ONCOLOGY

Isolation and Characterization of ICO-160 Monoclonal Antibodies to CD95(FAS/APO-1) Antigen That Mediates Apoptosis

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Isotype IgG2a monoclonal antibodies ICO-160, detecting CD95(Fas/APO-1) antigen, were isolated and characterized. They react with 26.8±15.6% donor lymphocytes in the indirect immunofluorescence test, do not react with granulocytes, erythrocytes, and platelets, and stain part of monocytes. Monoclonal antibodies ICO-160 induce apoptosis in CD95(Fas/APO-1)-positive cells.

Key Words: *apoptosis; monoclonal antibodies; double staining; DNA*

CD95(Fas/APO-1) antigen that mediates apoptosis was discovered at two laboratories using anti-Fas and APO-1 monoclonal antibodies (MAb) inducing apoptosis in some human cells [6,7]. Monoclonal antibodies anti-APO-1, anti-Fas, 7C11, and IPO-4 are referred to CD95 differentiation cluster. These MAb specifically react with murine 300-19 cells transfected with cDNA coding for human CD95 antigen and do not react with nontransfected cells. CD95 antigen is a member of the tumor necrosis factor superfamily including nerve growth factor and CD27, CD40, and OX40 antigens. CD95 antigen is a receptor for Fas ligand, a member of the ligand superfamily. CD95 (Fas/APO-1) antigen was detected in many normal and tumor cells [2,3,5]. The capacity of MAb to induce apoptosis attracts the attention of researchers to this antigen.

We isolated MAb to CD95(Fas/APO-1) antigen and studied their specificity.

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MATERIALS AND METHODS

Hybridoma producing ICO-160 MAb was obtained by somatic hybridization of splenocytes from mice immunized three times with phytohemagglutinin-activated donor lymphocytes with NS1 myeloma cells. After double cloning by the limiting dilutions method, a hybridoma, producing isotype IgG2a MAb denoted ICO-160 was obtained.

ICO-160 MAb were derived from ascitic fluid of mice with hybridomas in dilution 1:500 and MAb purified from ascitic fluid. The second antibody for the immunofluorescence test was fluorescein isothiocyanate (FITC)-labeled sheep antiserum to albino mouse immunoglobulins (MedBioSpektr Center, Moscow). Reference anti-CD95 MAb were IPO-4 MAb [4], a gift from Prof. D. F. Gluzman (Kiev), and 7C11 MAb. In the double staining test, MAb IPO-4 and 7C11 were stained with isotype-specific antimurine IgM-FITC conjugate (Sigma) and biotin-treated MAb ICO-160 with Streptavidine-Phicoerythrine (Str-PE, Sigma).

MAb were isolated from ascitic fluid on sepharose A as described previously [3]. Purified MAb

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were treated with biotin N-hydroxysuccinimide ester dissolved in dimethylsulfoxide in a concentration of 5 mg/ml.

Indirect superficial immunofluorescence and double staining tests were carried out as described previously [1]. The immunofluorescence test was read on a FACScan flow cytofluorometer (Becton Dickinson).

Raji cells (10^6) were incubated with purified ICO-160 MAb in a concentration of 10 μg/ml and IPO-4 MAb in a final dilution of 1:100 for 24-72 h at 37°C.

The percentage of apoptotic cells was determined by the cytofluorometric method for measuring hypodiploid DNA stained with propidium iodide (PI, Sigma). 2.5×10^5 cells were washed in phosphate buffer and resuspended in coed 70% alcohol for 1 h, after which the cells were precipitated by 7-min centrifugation at 500 rpm. Then the cells were carefully resuspended in 1 ml hypotonic fluorochromium solution (5 μg/ml PI, 0.1% sodium citrate, and 0.1% Triton X-100) and incubated in the dark for 15 min. Fluorescence of PI-stained DNA was measured by FACScan without further washing.

RESULTS

At the first stage of MAb characterization we compared the percentage of antigen-positive cells and histograms of immunofluorescent distribution of cells, stained with ICO-160 and IPO-4 MAb directed against CD95 antigen. Both MAb reacted with normal human blood lymphocytes detecting 26.8±15.6% (n=59) and 22.3±12.6% (n=56), respectively, and did not react with granulocytes, platelets, and erythrocytes. MAb ICO-160 reacted with 19.1±10.7% thymocytes of children (n=20) and MAb IPO-4 with 13.6±9.7% (n=21). Analysis of reactivity of MAb ICO-160 and IPO-4 with continuous cell strains showed that they react with T cells Jurkat, CEM, YT, B cell Raji strain, and monocytoid THP-1 strain and not react with T cell Molt-4 strain, monoblastoid U937, and erythroblastoid K562 cells. Histograms of immunofluorescent distribution of cells detected by ICO-160 MAb were similar to histograms of distribution of cells stained with MAb IPO-4 (Fig. 1).

