CHARACTERISTICS OF NK CELLS IN SYRIAN HAMSTERS WITH TUMORS DIFFERING IN METASTATIC AND RESISTANCE-INHIBITING ACTIVITY

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KEY WORDS: cytotoxicity; NK cells; natural resistance; metastatic activity.

Participation of effector cells of natural resistance (NR), namely natural killer (NK) cells and macrophages, in the control of tumor growth and metastasization in vivo has now been proved experimentally [1, 8-10]. Experiments with certain progressively growing tumors (Moloney's sarcoma of mice, mouse mammary gland adenocarcinoma) have shown that NK cells, infiltrating the tumor, unlike NK cells isolated from the organs of the same animals, have lower cytotoxic activity (CTA) against sensitive target cells in the cytotoxic test (CTT) [6]. However, in other experimental systems (rat tumors induced by methylcholanthrene) activity of NK cells in the tumor and organs was indistinguishable from normal [7]. According to Gorelick et al. [9, 10], tumor target cells, selected on the basis of resistance to CTA or NK cells, also possessed greater malignancy (higher metastatic activity). It has been shown in the writers' laboratory that highly metastatic forms of tumor cells, selected in vivo, also were able to inhibit the NR of the host to the tumor, by contrast with the parental cells of the strain [2, 3].

The aim of this investigation was to determine the effect of tumor cells of the same origin but differing in degree of malignancy on CTA of NK cells.

EXPERIMENTAL METHOD

A strain of hamster embryonic cells transformed spontaneously in vitro (HETR) and its highly metastatic version strain HETR-MLN-8 were used. Cells of both strains were injected subcutaneously into 3-month-old Syrian hamsters, and after tumor nodules measuring 3 × 4 cm had formed the animals were anesthetized with ether and NK cells were isolated from the tumor nodules, blood, spleen, and bone marrow. To isolate NK cells from tumor nodules, host cells infiltrating the tumor were separated from tumor cells by the method of Saksela et al. in 10 and 17% solutions of Percoll during centrifugation at 150 rpm for 10 min [11, 12]. Fractionation was repeated 2 or 3 times until the degree of contamination of the preparation with tumor cells did not exceed 1-2%. Adherent cells were then separated on a column with nylon wadding. The NK cells (large granular lymphocytes) were then isolated in a stepwise Percoll density gradient, using 24, 45, 55, and 60% Percoll in medium RPMI-1640 with 10% bovine serum. The Percoll gradient was made up in serologic tubes, by layering each fraction of Percoll in a volume of 1 ml, starting with the maximal concentration. The cell suspension for testing, containing $1 \cdot 10^7$-$5 \cdot 10^7$ cells, was layered above the gradient. The cell fraction isolated from 55% Percoll consisted to the extent of more than 50-70% of medium-sized and large granular lymphocytes and was used in the CTT as a suspension enriched with NK cells.

NK cells were isolated from blood, spleen, and bone marrow by the method described previously [4, 5]. The CTA of NK cells isolated from the tumor and organs of hamsters was investigated in a one-stage CTT with MOLT-4 target cells, labeled with ^{51}Cr. The ability of the isolated NK cells to be activated by interferon was determined by the method described previously after treatment of the NK cells with an interferonogen, namely Newcastle disease virus (NDV) [5].

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TABLE 1. Cytotoxic Activity of NK Cells Isolated from Hamsters with Transplanted HETR and HETR-MLN-8 Tumors, and Their Ability to be Activated by NDV (values of CTA are pooled for 19 hamsters)

NK cells isolated from:	Transplanted tumor	Mean values of CTA of inactivated NK cells		Mean values of CTA of activated NK cells	
		% ± SE	P*	% ± SE	P †
Tumor nodules	HETR	12,5±1,1	0,02	14,7±3,3	H.s.
	HETR-MLN-8	5,4±1,5		9,8±2,8	
Blood	HETR	19,8±3,2	N.s.‡	38,6±2,4	0,01
	HETR-MLN-8	20,5±3,8		36,7±4,4	0,02
Spleen	HETR	11,7±0,8	N.s.	22,8±2,0	0,02
	HETR-MLN-8	9,5±1,9		18,6±3,0	0,02
Bone marrow	HETR	3,5±0,4	N.s.	7,0±1,4	0,05
	HETR-MLN-8	4,6±1,4		4,9±0,6	N.s.

*Data for CTA of NK cells from hamsters with HETR and HETR-MLN-8 tumors are compared.

†CTA values for NK cells activated and not activated by NDV are compared.

‡N.s.) Not significant.

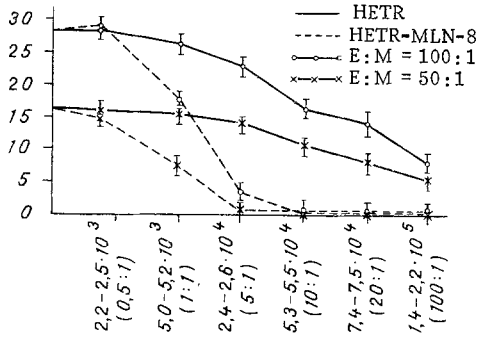

Fig. 1. Competitive inhibition of CTA of NK cells by tumor cells of strains HETR and HETR-MLN-8.

