EFFECT OF BONE MARROW, SPLEEN, AND PERITONEAL EXUDATE CELLS ON METASTASIS OF TUMOR CELLS IN THE LUNGS OF SYRIAN HAMSTERS

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Effector cells of the system of natural resistance (macrophages, natural killers and also, perhaps, certain other cells) are known to have a cytostatic and cytotoxic action on tumor cells in vitro and in vivo. However, the treatment of growing tumors by means of these cells has a number of limitations, among them the impossibility of creating optimal quantitative ratios in vivo between effector cells and target tumor cells. Closer-to-optimal quantitative relationships between these cells can evidently be created in the case of tumor cells circulating in the body of tumor-bearing animals and forming metastases. For instance, a reciprocal relationship has been found between the number of macrophages in the tissue of a growing tumor and the formation of spontaneous metastases [2, 6]. These observations served as the basis for attempts to prevent metastases in the lungs by repeated injection of activated syngeneic macrophages of normal [5] or tumor-bearing [3] mice into experimental mice. In both cases some decrease was found in the frequency of pulmonary metastases in the experimental animals, although the complexity of the macrophage activation procedure and the consequent losses of effector cells led to a search for other approaches and, in particular, to the use of factors activating macrophages in vivo actually in the body of tumor-bearing animals [4]. Meanwhile, in reactions of natural resistance to tumors in vivo, effector cells evidently function in cooperation with each other and with humoral factors. That is why artificial separation of the components involved in reactions of natural resistance to tumors, which is essential for analytical experiments in vitro, may lead to a decrease in the in vivo antitumor effect. So far, moreover, the ability of normal (i.e., not activated) effector cells to inhibit the development of metastases has not been adequately verified in vivo.

In the investigation described below an attempt was made to inhibit the development of pulmonary metastases in animals by the use of inactivated whole bone marrow (BM) cells, spleen cells (SC), and peritoneal exudate (PE) cells from normal animals as the sources of effector cells.

EXPERIMENTAL METHOD

A highly malignant metastatic variant of strain KhÉTR, namely KhÉTR-MLN-8, was used as the test tumor. Unlike the parental KhÉTR strain the cells of this line can inhibit the natural resistance of animals to the tumor, and this property of these cells correlates with their high metastatic activity [1]. KhÉTR-MLN-8 cells were injected into normal adult noninbred Syrian hamsters either subcutaneously, in a dose of 10^4-10^5 cells in experiments to study inhibition of spontaneous metastases in the lungs, or into the orbital venous sinus in experiments to study inhibition of colony formation (so-called experimental metastases) of tumor cells in the lungs. In the second case, the number of tumor cells injected was 0.5×10^5 to 5×10^5, which usually leads to the appearance of tens or hundreds of visible experimental metastases in the lungs. At different times before, after, or simultaneously with intraorbital injection of the tumor cells (and also with the appearance of palpable tumor nodules after subcutaneous transplantation of the tumor) into the experimental animals a freshly prepared suspension (different numbers) of cells of whole BM, SC, or PE was injected into the orbital sinus. The PE cells were obtained both from normal animals and from animals inoculated before-

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TABLE 1. Inhibition of Experimental Metastases in Lungs of Syrian Hamsters by PE Cells (single and subcutaneous injection in three experiments of the same kind)

Expt. No.	Intraorbital injection of cells		Experimental metastases in lungs	
	KhÉTR-MLN-8	PE	number of animals with metastases	number of metastases in each animal
1	$6,5 \cdot 10^4$	Control without PE	5 (n=5)	11, 24, 27, 246, 482
	$6,5 \cdot 10^4$	$4,0 \cdot 10^7$	2 (n=5)	0, 0, 0, 2, 2
2	$6,0 \cdot 10^4$	Control without PE	10 (n=10)	6, 31, 52, 121, 202, 310, 315, 342, 389, 440
	$6,0 \cdot 10^4$	$1,8 \cdot 10^7$	4 (n=9)	0, 0, 0, 0, 0, 3, 8, 38, 95
3	$5,0 \cdot 10^4$	Control without PE	5 (n=5)	12, 71, 73, 261, 327
	$5,0 \cdot 10^4$	$2,5 \cdot 10^7$	2 (n=9)	0, 0, 0, 0. 0, 0, 0, 1, 28

Legend. Here and in Tables 2-4, number of animals in group shown in parentheses.

hand (24-48 h before sacrifice) intraperitoneally with tryptose-phosphate broth. Since no differences could be found in the activity of PE cells obtained from normal animals or animals inoculated with broth, no distinction was subsequently drawn between these materials.

Intraorbital injection of KhÉTR-MLN-8 cells, labeled with [^{125}I]deoxyuridine, into Syrian hamsters in the present experiment showed that 15-25 min after injection about 100% of these cells were present in the lungs, about 1% in the liver, and about 0.5% in the spleen (the actual data are not given). To count the number of experimental and spontaneous pulmonary metastases 24-27 days after intraorbital injection of the tumor cells, the animals were autopsied 30-40 days after the appearance of palpable tumor nodes following subcutaneous transplantation of tumor cells, and the presence of metastases was noted in the lungs and other organs. For quantitative determination of the metastases the lungs of each animal were then divided into lobes and placed between two flat surfaces formed by glass Petri dishes. Metastases in the lungs were counted on the whole surface of the lung tissue by means of a magnifying glass with a magnification of 25-30 times.

