these reactions. It must be pointed out that antibodies to epithelial tissues and also to cell nuclei are not found in animals after injection only of PEL or of A-PSC with Freund's adjuvant. The reason for the absence of antibodies to nuclei during immunization with pepsin-treated streptococcus calls for further study. In the present investigation only a small number of sera reacting with thymus epithelium, and obtained a short time after the beginning of immunization, were studied.

We know [10] that the epithelium of the thymus constitutes a microenvironment in which maturation and differentiation of T lymphocytes take place. The earlier appearance of autoantibodies to the epithelium of the thymus during immunization with A-PSC, conjugated with PEL, was possibly the cause of development of the immunoregulatory disturbances leading to the appearance of autoantibodies to nuclei. The problem of the damaging action of autoantibodies to the CR-determinant of A-PSC on the epithelium of the thymus and also the causes of appearance of autoantibodies to cell nuclei require further study. Animals immunized with conjugates of A-PSC with PEL can be used as an experimental model with which to study these problems.

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MONOCLONAL ANTIBODIES TO ANTIGENS ON HUMAN NEUTROPHILS, ACTIVATED T LYMPHOCYTES, AND ACUTE LEUKEMIA BLAST CELLS

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Monoclonal antibodies (MCA) to differential antigens of human hematopoietic and lymphopoietic cells are currently widely used for marking leukocyte cell populations under normal conditions and in various pathological states, primarily in malignant diseases of the hematopoietic system [5, 14]. The use of these MCA provides extensive opportunities for the development of fundamentally new methods of diagnosis [5] and treatment [13] of hemoblastoses, and this, in turn, indicates the necessity of obtaining MCA to a broad spectrum of differential antigens of hematopoietic cells and a thorough study of the cellular and molecular specificity of the antigenic determinants revealed by them.

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TABLE 1. Reactivity of MCA Ta/Nph-1 and Ta/Nph-2 Relative to Healthy Human Cells

Cells	Ta/Nph-1		Ta/Nph-2	
	IFT	CTT	IFT	CTT
Lymphocytes	0/6	0/4	0/6	0/4
Adherent monocytes	0/5	—	0/5	—
Neutrophils	5/5 (86—95 %)	3/3 (80 %)	5/5 (84—91 %)	3/3 (80 %)
Erythrocytes	0/1	—	0/1	—
Lymphocytes activated by PHA	1/2 (75 %)	—	2/2 (73—81 %)	—
Alloantigens	0/1	—	0/1	—

Legend. Numerator, number of positive reactions; denominator, number of specimens tested. Percentage of antigen-positive cells given in parentheses.

This paper describes the production of two mouse hybridomas secreting MCA to antigenic determinants of the surface membranes of human neutrophils, activated T lymphocytes, and acute leukemic blast cells.

EXPERIMENTAL METHOD

BALB/c mice were immunized twice at an interval of 10 days with leukocytes ($10 \cdot 10^6$ and $5 \cdot 10^6$ cells) from a patient with acute lymphoblastic leukemia (ALL), 90% of whose blast cells were of neither the T nor the B phenotype (Ia^+, $T11^-$, $T10^-$, mIg^-). On the 4th day after the second immunization, $10 \cdot 10^6$ mouse spleen cells were hybridized with $20 \cdot 10^6$ mouse myeloma P3-NS1-Ag4-1 cells with the aid of a 45% solution of polyethylene-glycol (mol. wt. 1.5-1.7 kilodaltons, from Serva, West Germany). After fusion the cells were cultured in medium RPMI-1640 containing 20% embryonic calf serum, 1% sodium pyruvate, 0.4% gentamycin, and 1:50 HAT concentrate (Flow Laboratories, England) in microwells of a 96-well plate with a feeder layer consisting of mouse peritoneal macrophages, in an atmosphere with 5% CO_2. On the 8th day of culture aminopterin was removed from the culture medium and culture continued for a further 8 days, after which the hypoxanthine with thymidine was removed and culture continued without selective additives. Eight days later antibodies to the leukemic cells used for immunization were found (by the indirect immunofluorescence test) in the supernatants of two actively growing clones, subsequently named Ta/Nph-1 and Ta/Nph-2. These clones were recloned by the limiting dilutions method and adapted for culture in vitro and for growth in the form of ascites tumors in vivo in mice.

