Examination of the effect of PGE_2 on PEC of intact animals did not reveal any decrease in their CL activity. This may be due to the fact that it is M which are mainly responsible for CL of PEC from intact animals. Since M secrete PGE themselves, they may be more resistant to its immunodepressive effect.

Thus, the study performed demonstrated a substantial enrichment of PEC with N and a marked enhancement of their CL activity in inoculated animals independently of the type of material used. In addition, we revealed a much higher sensitivity of N to a wide spectrum of PGE_2 doses in comparison with M. Finally, it was shown that N exhibit different degrees of sensitivity to PGE_2 when stimulated by different agents.

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Role of E-Type Prostaglandins in Tumor Cells During Contact with NK Cells *in Vitro*

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It has been demonstrated in our previous studies that highly malignant tumor cells, in contrast to cells of low malignancy, when in contact *in vitro* with NK cells as well as macrophages and neutrophils, rapidly begin (for several min) to secrete into the culture medium E-type prostaglandins (PGE), which suppress the cytotoxic activity (CTA) of NK cells [1,2]. We observed the active PGE release by highly malignant cells of Syrian hamster sarcoma, selected *in vivo* or transformed by the Rous sarcoma virus after their contact with NK cells. Information is available on the greater resistance to the cytotoxic effect of macrophages and neutrophils as well as NK cells exhibited by malignant tumor cells selected *in vivo* as compared to parental variants [4,8,9]. It has been shown in other reports [7,10] as well as in our own [2] that the resistance of several types of tumor cells is conditioned by their ability to suppress NK cells activity due to PGE secretion. It is obvious that PGE secretion induced by effector cells of the system of natural resistance is one of the protective mechanisms of tumor cells [1,6,9].

It is of interest to us to study the following aspects of the mechanisms of the interaction between

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TABLE 1. Dynamics of PGE Release by HET–SR Tumor cells Depending on Tumor Cell:NK–Cell Ratio for Contact *in Vitro*

Tumor cell: NK-cell ratio in mixture	Treatment of tumor cells with indomethacin: + or -	PGE secretion in CF by HET-SR after contact with NK cells; time of contact, min				
		30	60	120	180	240
Control (C)	–	56/51(1.0)	52/52(1.0)	50/52(0.9)	48/50(0.9)	46/47(0.9)
1:1	–	57/48(1.1)	n.d.	50/49(1.0)	47/53(0.9)	n.d.
1:10	–	56/10(5.6)	59/30(2.0)	42/20(2.1)	48/22(2.1)	42/32(1.3)
1:10	+	56/49(1.0)	59/58(1.0)	59/58(1.0)	42/41(1.0)	42/42(1.0)
1:20	–	57/17(3.3)	58/36(1.6)	49/47(1.0)	50/46(1.0)	n.d.
1:20	+	57/51(1.0)	n.d.	49/49(1.0)	n.d.	n.d.

Note: *: number of tumor cells in all samples 1.7×10^6, C: control, CF from intact tumor cells. Numerator: % CTA of intact tumor cells; denominator: % CTA of NK cells treated with CF; in parentheses: ratio between CTA of NK cells in experiment and in control.

tumor cells and NK cells: 1) the duration of the active PGE secretion by tumor cells upon contact with NK cells; 2) the possibility of indomethacin inhibition of PGE secretion by tumor cells during the process of PGE release.

MATERIALS AND METHODS

A highly malignant strain of Syrian hamster embryonal fibroblasts transformed by the Rous sarcoma virus (strain HET-SR) was used in the experiments [5]. Cells were grown in an F-12 culture medium containing 10% calf serum and gentamicin. NK cells were isolated from human lymphomass (provided by D. M. Mkheidze at the Departament of Blood Transfusion of the Cancer Research Center, Russian Academy of Medical Sciences). Isolation was carried out in a Percall density gradient according to a technique described previously [3]. PGE secreted by HET-SR tumor cells upon their contact with NK cells *in vitro* was determined in the culture fluids (CF) of tumor cells by a bioassay developed by us on the basis of the immunodepressive effect of PGE on the CTA of NK cells [1]. For the assay tumor cells were removed from the glass with versene and mixed in a dose of $1.0\text{-}1.5\times10^6$ with NK cells in 1.0 ml of RPMI-1640 medium, as a rule, in a 1:10 ratio or other ratios as required. To enhance contact between tumor cells and NK cells the mixture was centrifuged for 90 sec at 1000 g. After 30 min contact at 37°C 0.2 ml of CF from each sample was added to fresh intact NK cells, after which the cytotoxic activity was tested by the standard technique using ^{51}Cr-labeled MOTL-4 target cells (effector:target ratio 50:1).