At the next stage of MAb ICO-160 characterization double staining was used. MAb ICO-160 and IPO-4 stain the same population of blood lympho-

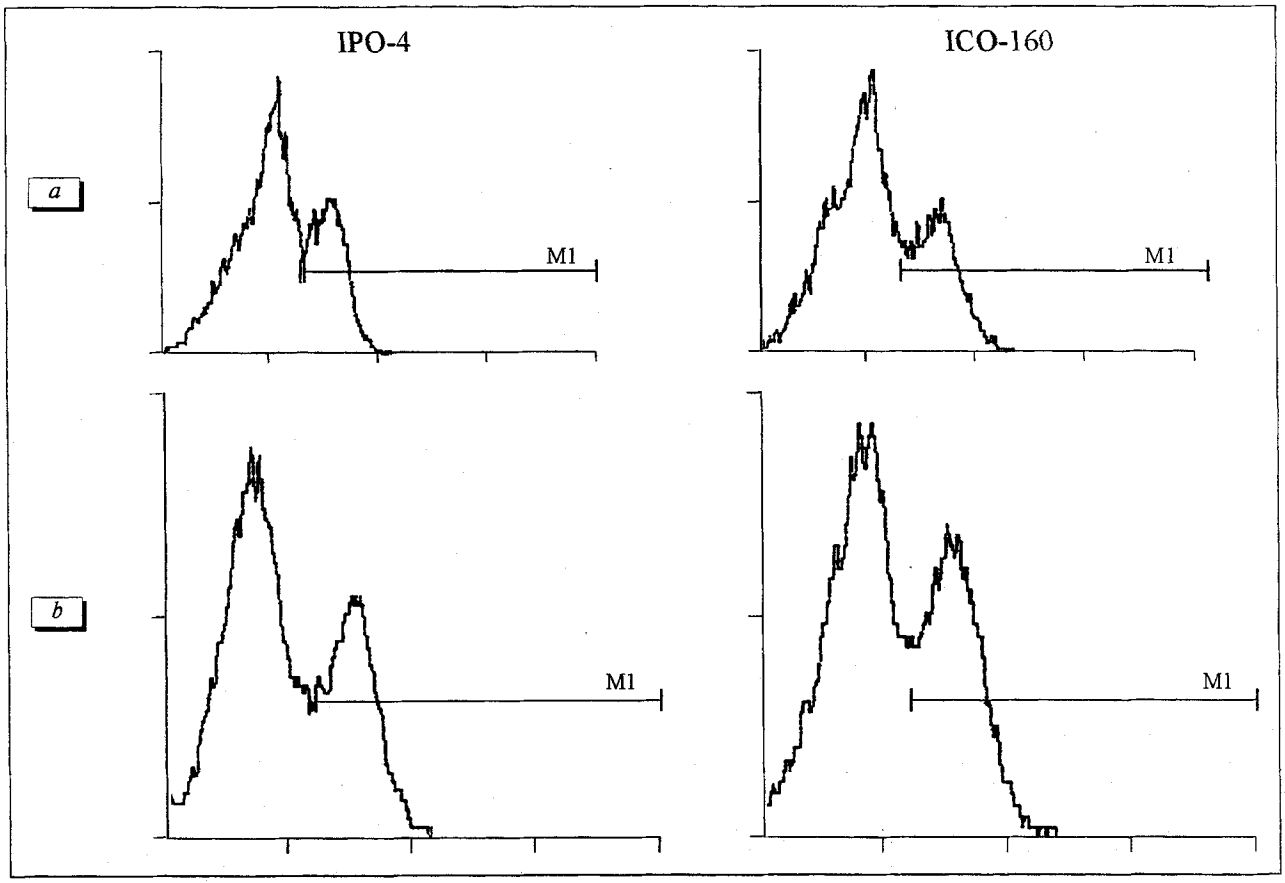

Fig. 1. Fluorescence profiles of cells stained with monoclonal antibodies ICO-160 and IPO-4. *a*) blast cells from patient K. with chronic myeloleukemia, blast crisis; *b*) leukemic cells from patient Kh. with acute lymphoblastic leukemia. Ordinate: cell number; abscissa: intensity.

