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EXPERIMENTAL MODEL FOR DEMONSTRATING LEUKEMIA-SPECIFIC ANTIGENS IN ACUTE HUMAN LEUKEMIAS

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The question of the existence of leukemia-specific antigens (LSA) is patients with acute leukemia is one of the most important in cancer immunology. To detect LSA, immunization of inbred animals with human leukemia cells followed by the study of the specificity of the developing antibodies, which may be directed not only toward the sought (LSA) antigens, but also to specific, tissue, differential, and so on, antigens, not to mention histocompatibility antigens which are also present in leukemia cells, is most frequently used [3, 6, 7].

All these factors create additional difficulties in the way of final interpretation of the results of the study and demonstration of the presence of LSA in a patient's leukemic cells.

In this paper we describe a new model for the demonstration of antigenic differences between leukemia cells and lymphocytes, by means of which we attempted to avoid these shortcomings.

EXPERIMENTAL METHOD

(CBA × C57Bl/6)F_1 mice were immunized intraperitoneally by two injections (with an interval of 10 days between them) of leukemia cells from the same patient with acute lymphoblastic leukemia, in a sessional dose of 10^8 cells per mouse. Serum was obtained 10 days after the last immunization of the animals. Intact mouse serum served as the control. The sera were tested for the presence of antibodies in the C'-dependent cytotoxic test (C'-CTT) in Terasaki plates parallel with leukemia cells used for imminuzation, and with lymphocytes obtained from the peripheral blood of the same patient during a remission (the method of isolating the cells was described previously [1]). The antibody titer in the sera was expressed as its final dilution causing 50% death of target cells. Immune sera were adsorbed with leukemia cells in the acute period and with "remission" leukocytes of the same patient in doses with which complete exhaustion of the serum from the corresponding antibodies was achieved (between 5×10^7 and 4.5×10^8 cells/ml). The conditions of adsorption were: incubation of cells in serum at 37°C for 20 min with shaking of the tube every 5 min,

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TABLE 1. Cytotoxic Activity of Sera

Target cells	Antibody titers in sera		
	before adsorption	after adsorption	
		by remission leukocytes	by leukemic cells
Leukemic cells	1 : 64	1 : 16	0
Remission lymphocyte	1 : 8	0	0

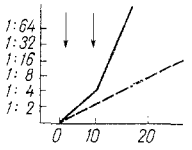

Fig. 1. Time course of cytotoxic antibody formation in mice immunized with leukemic cells. Abscissa, time from beginning of immunization (in days); ordinate, dilutions of serum. Continuous line, time course of accumulation of antibodies against leukemic cells; broken line — time course of accumulation of antibodies against remission lymphocytes. Arrows indicate times of immunization.

followed by separation of the serum from the dense cell residue by centrifugation of the suspension at 200g for 20 min.

EXPERIMENTAL RESULTS

The results showing cytotoxic activity of the mouse sera with leukemic cells and "remission" lymphocytes are given in Fig. 1. After reimmunization, the antileukemic activity of the serum was distinctly higher (1:64) than the antilymphocytic activity (1:8), and this provided a basis for the adsorption experiments.

Initially, to remove antilymphocytic antibodies its sera were adsorbed with "remission" leukocytes of the same patient. As a result, complete exhaustion of the serum from antilymphocytic antibodies was obtained (Table 1). The titer of antileukemic antibodies also fell at the same time, but the serum still retained residual cytotoxic activity toward autologous blast cells in a titer of 1:16.

To confirm the specificity of the residual antibodies in the serum, they were adsorbed with leukemic cells from the same patient in a dose of 5×10^7 cells/ml, which caused complete disappearance of the antileukemic antibodies (Table 1).

The results of these experiments thus show that the antigenic characteristics of leukemic cells and of lymphocytes in the genetically unified organism of patients with acute leukemias are not identical. Leukemic cells contain certain additional antigenic determinants on their surface which are absent on the patient's mature lymphocytes. These differences cannot be connected with histocompatibility antigens, for the target cells all belonged to the same patient. It was shown previously that the HLA-phentype of autologous leukemic cells and lymphocytes is identical and stable for each patient throughout his illness [2, 4, 5]. These differences cannot be connected either with species and tissue-specific antigens, for both these types of cells (both leukemic cells and "remission" lymphocytes) were tested simultaneously under the same conditions, as a result of which the hypothetical "extra" tests ought to have been exhibited against both types of cells. The most probable and most solidly

based view is that the antigens revealed are leukemia-specific antigens, characteristic of leukemic cells. However, under the experimental conditions used the possibility cannot be ruled out that they were antigens of yet another type — differential antigens, present on cells in the early stages of their development. The absence at present of methods of obtaining normal hematopoietic cells in sufficient quantities at the lymphopoietic precursor-cell stage prevents us from comparing the antigenic characteristic of blast cells in patients with acute leukemias with those solitary lymphoblasts which are present in the bone marrow of healthy subjects.

That is why the antigens demonstrated in the present experiments may be called leukemia-linked or leukemia-associated antigens, rather than leukemia-specific antigens, for they are characteristic of blast cells of patients with acute leukemias.

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