SENSITIVITY OF TUMOR CELLS TO THE CYTOSTATIC ACTION OF NATURAL KILLER CELLS AND THEIR ABILITY TO RELEASE PROSTAGLANDINS OF TYPE E

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Malignant tumor cells, on contact with natural killer (NK) cells in vitro or in vivo (in tumor nodules) largely inhibit their cytotoxic activity (CTA) [3, 11, 12]. The mechanism of this inhibition was not completely clear. Inhibition of CTA of NK cells can be caused by prostaglandins of type E (PGE) [8]. According to our own findings and data in the literature, inhibition of CTA of NK cells may be due to the active secretion (release) of PGE by tumor cells on coming into contact with NK cells in vitro [4, 5, 7, 10]. In our view, ability to release PGE on contact with NK cells in vitro is a property of highly malignant (highly tumorigenic, and also metastasizing) tumor cells [4, 5]. It was decided to investigate to what degree the active secretion of PGE by tumor cells determines their sensitivity to NK cells on contact in vitro.

EXPERIMENTAL METHOD

Altogether 12 strains of sarcomas of Syrian hamsters, obtained in the Writers' laboratory and characterized according to their degree of malignancy were studied [1, 2, 9]. Some of these strains were obtained by selection in vivo from parental cells of the STHE strain (embryonic Syrian hamster cells, spontaneously transformed in vitro), characterized by low tumorigenicity, and not metastasizing [1, 9]. In the present investigation, of all the variants of strain STHE, we studied three malignant cell lines (strains STHE-LM^6, STHE-LM^8, and STHE75/18) and four strains (STHE-PK^4, STHE-PK^5, STHE-LM^1, and STHE-LM^4) with less marked malignant properties. We investigated highly tumorigenic, but nonmetastasizing cells of strain STHE-SR, obtained by transformation of hamster embryonic cells in vitro by Rous sarcoma virus (RSV), and two of its variants selected in vivo: strains STHE-SR-4PK — a highly tumorigenic but nonmetastasizing variant, and STHE-SR-2PK-MLU — a highly tumorigenic metastasizing variant. Cells of strain OPH-SR, highly tumorigenic, but nonmetastasizing, were obtained from a tumor induced by RSV in a Syrian hamster. All strains of tumor cells were grown in vitro in tissue culture on Eagle's or F-12 medium with the addition of 10% bovine serum and 0.1 mg/ml of gentamicin. A cell culture aged 2-3 days was used.

Human NK cells were isolated from buffy coat obtained on an "Amico" blood cell separator at the bone marrow bank of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and they were generously provided by D. M. Mkheidze (Department of Blood Transfusion, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR). The NK cells were isolated by the method described previously, using a Percoll gradient [6].

PGE secretion by the tumor cells was induced by contact between them and NK cells in vitro, and the presence of PGE in the culture medium was determined by a biological test developed previously, based on the immunodepressive action of PGE on intact NK cells [4]. Tumor cells, removed with versene and numbering $(1\text{-}1.5) \cdot 10^6$, were joined to NK cells in the ratio of 1:10 in 1 ml nutrient medium. The cell mixture was centrifuged for 1.5 min at 1000 rpm. After exposure for 15-20 min at 37°C, resuspension of the cell mixture, and recentrifugation, the culture fluid (CF) from each sample of tumor cells was added to fresh, intact NK cells, whose cytotoxic activity was studied in the cytotoxic test (CT) with MOLT-4 target cells, labeled with ^{51}Cr

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TABLE 1. Characteristics of PGE-Inducing Activity of Strains of Tumor Cells Differing in their Degree of Malignancy and during Contact with NK Cells in Vitro (results of biological tests)

Strain	Characteristics of malignancy	Treatment with indomethacin (−) and (+)	Per cent CTA of NK cells (M±m, n = 3)	Number of times by which CTA reduced in experiment
STHE	Low	—	48,2±2,3	1,0
		+	47,4±3,2	1,0
STHE-LM1	»	—	30,7±3,6	1,6
		+	48,6±2,3	1,0
STHE-LM4	»	—	31,1±2,5	1,6
		+	49,2±1,6	1,0
STHE-PK4	»	—	25,7±4,2	1,9
		+	48,4±2,6	1,0
STHE-PK5	»	—	27,3±3,7	1,8
		+	49,7±2,4	1,0
STHE-75/18	High	—	14,9±3,5	3,3
		+	41,4±2,1	1,2
STHE-LM6	»	—	16,9±1,2	2,9
		+	46,7±2,3	1,0
STHE-LM8	»	—	17,5±3,1	2,8
		+	46,8±3,0	1,0
HTE-SR	»	—	14,0±2,7	3,5
		+	42,1±3,4	1,2
	»	—	15,4±1,8	3,2
HTE-SR-2PK-MLU		+	41,7±2,1	1,2
	»	—	16,4±3,6	3,0
STHE-SR-4PK		+	44,7±2,5	1,1
	»	—	12,0±3,2	4,1
OPH-S		+	38,1±3,5	1,3

*All characteristics of malignancy of test tumors given in publications of Deichman et el. [1, 2, 9]. 2) Percent CTA of NK cells in control (E:M = 50:1) 49.2 ± 3.5; 3) number of times by which percent CTA of NK cells was reduced by treatment of tumor cells with CF compared with control. Statistically significant differences with control ($p < 0.01$) correspond to the value of 1.7.

