ICO-20 MONOCLONAL ANTIBODIES TO HUMAN THYMOCYTE T10 ANTIGEN

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The introduction of immunologic methods of investigation into clinical practice for the diagnosis of hemoblastoses has led to the discovery of heterogeneous forms among morphologcially similar leukemias. The isolation of immunologic subvariants of leukemia and lymphosarcoma is currently based on determination of differential antigens on the surface of the tumor cells with the aid of monoclonal antibodies (MCAB) by the immunofluorescence test [1].

A commonly used marker of leukemic cells is the T10(SD38) antigen, expressed on all thymocytes and also on activated T lymphocytes and plasma cells [6].

The aim of this investigation was to obtain MCAB to the differential T10 antigen of thymocytes.

EXPERIMENTAL METHOD

Strain ICO-20 of hybrid mouse cells in culture was obtained by somatic hybridization of mouse myeloma P3X63Ag 8.653 cells and spleen cells of BALB/c mice, repeatedly immunized with human thymocytes.

Hybridoma ICO-20 produces MCAB of the IgG 2a class. The class of immunoglobulins was determined by Ouchterlony's precipitation test with antisera to different classes of murine Ig ("Meloy Laboratories," USA).

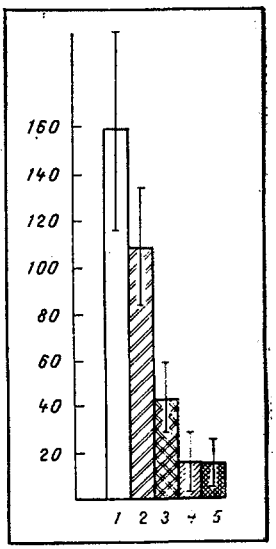

Fig. 1. Inhibition of formation of colonies of granulocytic-macrophagal series in agarized medium by ICO-20 MCAB. Ordinate, number of CFU per dish. 1) Intact control; 2) cells treated with complement; 3) cells treated with supernatant of ICO-20 hybridoma in dilution of 1:100; 4) cells treated with supernatant of ICO-20 hybridoma in dilution of 1:10; 5) cells treated with supernatant of ICO-20 hybridoma.

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TABLE 1. Reactivity of ICO-20 MCAB with Cells from Healthy Blood Donors (M ± m)

Type of cell	Number of positive cases	Number of donors tested	Frequency of expression of antigen, %	Percentage of antigen-positive cells
Thymocytes	20	20	100	68,7±4,7
Peripheral blood mononuclears	14	35	40,0	6,4±1,7
Granulocytes	0	20	0	0
T cells	5	18	27,8	7,0±1,6
Non-T cells	0	11	0	0
Monocytes	0	11	0	0
Normal bone marrow	0	3	0	0

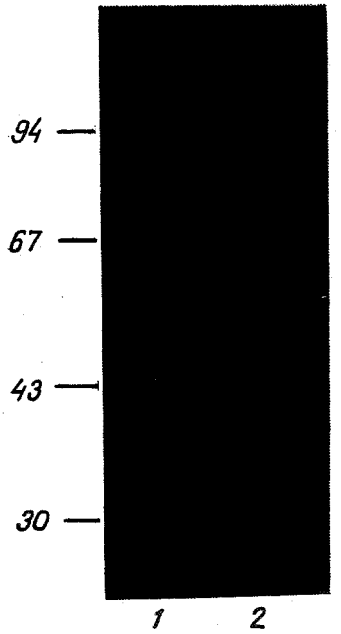

Fig. 2. Immunoprecipitation of T10 antigen by ICO-20 MCAB from human thymocytes labeled with ^{125}I. Numbers on left indicate mol. wt., kD. 1) ICO-20, 2) P3X63Ag 8.653.

Radioimmunoprecipitation was carried out with a lysate of ^{125}I-labeled thymocytes with the aid of lactoperoxidase ("Sigma") and H_2O_2 [5]. The thymocytes ($5 \cdot 10^7$ cells) were solubilized in 1 ml of lytic buffer containing 10 mM Tris-HCl, pH 8.1, 0.5% Nonidet P-40, 5 mM iodoacetamide, 1 ml of phenylmethylsulfonyl fluoride, and 0.14 M NaCl for 30 min on ice. Material solubilized by the detergent was centrifuged at 20,000g for 30 min at 4°C. The supernatant was incubated for 1 h on ice with protein A-sepharose 6MB ("Sigma"), covered with MCAB (0.1 ml of lysate of ^{125}I-labeled thymocytes to 0.2 ml of sepharose gel). The residue was washed 4 times with lytic buffer and dissolved in the course of 3 min at 100°C in "application buffer" (62 mM Tris-HCl, pH 6.8, 2% sodium dodecylsulfate, 5% 2-mercaptoethanol, 0.02% bromphenol blue, and 10% glycerol). Electrophoresis of the precipitates was carried out in a 5-20% polyacrylamide gel gradient in the presence of sodium dodecylsulfate in Laemmli's system [4]. The LMW kit ("Pharmacia," Sweden) was used as molecular weight markers. The gel was dried and exposed with ORWO HS11 radiographic film for 10 days at -70°C.

The specificity of the ICO-20 MCAB was determined in the indirect surface immunofluorescence technique (IFT) [2]. $F(ab)_2$-fragments obtained from commercial rabbit antiserum against albino mouse globulins, labeled with fluorescein isothiocyanate (N. F. Gamaleya Research Institute of Epidemiology and Microbiology), and adsorbed with liver powder, were used as labeled antibodies.

