SECRETION OF TYPE E PROSTAGLANDINS (PGE) BY SYRIAN HAMSTER TUMOR CELLS DURING CONTACT IN VITRO WITH NATURAL KILLER CELLS

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Data obtained during investigation of local interactions between tumor cells and natural killer cells (NKC) both in animal experiments [4, 10] and in clinical material [9] are evidence that tumor cells can inhibit the cytotoxic activity (CTA) of NKC. Local depression of activity of the effector cells of natural resistance is perhaps an essential condition for development of a primary tumor and its metastases [1, 2]. It has been shown that prostaglandins of types E_1 and E_2, synthesized by tumor cells, depress the cytotoxicity of NKC [6, 7]. Other workers have obtained data showing that highly malignant tumor cells, treated in vitro with laminin or fibronectin, produce arachidonic acid metabolites, including PGE_1 and PGE_2, more actively than less malignant cells [8]. It was considered important to discover whether stimulation of PGE secretion during contact between tumor cells and NKC in vitro takes place, and whether differences exist in PGE secretion between highly malignant (metastasizing) variants of tumor cells and their less malignant original parent cells.

The ability of two strains of hamster tumor cells of the same origin, but differing in malignancy, to secrete PGE on contact in vitro with human and Syrian hamster NKC was investigated.

EXPERIMENTAL METHOD

Two strains of tumor cells were used in the experiments in vitro: 1) strain HETR — hamster embryonic cells transformed spontaneously in vitro and possessing no metastatic activity; 2) a highly malignant metastatic version of strain HETR (strain HETR-MLN-8), selected in vivo [3]. The cells were grown in tissue culture on Eagle's medium with the addition of 10% bovine serum and gentamicin. Syrian hamster NKC were isolated from the blood of adult animals by the method described previously, using a stepwise Percoll density gradient [5]. In each experiment HETR and HETR-MLN-8 tumor cells, previously treated in a monolayer for 2 h with a solution of indomethacin (Sigma, USA) in a concentration of 20 μg/ml were used as the control.

To assess the direct action of tumor cells on CTA of NKC during contact in vitro, a suspension of native HETR and HETR-MLN-8 tumor cells ($1\text{-}2\cdot10^6$/ml) and a suspension of similar cells treated previously with indomethacin were added in equal volume to a suspension of hamster NKC ($1\text{-}2\cdot10^7$/ml). The resulting mixtures of cells and the corresponding control samples were transferred in a volume of 1 ml into three or four wells of 24-well plates for each variant of the experiment. After contact for 16-18 h at 37°C in an atmosphere of CO_2 the NK cells were separated from the tumor cells in a stepwise Percoll density gradient [4, 5] and their cytotoxicity was investigated in the cytotoxic test (CT) with MOLT-4 target cells labeled with ^{51}Cr.

To study the possible secretion of PGE by the tumor cells into the surrounding medium, a technique was developed whereby the presence of PGE could be determined in the cytotoxic test in vitro by depression of CTA of normal NKC by preparations of culture fluid (CF) of the test tumor cells before and after their contact with NKC in vitro. The scheme of this experiment is illustrated in Fig. 1. PGE were determined quantitatively in parallel tests on CF preparations. For this purpose the prostaglandins were extracted in acid medium with subsequent isolation of PGE on columns with silicic acid, and radioimmunoassay using kits from Clinical Assays (USA).

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TABLE 1. Secretion of PGE into Culture Medium by Malignant Syrian Hamster Tumor Cells during their Contact in Vitro with Hamster and Human NKC (data of CT)

Contacting cells		Preliminary treatment of tumor cells with indomethacin (+) or (–)	CTA of hamster NKC (after contact with culture fluid of test cells)	
			CTA, % (M ± m)[1]	MD[2]
Normal hamster embryonic cells	Control CF	–	24,2±2,6	0
	Syrian hamster NKC	–	22,8±3,2	0
	—	–	20,7±2,8	0
HETR	Control CF	+	21,4±3,1	0
		–	22,1±2,7	0
	Syrian hamster NKC	+	23,6±3,2	0
	Human NKC	–	23,2±3,3	0
HETR-MLN-8	Control CF	+	21,5±3,1	0
		–	19,8±3,3	0
		+	20,1±2,6	0
		–	7,2±3,2	3,4
	Syrian hamster NKC	+	20,5±2,0	0
		–	6,5±3,0	3,7
	Human NKC	+	23,1±3,5	0

Legend. [1]CTA of normal intact hamster NKC in control (without treatment with CF preparations) was 24.7 ± 2.7%.
[2]MD) Multiplicity of depression of CTA of NKC by preparations of CF of test normal or tumor cells (compared with CTA of intact NKC).

