SENSITIVITY OF HUMAN GLIAL TUMOR CELLS WITH DIFFERENT DEGREES OF ANAPLASIA TO LYSIS INDUCED BY NATURAL KILLER CELLS, DEPENDING ON GLYCOPROTEIN STRUCTURE OF THE TUMOR CELL MEMBRANES

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Individual lymphocyte subpopulations possess spontaneous cytotoxic activity against transformed cells (natural killer — NK — activity). The process of recognition of the tumor cell by the lymphocyte and the nature of its target structures have not been adequately studied [5, 11]. It has been shown that transition of target cells into a more highly differentiated state is accompanied in some cases by resistance to the action of lymphoid cells [6, 10, 14], which is evidently associated with a change in the composition of the membrane glycoproteins of the tumor cell during differentiation, and in the degree of their sialation [7, 8, 10, 13]. Meanwhile, the characteristics of sialation of human glial tumor cell membranes and their vulnerability to the killer action of lymphocytes have been inadequately studied.

Accordingly, the aim of this investigation was to study the sensitivity of freshly isolated glioma cells to lysis mediated by NK cells, depending on their degree of malignancy and the degree of sialation of their membranes.

EXPERIMENTAL METHOD

Material for determination of the sensitivity of the glioma cells to lysis induced by NK lymphocytes consisted of freshly isolated cells from tumors obtained at operations on 13 patients. The diagnoses were verified histologically in accordance with the International Histological Classification of Tumors of the Central Nervous System (World Health Organization) [1]. Depending on the degree of anaplasia, the cases were distributed as follows: astrocytomas — gliomas of the I-II degree of malignancy — four cases; anaplastic astrocytomas — gliomas of the III degree — three cases; glioblas-tomas — gliomas of the IV degree — six cases. Lymphocytes were obtained from the peripheral blood of a constant group of eight donors, by isolation on a Ficoll—Verografin gradient. The cells were washed 3 times with Hanks' solution and resuspended in medium 199 with the addition of 10% fetal calf serum. NK activity of the lymphocytes was determined by the usual method [2]. When the test was set up the ratio of effector:target was 5:1 and 10:1. Cells of the K-562 line were maintained in medium RPMI-1640 with the addition of 10% fetal calf serum ("Flow Laboratories," Great Britain), 300 μg/ml glutamine, and 80 μg/ml gentamicin. Human glial tumors (biopsy material) were treated with 0.2% Versene solution, disaggregated by pipetting, and filtered through a nylon filter. To reduce spontaneous lysis, living cells were separated from dead by additional centrifugation on a Ficoll—Verografin gradient for 20 min at 1500 rpm. Desialation of the tumor cell membranes was carried out in medium 199, containing 0.004 U/ml neuraminidase, for 1 h at 37°C, after which the cells were washed by centrifugation at 1000 rpm, and their viability determined by studying incorporation of 0.1% trypan blue. Target cells ($2-3 \cdot 10^6$) were labeled with ^{51}Cr (final concentration $3.7 \cdot 10^5$ Bq/ml) at 37°C for 1 h, washed 3 times by centrifugation in a large volume of medium at 1000 rpm for 5 min, and resuspended in complete culture medium. The reaction was carried out in 96-well planchets ("Lenmedpolimer," USSR). After introduction of the effector and target cells into the wells (final volume 200 μl) the planchets were centrifuged for 5 min at 1000 rpm and incubated for 4 h in an atmosphere containing

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TABLE 1. Sensitivity of Freshly Isolated Glial Tumor Cells to NK-Mediated Lysis by Donors' Lymphocytes

Degree of anaplasia	Number of tests	Lysis of tumor cells, %, 10:1
I-II:		
before neuraminidase treatment	22	16,1±4,0
after neuraminidase treatment	22	31,6±4,4**
III:		
before neuraminidase treatment	18	20,1±3,6
after neuraminidase treatment	18	28,6±4,5
IV:		
before neuraminidase treatment	27	36,2±10,1*
after neuraminidase treatment	27	37,4±14,1
Control - K-562 cells		
before neuraminidase treatment	33	18,1±3,6
after neuraminidase treatment	33	23,8±4,3

Legend. *) Difference statistically significant compared with control; **) difference between parameters within group before and after neuraminidase treatment is statistically significant.

