SECRETION OF PROSTAGLANDIN E BY HUMAN THYMOMA MOLT-4 CELLS AND THEIR SENSITIVITY TO THE CYTOTOXIC ACTION OF NK CELLS

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The writers showed previously that highly malignant cells of Syrian hamster sarcoma strain STHE-LM[8] (unlike the original tumor cells of the STHE strain, of low malignancy), on contact with natural killer (NK) cells and also with neutrophils and macrophages, begin to secrete prostaglandins of type E (PGE), which inhibits the cytotoxic action (CTA) of NK cells [1, 2], into the culture medium quickly (in the course of a few minutes). The sensitivity of tumor target cells to CTA of NK cells has been shown to depend on the ability of the tumor cells to release PGE on contact with NK cells: PGE-secreting cells of strain STHE-LM[8] were resistant to the CTA of NK cells, unlike the original cells of the STHE strain, which do not secrete PGE.

As we know, several different strains are used as target cells in standard tests for GTA of NK cells, and in the course of the reaction these cells are in contact with NK cells. among these strains, including standard and highly sensitive to the CTA of NK cells, are MOLT-4 (human thymoma) cells [4]. The aim of this investigation was to determine whether the cells of this strain are able to release PGE on contact with NK cells, and to what degree this property (or its absence) determines the sensitivity of MOLT-4 cells to the CTA of NK cells.

EXPERIMENTAL METHOD

A suspension culture of MOLT-4 cells was grown on medium RPMI-1640 with the addition of 10% bovine serum and gentamicin. A 2-3-day cell culture was used. Human NK cells were isolated from buffy coat obtained from human blood and generously provided by D. M. Mkheidze, of the Bone Marrow Bank, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. NK cells were isolated from buffy coat by the method described previously [3, 4]. Secretion of PGE by MOLT-4 cells was induced by their short-term contact in vitro with human NK cells, and subsequent determination of PGE in the culture fluid (CF) of these tumor cells was carried out by radioimmunoassay [6] and by biological methods [1]. The biological method of testing for PGE in preparations of tumor cell CF was based on the immunodepressive action of PGE on CTA of NK cells, and it consisted of the following procedures: a mixture of MOLT-4 tumor cells and normal NK cells in numbers of $(1\text{-}3) \cdot 10^7$ and $(0.5\text{-}1.0) \cdot 10^8$ respectively was centrifuged for 1.5-2 min at 800*g* and exposed for 20 min at 37°C. The mixture was then shaken and recentrifuged (5 min, 1000 g) and the supernatant CF was quickly transferred to test tubes with fresh intact NK cells. The NK cells were resuspended in CF and, after contact for 20 min at 37°C the CTA of these NK cells and of intact (control) NK cells was studied with effector cells and target cells in the ratio of (30-50):1. Contact with intact target cells (MOLT-4), labeled with ^{51}Cr, was carried out by the standard method. The presence of PGE in the CF preparations was judged by the degree of inhibition of CTA of NK cells treated with CF, compared with the CTA of intact NK cells. PGE was determined in the same CF preparations in parallel tests by radioimmunoassay, using kits from "Clinical Assays" (USA), by the method described previously [6]. To determine the PGE-producing activity of the MOLT-4 cells and its affect on sensitivity of

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TABLE 1. Appearance of Detectable Amounts of PGE in Culture Fluid Depending on Dose of MOLT-4 Tumor Cells, Their Contact with NK Cells in Vitro, and Their Treatment with Indomethacin (results of biological test)

Dose of tumor cells in contact with NK cells	Treatment with indomethacin	CTA of NK cells, per cent	Decrease in CTA of NK cells relative to control, number of times
$1{,}0 \cdot 10^6$	—	44,4/44,3	1,0
$3{,}4 \cdot 10^6$	—	44,4/44,1	1,0
$7{,}5 \cdot 10^6$	—	30,4/12,4	2,4
$1{,}6 \cdot 10^7$	—	32,9/10,2	3,2
	+	32,9/30,6	1,0
$3{,}0 \cdot 10^7$	—	40,6/12,0	3,1

Legend. Here and in Table 3: +) treatment carried out; —) not carried out. Numerator indicates control, denominator — after treatment by CF (from mixture of MOLT-4 tumor cells with NK cells).

the cells to the CTA of NK cells, besides native target cells we also used MOLT-4 cells treated beforehand for 2 h at 37°C with indomethacin ("Sigma"), an inhibitor of PG synthesis, taken in a dose of 20 μg/ml medium.

