cause of exacerbation of the disease may be expected in such cases. Apheresis stimulation of immune antibody production seems to be largely due to the cyclic pattern of changes occuring during such treatment, namely, a drop of the antibody level resulting from a removal of immunoglobulins along with the plasma, followed by antibody hyperproduction due to reparative regeneration of removed plasma proteins. These data suggest that brief courses of apheresis may stimulate to the greatest degree production of existing immune antibodies.

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Monoclonal Antibody ICO-166 Against CD45RA Antigen

E. A. Frolova, A. Yu. Baryshnikov, V. V. Novikov, and A. V. Syrkin

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Antigens belonging to the CD45 family of common leukocyte antigens exist in four isoforms that have molecular weights of 170 to 220 kD and are expressed in a cell-specific manner. Antigens of this family are subdivided into two main clusters. Anti-CD45 monoclonal antibodies (mAb) recognize epitopes shared by all molecules of the family, whereas anti-CD45R mAb recognize limited epitopes present only on the higher-molecular-weight species that are predominantly expressed on B lymphocytes and on a T-cell subset [3,5,7]. During the Fourth Workshop on Human White Cell Differentiation Antigens, held in Vienna in 1989, CD45R antigens were assayed on transfectant cells using a panel of anti-CD45R mAb, which led to the separation of these antigens into CD45RA, CD45RO, and CD45B [7]. CD45RA is an isoform of the 220-kD common leukocyte antigen.

Since no Russian-manufactured mAb to CD45RA antigen exist, the purpose of this study was the

Oncology Research Center, Russian Academy of Medical Sciences, Moscow; and Research Institute of Epidemiology and Microbiology, State Committee for Sanitary and Epidemiological Surveillance, Nizhnii Novgorod. (Presented by N. N. Trapeznikov, Member of the Russian Academy of Medical Sciences)

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production of a mAb to this antigen and its characterization.

MATERIALS AND METHODS

mAb ICO-166 (IgG1) was obtained by immunizing a BALB/c mouse with T cells from peripheral blood of healthy human donors followed by fusion of its spleen cells with X63Ag8.653 myeloma cells. This mAb was tested in an indirect immunofluorescence assay (IFA) read in a Leitz fluorescence microscope and a FACScan cytofluorimeter (Becton Dickinson, USA). As mAb, a cell culture supernatant in 1:10 dilution and ascitic fluidsfrom hybridoma-carrying mice in 1:500 dilution were used. As the second antibody, F(ab)2 fragments obtained from a commercial FITC-labeled anti-mouse Ig rabbit serum were employed.

mAb ICO-1 (1 mg/ml), purified on protein A-Sepharose (Pharmacia, Sweden), was biotinylated with the N-hydroxysuccinimide ester of biotin dissolved in dimethyl sulfoxide in a concentration of 5 mg/ml. For this, the mAb and biotin ester were mixed in a 10:1 ratio by volume in 0.01 mol/liter phosphate buffer, pH 7.4, incubated with stirring for 1 h, and dialyzed against 0.01 mol/liter phosphate buffer, pH 7.4, containing 0.15 mol/liter NaCl. Streptavidin (obtained from the Ferment Company, Russia) was conjugated to FITC by mixing them in a 500:1 ratio by weight and keeping the mixture in bicarbonate buffer, pH 9.2, for 18 h, followed by dialysis against 0.01 mol/liter phosphate buffer, pH 7.4, containing 0.15 mol/liter NaCl.

The double-label assay used in this study has been described by us previously [2]. For the double labeling of cells, we used a FITC- or phycoerythrin(PE)-labeled mAb and also FITC- or PE-labeled isotype-specific anti-mouse Ig sera kindly provided by Dr. T. M. Trishman (USA). The procedures for competitive inhibition of antibody binding and for radioimmunoprecipitation assay have been described previously [1].

