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MONOCLONAL ANTIBODIES AGAINST DIFFERENTIATING ANTIGENS OF HUMAN THYMOCYTES

A. Yu. Baryshnikov

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It has recently been found that monoclonal antibodies can be used to identify different levels of lymphoid differentiation and individual subpopulations of T lymphocytes. Common T-cell antigens [2, 4, 6], antigens of T-suppressors/cytotoxic cells [4, 7], of T-helpers/inducers [5, 7, 9], thymocytic antigens [8, 9], and so on, have been found. These antibodies are used both to study subpopulations and stages of differentiation of T cells and also for the immunodiagnosis of leukemia and lymphosarcoma.

The aim of this investigation was to obtain hybridomas producing monoclonal antibodies against differentiating thymocyte antigens, suitable for immunodiagnosis of leukemia and lymphosarcoma in man.

EXPERIMENTAL METHOD

Hybridomas were obtained by the method in [4]. BALB/c mice were immunized with human embryonic thymus cells treated with monoclonal IKO-1 antibodies against DR-antigens and IKO-5 antibodies against HLA antigens. One mouse received nine intravenous injections each of $2 \cdot 10^7$ cells in the course of 8 months, another received 3 injections in the course of 4 months. Splenocytes were fused with P3×63Ag653 cells with the aid of 50% polyethylene glycol with a molecular weight of 1500. After growth in selective medium and screening, the producing hybridomas were cloned twice by the limiting dilution method on feeder consisting of splenocytes and thymocytes from BALB/c mice. The antibodies were screened and tested in the indirect surface immunofluorescence test (IFT). The IFT with thymocytes was carried out on the cell suspension, and the other cells were attached to the glass by means of 50 μg of poly-L-lysine. The thymocytes were fractionated in an 8-step bovine serum albumin (BSA) gradient [3]; 10^9 thymocytes in 17% BSA solution were layered above a 19-33% BSA gradient and centrifuged at 1000g for 35 min at 4°C. More than 65% of the cells were contained in fractions 4-6. Early precursors of T cells and medullary thymocytes were sedimented in fractions 1-3, and cortical thymocytes in fractions 4-6 [3]. The fetal thymus was obtained during spontaneous abortion, the child thymus during open heart operations.

EXPERIMENTAL RESULTS

Two hybridomas producing monoclonal antibodies reacting with thymocytes from three 24-week-old fetuses and 9 children aged 5-15 years were obtained. Monoclonal IKO-11 antibodies reacted with 75.4 ± 1.6% of thymocytes, IKO-10 with 2.5 ± 0.06% (Table 1). IKO-10 antibodies reacted with 40-60% of thymocytes from fraction 1 of the BSA gradient (Fig. 1). Fractions 2 and 3, in which medullary thymocytes also were sedimented,

Laboratory of Clinical Immunology, Research Institute of Clinical Oncology, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 9, pp. 324-326, September, 1984. Original article submitted October 21, 1983.

TABLE 1. Reactivity of Monoclonal Antibodies in Relation to Cells from Normal Individuals and Leukemia Patients

Material tested	IKO 10		IKO-11	
	reactivity	per cent of antigen-positive cells	reactivity	per cent of antigen-positive cells
Thymocytes	12/12	2,5±0,05	12/12	75,4±1,6
Mononuclear cells from normal blood	0/20	0	0/11	3,5±0,9
Normal bone marrow	0/5	0	0/6	2,04±0,9
T lymphocytes	0/2	0	0/5	0
Granulocytes	0/11	0	0/8	0
B-CLL	2/9	15±3	0/11	0
ALL	3/28	59±26,7	3/23	59,3±20,3
Acute myeloblastic leukemia	0/5	0	0/4	0
Lymphosarcoma	0/5	0	1/4	86,5

Legend. Numerator – number of positive reactions, denominator – number of tests.