(E:M = 30-50:1). The presence of PGE in the preparations of CF was judged by the degree of inhibition of CTA of the NK cells treated with the CF preparation compared with CTA of intact control NK cells. In each experiment tumor cells in which PGE synthesis had been blocked beforehand with indomethacin (20 μg/ml medium, 2 h, 37°C), were used as the control.

The sensitivity of the test strains of tumor cells to the cytostatic action (CSA) of NK cells, depending on PGE secretion, was determined in the cytostatic test as follows: tumor cells (intact and treated with indomethacin), after contact with NK cells and removal of the supernatant culture fluid, were resuspended in nutrient medium with 10% bovine serum and ^{3}H-thymidine (0.5 μCi/ml). Each sample, 2 ml in volume, of cell suspension was poured into three scintillation flasks. The number of tumor cells tested in each flask varied from 3 to $5 \cdot 10^6$ cells in individual experiments. Incorporation of ^{3}H-thymidine into the cell nuclei was determined after 20 h of culture at 37°C. The results of the cytostatic test were assessed as a percentage of CSA of NK cells relative to the tumor target cells tested, by the equation:

$$\% \text{ CSA} = \frac{\text{cpm in control} - \text{cpm in expt.}}{\text{cpm in control}} \cdot 100.$$

Each cell variant was tested in three or more experiments. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Data characterizing the PGE-secretory activity of 12 cell strains, differing in their level of malignancy and in the conditions of their short-term contact with NK cells in vitro, are given in Table 1. They show that active release of PGE into the culture medium was observed only in the case of the highly malignant strains. This was shown both for malignant variants of the STHE strain (strains STHE-75/18, STHE-LM^6, and STHE-LM^8), and also for highly malignant strains HTE-SR, HTE-SR-2PK-MLU, HTE-SR-4PK, and OPH-SR. Preparations of CF of these seven strains lowered CTA of the NK cells by 2-4 times compared with the control relative to standard MOLT-4 test cells. The inhibitory activity of preparations of CF of tumor cells of strains STHE-PK-4 and STHE-PK-5 was somewhat lower but still considerable. For strains STHE-LM^1 and STHE-LM^4 the values of these parameters were not statistically significant. Just as in the previous investigations we found no release of PGE

TABLE 2. Sensitivity of Strains of Tumor Cells Secreting and not Secreting PGE on Contact with NK Cells in Vitro to CSA of NK Cells (results of cytostatic tests, labeling with ^{3}H-thymidine)

Strain	Preliminary treatment with indomethacin (−) and (+)	CSA of NK cells, per cent (M ± m)
STHE	−	69,2±2,4
	+	68,4±2,7
STHE-LM1	−	58,7±10,9
STHE-LM4	−	52,3±6,5
STHE-PK4	−	48,9±6,2
STHE-PK5	−	51,4±4,9
STHE-75/18	−	17,3±3,9
	+	67,3±4,8
STHE-LM6	−	21,4±3,1
	+	69,6±4,2
STHE-LM8	−	19,2±3,6
	+	63,5±2,1
HTE-SR	−	18,6±4,1
	+	67,4±4,2
STHE-SR-4PK	−	23,3±3,8
HTE-SR-2PK-MLU	−	22,0±3,2
	+	64,7±4,3
OPH-SR	−	18,9±4,4

on contact between cells of a strain of STHE with low malignancy and NK cells. Inhibition of PGE synthesis by preliminary treatment of the test tumor cells with indomethacin completely abolished the inhibitory action of the CF of these cells on CTA of NK cells.

Thus this investigation of 12 strains of tumor cells differing in their degree of malignancy revealed definite correlation between the degree of malignancy of these cells and their ability to actively secrete PGE in response to contact with NK cells in vitro. The ability of the transformed cells to release PGE on contact with NK cells is evidently a feature which creates selective advantages during their selection in vivo, as was seen particularly clearly when variants of strain STHE selected in vivo were tested. In this connection it was decided to study whether correlation exists between the ability of these cells to release PGE and the ability of NK cells to exert a cytostatic (cytotoxic) action on these cells. To investigate this problem, 12 cell lines characterized for their ability to release PGE were used as target cells (native, or treated beforehand with indomethacin) in the cytostatic test with NK cells. The results (Table 2) showed that the sensitivity of the test cells to the CSA of NK cells depended on their ability to release PGE. For instance, strains actively secreting PGE (STHE-75/18, STHE-LM[8], HTE-SR, and all its variants, as well as OPH-SR) were highly resistant to the CSA of NK cells, which amounted to 17-22%.