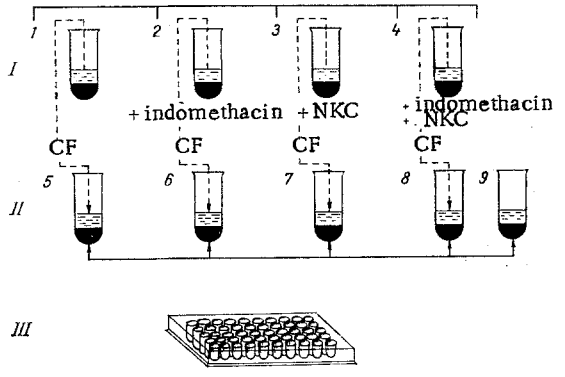

Fig. 1. Scheme of biological method of determining PGE secretion by tumor cells during contact with NKC in vitro. 1-4) Tube containing test cells in culture medium, 5-9) tube with normal intact NKC; I) contact of test cells (intact or treated with indomethacin) with NKC in suspension for 20 min at 37°C; II) transfer of culture fluid from tubes 1-4 into tubes 5-8, containing intact normal NKC; III) CT performed with NKC from tubes 5-9 and with ^{51}Cr-labeled MOLT-4 target cells.

EXPERIMENTAL RESULTS

The study of the direct effect of HETR and HETR-MLN-8 tumor cells on cytotoxicity of Syrian hamster NKC was carried out before and after contact in vitro for 16-18 h at 37°C. HETR tumor cells, both native and treated with indomethacin, had no significant effect on CTA of NKC. Meanwhile after contact with native HETR-MLN-8 tumor cells, CTA of NKC was reduced by more than two-thirds. The effect was abolished by treating the HETR-MLN-8 cells with indomethacin. It is thus evident that highly malignant tumor cells, unlike those of low malignancy, on contact with NKC are able to inhibit their CTA considerably, evidently on account of active production of PGE by these cells.

Taking the results into account, it was important to study activity of PGE secretion by the tumor cells during contact with NKC in vitro. By the technical approach indicated in Fig. 1, it was possible to determine PGE in CF of the tumor cells as reflected in their ability to inhibit CTA of normal NKC in CT. A parallel quantitative determination of PGE was carried out in the same preparations by radioimmunoassay. Data characterizing PGE secretion by tumor cells into the culture medium during contact with NKC in vitro are given in Table 1. According to the results of the biological test, activation of PGE secretion by malignant HETR-MLN-8 cells was observed, and was determined as early after contact for 20 min with NKC. Secretion of PGE by these cells was observed on contact both with Syrian hamster NKC and with xenogeneic human NKC.

We did not observe PGE secretion by HETR-MLN-8 cells during their contact with various normal and tumor cells (hamster and human erythrocytes, normal hamster fibroblasts, tumor cells of varied origin).

It will be clear from Table 1 that preparations of CF of native (but not indomethacin-treated) malignant HETR-MLN-8 cells depressed CTA of normal NKC more than threefold. According to the results of the biological test, no spontaneous secretion of PGE by the malignant variant of the tumor cells was observed outside contact with NKC. Quantitative determination of PGE in these CF preparations revealed a twofold increase in PGE in the preparations compared with that found in control preparations of CF of intact or indomethacin-treated tumor cells. Unlike highly malignant HETR-MLN-8 tumor cells, preparations of CF or normal hamster embryonic cells and of HETR tumor cells with low malignancy, tested after contact with NKC, did not depress CTA of normal NKC.

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