but also to normal spleen, and in addition they gave nonspecific fixation in a lesser titer

with antigens from the liver of healthy persons. The sera from rabbits immunized with leukemic leukocytes were more suitable. Treatment of these sera with normal formalinized tissues, by a method evolved in the laboratory [2], freed them from superfluous nonspecific antibodies, so that they could be used together with control organ-specific sera for the comparative study of leukemia and normal antigens.

Table 1 shows the result of one of the experiments in the comparative study of the antigens, and demonstrates that sera Nos. 945 and 28 gave a clear, positive reaction in dilutions to 1 : 320 with extracts from patients' leukocytes and did not react with antigens from donors' erythrocytes and healthy human tissues (liver and spleen). Organ-specific sera to liver and spleen fixed complement in the presence of homologous antigen.

We found no relationship between the results of the reaction and the blood group of the person from whom the tissues or cells were taken.

The experiments showed that leukocytes obtained from leukemic patients contain an antigen which is not found in the erythrocytes and tissues of healthy persons. However, the question of the specificity of this antigen is still undecided.

TABLE 2. Comparative Study of the Antigenic Properties of Normal and Leukemic Leukocytes

Serum	Dilution of serum	Antigens			
		from leukocytes of leukemic patients		from leukocytes of a healthy person	from healthy human liver
		M.	N.		
No. 945 to leukocytes of leukemic patient M.	1 : 80	++++	++++	++++	–
	1 : 160	++++	++++	++++	–
	1 : 320	+++	+++	++++	–
	1 : 640	+	±	++	–
No. 1 to healthy human liver	1 : 80	–	–	–	++++
	1 : 160	–	–	–	++++
	1 : 320	–	–	–	+++

Legend as in Table 1.

Experiments accordingly were undertaken in which, besides the antigens already mentioned, antigens from 8 healthy donors were also investigated. It can be seen from Table 2 that serum No. 945, obtained by immunization with leukocytes from a leukemic patient, reacted not only with antigens from leukemic leukocytes, but also with antigen from the leukocytes of a healthy person, while giving a negative reaction with antigen from healthy human liver tissue.

Similar results were obtained from tests of the leukocytes from other healthy donors.

Our experiments thus showed that the leukocytes obtained both from healthy persons and from leukemic patients contain antigens which are not present in the tissues of the liver and spleen and in erythrocytes, i.e., they possess a specific antigen structure peculiar to leukocytes. We were, however, unable to establish any qualitative antigenic difference between leukemic leukocytes and leukocytes from healthy persons.

SUMMARY

As shown by the complement fixation test leukocytes of healthy persons and those suffering from leukemia contain antigens absent in tissues of the liver spleen and erythrocytes of healthy individuals, i.e., possess their own specific antigenic structure peculiar only to leukocytes. However, no qualitative peculiarity was noted in the leukemic leukocytes as compared to the normal ones.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
