PHENOTYPIC CHARACTERISTICS OF A POPULATION OF NORMAL KILLER CELLS OF AUTOLOGOUS ERYTHROCYTES

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The study of membrane structures makes it possible to identify individual subpopulations of lymphoid cells more precisely and to discover the molecular components of membranes which play an important role in the mechanisms of cell interactions. Membrane structures of the population of normal killer cells of autologous erythrocytes (NKAE), which have a spontaneous selective killer action on old autologous erythrocytes in man and animals under normal conditions [9] have virtually not been studied. Meanwhile the NKAE population is similar from the functional point of view to T lymphocytes with respect to identification of and cytotoxic action on autologous and syngeneic erythrocytes [10, 11] and to their helper effect on the erythroid direction of proliferation and differentiation of hematopoietic stem cells [6, 8].

The aim of this investigation was to study phenotypic characteristics of the NKAE population and to compare them with those of T lymphocytes.

MATERIAL AND METHOD

Monoclonal antibodies (MCA) IKO-10 and IKO-11 were obtained from the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Antigen expression was based on the decrease in the number of NKAE in the cytotoxic test on treatment of cells with MCA in a dilution of 1:50 (MCA, undiluted and diluted 1:2 gave a prozone effect) in the presence of complement — fresh rabbit serum in a dilution of 1:10, exhausted with all human or mouse peripheral blood cells. Cells treated with medium 199 in the presence of similarly exhausted fresh rabbit serum served as the control. Xenogeneic antimouse brain serum (AMBS), directed against specifitieis SC-1 and Ihy-1 was obtained by repeated immunization of rabbits with a suspension of mouse brain tissue homogenate, inactivated for 30 min at 56°C, was absorbed with mouse erythrocytes and with a washed homogenate of mouse liver tissue [12]. The AMBS thus obtained, in a dilution of 1:10, killed 98% of thymocytes of $(CBA \times C57BL/6)F_1$ mice and was used in a dilution of 1:1.5. The same AMBS, but additionally exhausted with the immunizing antigen (mouse brain tissue homogenate), served as the control. Suspensions of cells from the organs were obtained in the cold by the usual method and their viability, estimated by staining with trypan blue, was not less than 95%. Fractionation of the cells into adherent and nonadherent was carried out by the method in [4]. The effect of phytohemagglutinin (PHA) on the cytotoxic activity of NKAE was studied by treating them with 50 μg of PHA ("Gibco," USA) for 1 h at 37°C, which was subsequently washed off. Peripheral blood mononuclears from clinically healthy individuals were isolated in a Ficoll—Verografin density gradient ($\rho = 1.076$ g/cm^3). NKAE were identified by the method described previously [9] in the plaque-formation test with autologous and syngeneic erythrocytes.

Experiments were carried out on female CBA and $(CBA \times C57BL/6)F_1$ hybrid mice weighing 18-22 g, obtained from the "Rappolovo" laboratory animals nursery. The results of the investigations were subjected to statistical analysis by Student's test of significance.

EXPERIMENTAL RESULTS

Fractionation of the cells on a column with viscose wadding showed that NKAE of mouse thymus and bone marrow are located entirely in the nonadherent fraction (Fig. 1). Treatment

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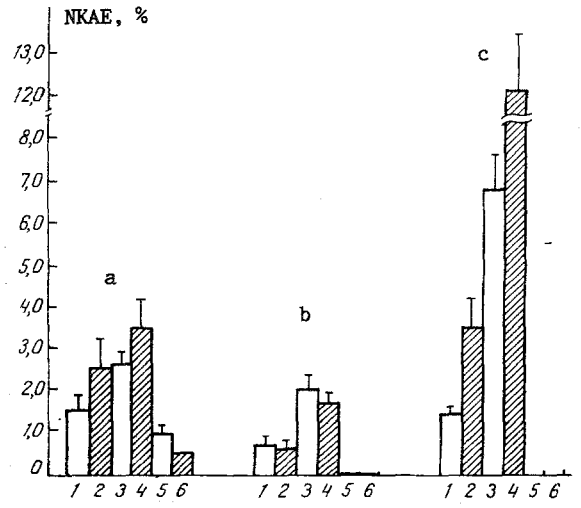

Fig. 1

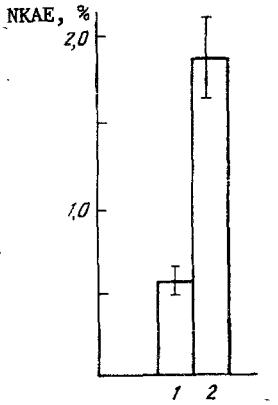

Fig. 2

Fig. 1. Effect of PHA on number of NKAE from different fractions of thymus, bone marrow, and spleen cells of CBA mice. Unshaded columns — cells not treated with PHA; shaded — cells treated with PHA. Sources of cells: a) spleen, b) bone marrow, c) thymus. 1, 2) NKAE of unfractionated cell populations; 3, 4) NKAE of fraction of nonadherent cells; 5, 6) NKAE of fraction of adherent cells.

