number of TC-II membrane receptors of the K-562 and HL-60 cell lines of human leukemia during their chemically induced differentiation by arabinoside-cytosine and dimethyl sulfoxide [11].

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ACTIVITY OF HUMAN NATURAL KILLER CELLS UNDER DIFFERENT EXPERIMENTAL CONDITIONS

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The traditional radiometric methods of estimating activity of human natural killer (NK) cells presuppose the use of nutrient media prepared with the addition of serum derived from various sources. Depending on the conditions of adaptation of a culture of K-562 cells, the traditional target cells (TC) for testing NK activity in vitro, additives to the medium may include: fetal calf serum (FCS) [6, 8, 14], bovine serum [2], human blood group IV serum [8], autologous plasma [1], and autologous serum [4]. In order to obtain high titers of production of cytotoxic NK factors, it has been shown that the cells must be incubated in serum-free medium [6]. A marked increase in NK activity was recorded in previous studies [1, 4] in the presence of autologous plasma and autologous serum respectively. The basis for this change in activity of NK cells when incubated under nonstandard experimental conditions is provided by the connection, which several workers have noted, of the level of natural cytotoxicity (NCT) with functioning of the HLA system [9, 11, 12], which is confirmed by the high activity of the cells obtained with the use of a xenogeneic model [7]. Meanwhile, the absence of any such connection was concluded from other investigations [5, 10, 15], and it was shown in [8] that for lymphocytes to realize their NK activity in the peripheral blood, a source of serum is not essential.

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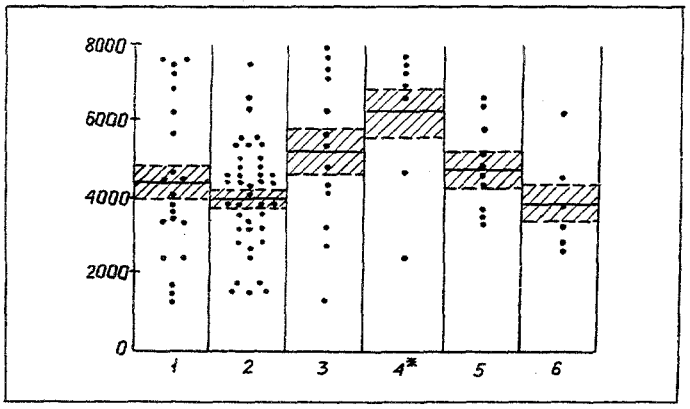

Fig. 1. Activity of human NK cells under different experimental conditions. Abscissa, No. of experimental plan; ordinate, NK activity (in conventional units). Individual levels of NK activity of healthy donors are shown. Horizontal line represents mean values of NK activity, broken lines show error of the mean in a group of observations, *$p < 0.05$ Compared with data obtained by plan 2.

The aim of this investigation was to study activity of human NK cells in the presence of various kinds of serum.

EXPERIMENTAL METHOD

Mononuclear cells (MNC) were isolated from venous blood of 63 healthy blood donors (27 men and 36 women) aged from 18 to 61 years, in 2 one-step Ficoll–Paque density gradient ("Pharmacia Fine Chemicals") The cytotoxic activity of the NK cells was determined by a radiometric method [3], against strain K-562 of human erythromyeloleukemic cells, labeled with ^{3}H-uridine in a dose of 3 μCi/ml. The initial suspensions contained: MNC $10 \cdot 10^6$, TC $10 \cdot 10^4$ cells in 1 ml of complete nutrient medium, prepared on the basis of RPMI-1640 ("Amimed") with the addition of 12% FCS (from the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences), 10 mM HEPES ("Serva"), 2 mM glutamine, and 40 μg/ml gentamicin ("Pharmachim"). After the cells had been poured into wells of 96-well round-bottomed microplanchets (0.1 ml of each suspension per well) combined incubation of MNC and TC was carried out for 14 h at 37°C in a humid atmosphere containing 5% CO_2. At the end of incubation and sedimentation of the cells on glass-fiber filters with a pore diameter of 2.5 μ ("Whatman") by means of a Dynatech 12-channel biological fraction harvester, residual radioactivity on the filters was determined with the aid of "Packard" or "Mark 2" scintillation β-counters. Each sample was counted for 1 min. The cytotoxic index (CTI) for each effector:target ratio tested (100:1, 50:1, 25:1, and 12:1) was determined in two or three parallel wells of the microplanchets, and calculated by the equation:

$$\text{CTI} = \left(1 - \frac{\text{number of counts in well}}{\text{number of counts in control}}\right) \times 100\ \%.$$

The control for the cytotoxic test consisted of TM incubated under the same conditions as the experimental cells, but without MNC. The area below the cytotoxicity curve, expressed in conventional units [13], was calculated as a measure of NK cell activity. The test serum was obtained from the donors' peripheral venous blood by centrifugation at 400g for 10 min at 20°C and was used for an experiment on the same day. To study the effect of the serum on cytotoxic activity of the MNC incubated with it, the following experimental plans were used: 1) FCS (12%) – 14 h of incubation; 2) FCS (12%) – 14 h of incubation + FCS (12%) – 1 h of preliminary treatment; 3) FCS (12%) – 14 h of incubation + autologous serum (ALS, 12%) – 1 h of preliminary treatment; 4) FCS (12%) – 14 h of incubation + homologous serum (HLS, 12%) – 1 h of preliminary treatment; 5) ALS (6%) – 14 h of incubation with 6% FCS; 6) ALS (6%) – 14 h of incubation with 6% FCS + ALS (12%) – 1 h of preliminary treatment. As the

above experimental plans show, the culture medium invariably contained a certain quantity of FCS, for the test strain K-562 was adapted to this type of serum. Preliminary treatment of the cells for 1 h in all experiments was carried out at 37°C in an atmosphere containing 5% CO_2.