To investigate the effect of tumor cells on CTA of the NK cells in vitro, the competitive inhibition of CTA test with ^{51}Cr-labeled MOLT-4 target cells was used, by the method described previously [4].

EXPERIMENTAL RESULTS

Table 1 gives data on CTA of NK cells isolated from tumor nodules, blood, spleen, and bone marrow of hamsters with subcutaneous HETR and HETR-MLN-8 tumors, and on their ability to be activated. It will be clear from Table 1 that NK cells isolated from HETR tumors and, in particular, from HETR-MLN-8 tumors, possessed lower CTA than blood NK cells, and they were virtually unable to be activated by interferon.

NK cells isolated from blood possessed marked cytotoxicity and remained capable of activation. Low values of cytotoxicity of NK cells were found in the spleen and bone marrow; the bone marrow NK cells from animals with HETR-MLN-8 tumors had completely lost their ability to undergo activation by interferon.

The effect of the HETR and HETR-MLN-8 cells on cytotoxicity of NK cells also was compared in vitro in a test of competitive inhibition of the CTA of normal NK cells relative to ^{51}Cr-labeled target cells. For this purpose, in a standard CTT, unlabeled test tumor cells were injected in different ratios with labeled MOLT-4 target cells. The results of four analogous experiments in which this approach was used (Fig. 1) showed that HETR-MLN-8 cells can inhibit CTA of NK cells when used in the reaction in doses 10 times less than those of HETR cells.

The results thus showed that NK cells infiltrating the tumor in tumor-bearing animals (and, evidently, entering the tumor from the blood stream) were distinguished by low CTA and were not activated by interferon, possible evidence of a direct inhibitory action of the tumor or its product on CTA of NK cells. It was also noted,

just as in the writers' previous investigation [4], that the ability of bone marrow NK cells to undergo activation is exhausted, whereas this activity of NK cells is preserved in the blood and spleen of the same animals. The results can be taken as evidence of active release of NK cells and their precursors from the bone marrow tissue of tumor-bearing animals.

The results of the competitive inhibition of CTT test in vitro showed that HETR-MLN-8 cells, which can inhibit NR of the host to tumors in vivo, also have much greater ability to inhibit the cytotoxicity of normal NK cells in vitro compared with the parental HETR cells. The results of this series of experiments suggest that this effect may perhaps be dependent on the degree of malignancy of the tumor cells.

LITERATURE CITED

1. G. I. Deichman, in: Tumor Growth as a Problem of Biological Development [in Russian], Moscow (1979), pp. 208-230.
2. G. I. Deichman, T. E. Klyuchareva, L. M. Kashkina, et al., Byull. Éksp. Biol. Med., No. 11, 596 (1981).
3. G. I. Deichman, T. E. Klyuchareva (T. E. Kluchareva), L. M. Kashkina, et al., Int. J. Cancer, 30, 319 (1982).
4. T. E. Klyuchareva and V. A. Matveeva, Byull. Éksp. Biol. Med., No. 9, 86 (1983).
5. T. E. Klyuchareva and V. A. Matveeva, Byull. Éksp. Biol. Med., No. 10, 86 (1983).
6. J. Gerson, H. Holdan, and R. Herberman, J. Natl. Cancer Inst., 65, 458 (1980).
7. K. Moore and M. Moore, Br. J. Cancer, 39, 636 (1979).
8. J. S. Haskill, J. Proctor, and Y. Yamamura, J. Natl. Cancer Inst., 54, 387 (1975).
9. E. Gorelick, M. Fogel, S. Segal, et al., J. Natl. Cancer Inst., 63, 1397 (1979).
10. E. Gorelick, S. Segal, and M. Fridman, Cancer Immunol. Immunother., 12, 105 (1982).
11. T. Timonen, C. W. Reynolds, and J. R. Ortaldo, et al., J. Immunol. Methods, 51, 269 (1982).
12. E. Sakasala, T. Timonen, A. Ranki, et al., Immunol. Rev., 44, 71 (1979).

EXPRESSION OF ANTIGENS IMMUNOLOGICALLY RELATED TO MOUSE MAMMARY GLAND CARCINOMA VIRUS ANTIGENS IN HUMAN BREAST TUMOR TISSUE

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Because of the similarity between certain features of neoplastic processes in the human and mouse mammary gland, frequent attempts have been made to discover an agent analogous to mouse mammary tumor virus (MMTV) in man. Virus particles morphologically similar to MMTV have been found in human milk, and in ultrathin sections through cells sedimented from it [5]. However, their budding from the cell surface has not been observed, nor have any cell systems capable of growing them been discovered. The principal searches by investigators have been aimed at finding nucleotide sequences homologous with sequences of MMTV in the genome of human cells and at studying antigens cross-reacting with MMTV antigens in human material. The results of research in this direction cannot be unequivocally interpreted, because they are very contradictory [6]. In our view the reason for this is the inadequacy of the methods used and, in particular, their lack of sensitivity.

In the present investigation two types of indirect solid-phase immunologic tests were used to look for antigens immunologically related to MMTV antigens in human breast tumor (HBT) tissues.

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