Fig. 2. Effect of PHA on number of NKAE among peripheral blood mononuclears of clinically healthy persons (n = 26). 1) Mononuclears treated with medium 199, 2) mononuclears treated with 50 μg PHA.

TABLE 1. Effect of IKO-10 and IKO-11 MCA on Number of NKAE from Mouse Organs and Healthy Human Peripheral Blood

MCA	Cells	Number of positive reactions/number of blood samples tested	Inhibition of number of NKAE (in %) relative to control (medium 199)
IKO-10	Mouse thymocytes	8/10	69 ($p<0,02$)
IKO-10	Nonadherent mouse bone marrow cells	7/10	59 ($p\geqslant 0,05$)
IKO-10	Human peripheral blood mononuclears	6/9	66 (73 % in block test)
IKO-11	The same	9/11	52

of this fraction with PHA activated the cytotoxic ability of thymus NKAE, which was reflected in an increase in the number of NKAE. PHA had no such action on NKAE of the nonadherent fraction of bone marrow cells. The activating effect of PHA also was observed relative to the cytotoxic ability of NKAE of human peripheral blood mononuclears (Fig. 2). These data are evidence of the heterogeneity of bone marrow and thymus NKAE with respect to expression of the factor determining sensitivity of NKAE to that particular lectin.

Treatment of the nonadherent fraction of bone marrow cells with AMBS, directed against specificities SC-1 and Ihy-1, led to a significant reduction in the number of NKAE (0.09 0.04%; n = 14) compared with the control (0.68 ± 0.24, n = 15; inhibition by 86.8%). Since on the majority of mouse bone marrow cells SC-1 and Ihy-1 antigens are represented on the same cells, and only a very small proportion of the cells express antigen SC-1 only [5], the results suggest that both antigens are expressed on NKAE of bone marrow, and also on the majority of SC-1^+, Ihy-1^+ bone marrow cells. Evidence of expression of antigen Ihy-1 on NKAE of thymocytes is given by data on the discovery of up to 4.3 ± 0.6 and 1.4 ± 0.3% of NKAE respectively among CBA and (CBA × C57BL/6)F_1 mouse thymocytes, with a mortality of 100% after their treatment with AMBS and complement [7]. Antigen Ihy-1, expressed on NKAE of mouse thymocytes and bone marrow cells, with a high degree of homology and, in particular, domains of variable and constant regions of immunoglobulin, and considered to belong to the superfamily of immunoglobulin molecules [15], may play an initiating role in the mechanism of interaction of NKAE with their targets (autologous erythrocytes), for the function of these molecules is associated with recognition, and for antigen Ihy-1, with recognition of structures of autologous erythrocytes inhibited by rabbit AMBS and MCA to antigen Ihy-1 [14].

The antigenic structure of NKAE was also investigated with the aid of MCA IKO-10 and IKO-11. Although no data are yet available on the nature of antigen IKO-10, we know that IKO-10 MCA are directed against precursors of human T lymphocytes and reveal the antigen on cells of fraction 1 in a 19-33% gradient of bovine serum albumin in thymocytes [1]. In a function test, on treatment of human peripheral blood mononuclear with IKO-10 MCA in the presence of complement we observed a significant decrease in the number of NKAE. This effect also was well marked when human peripheral blood mononuclears were treated in a block test (Table 1). Treatment of mouse thymocytes, and also of the nonadherent fraction of mouse bone marrow cells, with IKO-10 MCA had a similar inhibitory action on the number of NKAE (Table 1). These data are evidence that antigen IKO-10 is represented on some NKAE of human peripheral blood mononuclears, of the nonadherent fraction of mouse bone marrow cells and thymocytes. Comparison of data on the number of NKAE of the nonadherent fraction of mouse bone marrow cells, expressing antigens SC-1 and Ihy-1, and on the number of NKAE expression antigen IKO-10, shows a considerable degree of overlapping between them, evidence either of expression of these antigens on the same cells or of possible homology between antigen IKO-10 and antigen Ihy-1, which requires experimental verification.