5% CO_2 at 37°C. The radioactivity in the supernatant was measured on a BDBS 3-1 eM gamma-counter (USSR) in a volume of 100 μl. The percentage lysis was determined by the formula:

Spontaneous lysis was monitored as the release of ^{51}Cr into the supernatant in the absence of effector cells. Maximal lysis was equivalent to half the radioactivity introduced into each well. The results of only those experiments in which spontaneous lysis of the target cells did not exceed 30% of maximal were taken into consideration. The significance of differences between the parameters was established by the Wilcoxon—Mann—Whitney test.

EXPERIMENTAL RESULTS

On parallel determination, sensitivity of K-562 cells, freshly isolated glioma cells, and desialated tumor cells to NK lysis depended on the degree of malignancy of the tumor (Table 1). Tumors of the IV degree of malignancy were most sensitive to NK-lysis, namely glioblastomas (percentage lysis 36.2 ± 10.1); the sensitivity of tumors of the I-II and III degrees of malignancy was lower and was comparable with the sensitivity of the standard target of NK cells, namely line K-562 (percentage lysis 18.1 ± 3.6). Thus, human tumor cells of different degrees of malignancy differ in their sensitivity to NK-lysis.

Treatment of the tumor cells with neuraminidase resulted in significant ($p < 0.05$) intensification of sensitivity only of benign gliomas (degree I-II of malignancy) to lysis — the percentage lysis before neuraminidase treatment was 16.4 ± 4.0 and after treatment 31.6 ± 4.4. Treatment of cells of gliomas of degree III and IV of anaplasia, and also cells of the K-562 line with neuraminidase as a rule did not change their sensitivity to NK lysis. These results are evidence that membranes of glial tumor cells are desialized to the greatest degree in patients with the most malignant tumors — degree III and IV of anaplasia.

The overwhelming majority of antigens associated with tumors are glycoconjugates. Tumor cells have their own composition of terminal carbohydrate residues of glycoconjugates of cell membranes [9], due to failure to complete the final stages of biosynthesis of carbohydrate-containing polymers, specifically with the absence of masking of terminal residues of the glycoconjugates by sialic acids [4, 9]. The important signs of malignant transformation are an increase in the content of D-galactose in tumor cell membranes and atypism of its localization, combined with a decrease in the content of N-acetyl-D-glucosamine and of sialic acids, as has been shown for tumors of different histological types and different

degrees of anaplasia [4]. Meanwhile, the view has been expressed that malignant transformation is accompanied by elevation of the sial-transferase level and of neuraminic acid production [7]. This tendency has been shown in investigations using transformed cell lines subcultured in vitro and in vivo [7].

Our results obtained with the use of freshly isolated cells from gliomas with different degrees of anaplasia as the target cells in NK-mediated cytolysis showed that the efficiency of lysis of these tumor cells depends on the degree of sialation of their membranes, for removal of sialic acids unmasks the binding sites with killer cells. Under these circumstances, in the most malignant tumors, namely gliomas with the III and IV degrees of anaplasia, binding sites on the cell membrane are completely unmasked, for neuraminidase treatment did not affect their sensitivity to lysis through the action of killer cells. On the other hand, the results indicate that NK cells react only with certain membrane structures of brain gliomas, which in the normal individual and in tumors of low malignancy are evidently masked, which is a characteristic feature of other types of tumors as well.

It can be concluded from these investigations that in brain tumors the level of anaplasia of the tumor cells determines their sensitivity to the action of NK lymphocytes in vivo. The most marked disturbances in the mechanisms of membrane sialation, it can be postulated, are observed in gliomas of the IV degree of anaplasia.

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