Lymphocytes were obtained by gradient centrifugation on Hypaque–Ficoll [3] and subsequent removal of adherent monocytes from the suspension after incubation on plastic. Neutrophils were obtained from the residue of cells passing across the gradient, after removal of erythrocytes from them by means of 0.83% NH_4Cl solution. Leukemia cells were isolated from the heparinized peripheral blood of leukemia patients after hemolysis of the erythrocytes. Frozen stored cells from leukemia patients, receiving treatment in the No. 1 Hematology Department of the Central Research Institute of Hematology and Blood Transfusion (Head, Professor F. E. Fainshtein), and in the Professorial Department of Pediatrics of the N. I. Pirogov Second Moscow Medical Institute (Head, Corresponding Member of the Academy of Medical Sciences of the USSR N. S. Kislyak) were tested.

Immunological testing of supernatants of the hybridomas was carried out by the indirect immunofluorescence test (IFT) in a micromodification. A suspension of isolated cells in a volume of 20 µl and a concentration of $5 \cdot 10^6$ cells/ml medium was applied to a glass microwell surface treated with 0.0025% solution of poly-L-Lysin (mol. wt. 70-15 kilodaltons, from Sigma,

USA), incubated for 30 min at 22°C, after which 20 µl of supernatant of the hybridoma cultures with 0.1% NaN_3 was applied to the adherent cells, and the sample was incubated for 30 min at 4°C. After two washes, 20 µl of fluorescein isothiocyanate-labeled antibodies against mouse Ig (1:20) was introduced into the wells, followed by incubation for a further 30 min at 4°C. Next, 5 µl of 50% glycerin solution was applied to the washed cells and the microwells were covered with a coverslip and sealed. The results of the test were read with a fluorescence microscope. Immunologic phenotyping of the blast cells from the patients with leukemia was carried out in the indirect IFT using MCA against differential leukocyte antigens OKT3, OKT4, OKT8, OKT10, and OKT11 (from Ortho, USA), IKO-1 and IKO-11 (obtained in the All-Union Oncologic Research Center, Academy of Medical Sciences of the USSR, by A. Yu. Baryshnikov [1, 2]), and also with polyclonal anti-Ig (H + L-chains)-antibodies (from Behringwerke, West Germany) in the direct IET. The complement-dependent cytotoxicity test (CTT) was carried out by a micromethod [15].

Lymphocytes were stimulated in vitro with phytohemagglutinin (PHA-P) or with alloantigens (in mixed lymphocyte culture) in culture tubes with 1 ml of medium RPMI-1640 containing 10% healthy human serum of blood group AB(IV). Stimulated and control lymphocytes were collected on the 7th day and tested with MCA in the IFT. The degree of lymphocyte stimulation was estimated from incorporation of ^3H-thymidine with parallel microculture.

EXPERIMENTAL RESULTS

MCA of supernatants of hybridoma cultures Ta/Nph-1 and Ta/Nph-2 reacted in both IFT and CTT with surface membrane antigens on the majority of neutrophils and PHA-activated peripheral blood lymphocytes from healthy subjects, but did not give positive reactions with unactivated lymphocytes, adherent monocytes, erythrocytes, and alloantigen-stimulated lymphocytes (Table 1). A very small number (not more than 4%) of Ta/Nph-1^+- and Ta/Nph-2^+-lymphocytes were found in the blood of some healthy subjects in IFT, but the intensity of fluorescence in this case was weak.

MCA Ta/Nph-1 and Ta/Nph-2 also reacted with 36-93% of the peripheral blood lymphocytes of five patients with various disturbances of myelopoiesis, which contained antigens characteristic of a state of activation ($T10^+$, $T9^+$, Ia^+).

It was also found that the antigen detected by MCA Ta/Nph 1 was present on blast cells of some patients with the non-T-non-B-, the pre-T-, and T-types, and the activated T-subtype of ALL (Table 2). Of 11 cases of acute nonlymphatic leukemias, the blast cells of only one patient were found to be Ta/Nph-1^+. MCA Ta/Nph-2 reacted with the blast cells of most (85%) patients with ALL of the non-T-non-B-type and of some patients with ALL of pre-T- and T-types.

