not only interesting for the experimental study of human tumors, but they can also be used for production purposes. For example, strain LB-N can produce human interferon, and strain LB-P can produce Epstein-Barr virus.

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CYTOTOXIC ACTION OF NATURAL KILLER CELLS ON HUMAN TUMOR CELLS

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An important role is nowadays ascribed in antitumor surveillance to cell-mediated natural cytotoxicity [3-5]. The principal property of natural killer cells (NKC) is their ability to exert a cytotoxic action on a broad spectrum of target cells, including malignant, virus-infected, as well as certain normal cells, spontaneously (without previous sensitization). In most investigations NKC activity has been detected on the basis of their action on leukemic and lymphomatous target cells. There have been very few studies of natural killer activity against cells of solid tumors [9-11].

Meanwhile data have been obtained on heterogeneity of the NKC population and the existence of NKC subpopulations both producing lysis mainly of lymphoma and leukemia cells (natural killer (NK) cells) and those having a cytotoxic action mainly on tumors cells of nonlymphoid origin (natural cytotoxic (NC) cells). In this connection the comparative study of the cytotoxic action of NKC on leukemia cells and on cells of human solid turmors is of great interest.

For this purpose the cytotoxic action of blood monocytes on cells of human tumor lines in culture was investigated in healthy donors: leukemic line K562 and line AKL of adenocarcinoma of the lung.

EXPERIMENTAL METHOD

Blood monocytes from healthy blood donors, obtained by centrifugation in a Ficoll-Verografin gradient and suspended in medium RPMI-1640 with 10% inactivated human serum (group IV), glutamine, and HEPES buffer (20 mM, complete medium RPMI-1640) in a concentration of 5×10^6 cells/ml, were used as effector cells. K562 and AKL cells were used as target cells. The K562 cells were grown in medium RPMI-1640 with the addition of 10% calf fetal serum, glutamine, and monomycin. Monolayer line AKL was grown in Eagle's medium with the additives mentioned above [2]. Target cells (2×10^6 cells in 1 ml of complete medium RPMI-1640) were incubated for 1 h at 37°C with 100 μCi of $Na_2{}^{51}CrO_4$ (USSR origin, specific radioactivity > 3 mCi/ml), after which the cells were washed 3 times and their concentration adjusted to 10^5 cells/ml with medium RPMI-1640. The cytotoxic test was carried out in plates for 16-18 h. To 0.1 of monocytes 0.1 ml of labeled target cells was added (ratio 50:1), and the mixture was centrifuged at 1000 rpm for 2 min. After the end of the reaction the plates were again centrifuged and radioactivity of 0.1 ml of supernatant determined on a "Gammacord" counter (Ames,

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TABLE 1. Inhibition of Lysis of Labeled K562 Cells after Addition of "Cold" ACL and K562 Cells

Expt. No.	Ratio of labeled K562 cells to "cold" ACL cells	PIL, %	Ratio of labeled K562 cells to "cold" K562 cells	PIL, %
1	1:1	42,49[a]	1:1	73,37[a,b]
	1:5	65,16[a]	1:5	100[a,b]
2	1:5	40,41[a]	—	—
3	1:5	69,4[a]	—	—
4	5:1	11,7	5:1	30,2[a,b]
	1:1	19,6[a]	1:1	45,6[a,b]
	1:5	31,7[a]	1:5	69,5[a,b]

Legend. Here and in Table 2: a) significant values of PIL ($P_u \leqslant 0.05$), b) significant differences between inhibitory effect of "cold" K562 and ACL cells.

USA). The percentage of specific release of ^{51}Cr (cytotoxic index — CTI) was calculated by the equation:

$$CTI = \frac{\text{Number of counts in experimental samples} - \text{spontaneous release of } ^{51}Cr}{\text{Total release of } ^{51}Cr - \text{spontaneous release of } ^{51}Cr.} \times 100\%.$$

Spontaneous releast of ^{51}Cr was determined by incubation of 10^4 labeled target cells in the absence of effector cells; total release of ^{51}Cr by incubating the same number of target cells in the presence of a 2% solution of Triton X-100. The percentage of spontaneous release of ^{51}Cr was 14.8-22.8 for K562 cells and 13.5-32.2 for ACL cells. No fewer than three parallel samples were used in the reaction. The results were subjected to statistical analysis by Student's t test and the Wilcoxon—Mann—Whitney P_u test. When the competitive inhibition method was used [6], unlabeled "cold" cells of the same or a different type were added to the labeled target cells in the ratios of 5:1, 1:1, 1:5, and 1:10 and the percentage of inhibition of lysis (PIL) was calculated by the equation:

$$PIL = \frac{\text{CTI in absence of cold target cells} - \text{CTI in presence of cold target cells}}{\text{CTI in absence of cold target cells.}} \times 100\%$$