The effect of mAb ICO-166 on the lymphocyte blast transformation reaction (LBTR) to phytohemagglutinin (PHA) was tested using a mAb ICO-166 purified with a staphylococcal reagent

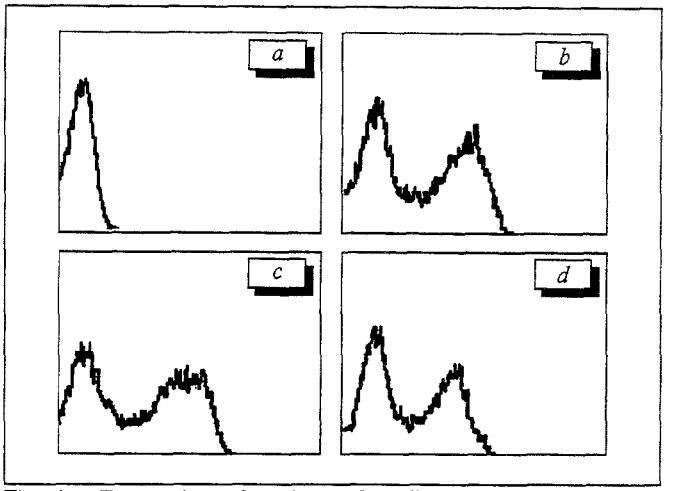

Fig. 1. Expression of antigen identified by mAb ICO−166 on human peripheral blood lymphocyte subsets *a*) T cells; *b*) B cells; *c*) T helpers; *d*) NK cells; *e*) NK and cytotoxic cells; *f*) T suppressors

(provided by the Pasteur Research Institute of Experimental Medicine, St. Petersburg). Activated lymphocytes were obtained by incubation of peripheral blood mononuclear cells from healthy human donors in 30 ml RPMI 1640 supplemented with 10% fetal calf serum, in the presence of 1 μg/ml PHA or 120 U/ml interleukin-2 (IL-2) or both. These cells were sampled at various times during the 10-day incubation period for the indirect IFA. On day 5, 120 U/ml of IL-2 were added to the culture vials. The activated cells were washed three times with PBS and used in the IFA.

RESULTS

In the indirect IFA read in the Leitz fluorescence microscope, mAb ICO-166 detected 36.2±3.3% of human peripheral blood mononuclears and did not bind to thymocytes or granulocytes. With cytofluorimetry antigen was identified on cell surfaces of cell lines derived from Burkitt's lymphoma (Raji, Daudi, Namalva, and EB3 cell lines) and also on those of the JY line (Epstein-Barr virus-transformed human lymphocytes). mAb ICO-166 also bound to the T-cell lines Jurkat (malignant non-Hodgkin's T-cell lymphoma) and MOLT4 (T-cell variant of acute lymphatic leukemia) and to

TABLE 1. Reactivity of mAb ICO−166, 4KB5, F8−11−13, HD28, and HD66 with Human Blood Cells

Cell type	ICO-166	4KB5	F8-11-13	HD28	HD66
Lymphocytes	54.1±1.9	54.3	51.2	18.3±3.7	18.0
Monocytes	15.2±2.3	17.9	19.7	29.2±4.3	–
Granulocytes	0.7±0.2	0.4	0.6	55.2±9.9	–
Platelets	1.0±0.7	0	0	6.8±0.2	–

Note. Here and in Tables 2 and 3 the values are percentages of antigen−positive cells (mean±SEM).