PGE in CF was determined by measuring the degree of suppression of NK cells CTA and the inhibitory effect of indomethacin. PGA secretion by tumor cells was arrested by indomethacin (Sigma) in a concentration of 20 μg.

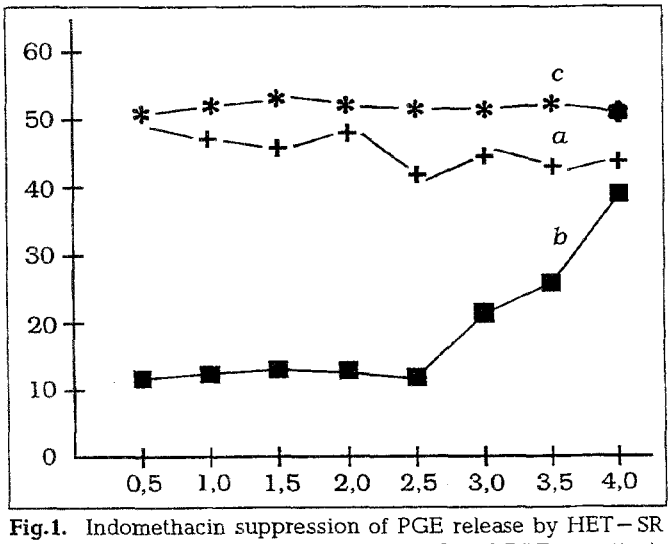

Fig. 1. Indomethacin suppression of PGE release by HET–SR tumor cells (at different times of NK–induced PGE secretion). *a*) control of CTA of intact NK cells; *b*) PGE release by tumor cells induced by NK cells (from data on suppression of CTA of NK cells), HET–SR:NK–cell ratio 1:10; *c*) indomethacin suppression of PGE release by tumor cells.Abscissa: PGE secretion after contact with NK cells (h); ordinate: CTA of NK cells (%).

RESULTS

For a study of the dynamics of PGE secretion by HET-SR tumor cells induced by NK cells, samples of CF from the cell mixtures were taken 30 min after contact, and then at hourly intervals. The medium was changed after each run. The samples were tested immediately for PGE presence by the bioassay. We have previously reported in studies relating the development of this test that active PGE release by tumor cells is detected in all cases provided that the tumor cell:NK-cell ratio is 1:10. The data on the dynamics pf PGE secretion for various cell ratios are presented in Table 1. PGE secretion in CF (tumor cell:NK-cell ratio 10:1 or 20:1) was shown to start after just 30 min contact; moreover, in the case of a 20:1 ratio the maximum PGE release seems to be attained even before this time. Accordingly, PGE secretion by HET-SR tumor cells for the maximum tumor cell:NK-cell ratio was shown to end 1 h after contact, whereas in the case of 10:1 ratio PGE se-

cretion was detected in the samples taken 2 h after contact. No PGE release was found for a 1:1 ratio during the entire period of observation. PGE release in the samples was arrested by a preliminary treatment of the tumor cells with indomethacin. No spontaneous PGE secretion was detected in the control (see Table 1).

The results obtained suggest that the duration of PGE secretion depends on both the amount of tumor cells and the ratio between the tumor cells and the NK cells inducing the PGE secretion.

It has been previously reported [1] that direct contact between tumor cells and NK cells is required to cause PGE release; this is achieved by centrifugation of the cell mixture for several min. In the case of a low NK-cell:tumor cell ratio only some of the tumor cells seem to enter into contact with the NK cells, and repeated cycles of the contact lead to PGE release by tumor cells not engaged in contact earlier. Accordingly, in such samples the PGE secretion process is drawn out. Increasing the NK-cell:tumor cell ratio provides for a simultaneous contact of the NK cells with the majority of the tumor cells, and consequently leads to a rapid dwindling of the PGE secretory activity of these cells. Therefore, it is evident that PGE secretion by the tumor cells starts from the first minutes of their contact with NK cells and is completed after 1 to 1.5 h.

It was of interest to study the possibility of the suppression of PGE release by HET-SR tumor cells induced by NK cells (1:10) ratio. For this purpose, a suspension of tumor cells was treated with indomethacin for 1.5 h prior to their contact with NK cells or at different moments after this contact during the entire period of PGE secretion (3-4 h). It was shown (see Fig.1) that PGE secretion may be suppressed by indomethacin at any time during the entire period of its release.

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