EXPERIMENTAL RESULTS

The cytotoxic action of monocytes on K562 and ACL tumor cells was investigated in 37 donors. The mean value of CTI for the ACL cells was 40.78 ± 3.43%, and for K562 cells 36.13 ± 3.23%; the differences were not statistically significant ($t = 0.998$, $P > 0.05$). The mean level of cytotoxic action of the monocytes from healthy donors against both K562 and ACL cells was therefore virtually identical. These results are evidence of the vulnerability of ACL cells to the lytic action of NK cells; the sensitivity of the ACL cells to the cytotoxic action of NK cells was virtually the same as the sensitivity of K562 cells, which are used as the standard target for evaluation of human NK cells.

The cytotoxic action of the monocytes on K562 and ACL cells was determined simultaneously in 23 donors. Analysis of the parameters of cytotoxic action of monocytes from invidual donors revealed considerable fluctuations of CTI: from 85.5 ± 0.7 to 14.2 ± 1.35% for ACL cells and from 68.8 ± 3.45 to 3.7 ± 0.55% for K562 cells. In 9 donors (39.1%) the lytic activity of the monocytes was virtually identical against both cell lines (differences not statistically significant). In this group individuals were found with both reduced (3 donors) and increased NK cell activity (4 donors) compared with the average level. In 14 cases (60.1%) the cytotoxic action of the monocytes on the two cell lines differed significantly. Monocytes of half of the donors (7 cases) had a stronger cytotoxic action against cells of the solid ACL tumor, whereas in the other 7 donors it was stronger against K562 leukemic cells. In most donors lytic activity of the monocytes was thus greater against one of the types of tumor cells.

TABLE 2. Inhibition of Lysis of Labeled ACL Cells after Addition of "Cold" K562 and ACL Cells

Expt. No.	Ratio of labeled ACL cells to "cold" K562 cells	PIL, %	Ratio of labeled ACL cells to "cold" K562 cells	PIL, %
1	1:1	15,65	—	—
	1:5	40,05[a],	1:5	30,77[a]
	1:10	64,72[a]	—	—
2	1:1	39,47[a]	—	—
	1:5	78,29[a]	—	—
	1:10	100[a]	—	—
3	1:1	30,98	—	—
	1:5	77,65[a],	1:5	32,94[a]
4	1:1	24,78	—	—
	1:5	52,65[a],	1:5	38,5[a]

These results suggest the existence of at least two types or subpopulations of cells, detectable with about equal frequencies, and differing in selectivity of their natural cytotoxic action on the target cells studied. The ratio between these subpopulations of cells varied very considerably among different individuals under normal conditions.

The use of the competitive inhibition method showed that addition of "cold" ACL cells to labeled K562 cells inhibited lysis of the latter (Table 1); addition of the same numbers of "cold" K562 cells, as might be expected, inhibited lysis of labeled K562 cells by a much greater degree. A direct relationship was noted between the number of "cold" cells and the intensity of inhibition of lysis. Experiments with labeled ACL cells showed that in this case also "cold" K562 cells inhibited lysis of ACL cells more effectively than "cold" ACL cells (Table 2). Similar results, incidentally, are reported by Ortaldo and Herberman [7] who found, in particular, that adsorption of donors' monocytes on a tumor cell monolayer inhibited lysis of analogous cells by a lesser degree, in certain cases, than adsorption on a K562 monolayer. The fact that interaction betwwen K562 and NK cells was more effective than with ACL cells can be explained by the larger number of receptors for NK cells on the surface of K562 cells. Other data have been published [8] to show that K562 cells possess surface target structures for several NK cell subpopulations, whereas other tumor cells possess receptors for only one NK cell subpopulation.

NK cells thus interact with both K562 and ACL cells. The degree of interaction of NK cells with the tumor target cells studied varies very considerably among healthy donors. The results evidently indicate heterogeneity of the human NK cell populations capable of effecting surveillance both on leukemic cells and on cells of solid tumors.

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