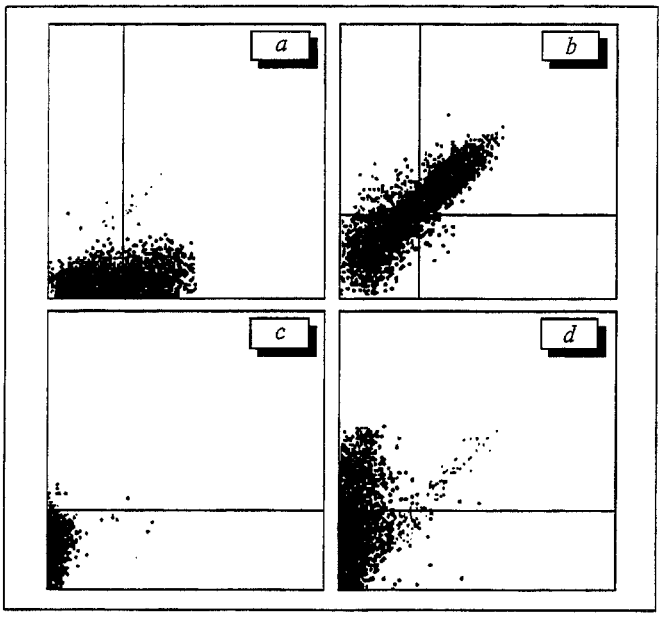

Fig. 2. Immunofluorescence profiles of human peripheral blood lymphocytes stained with anti-CD45R mAb. Abscissa: fluorescence intensity; *ordinate*: number of cells. a) control; *b*) 4KB5; *c*) F8-11-13; *d*) ICO-166

the monoblastoid cell line U937. It reacted weakly with a small percentage of cells from the pre-B-cell line Reh and failed to react with cells of the promyelocytoid cell line HL60.

In the indirect IFA read with the cytofluorimeter, mAb ICO-166 also reacted with 54.1±1.9% of human peripheral blood lymphocytes and with 15.2±2.3% of monocytes, but virtually failed to react with granulocytes and/or platelets from peripheral blood (Table 1). In normal human bone marrow, it detected 4.7% of antigen-positive cells. Analysis of its reaction with bone marrow cells using Paint-a-Gate software showed that ICO-166-positive cells occurred in the light-scattering areas of lymphocytes and late nucleus-containing erythroid cells but were absent from areas where precursor cells of various lines and differentiation lineages and levels were located.

Expression of the mAb ICO-166-identified antigen on human peripheral blood T and B lymphocytes, NK cells, and functional T-lymphocyte subsets was examined by the double labeling technique (Fig. 1), which showed that the mAb detected all B lymphocytes, all NK cells, and 31% of mature T lymphocytes. This antigen was carried by 55% of CD8-positive suppressor cells and by only 21% of CD4-positive helper cells (Fig. 1).

For the further characterization of mAb ICO-166, we compared the expression of the antigen detected by it with that of the antigens CD37, CD45R, and CD76 detectable by the respective mAb. These particular mAb were selected because

TABLE 2. Binding of mAb ICO-166 to Cells Activated by PHA, IL-2, or Both

	Days	NS1	ICO-166
PHA	0	5.3	56.9
	3	5.4	63.0
	5	4.8	50.0
	7	4.9	51.5
	10	7.7	24.5
IL-2	0	4.5	58.1
	3	5.8	68.1
	5	5.6	34.9
PHA+IL2	0	5.6	66.6
	3	7.9	69.5
	5	5.1	67.3
	7	6.1	72.0
	10	10.6	30.6

they have been reported to be reactive also with all B lymphocytes and a proportion of T lymphocytes [2, 4, 6]. The anti-CD76 mAb HD66 and anti-CD37 mAb HD28 bound to a much smaller number of T cells than did mAb ICO-166 (Table 1). In addition, mAb HD28 reacted well with granulocytes.

With human blood cells, mAb ICO-166 showed reactivity patterns similar to those of mAb 4KB5 and F8-11-13, which are directed against

