ONCOLOGY

Prostaglandin E_2 Release by Human and Syrian Hamster Tumor Cells and Their Sensitivity to Cytostatic Activity of Natural Killers

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 131, No. 2, pp. 191-194, February, 2001
Original article submitted November 24, 2000

The release of prostaglandin E_2 by human and Syrian hamster tumor cells in response to contact with natural killers and their sensitivity to cytostatic activity of natural killers were studied. A previously reported correlation between prostaglandin E_2 release by Syrian hamster tumor cells and their resistance to cytostatic activity of natural killers was confirmed. This resistance can be cancelled by inhibition of prostaglandin E_2 release with indomethacin. Unlike malignant tumor cells of Syrian hamsters, only few human tumor cells resistant to cytostatic activity of natural killers secrete prostaglandin E_2. The resistance of these cell strains to cytostatic activity of natural killers could be cancelled by indomethacin treatment. Possible role of prostaglandin E_2 release as a mechanism of tumor cell protection from effector cells of this type of natural (congenital) immunity is discussed.

Key Words: *natural killers; prostaglandin E_2; human and Syrian hamster tumor cells*

One of the most important aspects in tumor-host cell relationships are the mechanisms protecting tumor cell from effectors of natural (congenital) immunity: macrophages, dendritic cells, neutrophils, and natural killers (NK). These mechanisms allow tumor cells to resist defense reactions of host organism and are determined by many factors, including tumor cell secretion of prostaglandin E_2 (PGE_2) suppressing cytotoxic (CTA) and cytostatic (CSA) activities of NK and T lymphocytes [4,6,10]. Previously we showed that some (but not all) malignant tumor cells release PGE_2 upon contact with NK, which leads to suppression of NK CTA [2,3,7]. Our very first studies showed that *in vitro* spontaneously transformed embryonic fibroblasts of HETR Syrian hamster and their *in vivo* selected low-malignant variants do not release PGE_2 upon contact with NK and are sensitive to CTA and CSA produced by these cells. At the same time, all highly malignant variants of HETR tumor cells release PGE_2 upon contact with NK and are resistant to NK CSA [2,3]. These *in vivo* selected variants acquired resistance to cytotoxic products of macrophage and neutrophil oxygen burst, in particular, H_2O_2 and rapidly catabolized these products. High H_2O_2-catabolizing activity ($H_2O_2^{CA}$) and PGE_2 release are new stable characteristics of tumor cells, acquired simultaneously as a cluster of signs, which allowed us to denote them as $H_2O_2^{CA}$+PGE_2. Expression of this phenotype determined a series of biological properties developing in tumor cells during carcinogenesis, in particular the level of their tumorogenicity [1]. Here we investigated whether human tumor cells characterized by a high level of antioxidant defense [10], release PGE_2 upon contact with NK and how their sensitivity or resistance to NK CSA depends on PGE_2 release.

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0007-4888/02/1312-0156$25.00

MATERIALS AND METHODS

Nine strains of Syrian hamster tumor cells obtained in our laboratory were studied: low malignant *in vitro* spontaneously transformed HETR embryonic fibroblasts highly sensitive to NK CSA (parent cells) and 8 *in vivo* selected variants. Four of these strains (HETR-70/18, HETR-128, HETR-162, and HETR-MLN-1) were previously characterized as low tumorogenic and 4 (HETR-74/18, HETR-83/20, HETR-MLN-6, and HETR-MLN-8) as highly tumorogenic [5]. Tumorogenic activity of strains HETR-70/18, HETR-128, HETR-162, and HETR-MLN-1 varied from 2.7 to 3.4 log 50% transplantation dose, while that of strains HETR-74/18, HETR-83/20, HETR-MLN-6, and HETR-MLN-8 from 0.7 to 1.8 log 50% transplantation dose, *i.e.* 10-100 times higher. The cells were cultured in DMEM medium with 10% bovine serum and 100 U/ml gentamicin. Nine *in vitro* cultured human tumor cell strains were studied: teratocarcinoma NT2/D1, KC astrocytoma, breast cancer MCF-7, two melanoma strains (MS and MeWo), rhabdomyosarcoma RD, and three rectal cancer strains (SW-480, Lim 1215, and HT-29). Human tumor cells were cultured in DMEM or RPMI-1640 with 10% ETC and 100 U/ml gentamicin. Human NK were isolated in Percoll density gradient [9] from donor lymph mass (a gift from Dr. D. M. Mkheidze, Bone Marrow Bank, Department of Blood Transfusion, Cancer Research Center). One of controls for each experiment were cells pretreated with indomethacin (Sigma) in a dose of 20 μg/ml for 2 h.

The capacity of tumor cells to release PGE_2 (PGE^+ phenotype) was evaluated by a previously developed indirect biological test based on suppression of NK and T lymphocyte CSA by PGE_2 [2-4,6,7]. The release of PGE_2 by tumor cells was induced by their direct *in vitro* contact with NK, after which PGE_2 were detected in culture medium by immunosuppressive effect of PGE_2 on intact NK CSA [7]. To this end, tumor cells ($2.0\text{-}3.0\times10^6$/ml) untreated or pretreated with indomethacin were mixed 1:10 with NK and centrifuged at 500 rpm for 2-3 min. This procedure enables immediate contact of examined tumor cells with NK (inductors of PGE_2 release) for 20 min at 37°C. Then the supernatant was added to fresh NK suspension, and after 30 min CSA of intact and treated NK towards highly sensitive target HETR (or MOLT-4) cells was evaluated by the standard tests. HETR cells were cultured in 96-well plates (2.0×10^4/well). NK tested for CSA were layered onto them. After 20-h contact with NK (native or treated with culture medium), ^{3}H-thymidine (0.25 mCi/0.2 ml medium) was added into wells, and after 4 h the cells were washed and cell lysates were transferred with a harvester onto paper filters. Label incorporation was evaluated on a β-counter. These data allowed us to compare CSA of intact NK and NK treated with culture medium of tumor cells potentially producing PGE_2 towards label incorporation in intact of indomethacin-treated tumor cells. NK CSA was estimated by the formula:

