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ICO-45 MONOCLONAL ANTIBODIES TO A NEW EPITOPE OF CD38 ANTIGEN

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Monoclonal antibodies (McAb) against differential antigens of hematopoietic cells are widely used in the immunodiagnosis of hemoblastoses and in the study of fundamental problems in hematopoiesis [5]. New differential surface antigens of blood cells are continually being described and are broadening our ideas about the cell surface [3, 4, 6]. An important marker of leukemia cells is the T10 antigen, which was classed at the International Working Conference on Differential Antigens of Human Leukocytes with the CD38 cluster. In its structural organization, CD38 antigen very closely resembles antigens of the human class I major histocompatibility complex, but differs from it in the character of its distribution on hematopoietic cells. The T10 antigen is expressed on all thymocytes, null cells, NK cells, and also on activated T lymphocytes and plasma cells [7].

The aim of this investigation was to characterize ICO-45 McAb, directed against a new epitope of the CD38 antigen.

EXPERIMENTAL METHOD

Blood cells were fractionated by standard methods. Thymocytes were isolated from the thymus of children aged from 1 to 14 years, undergoing open heart operations. Expression of the antigen was investigated by the indirect surface immunofluorescence test (IFT) on living cells [2]. The reaction was read by means of a "light" fluorescence microscope and a "Spectrum 111" flow cytofluorometer. To study the effect of McAb on NK-cell activity, mononuclear cells from healthy blood donors were preincubated with McAb and rabbit complement, washed off, and used in the NK test against ^{51}Cr-labeled K-562 target cells. To determine the effect of the McAb on the blast transformation reaction, peripheral blood mononuclear cells from healthy donors were preincubated for 30 min with ICO-45 McAb, PHA was added, and the sample was then incubated for 48 h at 37°C in an atmosphere of 5% CO_2. ^{3}H-thymidine was then added. After 24 h the cells were transferred to filters and incorporation of ^{3}H-thymidine into the cell DNA was determined [1]. Competitive blockade was carried out with the aid of ^{125}I-labeled McAb and with human thymocytes, fixed with glutaraldehyde. The effect of McAb on secretion of active forms of oxygen by human neutrophils and

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TABLE 1. Reactivity of ICO-45 McAb with Cells of Healthy Blood Donors

Type of cells	Number of positive cases	Number of cases tested	Percentage of antigen-positive cells (M ± m)
Mononuclear blood cells	13	13	50,3±12,5
Granulocytes	5	5	95,7±3,0
T cells	5	5	46,2±5,2
Bone marrow cells	5	6	7,9±3,0
Thymocytes	5	5	77,1±12,0
Monocytes	3	3	68,1±8,7
Platelets	0	3	0
Erythrocytes	0	5	0

TABLE 2. Response of ICO-45 McAb with Blood Mononuclear Cells from Patients with Stages II and III of Breast Carcinoma

Group of subjects	ICO-45-positive cells, %	*p*	OCT4/OCT8
Blood donors (n=13)	50,3±12,5	—	2,0
Patients before treatment (n = 14)	29,3±3,9	<0,05	1,57±0,48
Patients 1-3 weeks after mastectomy (n = 5)	27,1±2,3	<0,05	1,70±0,77
Patients 3-4 months after mastectomy (n = 5)	53,2±1,9	>0,05	0,63±0,29

monocytes during phagocytosis was evaluated in phagocyte-dependent chemiluminescence. The molecular weight of the antigen was determined by radioimmunoprecipitation from a lysate of ^{125}I-labeled thymocytes.

EXPERIMENTAL RESULTS

ICO-45 McAb revealed the antigen on 50% of blood mononuclear cells from healthy donors, on 95% of granulocytes, 46% of T lymphocytes, 59% of non-T cells, 77% of thymocytes, and 68% of monocytes but did not bind with platelets or erythrocytes (Table 1).

Comparison of histograms of distribution of thymocytes and lymphocytes, stained with ICO-20 McAb against CD38 antigen and the histograms of distribution of these same cells, stained with ICO-45 McAb, obtained by the flow cytofluorometry technique, showed that they were identical (Fig. 1). However, the ICO-45 McAb reacted with granulocytes, whereas the ICO-20 McAb and their analogs did not bind with polymorphonuclear neutrophils.

ICO-45 McAb precipitated an antigen with mol. wt. of 45,000 daltons in the IFT from a lysate of ^{125}I-labeled platelets (Fig. 2), corresponding to the molecular weight of CD38 antigen. Preliminary adsorption of the ^{125}I-labeled platelet lysate with ICO-20 McAb abolished immunoprecipitation of the antigen by ICO-45 McAb. Meanwhile, preincubation of the lysate of ^{125}I-labeled platelets with ICO-45 McAb abolished immunoprecipitation of the antigen by ICO-20 McAb. These experiments showed that ICO-45 McAb are directed against the CD38 antigen revealed by ICO-20 McAb and their analogs.

The cross blockade experiment showed that ICO-45 did not block binding of ICO-19 McAb, directed abainst CD38 antigen, with the surface of ^{125}I-labeled platelets, whereas 8 other McAb to the CD38 molecule blocked binding of ICO-19 McAb. This indicates that ICO-45 McAb recognized an epitope of the CD38 antigen which differs from the epitope recognized by ICO-20 McAb and their analogs.