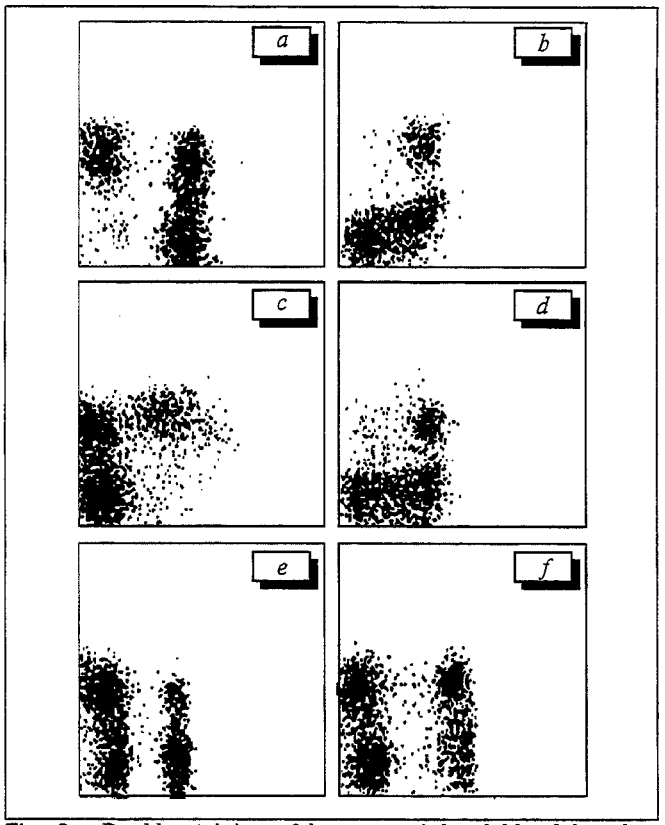

Fig. 3. Double staining of human peripheral blood lymphocytes with mAb ICO-166 and mAb ALB11 against CD45R antigen. *a*) ALB11-FITC; *b*) ALB11-FITC; *c*) aIgG-PE; *d*) ICO-166-aIgG-PE

TABLE 3. Expression of Human Leukocyte Differentiation Antigens in Patients with Chronic Lymphatic Leukemia

Case №	Lympho-cytes, %	Markers of human lymphocyte differentiation										
		HLA-DR	HLA-DQ	HLA-DP	CD19	CD20	CD45RA	CD3	sIgM	CD37	CD5	CD3
1	—	92	71	47	78	—	66	0	4	85	—	15
2	47	34	12	4	47	48	16	5	4	30	25	5
3	82	87	32	1	97	68	8	2	4	60	10	2
4	95	61	1	37	—	70	10	5	0	53	74	—
5	82	88	34	3	98	97	17	16	1	88	89	37
5	—	70	22	6	89	86	13	4	3	72	96	17
6	77	71	29	13	—	91	6	38	0	62	78	30
7	—	84	16	87	—	80	58	0	—	58	40	1
7	69	71	59	36	—	90	16	40	0	68	82	69
8	—	60	14	28	—	—	0	22	—	30	—	25
9	—	53	13	9	—	—	0	39	—	13	—	52

CD45R antigen. This finding, and also the similarities between the fluorescence profiles of human lymphocytes stained with these antibodies, suggested that mAb ICO-166 detects CD45R antigen (Fig. 2). Support for this came from the observation that the same cell population was identified among human lymphocytes by mAb ICO-166 and mAb ALB11, which is directed against CD45R (Fig. 3).

Tests were also run in which the binding of FITC-labeled mAb ALB11 with human lymphocytes was blocked by treating these cells with mAb ICO-166 in various concentrations. In control tests, mAb ALB11 and mAb F8-11-13 were added to human lymphocytes instead of mAb ICO-166 as positive controls, while mAb ICO-101, which is directed against rat cells, was added as a negative control. It was found that the treatment of human lymphocytes with mAb ALB11 blocked the binding of ALB11-FITC to these cells by 85% on average, and that their treatment with mAb ICO-166 and mAb F8-11-13 blocked the binding of ALB11-FITC by 66% and 68%, respectively. mAb ICO-101, which has the same isotype as mAb ICO-166, failed to block ALB11-FITC binding. The use of mAb ICO-166 in various dilutions demonstrated that the blocking of mAb ALB11-FITC binding by this monoclonal was dose dependent.