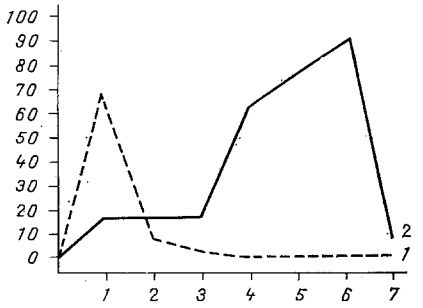

Fig. 1. Distribution of thymocytes reacting with monoclonal IKO-10 and IKO-11 antibodies in 8-step BSA gradient. Abscissa, number of antigen-positive cells (in percent); ordinate, fractions of BSA gradient (7 denotes residue). 1) Thymocytes containing IKO-10 antigen; 2) thymocytes expressing IKO-11 antigen.

contained single cells which reacted with IKO-10 antibodies. IKO-10 antibodies did not react with cortical thymocytes. Conversely, IKO-11 antibodies reacted only with a small percentage of cells in fractions 1-3 and recognized on 60-90% of cells in fractions 4-6 (Fig. 1).

IKO-10 antibodies did not react with peripheral blood mononuclear cells, T lymphocytes, granulocytes, or bone marrow cells from normal adults (Table 1). IKO-11 antibodies likewise did not react with T-lymphocytes and granulocytes, but revealed a small percentage of antigen-positive cells in bone marrow and peripheral blood. IKO-11 antibodies did not react with cells of 11 patients with B-cell chronic lymphatic leukemia (B-CLL). However, IKO-10 antibodies stained 12 and 18% of cells from 2 of 9 patients with B-CLL.

IKO-10 antibodies reacted with cells from 3 of 28 patients, and IKO-11 antibodies reacted with cells from 3 of 23 patients with acute lymphoblastic leukemia (ALL). Patients with ALL whose cells reacted with IKO-10 or IKO-11 antibodies had T-cell ALL. Under these circumstances, if IKO-10 antibodies reacted, there was no reaction with IKO-11 antibodies, and vice versa. IKO-11 antibodies reacted with tonsillar cells of a patient with T-cell lymphosarcoma. In this case also there was no reaction with IKO-10.

IKO-10 and IKO-11 antibodies did not react with cells from normal lymph nodes.

IKO-10 antibodies reacted with T-cell lines Molt-4 and SKCO-3 but did not react with cell line Reh-6. There was no reaction with K-562 erythroid cells, with plasmacytoma HMy2, with cells of normal fibroblasts of line NC87, and cells of mammary gland tumor MuTu and melanoma. IKO-11 antibodies reacted with none of the cell lines tested (Table 2).

TABLE 2. Reactivity of Monoclonal Antibodies Relative to Transplantable Cell Lines (percent of antigen-positive cells)

Line	Origin	IKO-10	IKO-11
Molt-4	T-ALL	90%	0
SKCO-3	T-ALL	60%	0
Reh-6	ALL (different from T- and B-ALL)	50%	0
K-562	Erythroblastoid cells	0	0
HMy2	Plasmacytoma	0	0
NC87	Normal fibroblasts	0	0
MuTu	Mammary gland tumor	0	0
MeWo	Melanoma	0	0

The tissue distribution of antigen determined by monoclonal IKO-11 antibodies is similar to that of antigen determined by monoclonal antibodies HT1 [8], OKT-6 [9], and D47 [1]. All these antibodies detect antigen of cortical thymocytes.

Monoclonal IKO-10 antibodies determine antigen on early precursors of T lymphocytes. The reaction of IKO-10 antibodies with Reh-6 cells and cells of patients with B-CLL is evidently not accidental, for Leu1 cells also recognize T-cell antigen on these same cells [10].

The different reactivity of IKO-10 and IKO-11 antibodies relative to thymocytes, T-cell lines, cells from patients with T-cell ALL, and lymphosarcoma is evidence that antigens are expressed on cells in different stages of differentiation. These antibodies can be used for the diagnosis of T-cell acute lymphatic leukemias and lymphosarcoma.

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