TABLE 2. Reactivity of MCA Ta/Nph-1 and Ta/Nph-2 Relative to Malignant Cells from Patients with Various Forms of Hemoblastoses

Leukemic cells from patients with various forms of hemoblastoses	Antigenic phenotype of leukemic cells	Ta/Nph-1		Ta/Nph-2	
		IFT	CTT	IFT	CTT
ALL					
Non-T-non-B-variant	$Ia^{+/-}$, T11-, T3-, T10-, IKO-11-, mIg-,	1/10	2/6	6/10	5/6
Pre-T-variant	Ia+, $T11^{+/-}$, T10+, T3-, IKO-11+, mIg-	0/4	1/2	0/4	2/2
T-variant	Ia-, T11-, T10+, $T3^{+/-}$, IKO-11+, mIg-	1/7	1/3	0/5	1/3
Activated T-variant	Ia+, T11+, T3+- T10+, IKO-11+, mIg-	1/2	0/1	2/2	0/1
B-variant	Ia+, T11-, T10-, T3-, IKO-11-, mIg-	0/1	—	0/1	—
Acute nonlymphatic leukemias	Ia+, T11-, T3-, IKO-$11^{+/-}$, $mIg^{+/+}$	1/11	1/3	1/11	1/3
Chronic lymphatic leukemia					
B type	Ia+, T11-, T10-, T3-; IKO-11-, mIg+	0/4	0/2	0/4	1/2
T type	Ia-, T11+, T10-, T3+, IKO-11+, mIg-	0/1	—	0/1	—

Legend. Numerator) Number of positive reactions (sample contained more than 10% of positive blast cells); denominator) total number of patients tested.

By analogy with Ta/Nph-1, Ta/Nph-2^+-blast cells were found in only one patient with acute non-lymphatic leukemia.

An investigation conducted jointly with workers at the Laboratory of Clinical Immunology, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR (Head, Professor Z. G. Kadagidze) showed that MCA Ta/Nph-1 belong to the IgM class, whereas the Ta/Nph-2 belong to the mouse IgG class.

Thus both types of MCA which we obtained reacted with peripheral blood neutrophils, with lymphocytes activated in vitro and in vivo, but not with monocytes, with unactivated lymphocytes, or with erythrocytes from the peripheral blood. Meanwhile, testing with leukemic cells revealed certain differences in their cellular specificity. MCA Ta/Nph-1 reacted only with blast cells of individual patients with ALL, whereas Ta/Nph-2 reacted with blast cells from most patients with non-T-non-B ALL, and only in a few cases from patients with pre-T- and T-ALL. According to the spectrum of cellular specificity, the distribution of the antigen revealed by MCA Ta/Nph-2 was similar to that of the antigen of early lymphoid precursors with ALLA [6], also present on peripheral neutrophils [4], PHA-activated lymphocytes [10], and lymphocytes activated in vivo during bone marrow regeneration [7], blast cells from the majority (about 85%) of patients with non-T-non-B-ALL, and also a small proportion of patients with T-ALL and with B-type lymphomas [7, 11]. Ta/Nph-1, defining the antigenic determinant contained on neutrophils and activated T lymphocytes, are evidently an original kind of MCA, for specificity relative to activated T lymphocytes was not found in MCA to human neutrophils obtained by other workers [12]. Meanwhile existing MCA to activated T lymphocytes (for example, I2 [9], 4F2 [8], etc.) do not react with the surface membranes of neutrophils.

The spectrum of cellular specificity of MCA Ta/Nph-1 and Ta/Nph-2 discovered in this investigation indicates that it may be possible to use them to determine subpopulations of activated T lymphocytes in various pathological states accompanied by the appearance of activated lymphocytes in vivo, including disturbances of myelopoiesis, bone marrow transplantation, and malignant lymphoproliferative diseases. Meanwhile, considering the complement-fixing properties of these MCA, they may perhaps be used in the future in the treatment of pathological states, in which a definite pathogenetic role may be played by activated T lymphocytes.

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