The purpose of the next stage in the characterization of mAb ICO-166 was estimation of the molecular weight of the antigen detected by it. ICO-166 immunoprecipitated a protein band of 220 kD from lysates of human peripheral blood mononuclear cells, and this finding, together with the observations that mAb ICO-166 and mAb F8-11-13, which is known to detect the CD44RA molecule, display similar patterns of reactivity with various types of human blood cells, and that these two mAb both block mAb ALB11-FITC binding to such cells, led us to conclude that mAb ICO-166 detects the CD45RA molecule.

Examination of mAb ICO-166 binding to activated cells demonstrated that the activation did not alter significantly the number of cells expressing the antigen detected by this antibody (Table 2). Not until the 10th day of incubation of the cells with the activating agent did the percentage of antigen-positive cells decrease. This result agrees with the report that the percentage of CD45R cells remains unchanged after a 3-day incubation with PHA.

Finally, we studied the expression of mAb ICO-166-detected CD45RA antigen on B cells from patients with chronic lymphatic leukemia (CLL). CD45RA antigens were found to be present on a proportion of cells in 7 out of the 9 CLL cases tested (Table 3). Lymphocytes from the patients all carried HLA-DR antigens, the pan-B-cell antigen CD19, and/or CD20, an antigen of mature B cells. This suggested to us that CD45RA antigens were acquired by cells somewhat later than HLA-DR, CD19, and CD20. That CD45RA appear at a discrete stage of B-cell differentiation and are not present on early B-cell precursors is also indicated by the very weak reaction of mAb ICO-166 with the pre-B-cell line Reh.

The results of this study thus indicate that mAb ICO-166 detects the human CD45R molecule and may therefore be used as an additional marker that can help in the differentiation of functionally distinct lymphocyte subpopulations in various immune disorders; it also may be usefully employed to study hemopoiesis in cases of hemoblastosis.

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Effects of Monoclonal Antibodies on Evoked Potentials and on the Development of Long-Term Posttetanic Potentiation of the Hippocampus

T. M. Pankova, M. V. Starostina, P. N. Lyuboslavskaya, V. I. Kharchenko, and M. B. Shtark

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Studies of "simple" neuronal systems have shown that monoclonal antibodies (mAb) can be used for analyzing elementary physiological functions. In studies where mAb were applied to examine the involvement of brain-specific proteins in the establishment of plasticity in the central nervous system, long-term potentiation (LTP) of the hippocampus after tetanization was used as a model for this purpose. Hippocampal LTP is thought to share common molecular mechanisms with long-term memory [11], and some information has also been reported on the role played by brain-specific proteins in the origin of the LTP phenomenon [7,8].

In this study, we tested mAb that had been screened by immunohistochemical assays for their ability to serve as markers of particular nerve cell structures with a view to detecting in them not only marker properties but also inhibitory properties at the level of electrical characteristics of the hippocampus.

Department of Medical and Biological Cybernetics, Institute of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

MATERIALS AND METHODS

To obtain mAb, BALB/c mice were immunized with the synaptosomal fraction of rat cerebral cortex isolated as described previously [2], introducing 150 μg of this antigen (in terms of total protein) per injection. For hybridization, the method described by Koller and Milstein [5] was employed, and the cells were cloned by the limiting dilution technique. The hybridomas were screened by dot enzyme immunoassay [3]. The antigens identified by mAb were detected in cryostated and paraffinized sections of rat brain, using second horseradish peroxidase-conjugated antibodies to murine Ig. As the substrate for the peroxidase reaction, 3,3'-diaminobenzidine was used, and the intensity of staining was enhanced by adding sodium chloride [4].

For electrophysiological tests, Wistar rats were fixed in stereotaxic apparatus under Nembutal anesthesia (50 mg/kg), and monopolar recording electrodes and exciting bipolar electrodes 150 μ in diameter made of steel wire in Teflon insulation, were implanted into their brain ventricles together

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