Preliminary treatment of cells of several strains with indomethacin significantly increased their sensitivity to the CSA of NK cells — up to 63-69%. Treatment of STHE cells with indomethacin, however, did not change their sensitivity to the CSA of NK cells (68%).

The investigation thus showed that one mechanism protecting tumor cells against the CTA of NK cells is their ability to release PGE on contact with NK cells and inhibition of their CTA.

LITERATURE CITED

1. G. I. Deichman and E. L. Vendrov, Dokl. Akad. Nauk SSSR, **284**, No. 6, 1510 (1985).
2. G. I. Deichman and E. A. Kashleva, Dokl. Akad. Nauk SSSR, **232**, 473 (1987).
3. T. E. Klyuchareva and V. A. Matveeva, Byull. Éksp. Biol. Med., No. 6, 732 (1985).
4. T. E. Klyuchareva, V. A. Matveeva, L. S. Bassalyk, and N. E. Kushlinskii, Byull. Éksp. Biol. Med., No. 2, 204 (1988).
5. T. E. Klyuchareva, V. A. Matveeva, L. M. Kashkina, et al., Éksp. Onkol., **11**, No. 1, 53 (1989).
6. V. A. Matveeva and T. E. Klyuchareva, Byull. Éksp. Biol. Med., No. 4, 457 (1986).
7. V. Yu. Umanskii, T. E. Klyuchareva, and V. A. Matveeva, Byull. Éksp. Biol. Med., No. 4, 438 (1989).
8. M. Brunda, R. Herberman, and H. Holden, J. Immunol., **124**, 2682 (1980).
9. G. I. Deichman and E. L. Vendrov, Int. J. Cancer, **37**, 401 (1986).

10. M. Droller, M. Shneider, and P. Perelmann, Cell. Immunol., **39**, No. 1, 165 (1978).
11. P. May, E. Holmes, and S. Golub, Cancer Res., **45**, No. 1, 57 (1985).
12. B. Perussia and G. Trinchieri, J. Immunol., **126**, No. 2, 754 (1981).

COMPARATIVE ACCUMULATION OF THE FLUORESCENT PROBE HOECHST 33258 IN LEUKEMIA P388 CELLS SENSITIVE AND RESISTANT TO DOXORUBICIN

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Experimental studies have demonstrated a decrease in accumulation of certain preparations in tumor cells resistant to cytostatics of natural origin (doxorubicin, vincristine, actinomycin D, colchicine) compared with sensitive cells in systems both in vitro and in vivo [4, 10]. A decrease in doxorubicin accumulation in leukemia P388 cells with induced resistance to this anthracycline antibiotic was demonstrated previously [1]. However, on determination of the total content of the cytostatic in tumor cells, the question of the relative accumulation of the preparation actually in their nuclei remained unexplained, i.e., nothing could be stated about the accessibility of the nuclear DNA for the preparation in resistant and sensitive cells. It has been suggested that the cytostatic is located in resistant cells mainly in the cytoplasm [6]. There is evidence in the literature of an equal cytotoxic action of doxorubicin on resistant cells if present in equal intranuclear concentrations [5]. It is technically difficult, however, to measure penetration of doxorubicin inside the nucleus, for when it interacts with biological macromolecules, quenching of the fluorescence takes place.

We know that multiple drug resistance of tumor cells to cytostatics can be overcome with the aid of detergents in low concentrations [3]. In our own experiments, to overcome the drug resistance of P388 cells with induced resistance to doxorubicin, we used Triton X-100 in concentrations not destroying cell membranes.

In the present investigation we studied penetration of the fluorescent probe Hoechst 33258 into sensitive and resistant leukemia P388 cells. The reason for the investigation was as follows: 1) the Hoechst probe fluoresces only on interaction with the DNA molecule [2]; 2) the probe molecule is a heterocyclic compound, i.e., the Hoechst dye is similar in its molecular structure to the group of cytostatics of natural origin to which the tumor cells acquire crossed resistance; 3) doxorubicin and Hoechst 33258 are known to be competitive with one another for binding sites with purified DNA [9].

EXPERIMENTAL METHOD

Leukemia P388 cells within induced resistance to doxorubicin (P388/DXR) were obtained by selection from leukemia P388 cells (P388/0 — the initial strain, from the tumor strain bank of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR) during treatment of animals with small doses of the antibiotic. To induce resistance, 35 passages were needed. To maintain the leukemia P388/0 and P388/DXR, male DBA/2 mice aged 2-3 months were used. The tumor cells were transplanted intraperitoneally in a dose of $1.0 \cdot 10^6$ cells in 0.2 ml of medium 199. The leukemia cells were isolated from

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