$$\text{CSA}=\frac{C\text{-}NK_O}{C\text{-}NK_K}\times100\%,$$

where C, NK_K, and NK_O correspond to label incorporation in HETR target cells, in target cells after their contact with intact NK, and in target cells after contact with NK pretreated with tumor cell culture medium, respectively. PGE_2 released into culture medium inhibits CSA of NK, hence ^{3}H-thymidine incorporation in tumor cells is higher compared to tumor cells incubated with NK with preserved CSA. Pretreatment of tumor cells with indomethacin suppressed PGE_2 release, and therefore differences in CSA of intact NK and NK treated with culture medium were leveled. Differences in CSA of NK treated with intact and indomethacin-treated tumor cells are indicative of PGE_2 release by tumor cells; values of at least 1.7 are significant, highly reproducible, and indicate PGE_2 release upon contact with NK [3,7].

The sensitivity of studied tumor cell strains to NK CSA depending on their PGE_2 release was evaluated in the direct standard cytostatic test [3], for which tumor cells (intact and indomethacin-treated) were put in 96-well plates (2×10^4/well, 1 ml) and after adhesion of tumor cells, NK in an equal volume ($2\text{-}2.5\times10^5$) were added (1:10 ratio). After 20-h culturing, ^{3}H-thymidine was added and the mixture was incubated for 4 h. NK CSA to tumor cells was evaluated by the formula:

$$\text{CSA}=\frac{C\text{-}O}{C}\times100\%,$$

where C and O are label incorporation in intact tumor cells and tumor cells cultured with NK, respectively.

The studied tumor cells were considered sensitive, if intact NK CSA surpassed 40% and highly resistant at intact NK CSA was below 36%.

RESULTS

Parent HETR cells and their *in vivo* selected low-tumorogenic variants HETR-70/20, HETR-128, HETR-162, and HETR-MLN-1 did not release PGE_2 upon contact with NK. By contrast, variants HETR-74/18, HETR-83/20, HETR-MLN-6, and HETR-MLN-8 released PGE_2 into the medium and suppressed NK CSA. The sensitivity of Syrian hamster tumor cells

TABLE 1. Sensitivity to NK CSA (in %) in Syrian Hamster Tumor Cells Releasing and Not Releasing PGE_2

Cell strains	Intact	Treated with indomethacin
Not secreting PGE_2		
HETR (parent cells)	69.2	68
HETR-70/20	65.4	62.3
HETR-128	57.3	56.1
HETR-162	65.5	62.7
HETR-MLN-1	59	55.1
Secreting PGE_2		
HETR-74-18	28.6	63
HETR-83/20	27.1	72.5
HETR-MLN-6	21.4	69.6
HETR-MLN-8	19.2	62.2

TABLE 2. Sensitivity to NK CSA (in %) in Human Tumor Cells Releasing and Not Releasing PGE_2

Cell strains	Intact	Treated with indomethacin
Not secreting PGE_2		
MeWo melanoma	15.6	21.5
Rectal cancer:		
SW-480	42.8	35.5
Lim-1215	50	53.2
HT-29	48.9	48.9
RD rhabdomyosarcoma	70.4	67
Secreting PGE_2		
NT2/D1 teratocarcinoma	25.4	57.8
KC astrocytoma	34.7	69.7
MCF77 breast cancer	10.9	50
MS melanoma	14.2	36.4

to NK CSA correlated with their capacity to secrete PGE_2: cells not releasing PGE_2 were highly sensitive to NK CSA, and indomethacin treatment did not modify their sensitivity. Tumor cells releasing PGE_2 were resistant to NK CSA, and indomethacin treatment cancelled this resistance and restored their sensitivity to NK CSA to the level of the parental HETR strain (Table 1). Hence, the capacity of Syrian hamster tumor cells to release PGE_2 upon contact with NK seems to be the main mechanism protecting these cells from NK CSA and the main factor determining their resistance to NK CSA.

Melanoma MS, teratocarcinoma NT2/D1, astrocytoma KC, and breast cancer MCF-7 cells released PGE_2 during contact with NK, the release being the highest in MCF-7 and the least in melanoma MS cells. Melanoma MeWo, rhabdomyosarcoma RD, and three rectal cancer strains did not release PGE_2. Three of four PGE_2-secreting human tumor cell strains were highly resistant to NK CSA, while astrocytoma KC cells were somewhat more sensitive. The sensitivity of all these strains to NK CSA was restored by pretreatment with indomethacin (Table 2). Three of five human tumor cell strains not secreting PGE_2 were highly sensitive to NK CSA. Two strains, particularly MeWo and less so rectal cancer SW-480 were more resistant to NK CSA, but indomethacin treatment of all 5 strains did not essentially modify their sensitivity or resistance to NK CSA. Hence, high resistance of MeWo, NT2/D1, MCF-7, and MS cells to NK CSA correlates in the majority of cases with their capacity to release PGE_2, while high resistance of Syrian hamster tumor cells to NK CSA is determined by their capacity to release PGE_2 in all cases, as was shown in our previous study and now.

Hence, despite essential differences in histogenesis and *in vivo* evolution of compared human and Syrian hamster tumor cells, the capacity of resistant human tumor cells to protect themselves from NK CSA by releasing PGE_2 is apparently one of the main common mechanisms of tumor cell defense from NK.

The study was supported by the Russian Foundation for Basic Research (grant No. 99-04-48358).

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