The significance of differences between mean values was determined by Student's t test.

EXPERIMENTAL RESULTS

Testing MCT by the traditional method in medium with added FCS enables a wide scatter of values of NK activity of healthy donors to be recorded (from $1.44 \cdot 10^3$ to $7.61 \cdot 10^3$ conventional units), with a mean cellular activity of $4.39 \cdot 10^3$ conventional units (Fig. 1, plan 1). Replacing half of the FCS by ALS reduced the scatter of the values in the NCT test system. The range was now from $3.12 \cdot 10^3$ to $6.57 \cdot 10^3$ conventional units. The mean value of NK cell activity was virtually unchanged at $4.71 \cdot 10^3$ conventional units (plan 5).

Addition of a further 1 h of incubation of MNC in the presence of both FCS and ALS made no significant changes to the resulting NK activity and to the scatter of values determined in the presence of the corresponding comparison serum. The level of cytotoxicity after incubation of MNC for 1 h in medium prepared in FCS, followed by carrying out the NCT test in medium of similar composition, was $4.06 \cdot 10^3$ conventional units, with scatter from $1.51 \cdot 10^3$ to $7.48 \cdot 10^3$ (plan 2) NK activity tested by the same plan, but in the presence of ALS, was $3.87 \cdot 10^3$ conventional units with scatter from $2.83 \cdot 10^3$ to $6.24 \cdot 10^3$ (plan 6). In both cases only a very small decrease in resulting cytotoxicity was recorded, and the scatter of the values remained at the control level.

Estimation of NK activity in the presence of FCS, preceded by incubation of the MNS for 1 h in medium containing ALS, revealed an increase of cytotoxicity to $5.21 \cdot 10^3$ conventional units, with scatter from $1.3 \cdot 10^3$ to $7.96 \cdot 10^3$ (plan 3), although the increase is not statistically significant. It will be noted that in eight of 13 observations the level of NK activity exceeded mean values of cytotoxicity estimated in a similar plan, but with preliminary incubation of MNC for 1 h in medium prepared with FCS.

Investigation of NK activity in the presence of FCS, and with preliminary incubation of MNC in medium containing HLS revealed a marked increase of cytotoxicity to 6.25 ± 10^3 conventional units, with scatter from 2.18 ± 10^3 to $7.67 \cdot 10^3$ (Fig. 1, plan 4), which is statistically significant ($p < 0.05$) compared with the level of cytotoxicity estimated in a similar system, but with preincubation of the MNC for 1 h in medium prepared with FCS. In six of seven cases NK activity exceeded the mean values obtained in the corresponding control group.

The results show that the cytotoxic activity of NK cells depends essentially on the serum composition of the nutrient medium in which NCT is tested, for it rises in a heterogeneous system containing FCS + ALC, and by an even greater degree, in one containing FCS + HLS, maintained for 1 h. This dependence was found only in the case of short-term preincubation of the cells. Under conditions when combined incubation of MNC and TC was carried out in medium with a heterogeneous composition throughout the period of NK cell-mediated cytolysis, NK activity was unchanged (plans 5-6) Considering that changes in NK activity were observed only when an additional step of preincubation of the MNC was present in a heterogeneous system, before they were joined to TC which had not been in contact with ALS or HLS, and also the absence of any significant changes in NK activity under conditions when TC were incubated with MNC throughout the period of their interaction in a geneous serum system, it can be concluded that this effect is exclusively connected with recognition of certain serum factors, carrying the donor's individual genotypic information, by the surface structures of the NK cells.

The absence of any changes except scatter of the cytotoxicity values, when the efficacy of cytolysis in ALS and FCS is compared (plans 1 and 5), is an important fact. The situation arises when, on the one hand, realization of the cytotoxic potential of the NCT system is definitely connected with functioning of the HLA system [7, 9, 11, 12] and homologous serum stimulates NK activity of human MNC, and on the other hand, FCS, which relative to human MNC is heterologous, is unable to change NK activity significantly, in agreement with data in [5, 8, 10, 15], evidence of the nonrestricted nature of the NCT system with respect to the major histocompatibility complex. The resulting divergence can be explained by the ability of so-called damper proteins of fetal serum to block recognition of serum factors carrying individual signals in the heterologous system by the surface structures of human NK cells.

The results of this investigation show that estimation of the response of NCT in a system containing FCS and ALS, after preliminary incubation of the MNC, must be accompanied by monitoring of the effect of the serum

composition of the medium on cytotoxicity of NK cells. The use of HLS for the purpose of positive controls to natural cytotoxicity tests is contraindicated.

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