MONOCLONAL ANTIBODIES TO MYELOID CELL ANTIGEN

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The surface structures of myeloid cells have been studied only comparatively recently with the aid of monoclonal antibodies (MCAB). However, even in this short time many MCAB to antigens of granulocytes have been obtained and characterized [1, 3]. Most of the MCAB which have been described have been characterized inadequately or with bias, and it is therefore difficult to compare these antibodies with one another and to state whether they reveal the same antigen or different antigens. MCAB to antigens of myeloid cells have been widely used to study functionally significant structures on the surface of myeloid cells, to detect subpopulation heterogeneity of neutrophils, and for the immunodiagnosis of nonlymphocytic leukemias [3]. The WHO Committee on Nomenclature distinguishes 15 clusters for differentiation of the antigens of human leukocytes, which include antigens of myeloid cells [4]. If this nomenclature is followed, MCAB ICO-G-2 belong to 15 clusters and can be described as CDw 15 (G, gp 100) ICO-G-2.

EXPERIMENTAL METHOD

BALB/c mice were immunized twice, intravenously, with leukocytes from the blood of a patient with acute myelomonoblastic leukemia (AMML) in a dose of $2 \cdot 10^7$ cells with an interval of 4 weeks. On the 4th day after the second immunization somatic hybridization of the spleen cells was carried out with mouse myeloma P3X63Ag 8.653 cells with the aid of 50% polyethyleneglycol, with mol. wt. of 1.5 kilodaltons (Schuchardt, West Germany). The hybridoma, passed through selective medium, was cultured in medium RPMI 1640 (Serva, West Germany) with 20% embryonic calf serum (ECS, from Flow Laboratories, England). The ICO-G-2 hybridoma, after being twice cloned by the limiting dilutions method, produced antibodies of the IgG 2a class. The class of antibodies was determined by the double radial immunodiffusion test after Ouchterlony [2] with antisera against classes of mouse immunoglobulins (Meloy Laboratories, USA).

Specificity of the ICO-G-2 MCAB was determined by the indirect surface immunofluorescence method. $F(ab')_2$-fragments obtained from commercial rabbit antiserum against albino mouse globulins, labeled with fluorescein isothiocyanate (N. F. Gamaleya Research Institute of Epidemiology and Microbiology), and absorbed with liver powder, were used as labeled antibodies. Granulocytes and mononuclears of healthy blood donors were isolated on a Ficoll–Verografin density gradient.

Reactivity of the ICO-G-2 MCAB with bone marrow cells was estimated by enzyme immunoassay on dried bone marrow films. The films were fixed in methanol for 10 min and then incubated in a solution of MCAB for 1 h at 37°C. The films were washed three times to remove excess of MCAB in 0.05% Tween in phosphate buffer, pH 7.2, and then incubated with conjugate (rabbit serum against albino mouse globulins, labeled with perioxidase). Films were again washed three times and exposed for 15-20 min at room temperature (in darkness) with freshly prepared peroxidase reagent (diaminobenzidine). After incubation the films were twice washed to remove excess of reagent and counterstained with azure-eosin.

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TABLE 1. Reaction of ICO-G-2 MCAB with Blood Cells from Normal Subjects and Blood/Bone Marrow Cells from Patients with Leukemia and Lymphosarcoma (M ± m)

Type of cells tested	Number of antigen-positive cases	Number of persons tested	Frequency of detection of antigen, %	% of antigen-positive cells
Polymorphs	11	11	100	90—100
Eosinophils	5	5	100	90—100
Healthy human blood mononuclears	0	16	0	2,1±0,6
Erythrocytes	0	5	0	0
Platelets	0	5	0	0
CML, chronic phase	27	27	100	47,6±5,3
CML, blast crisis	5	12	42	65,8±11,1
AML	9	28	32	54,3±7,2
AMML	2	12	16	57,5
AMonL	0	11	0	0
CMonL	0	4	0	0
ALL	4	49	8	69,0±5,7
B-CLL	0	10	0	0
Lymphosarcoma	0	31	0	0