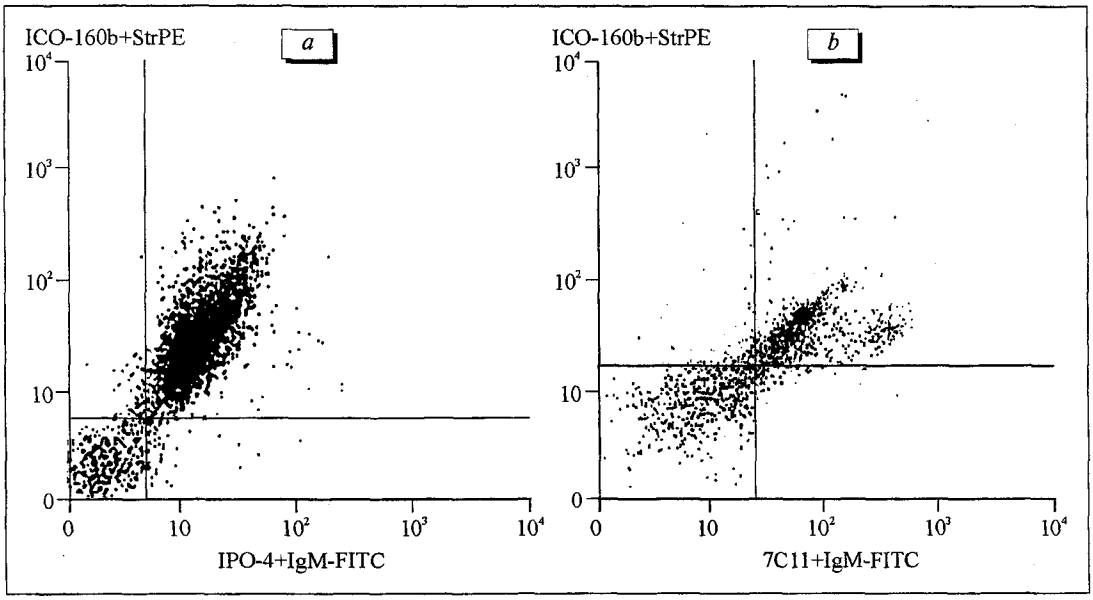

Fig. 2. Double staining of lymphocytes from patient with multiple myeloma by monoclonal antibodies (MAb) ICO-160 and IPO-4 (*a*) and of donor lymphocytes by MAb ICO-160 and 7C11 (*b*).

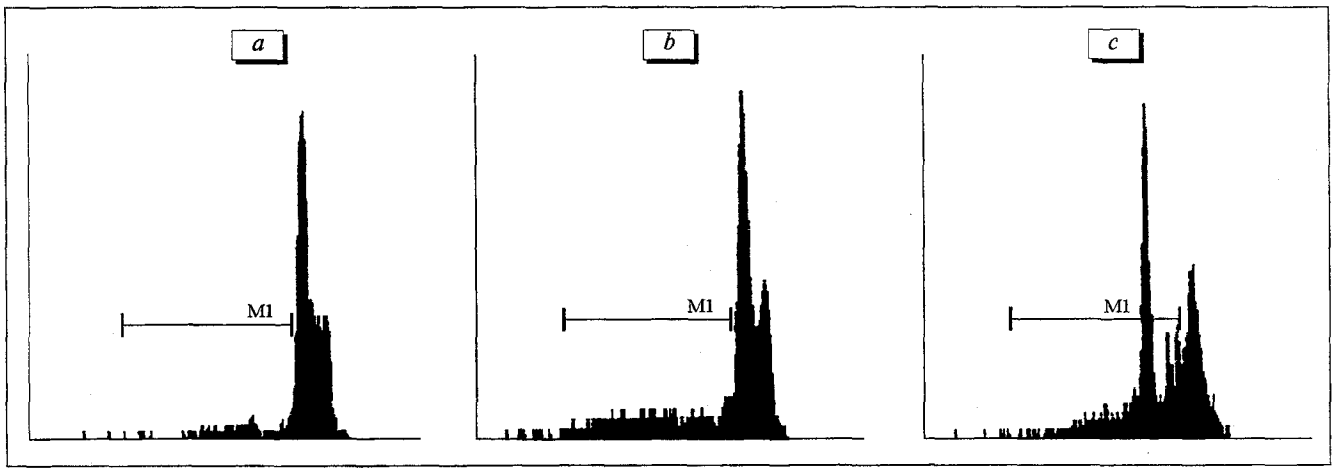

Fig. 3. Apoptosis induction in continuous Raji cells by monoclonal antibodies IPO-4 (*c*) and ICO-160 (*b*) and in the control (*a*). M1: hypodiploid zone.

cytes localized in quadrant 2. There were no cells stained with MAb ICO-160 or IPO-4 alone (Fig. 2, *a*). Similarly, ICO-160 and 7C11 MAb, directed against CD95 antigen bound to the same blood lymphocytes (Fig. 2, *b*). An important property of MAb to CD95 (Fas/APO-1) antigen is the capacity to induce apoptosis. We induced apoptosis in B cell Raji strain by MAb ICO-160, IPO-4, and 7C11. Apoptosis was assessed by changed content of hypodiploid DNA, which was measured by flow (FACScan) cytofluorometry. Raji cell incubation with MAb ICO-160, IPO-4, or 7C11 induced apoptosis of 30-60% cells on day 3 in comparison with 7-10% spontaneous apoptosis (Fig. 3).

Therefore, MAb ICO-160 are similar to MAb IPO-4 and 7C11 to CD95(Fas/APO-1) antigen. They detect similar percentage of antigen-positive cells, their histogram distributions are similar, and they react with the same cell population. Incubation of MAb ICO-160 with antigen-positive cells induced their apoptosis. These signs permit a conclusion that MAb ICO-160 are directed to CD95(Fas/APO-1) antigen mediating apoptosis.

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