It can thus be tentatively suggested on the basis of these different activities of MP in health and disease that a definite and adequate relationship is found in healthy individuals between the stimulating and suppressor activity of MP, but in disease one of the effects may become predominant. In AGG, for example, the suppressor effect predominates, whereas in multiple myeloma, besides an increase in B-activin activity, increased mobility of the target cells also is observed under the influence of the patients' BMC.

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MONOCLONAL ANTIBODIES AGAINST THE α-CHAIN OF HUMAN LYMPHOCYTE FUNCTION-ASSOCIATED ANTIGEN (LFA-1)

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Human lymphocytic functional-associative antigen (LFA-1) is a noncovalently bound heterodimer membrane glycoprotein consisting of α- and β-chains with molecular weights of 175-180 and 93-95 kD respectively, which is a structural analog of the mouse LFA-1 antigen described previously [4, 5, 8].

A study of the function of mouse LFA-1 antigen revealed its important role in the initial recognition-adhesive phase of the reaction of cytotoxic T lymphocytes (CTL) [6]. The α-chain is unique for LFA-1 whereas the β chain is also a component of the CR3 antigen, which is a receptor for the inactivated C3b component of complement (C3bi) and of antigen

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TABLE 1. Reaction of ICO-11 MCAB with Cells from Healthy Blood Donors and Patients with Leukemia and Lymphosarcoma

Material tested	No. of positive cases / Number of persons tested	Frequency of antigen expression, %	Frequency of antigen-positive cells
Thymocytes	46/46	100	69,9±2,6
Mononuclear cells from healthy human peripheral blood	64/64	100	12,4±1,4
Monocytes	0/20	0	0
T lymphocytes	12/22	54,5	12,9±3,5
B lymphocytes	6/11	54,5	11,2±3,0
Granulocytes	0/31	0	0
Normal bone marrow cells	14/20	70	9,9±3,7
ALL	33/187	14,1	50,1±4,8
LSA	9/44	20,5	52,1±10,3
AMEL	5/46	10,9	55,0±15,2
AMML	8/20	40,0	50,0±9,5
AML	8/19	42,1	42,3±6,9
ALL	1/11	9,1	21,0±0
CML BC	2/35	5,7	46,7±36,2
CLL	0/63	0	0

p150/95, which is probably responsible for platelet adhesion [3]. The molecular weight of the α-chain of CR3 antigen is 165 kD, and that of antigen p150/95 is 150 kD. Monoclonal antibodies (MCAB) to β-chain caused immunoprecipitation of a protein with mol. wt. of 95 kD, and also another three chains with mol. wt. of 170-180, 165, and 150 kD. MCAB to the three different α-chains give immunoprecipitation of proteins with mol. wt. of 175-180 and 95, 165 and 95, or 150 and 95 kD.

The aim of this investigation was to characterize antigens revealed by ICO-11 antigens obtained by the writers previously [1].

EXPERIMENTAL METHOD

Expression of the antigens was determined in the indirect surface immunofluorescence test (IFT) on living cells by the method described previously [2].

To determine the effect of MCAB on natural killer cell (NKC) activity, blood mononuclears from healthy donors were incubated with the MCAB, then washed, after which NKC activity was determined against K-562 and Molt-4 target cells [2].

To determine the effect of MCAB on the lymphocyte blast transformation reaction to phytohemagglutinin (PHA) mononuclears were preincubated for 30 min with serial dilutions of MCAB, and PHA was then added. The reaction was carried out by the standard methods.

To determine the effect of MCAB to CTL, they were incubated in a 7-day mixed lymphocyte culture (MLC). The target cells were mononuclears used for stimulation, and cultured in the presence of concanavalin A. Cells from MLC were preincubated with MCAB, ^{51}Cr-labeled target cells were added to them, and 6 h later the level of radioactivity in the supernatant was determined [4].

For the immunoblotting test mononuclears or thymocytes were washed 3 times in phosphate-buffered physiological saline (pH 7.4) and $5 \cdot 10^7$ cells were lysed in 1 ml for 30 min on ice in buffer: 0.15 M NaCl, 0.01 M Tris-HCl (pH 8.1), 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 100 kallikrein inhibition units/ml of trasylol, and 2 mM iodoacetamide. The lysate was centrifuged at 20,000 rpm for 60 min at 4°C.

The lysate of thymocytes or blood mononuclears was subjected to electrophoresis in a 5-20% gradient of a thin layer of polyacrylamide gel in the presence of sodium dodecylsulfate (PAG-SDS) in Laemmli's system [7]. Proteins were transferred from the gel to nitrocellulose filters (Millipore) by the method in [9].