TABLE 2. Reactivity of ICO-20 MCAB with Cells from Patients with Leukemia and Lymphosarcoma (M ± m)

Diagnosis	Number of positive cases	Number of persons tested	Frequency of expression of antigen, %	Percentage of blast cells	Percentage of antigen-positive cells
T-1 ALL	1	3	33,3	88,2±8,2	82,0±0
T-2 ALL	4	4	100,0	77,4±12,3	40,0±12,8
T-3 ALL	4	5	80,0	81,7±6,6	40,8±4,6
"Total" ALL	1	17	5,8	97,0±1,4	55,0±0
Ia- ALL	0	6	0	77,1±11,8	0
Pre-B-ALL	1	8	12,5	92,3±4,0	37,0±0
B-ALL	0	1	0	100	0
CLL	0	10	0	0	0
AML	6	12	50,0	87,8±5,9	44,5±13,2
CML BC	4	11	36,4	59,3±15,1	31,5±2,7

Legend. CLL) Chronic lymphatic leukemia, AML) acute myoblastic leukemia, CML BC) chronic myeloid leukemia in the blast crisis stage.

Thymocytes were isolated from the thymus of children aged between 1 and 14 years, undergoing open heart surgery. Peripheral blood mononuclear cells were isolated from heparinized donated blood in a Ficoll–Verografin density gradient (d = 1.076 g/cm^3). Monocytes were isolated by adhesion to a plastic surface from a suspension of mononuclear cells. Mononuclear cells ($3 \cdot 10^7$) were incubated on Petri dishes 60 mm in diameter ("Falcon Plastics," USA) in medium RPMI-1640 ("Flow Laboratories," Great Britain) with 10% embryonic calf serum ("Flow Laboratories") and 0.005 M HEPES buffer. Incubation was carried out for 1 h at 37°C in an atmosphere of 5% CO_2.

T lymphocytes were isolated by the method of E-rosette formation with sheep's erythrocytes treated with neuraminidase. Granulocytes were isolated from a leukocyte suspension in a Ficoll–Verografin density gradient, and the granulocytes were withdrawn from the residue. Bone marrow cells were obtained by sternal puncture.

To determine the character of expression of the antigen revealed by ICO-20 MCAB on colony-forming cells, healthy human bone marrow was treated with ICO-20 antibodies and complement. Culture fluid with a titer of 1:100 was used as the antibodies, and a pool of nontoxic rabbit sera, diluted 1:4, as complement. Bone marrow cells ($5 \cdot 10^6$) were incubated with 0.5 ml of antibodies at 20°C for 30 min, after which 0.5 ml of complement was added and the mixture was incubated for 1 h at 37°C. Next the cells were washed 3 times and cultured in agarized medium. Colonies were counted under an inverted microscope and their composition studied morphologically [3].

EXPERIMENTAL RESULTS

ICO-20 MCAB reacted in IFT with thymocytes, to reveal 68.7 ± 4.7% of antigen-positive cells (Table 1). Antigen diagnosed by ICO-20 MCA was present in the peripheral blood of healthy blood donors in a small proportion of T cells. The antigen was absent on granulocytes, monocytes, non-T cells, and normal bone marrow cells.

ICO-20 MCAB caused lysis of 60.8 ± 4.2% of thymocytes in the complement-dependent cytotoxic test.

Antigen detected by ICO-20 MCAB was expressed on colony-forming bone marrow cells. Treatment of normal bone marrow with ICO-20 MCAB and complement inhibited the formation of colonies of the granulocytic-macrophagal series in three of three experiments (Fig. 1).

ICO-20 MCAB caused immunoprecipitation of an antigen with mol. wt. of 45 kD from a lysate of ^{125}I-labeled thymocytes (Fig. 2).

Expression of the antigen revealed by ICO-20 MCAB on blast cells from the blood and bone marrow of 85 patients with leukemia was studied. ICO-20 MCAB reacted heterogeneously

with the blood and bone marrow cells of leukemia patients. Antigen detected by ICO-20 MCAB was expressed on blast cells of one of three patients with the T-1 subvariant of acute lymphoblastic leukemia (ALL), of four of four patients with the T-2 subvariant of ALL, one of seventeen patients with the "total" variant of ALL, and one of eight patients with the Pre-B variant of ALL. ICO-20 MCAB did not react with the blast cells from six patients with the 1a-subvariant of ALL and one patient with B-cell All (Table 2).

Antigen revealed by ICO-20 MCAB was represed on blast cells of 6 of 12 patients with acute myeloblastic leukemia and 4 of 11 patients with chronic myeloid leukemia in the blast crisis stage. ICO-20 MCAB did not react with lymphocytes of 10 patients with chronic lymphatic leukemia.

Comparison of ICO-20 MCAB with W-155 MCAB by flow cytometry, carried out in the Monoclonal Antibodies Laboratory (Director, Dr. L. Baumsell), of the Hospital St.-Louis (Paris), confirmed their identity. It was found by flow cytometry that ICO-20 MCAB determine the antigen on 100% of thymocytes.

ICO-20 MCAB thus determine an antigen with mol. wt. of 45 kD expressed on thymocytes, and on a small percentage of peripheral blood T cells and blast cells of leukemia patients. It must be emphasized that expression of the antigen was confined to T-cell ALL. The character of expression of the antigen, its molecular weight, and the identity of the reaction with W-155 MCAB indicate that ICO-20 MCAB determine T10 antigen.

ICO-20 MCAB can be used to characterize blast cells of leukemic patients.

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