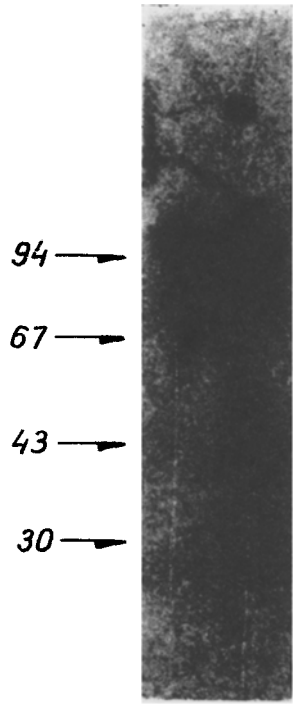

Fig. 1. Molecular weight of antigen revealed by ICO-G-2 MCAB. Ordinate, molecular weight (in kilodaltons). Autoradiography after SDS-PAGE of precipitate from lysate of ^{131}I-labeled granulocytes.

To discover the functional role of the antigen revealed by ICO-G-2 MCAB, rosette formation of peripheral blood granulocytes was carried out with sheep's red blood cells (SRBC), conjugated with C3b- and C3bi-fragments of complement (SRBC C3b and SRBC C3bi respectively) and with bovine erythrocytes loaded with IgG [6]. The SRBC C3b and SRBC C3bi conjugates were prepared by the method in [7, 9].

The molecular weight of the antigen revealed by ICO-G-2 MCAB was determined by the radioimmunoprecipitation test [8].

EXPERIMENTAL RESULTS

ICO-G-2 MCAB revealed an antigen with mol. wt. of 100 kilodaltons on the surface of peripheral blood polymorphs and eosinophils (Fig. 1). This antigen was not present on other peripheral blood cells (Table 1).

In normal bone marrow (patients with peptic ulcer) the antigen was expressed on polymorphs and stab cells, metamyelocytes, myelocytes, and promyelocytes. Because of limitations of the method it is difficult to speak of reactivity of the MCAB with myeloblasts. This antigen was absent on colony-forming cells of the granulocytic-macrophagal series. Among bone marrow cells, the antigen revealed by ICO-G-2 MCAB was expressed also on certain (27.3 ± 3.6) normocytes.

The functional role of this antigen is not clear, and all that we know is that it is not a receptor for C3b- and C3bi-components of complement and IgG, although this possibility cannot be completely ruled out because the antibodies may bind not with the binding site of these receptors, but with other antigenic determinants.

Testing the ICO-G-2 MCAB on cells of transplantable lines of different origin (T- and B-cell, "zero," erythro- and myeloblastic) showed that the MCAB react with cells of only three lines: K-562 (erythroblastic B-cell chronic myeloid leukemia — CML), Hα-1 (acute AMML), and Hα-60 (promyelocytic leukemia).

ICO-G-2 MCAB reacted heterogeneously with cells from patients with leukemia and lymphosarcoma. The G-2 antigen was found on cells from patients with nonlymphocytic leukemias. It was present in 32% of patients with acute myeloid leukemia (AML), in 16% of patients with AMML, in all patients with chronic myeloid leukemia (CML) in the chronic phase of the disease, and in five of 12 patients with CML in the blast crisis stage (BC CML). All patients studied with the myeloid variant of BC CML and one patient with erythroblastic BC CML were included in the group of antigen-positive cases (Table 1). The number of antigen-positive cells in these patients varied from 29 to 83%. The number of blast cells in the preparation as a rule was greater than the number of antigen-positive cells. The intensity of fluorescence of the cells varied not only in different patients, but also within the cell population of the same patient. These data are evidence of the heterogeneity of the blast cells of patients with nonlymphocytic leukemias with respect to expression of the G-2 antigen.

ICO-G-2 MCAB did not react with cells from patients with acute and chronic monocytic leukemias (AMonL, CMonL). In addition these MCAB did not react with blast cells from patients with lymphosarcoma, with lymphocytes from patients with chronic lymphatic leukemia (CLL), and with lymphoblasts from the majority of patients with acute lymphatic leukemia (ALL). It must be pointed out that in three of the four patients with ALL, whose blast cells carried the G-2 antigen, an undifferentiated leukemia was diagnosed on the basis of clinical and morphocytochemical investigations.

The characteristics described above thus indicate that ICO-G-2 MCAB reveal an antigen present on cells of the myeloid series and on some normocytes. Among the hemoblastoses, the MCAB react only with cells from patients with myeloid leukemias, so that these MCAB can be used for the differential diagnosis of leukemias and also to detect immunologically myeloid variants among leukemias undifferentiated by morphocytochemical methods.

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