IKO-11 MCA, blocking natural killer cell activity, have been shown to interact with the α-chain of human lymphocytic functionally associated antigen (HLFA-1) and its structural analog (LFA-1) in mice, which plays an important functional role in the initial reaction of recognition and adhesion of cytotoxic T lymphocytes [2, 3]. Treatment of human peripheral blood mononuclears with IKO-11 MCA had an inhibitory effect on the number of NKAE (Table 1). These data suggest that antigen IKO-11, which plays an important role in mechanisms of interaction of NKAE with autologous erythrocytes, is represented on some human peripheral blood NKAE. Blockade of the adhesive-recognizing function of antigen LFA-1 by means of IKO-11 MCA leads to disturbance of intercellular interactions, which is expressed in the function test as a decrease in the number of NKAE.

It has been shown that antigens IKO-10 and IKO-11, especially on human thymocytes, are present on the surface of cells of different populations, which are at different stages of differentation [1]. These antigens, on NKAE of human peripheral blood mononuclears, are perhaps also represented on different cell populations, as may be indirectly confirmed by the virtual inability of overlapping of IKO-10^+ NKAE and IKO-11^+ NKAE populations. Evidence of the heterogeneity of NKAE is given by data on their presence among precursors of mouse bone marrow T lymphocytes (SC-1^+-, Ihy-1^+-cells), not responding to PHA, and also among more mature forms of T lymphocytes: mouse thymocytes and spleen cells and human peripheral blood mononuclears, responding to PHA by increased cytotoxic activity.

The close resemblance between NKAE and T lymphocytes is manifested not only in their phenotypic characteristics, but also in their functional activity, for T lymphocytes from lymphoid organs of normal mice can recognize autologous erythrocytes and interact with them [10], and they also possess cytotoxic properties against syngeneic erythrocytes [11]; some workers consider that some T lymphocytes with cytotoxic function are normal killer cells [13]. It can also be concluded from the results that a certain fraction of T lymphocytes possesses, in particular, NKAE activity and plays an important regulatory role in homeostasis of the erythroid branch of normal hematopoiesis, by exerting a selective identifying and destructive action on old autologous erythrocytes, terminating with erythrophagocytosis in the spleen and inducing subsequent erythropoiesis.

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EFFECT OF GENTAMICIN ON NEUTROPHIL–STAPHYLOCOCCUS

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The autoradiographic investigation of neutrophil–microorganism interaction [2, 3] has enabled the intra- and extracellular bactericidal ability of neutrophils and its change under the influence of humoral factors to be demonstrated and compared [6] under normal conditions and in burns. There is no doubt about the fact that the principal method of treatment of infection at the present time, namely with antibiotics, modifies the natural course of interaction of neutrophils with bacteria. The method suggested has enabled these changes to be analyzed by comparing the degree of viability of bacteria existing in various topographic relationships with neutrophils with the ultrastructural changes taking place in the bacteria themselves and in the neutrophils.

This communication gives the results of experiments with gentamicin, an antibiotic which does not penetrate [8] into neutrophils.

EXPERIMENTAL METHOD

Blood samples were obtained when blood was taken from donors at a blood transfusion station (14 samples) and also in the course of bacteriologic and other diagnostic analyses on patients in the burns department (10 samples). The blood was collected in tubes containing 1 ml of 3% neutralized EDTA solution, to prevent clotting, and 1 ml of 10% gelatin solution, to accelerate erythrocyte sedimentation, to 10 ml of blood. After sedimentation of the erythrocytes for 30 min in an incubator, the layer of plasma with leukocytes was withdrawn, and the leukocytes were washed twice with medium 199 and added to a suspension of bacteria in the same medium. The bacteria consisted of a clinically isolated strain of *Staphylococcus aureus*, sensitive to gentamicin. During work with this strain the maximal allowable concentration (MAC) of gentamicin was determined 5 times, and with a concentration of bacteria of 10^7 cells/ml it was found to vary from 0.156 to 1.56 μg/ml. A culture seeded 24 h before the experiment on agar and washed off with 0.45% sodium chloride solution was used. After sedimentation in hypotonic solution the bacteria were resuspended in medium 199 and the concentration adjusted to the required value. A mixture of leukocytes and bacteria in the ratio of 1/10 was incubated in the presence of autologous serum (1/10 of the volume of the mixture) or without serum at 37°C, the tubes being inverted every 5 min. Before the beginning of incubation gentamicin (2 μg/ml) was added to the medium. After 60 min of incubation ^{3}H-uridine was added in a dose of 5 μCi/ml and incubation continued for a further 5 min. The incubation mixture was then

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