A milder course of experimental staphylococcal infection was thus observed in guinea pigs undergoing splenectomy followed by autografting of the decapsulated splenic fragments.

Culturing a homogenate of transplanted splenic fragments after 24 h revealed more than $3 \cdot 10^3$ CFU/g of staphylococci, evidence of the commencing vascularization of the graft and, in conjunction with the results of immunologic investigations, of partial restoration of the function of the grafted splenic fragments.

The results are evidence that the technique of autografting of splenic fragments is a promising method of compensating the functions of the organ lost by virtue of the operation.

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PREPARATION AND CHARACTERISTICS OF MONOCLONAL IKO-GM-1 ANTIBODIES

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UDC 612.112.95.017.1

KEY WORDS: monoclonal antibodies; myelomonocytic antigen; leukemias.

With the discovery of the important role of the mononuclear phagocytic system (MPS) in specific immunity interest has risen in the cells of this system [1]. This is particularly the case with the study of the surface structures of cells forming MPS, which has become possible mainly thanks to the use of monoclonal antibodies (MA). A large group of MA, detecting antigens on the surface of mononuclear phagocytes, and also cells of the myeloid series, has now been described. Among them four groups of MA can be distinguished.

Group 1 consists of MA detecting differential antigens on MPS cells, group 2 includes antibodies detecting differential antigens of myeloid cells, group 3 includes antibodies detecting common antigens of myeloid cells and macrophages. Group 4 includes MA with an even wider spectrum of specificity, which mark myeloid cells, MPS cells, and cells of other types.

These antibodies are widely used to study surface structures, differentiation, and functional heterogeneity of macrophages and myeloid cells, and also for the differential diagnosis of nonlymphoid leukemias [2]. However, no information on how to obtain MA detecting differential antigens of MPS and myeloid cells could be found in the Soviet literature.

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 6, pp. 721-723, June, 1985. Original article submitted August 1, 1984.

0007-4888/85/9906-0780$09.50

TABLE 1. Reaction of IKO-GM-1 Monoclonal Antibodies with Healthy Human Cells

Test material	Result of determination	Frequency of expression of antigen, %	% of antigen positive cells
Polymorphonuclear neutrophils	10/10	100	100
Monocytes	13/13	100	57,4±3,1
Healthy human blood mononuclears	14/14	100	25,9±2,4
T lymphocytes	14/14	100	11,1±1,0
T lymphocytes with Fc-receptor for IgG	5/5	100	14,6±1,6
Enriched population of B lymphocytes and "null" cells	4/4	100	49,3±8,2
Thymocytes	0/4	0	0

Legend. Here and in Table 2, numerator indicates number of positive cases, denominator number of subjects studied.

EXPERIMENTAL METHOD

BALB/c mice were immunized intravenously, twice, 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 of spleen cells with cells of mouse myeloma P3×63 Ag 8.653 was carried out with the aid of 50% polyethylene-glycol with molecular weight of 1500 (Schuchardt, West Germany). The hybridoma, passed through selective medium, was cultured in medium RPMI 1640 (Serva, West Germany) with 20% fetal calf serum (FCS) (Flow Laboratories, Great Britain). The IKO-GM-1 hybridoma, cloned twice by the limiting dilutions method, produces antibodies of the IgG2a class. The class of antibodies was identified by Ouchterlony's double radial immunodiffusion method [4] with antisera against classes of mouse immunoglobulins (Meloy Laboratories, USA).

Specificity of IKO-GM-1 MA was determined by the method of indirect surface immunofluorescence. $F(ab')_2$-fragments obtained from commercial rabbit antiserum against albino mouse globulins, labeled with fluorescein isothiocyanate (from the N. F. Gamaleya Institute of Epidemiology and Microbiology), and absorbed with liver powder, were used as labeled antibodies. Mononuclear cells were isolated from healthy blood donors on a Ficoll–Verografin density gradient. Monocytes were isolated by adhesion to a plastic surface from a suspension of monocytes. The monocytes ($3 \cdot 10^7$ cells) were incubated on Petri dishes with a diameter of 60 mm (Falcon Plastics, N3002, USA) in medium RPMI 1640 with 10% FCS and 0.005 M HEPES buffer. Incubation was carried out for 1 h at 37°C in an atmosphere with 5% CO_2. T lymphocytes were isolated by rosette formation with sheep's red blood cells, treated with neuraminidase. The fraction of B lymphocytes and "null" cells was obtained from a suspension of cells nonadherent to plastic by removal of E-rosette-forming cells. The purity of isolation of the monocytes was verified morphologically by staning by the Romanovsky–Giemsa method and cytochemically by staining for nonspecific esterase.

To determine the effect of IKO-GM-1 MA on EAC-cell activity 0.5 ml of antibodies was incubated with $5 \cdot 10^6$ mononuclears from healthy subjects at 20°C for 0.5 h, after which 0.5 ml of complement was added and the mixture was incubated for 1 h at 37°C. The cells were then washed three times and used to determine EAC-cell activity. Intact cells, incubated under the same conditions in medium 199, incubated with complement, and treated only with IKO-GM-1 antibodies without complement, were used as the control. Culture fluid with a titer of 1:10 was used as antibodies and the source of complement was a pool of nontoxic rabbit sera, diluted 1:2. EAC-cell activity was determined by measuring release of ^{51}Cr from K-562 and Molt-4 target cells [6], with effector cells and target cells in the ratios of 25:1 and 50:1. To determine the character of expression of antigen revealed by IKO-GM-1 MA on colony-forming cells, healthy human bone marrow was treated with IKO-GM-1 antibodies and complement in the same way as the healthy human mononuclears were treated to determine the effect of MA on EAC-cell activity. The bone marrow cells were then cultured in agar medium. The colonies were counted under an inverted microscope and their composition studied morphologically [3].