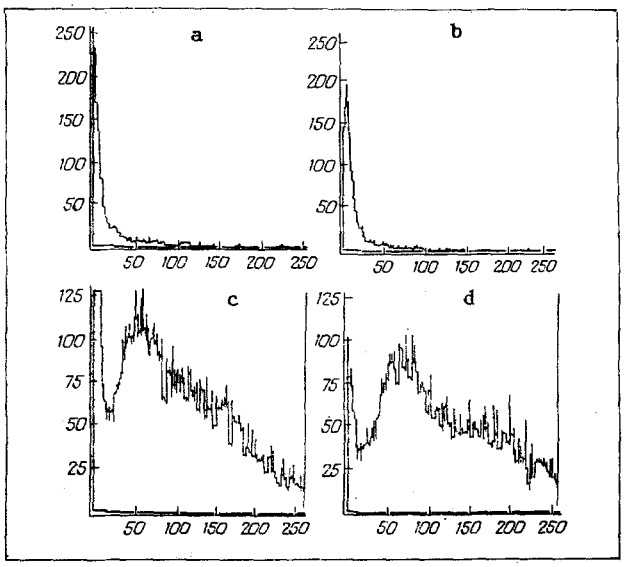

Fig. 1. Histogram of distribution of lymphocytes (a, b) and thymocytes (c, d), taken from healthy blood donors and stained with ICO-45 (b, d) and ICO-20 (a, c) McAb, relative to intensity of fluorescence. Abscissa, intensity of fluorescence; ordinate, number of cells.

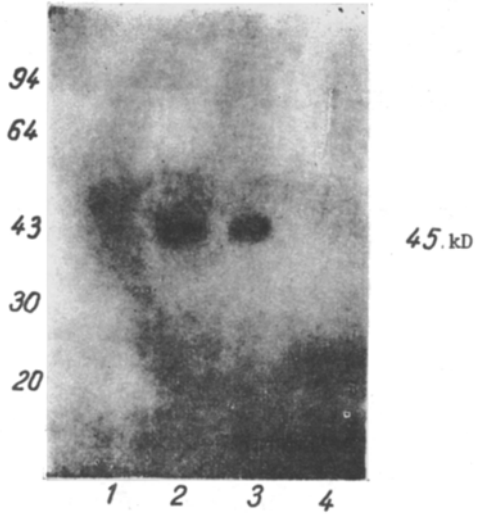

Fig. 2. Autoradiograph of electrophoretograms of precipitates of ICO-20 and ICO-45 McAb to CD38 antigen from ^{125}I-labeled human platelet lysate. 1) Immunoprecipitate of ICO-20 McAb after exhaustion of platelet lysate by ICO-45 McAb; 2) immunoprecipitate of ICO-45 McAb; 3) immunoprecipitate of ICO-20 McAb; 4) immunoprecipitate of ICO-45 McAb after exhaustion of platelet lysate with ICO-20 McAb.

In the presence of complement, ICO-45 McAb inhibited NK-cell activity of blood mononuclear cells for healthy blood donors. However, unlike the analogs, ICO-45 McAb blocked the blast transformation reaction of lymphocytes to PHA. It was also found that ICO-45 McAb inhibits the secretion of active forms of oxygen by human peripheral blood monocytes and neutrophils in the phagocyte-dependent chemiluminescence test by 28 and 62%, respectively. Meanwhile, ICO-20 McAb and their analogs did not affect the respiratory burst.

Expression of the antigen revealed by ICO-45 McAb on blood lymphocytes from patients with stages II and III of breast carcinoma were studied by flow cytofluorometry (Table 2). The percentage of ICO-45-positive cells was reduced statistically significantly in the patients before treatment and during the first weeks after removal of the tumor. The ratio OCT4/OCT8 also indicates the presence of immunodepression. From 3 to 4 months after the operation the percentage of ICO-45-positive cells was the same as in healthy subjects (Table 2). Meanwhile, the OCT4/OCT8 ratio may suggest immunodepression. However, OCT8 McAb determined two populations of T cells – suppressor and cytotoxic cells, which perform diametrically opposite functions: they inhibit immunity and kill tumor cells. Investigation of the lymphocytes of these patients by the aid of ICO-11 and ICO-20 McAb, which determines antigens to NK- and cytotoxic cells, showed that the number of cytotoxic cells, but not the number of suppressor cells, was in fact increased in the patients of this group. Thus, the true state of the immune response can be established with the aid of ICO-45 even without the use of additional methods of testing.

ICO-45 thus reveal a new epitope of the CD38 antigen expressed on thymocytes, granulocytes, and some lymphocytes and monocytes, but absent on platelets and erythrocytes. Blockade of the lymphocyte blast transformation reaction to PHA and of the respiratory burst of neutrophils and monocytes by ICO-45 McAb indicates the important functional role of CD38 antigens. The facts will be noted that OCT10 and ICO-20 McAb and their analogs have no inhibitory action on the functions of immunocompetent cells, although the presence of this antigen on effector cells of the immune system suggests that they have an important role in realization of the immune response.

We obtained ICO-45 McAb to the CD38 antigenic determinant which plays a direct role in function of the CD38 molecule. Heterogeneity in the expression of CD38 epitope may be due to different degrees of glycosylation of the CD38 antigen and also to the possible participation of glycolipids in the formation of the antigenic determinant. However, ICO-20 and ICO-45 McAb do not react directly with thymocyte glycolipids, extracted with methanol. Treatment of thymocytes with 25 mM sodium periodate reduced binding of ICO-20 and ICO-45 McAb with thymocytes.

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