To detect the necessary protein on the filter with the aid of the peroxidase-antiperoxidase (PAP) immunoenzyme technique, dilutions of ascites fluid from mice with hybridomas (1:10,000) and rabbit antiserum to albino mouse globulins (1:200), obtained from the N. F. Gamelya Research Institute of Epidemiology and Microbiology, were used. Conditioned medium from

TABLE 2. Effect of ICO-11 MCAB on NKC Activity of Healthy Human Blood Mononuclears against K-562 and Molt-4 Target Cells

K-562				Molt-4			
	percentage cytotoxicity				percentage cytotoxicity		
No. of test	intact effectors	effectors treated w/ MCAB	percentage of inhibition	No. of text	intact effectors	effectors treated w/ MCAB	percentage of inhibition
1	29	3	89,7*	1	53	56	0
2	69	31	55,1*	2	7	9	0
3	20	0	100*	3	14	0	100*
4	6	3	50	4	29	14	51,7
5	27	25	7,4	5	39	50	0
6	20	53	0	6	95	122	0
7	29	39	0	7	21	23	0
8	13	8	38,5	8	11	53	0
9	71	60	15,5*	9	45	23	48,8
10	23	9	60,9*	10	13	38	0
11	41	69	0	11	20	24	0
12	45	45	0	12	50	0	100*
13	6	4	33,3				
14	46	37	19,6*				
15	42	52	0				
16	37	29	21,6*				
17	45	57	0				
18	43	39	9,3				
19	42	29	30,9				

Legend. Asterisk indicates statistically significant inhibition. Ratio of effectors to targets 25:1.

hybridoma cultures producing mouse MCAB to horseradish peroxidase, containing 80 μg/ml of horseradish peroxidase, was used as the PAP complex (the hybridoma was obtained in the Laboratory of Immunochemistry, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR: Director, Professor G. I. Abelev).

EXPERIMENTAL RESULTS

The ICO-11 MCAB were obtained by immunizing a mouse with thymus cells from a 24-week human fetus. ICO-11 of the IgG3 isotype reacted in the IFT with 67.6% of thymocytes, 12.4% of peripheral blood mononuclears from healthy donors, and 9.9% of bone marrow cells from healthy donors. ICO-11 did not react with granulocytes or monocytes (Table 1). The ICO-11 also reacted with thymocytes and spleen and lymph node cells from AKR mice. When the reactivity of these MCAB was tested with cells from patients with leukemia and lymphosarcoma, expression of the antigen on the blast cells was found in acute lymphoblastic leukemia (ALL), acute monoblastic leukemia (AML), acute myelomonocytic leukemia (AMML), and lymphosarcoma (LSA). The ICO-11 reacted only rarely with cells from patients with acute myeloblastic leukemia (AMEL) and with chronic myeloid leukemia in the blast crisis stage (CML BC) (Table 1). In these cases antigen-positive cells had certain cytochemical characteristics of monoblasts. ICO-11 in the presence of complement depressed colony formation of the granulocytic-macrophagal series (CFU-GM) in semisolid agar.

Functional tests showed that ICO-11, in the absence of complement, block NKC activity, the lymphocyte blast transformation reaction to PHA, cytotoxic activity of CCL induced in a 7-day MLC, and also E_n-rosette formation.

The ICO-11 blocked NKC activity of healthy human blood mononuclears against K-562 and Molt-4 target cells, and this effect was manifested more clearly against the K-562 than the Molt-4 cell line, as shown by the larger number of positive tests (Table 2).

ICO-11 present in the test medium blocked activity of CTL induced in MLC in four of five experiments, but washing the effector cells after their incubation with MCAB led to a sharp increase in cytolytic activity. ICO-11 depressed the lymphocyte blast transformation reaction to PHA. This effect was dose-dependent. ICO-11 inhibited the formation of E_n-rosettes of healthy human blood mononuclears by 65%. Incidentally, the E_n-rosettes formed after treatment with MCAB contained fewer sheep's erthyrocytes than in the control.

Determination of the molecular weight of the antigens by the immunoblotting technique showed that ICO-11 revealed a polypeptide with mol. wt. of 180 kD in lysates of human blood mononuclear cells and thymocytes.

The spectrum of cells expressing an antigen revealed by ICO-11 MCAB, blockade of NKC activity in the lymphocyte blast transformation reaction to PHA, and the molecular weight of the antigen thus indicate that ICO-11 are directed against the α-chain of human lymphocytic functionally-associated antigen (LFA-1).

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ACTIVATION OF MACROPHAGES BY A SYNTHETIC ANTIOXIDANT

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Much research in recent years has been devoted to the general rule that acitvation of macrophages (MPh) is associated with a metabolic (oxidative) burst, with activation of the glucose monophosphate shunt (GMPS), with the production and secretion of highly active unstable products of oxygen reduction, namely superoxide anions O_2^-, hydrogen peroxide (H_2O_2), OH^- radicals, and singlet oxygen (O_2) [5].

The excess of toxic superoxide radicals formed under these circumstances, and also lipoperoxides accumulating in phagosomes of MPH during phagocytosis, may be responsible for oxidative damage to cell membranes and associated depression of MPh functions. An intrinsic system of antioxidative protection, including superoxide dismutase, which removes the excess of superoxide radicals, and also glutathione peroxidase and NADP-dependent glutathione reductase, which neutralize lipoperoxides [2, 8], has been described in MPh. However, if endogenous antioxidants are deficient, disturbances of MPh function may arise. It was shown previously [4] that alkyl-substituted derivatives of 3-hydroxypyridine (3-HP), which has moderate antioxidative action, are effective inhibitors of free-radical reactions and can be used to protect against the destructive effect of free radicals.

The aim of this investigation was to study the effect of synthetic antioxidants of MPh functions. Of all the various synthetic derivatives of 3-HP we chose 2-tert-butyl-3-hydroxypyridine (TBHP), whose ability to stabilize erythrocyte membranes was described previously [3].

EXPERIMENTAL METHOD

Mouse peritoneal MPh, removed by intensive irrigation of the peritoneal cavity of mice with Hanks's solution containing heparin (5 U/ml), were used as the target cells. The re-

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