EXPERIMENTAL RESULTS

Antigen detected by IKO-GM-1 MA is present in peripheral blood on polymorphonuclear neutrophils, on most monocytes, on a few T cells carrying a receptor for the Fc-fragment of IgG, and on "null" cells, for the

TABLE 2. Reaction of IKO-GM-1 Monoclonal Antibodies with Cells from Patients with Leukemia and Lymphosarcoma

Diagnosis	Result of determination	Frequency of expression of antigen, %	% of blast cells	% of antigen positive cells
AMonL	3/3	100	75,7±10,9	57,3±9,6
AMML	2/4	50	91,7±4,4	72,5
AML	3/8	37,5	89,0±5,3	30,0±7,3
BC CML	3/4	75	52,0±9,1	51,9±9,6
Chronic phase of CML	7/7	100	0	55,9±7,9
ALL	0/5	0	88,0±7,5	0
LSA	0/4	0	93,5±5,9	0
CLL	0/10	0	0	0

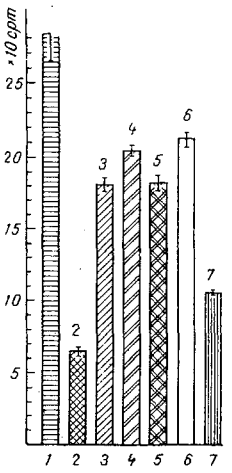

Fig. 1. Inhibition of natural killer activity of mononuclears from healthy blood donors by IKO-GM-1 monoclonal antibodies (effector to target ratio 25:1). Ordinate, quantity of label released (cpm · 10); 1) Molt-4 cells treated with Triton (without healthy human mononuclears – HHM); 2) Molt-4 cells in medium (without HHM); 3) HHM; 4) HHM treated with normal mouse serum; 5) HHM treated with complement; 6) HHM treated with IKO-GM-1 MA; 7) HHM treated with IKO-GM-1 MA and complement.

antibodies did not react with B lymphocytes from patients with chronic lymphatic leukemia (CLL) (Table 1).

It must be noted that the antigen is present not on all peripheral blood monocytes, for the number of antigen-positive monocytes detectable by MA is less than their total number in the preparation ($P < 0.001$).

Treatment of healthy human mononuclears with IKO-GM-1 antibodies and complement blocked their EAC-cell activity against Molt-4 cells in three of three cases, and in two of the three cases differences between the experiment and control were statistically significant (Fig. 1). When K-562 cells were used as target cells no blocking effect on EAC-cell activity was observed.

Antigen detected by IKO-GM-1 MA was not expressed on colony-forming cells. Treatment of normal bone marrow with antibodies and complement did not inhibit the formation of colonies of the granulocytic-macrophagal series in agar medium.

Antigen detected by ILO-GM-1 MA was expressed on blast cells of three of the three patients with acute monoblastic leukemia (AMonL), on leukocytes of patients in the chronic phase of chronic myeloid leukemia (CML), in three of four patients with a blast crisis of CML (BC CML). In a patient with BC CML on whose blast cells the antigen was not expressed, a lymphoid variant of BC CML was diagnosed. Antigen detected by IKO-GM-1 MA was represented on blast cells of three of the eight patients with acute myeloid leukemia (AML) and two of four patients with AMML.

IKO-GM-1 antibodies did not react with blast cells of five patients with acute lymphoblastic leukemia (ALL), four patients with lymphosarcoma (LSA), and with the B lymphocytes of 10 patients with CLL (Table 2); they likewise did not react with blast cells from a patient with acute promyelocytic leukemia. The antigen was well represented on monocytes of a patient with chronic monocytic leukemia.

The characteristics described above indicate that antigen detected by IKO-GM-1 MA is presented on mature cells of the myeloid series and on monocytes at all stages of differentiation. This character of expression of the antigen on blast cells of patients with AML and AMML can be explained by heterogeneity of the pool of leukemic cells, in which both early precursors of the myeloid series and also more mature cell forms are represented [5].

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ACTION OF HYDROCORTISONE ON CYTOTOXIC REACTIONS IN DELAYED-TYPE HYPERSENSITIVITY TO MICROBIAL ANTIGENS

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UDC 612.112.94.017.4-06:615.357:577.175:53]-08

KEY WORDS: delayed-type hypersensitivity (DTH); cytotoxic effect; lymphotoxin; macrophagotoxin; hydrocortisone.

In delayed-type hypersensitivity (DTH) sensitized lymphocytes in the presence of specific antigens and also macrophages activated by lymphokines have a cytotoxic action on target cells and secrete soluble factors (lympho- and macrophagotoxins), with cytotoxic properties [2, 7, 8, 14]. It has been shown that secretion of macrophagotoxin, like that of lymphotoxin, takes place under the influence of specific antigens to which the animals are sensitized. It has been shown that macrophagotoxin differs from lymphotoxin in certain of its parameters [1]. The mechanisms of the cytotoxic action of lymphotocytes and of lympho- and macrophagotoxins on target cells evidently have certain special features and may differ in their sensitivity to the inhibitory action of steroid hormones. It has been shown that corticosteroids inhibit secretion of certain lymphokines by lymphocytes [4, 13] and also the action of lymphokines on macrophages [6]. Meanwhile the inhibitory action of hydro-

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 6, pp. 723-725, June, 1985. Original article submitted October 12, 1984.