# ICO-13 MONOCLONAL ANTIBODIES TO DIFFERENTIAL ANTIGEN OF HUMAN HEMATOPOIETIC CELLS

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Differentiation of human hematopoietic cells can now be studied with the aid of a large number of monoclonal antibodies (MAB). MAB identifying common T-cell antigens SD5 and SD7 [1, 2], antigens of suppressor/cytotoxic T cells SD8, and of helper/inducer T cells SD4 [3], and thymocytic antigens SD1 and SD38 [4, 5], B-cell antigens, etc., have been obtained. These MAB can be used to study subpopulations and stages of differentiation of human hematopoietic cells, and for immunodiagnosis of human lymphoproliferative diseases.

The aim of the investigation was to obtain a strain of hybrid mouse cells in culture, producing MAB to differential antigens of hematopoietic cells.

## EXPERIMENTAL METHOD

Hybrid mouse cells of strain ICO-13 in culture were obtained by somatic hybridization of mouse myeloma P3X63Ag8.653 cells and spleen cells of BALB/c mice, repeatedly immunized with human thymocytes.

Hybridoma ICO-13 produces MAB of the IgM class. The class of immunoglobulins produced by hybridoma ICO-13 was determined by Ouchterlony's precipitation test with antisera to a class of mouse immunoglobulins (Meloy Laboratories, USA).

The specificity of ICO-13 MAB was determined in the indirect surface immunofluorescence test (IFT). $F(ab)_2$-fragments obtained from commercial rabbit antiserum to albino mouse globulins, labeled with fluorescein isothiocyanate (N. F. Gamaleya Research Institute of Epidemiology and Microbiology), and adsorbed with human liver powder, were used as labeled antibodies. Thymocytes were isolated from the thymus of children aged from 1 to 14 years, undergoing open-heart operations. Peripheral blood mononuclears were isolated from heparinized donated blood in a Ficoll-Verografin density gradient. Monocytes were separated from the suspension of mononuclears by adhesion to a plastic surface. T lymphocytes were isolated by E-rosette formation with sheep's red blood cells, treated with neuroaminidase. Granulocytes were isolated in a Ficoll-Verografin density gradient from a suspension of leukocytes, and the granulocytes were harvested from the residue.

## EXPERIMENTAL RESULTS

ICO-13 MAB reacted in ITF with 88.6 ± 3.4% of thymocytes from 26 of the 33 children, in 11 of 47 cases with 4.8 ± 1.8% of peripheral blood mononuclears from healthy donors, and in 13 of 25 cases with 7.1 ± 2.4% of peripheral blood T cells (Table 1). ICO-13 antibodies did not react with granulocytes, monocytes, and non-T cells from the peripheral blood of healthy donors. An antigen identified with ICO-13 MAB was absent on T cells activated by phytohemagglutinin (PHA).

Expression of the antigen on the surface membrane of the cells was labile. The antigen disappeared if the cells were kept at 4°C overnight or at −196°C in liquid nitrogen.

Adsorption of ICO-13 MAB by thymus gangliosides did not affect the reactivity of MAB. Treatment of the thymocytes with neuraminidase did not change antigen expression.

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TABLE 1. Reactivity of ICO-13 MAB with Blood Cells from Healthy Donors (M ± m)

| Type of cells | Number of positive cases | Number of donors tested | Frequency of expression of antigen, % | Percentage of antigen-positive cells |
|---|---|---|---|---|
| Thymocytes | 26 | 33 | 81,2 | 88,6±3,4 |
| Mononuclears | 11 | 47 | 23,4 | 4,8±1,8 |
| Monocytes | 0 | 11 | 0 | 0 |
| Granulocytes | 0 | 38 | 0 | 0 |
| T cells | 13 | 25 | 52,0 | 7,1±2,4 |
| Non-T cells | 0 | 10 | 0 | 0 |

TABLE 2. Reactivity of ICO-13 MAB with Cells from Patients with Leukemia and Lymphosarcoma (M ± m)

| Diagnosis | Number of positive cases | Number of patients tested | Frequency of expression of antigen, % | Percentage of antigen-positive cells |
|---|---|---|---|---|
| "Common" ALL | 0 | 10 | 0 | 0 |
| Ia-ALL | 3 | 8 | 37,5 | 26,0±11,0 |
| pre-B-ALL | 0 | 6 | 0 | 0 |
| B- ALL | 1 | 2 | 50,0 | 33,0±0,8 |
| T-1-ALL | 3 | 7 | 42,9 | 39,3±0,8 |
| T-2-ALL | 2 | 3 | 66,7 | 15,7±2,7 |
| T-3-ALL | 1 | 3 | 33,3 | 38,2 |
| T-I-LSA | 1 | 2 | 50,0 | 38,0 |
| T-3-LSA | 0 | 1 | 0 | 0 |
| Ia-LSA | 0 | 1 | 0 | 0 |
| B-LSA | 2 | 2 | 100,0 | 31,5±13,5 |
| "Common" LSA | 0 | 1 | 0 | 0 |
| CML BC | 0 | 15 | 0 | 0 |
| CLL | 0 | 10 | 0 | 0 |
| ANL | 4 | 21 | 19,0 | 31,4±9,1 |

Legend. Explanation in text.

Investigation of the immunologic phenotype of the blast cells in 92 patients with leukemia and lymphosarcoma (Table 2) showed that ICO-13 MAB reacted with blast cells of half of the patients with T- and B-cell acute lymphoblastic leukemias (ALL) and lymphosarcomas (LSA). The antigen was absent in chronic lymphatic leukemia (CLL) and in a blast crisis of chronic myeloid leukemia (CML BC).

However, ICO-13 MAB reacted with blast cells of 4 of 21 patients with acute nonlymphoblastic leukemia (ANL). ICO-13 MAB did not react with blast cells in the early stages of differentiation of B cells — "common" ALL and pre-B-ALL. No reaction of ICO-13 MAB with B cells could be found on frozen sections of reactive lymph nodes.

The molecular weight of the antigen could not be determined either by immunoblotting or by radioimmunoprecipitation.

The character of antigen expression is evidence that ICO-13 MAB determine a differential antigen that is expressed at a certain stage of T-cell (thymocytes) and B-cell (perhaps bone-marrow B cells) differentiation, and is absent in less mature and more highly differentiated T and B cells. Disappearance of the antigen from the cell surface during keeping and the absence of reaction with thymocytes in some cases indicate that ICO-13 MAB can recognize an antigen adsorbed from blood serum and bound with a certain receptor on the cell membrane, which appears at a certain stage of differentiation of T and B lymphocytes.

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