EXPERIMENTAL RESULTS

In our previous experiments with cells of highly malignant Syrian hamster sarcomas, we found that amounts of PGE detectable in radioimmunoassay and biological tests could appear in CF after contact between not less than $1 \cdot 10^6$ tumor cells and NK cells (in the ratio of 1:10). Preliminary experiments with cells of the MOLT-4 strain showed that these doses of tumor cells, on contact with NK cells in the same ratio, were insufficient for secretion of detectable amounts of PGE in CF. After a 7-10 fold increase in the dose of MOLT-4 cells (to $7.0 \cdot 10^6$ or higher) in the contact mixture with NK cells, we regularly found PGE secretion by these cells into the culture medium (Table 1). Preliminary treatment of the PGE-producing tumor cells with indomethacin abolished the inhibitory CTA of the NK cells, evidence that the effect of the CF preparations in inhibiting CTA of the NK cells was due to the appearance of PGE in the medium. Correlation was found between the results of determination of PGE in CF preparations by radioimmunoassay and biological tests (Table 2). Spontaneous secretion of PGE with the same dose of MOLT-4 cells, but without contact with NK cells, could not be detected.

Thus the data in Tables 1 and 2 showed that human thymoma MOLT-4 cells in doses 7-10 times higher than cells of hamster sarcoma STHE-LM[8] in contact with NK cells in vitro, actively secrete detectable amounts of PGE.

MOLT-4 thymoma cells were found to be highly sensitive to the CTA of NK cells. It was interesting to study to what degree the PGE secreted by these cells determines the sensitivity (or resistance) of MOLT-4 cells to the CTA of NK cells. For this purpose we studied native MOLT-4 cells and the same cells treated with indomethacin, as target cells in different doses ranging from standard $(0.6\text{-}1.2 \cdot 10^5$/ml) to $1.2 \cdot 10^6$/ml. It must be emphasized that the highest dose of MOLT-4 cells used in this series of experiments did not allow PGE secretion into the medium to be detected after contact for 20 min between them and NK cells in vitro in the biological test. The results of one series of analogous experiments, but using contact for 4 h and several ratios of human NK cells for each dose of target cells (60:1, 30:1, and 15:1) are shown. With an increase in dose of the native MOLT-4 cells in the experiment the cytotoxic activity (CTA) of the NK cells was sharply reduced. Spontaneous release of label in this case varied but not significantly (from 7.3 to 15.9%). Target cells treated with indomethacin, in which PG synthesis was suppressed, even with an increase in their dose to the maximum, remained highly sensitive to the cytolytic action of the effector cells, and CTA of the NK cells was preserved at a high level. Conversely, sensitivity of intact MOLT-4 cells to the CTA of the NK cells was reduced with an increase in the absolute number of tumor cells used in the test (with the same ratios to NK cells). For instance, if CTA of the NK cells in a ratio of 60:1 or the maximal dose of native MOLT-4 cells was 16.2%, if the same doses of target cells treated with indomethacin were used, the cytotoxic activity of the NK cells was three times higher (52.2%) and was indistinguishable from values obtained for NK cells tested with the minimal (20 times less) standard dose of native MOLT-4 target cells of $(0.5\text{-}1.0) \cdot 10^5$/ml. A decrease in sensitivity of the MOLT-4 cells to the cytotoxic action of NK cells and, simulta-

TABLE 2. PGE secretion by Human Thymoma MOLT-4 Tumor Cells during Contact with NK Cells

Test preparations of CF	Method of determination of PGE, radioimmunoassay (PG/ml of CF)	Biological testing of CTA of NK cells, per cent	
Nutrient medium (control)	1013	40,6	(—)
NK cells	1026	41,2	(0,9)
MOLT-4 cells	2451	38,9	(1,0)
mixture of NK cells MOLT-4 cells	9.845	12,8	(3,1)

Legend. CTA of NK cells in control with effector and target cells in the ratio of 50:1. Decrease in CTA of NK cells relative to control shown in parentheses as the number of times.

neously, a decrease in activity of NK cells were observed with all ratios of effector to target cells tested, when the absolute number of MOLT-4 cells in the cytotoxic test reached $5 \cdot 10^5$ or exceeded it. With low doses of target cells (standard for the test), preliminary treatment with indomethacin increased their sensitivity to the CTA of NK cells by 20-30%.

It can thus be postulated on the basis of these results that the known high sensitivity of MOLT-4 cells to CTA of NK cells in the cytotoxic test is due to the relatively reduced PG-secreting activity of these cells and to the use of comparatively low doses of cells in the standard tests, insufficient for the secretion of amounts of PGE inhibiting the CTA of NK cells. However, the decrease in cytotoxic activity of NK cells with an increase in the absolute number of MOLT-4 target cells in the cytotoxic tests and the abolition of this effect by indomethacin are evidence that MOLT-4 cells possess (although in reduced form) the same distinctive mechanism of defense against NK cells as that which we demonstrated previously for hamster sarcoma cells, namely PGE release in response to contact with NK cells.

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