EXPERIMENTAL RESULTS

In the first three experiments normal PE cells and tumor target cells were injected intraorbitally into animals simultaneously but separately (into the two eyes). As Table 1 shows, after injection of 5×10^4 to 6.5×10^4 tumor cells experimental metastases were found in all the control animals, whereas in many experimental animals no metastases were discovered. Differences in the overall frequency of onset of metastases between the experimental and control animals in these three experiments were statistically significant ($P < 0.001$; chi-square test). A similar inhibitory action on metastases was exhibited by normal BM cells, but not by SC (even if injected repeatedly).

It was necessary to discover to what degree the effectiveness of inhibition of metastases of the tumor cells in the lungs depended on the number of effector cells. For this purpose different doses (differing by a factor of two) of normal BM cells were injected intraorbitally, once, and simultaneously with injection of the same dose of test tumor cells. The ratio between the numbers of BM cells and tumor cells was 340:1, 170:1, and 85:1 respectively. The results of this experiment (Table 2) showed that the effectiveness of inhibition of growth of colonies of tumor cells in the lungs may perhaps depend on the dose of effector BM cells injected.

The experiments showed that injection of PE or BM cells inhibited growth of the tumor cells in the lungs completely in some animals, in others it significantly reduced the number of colonies in the lungs, and in a third group of animals it was ineffective. Because of significant differences in the frequency of metastases in the lungs of individual animals in the group, it was decided that it was pointless to determine the mean number of metastases in each group. It was important to discover whether growth of metastases in the lungs could be

TABLE 2. Inhibition of Experimental Metastases in Lungs of Syrian Hamsters Inoculated with Different Doses of BM Cells (single and simultaneous injection)

Intraorbital injection of cells			Experimental metastases in lungs	
KhÉTR-MLN-8	BM	Ratio*	No. of animals with metastases	Number of metastases in separate animals
$1{,}3 \cdot 10^5$	Control without BM	—	5 (n=5)	11, 36, 45, 68, 212
$1{,}3 \cdot 10^5$	$4{,}5 \cdot 10^7$	340:1	1 (n=5)	0, 0, 0, 0, 55
$1{,}3 \cdot 10^5$	$2{,}2 \cdot 10^7$	170:1	2 (n=5)	0, 0, 0, 7, 85
$1{,}3 \cdot 10^5$	$1{,}1 \cdot 10^7$	85:1	3 (n=5)	0, 0, 18, 31, 86

*Ratio between number of BM cells and tumor cells injected intraorbitally into each animal in the group

TABLE 3. Inhibition of Experimental Metastases in Lungs of Animals Inoculated with PE Cells Once at Different Times after Injection of Tumor Cells

Intraorbital injection of cells			Experimental metastases in lungs	
KhÉTR-MLN-8	PE			
	dose	time of injection after inoculation of tumor cells, days	number of animals with metastases	number of metastases in separate animals
$2{,}2 \cdot 10^5$	Control without PE	—	19 (n=19)	12, 24, 25, 41, 69, 80, 88, 91, 105, 106, 125, 140, 146, 158, 177, 208, 265, 287, 388
$2{,}2 \cdot 10^{-5}$	$9{,}0 \cdot 10^6$	Simultaneously	5 (n=10)	0, 0, 0, 0, 0, 2, 96 122, 238, 281
$2{,}2 \cdot 10^5$	$1{,}9 \cdot 10^7$	2-nd	5 (n=9)	0, 0, 0, 0, 0, 2, 4, 6, 9, 166
$2{,}2 \cdot 10^5$	$1{,}1 \cdot 10^7$	5-th	4 (n=10)	0, 0, 0, 0, 0, 0, 2, 4, 35, 111
$2{,}2 \cdot 10^5$	$2{,}7 \cdot 10^7$	8	7 (n=10)	0, 0, 0, 1, 5, 8, 44 91, 109, 254
$2{,}2 \cdot 10^5$	$3{,}0 \cdot 10^7$	9	4 (n=10)	0, 0, 0, 0, 0, 0, 12, 45, 190, 228
$2{,}2 \cdot 10^5$	$3{,}4 \cdot 10^7$	13	8 (n=10)	0, 0, 1, 2, 4, 7, 21, 24, 98, 291

inhibited by a single injection of effector cells, not only simultaneously with the tumor cells but also at different times after their injection, i.e., during the period of active multiplication of the tumor cells in the lungs. The data in Table 3 show that a maximal and approximately equal effect of inhibition of experimental metastases in the lungs by PE cells was observed when they were transplanted on the 2nd-9th day after injection of the tumor cells; the effect of inhibition of metastases by PE cells on the 13th day after injection of the tumor cells was somewhat weaker. In another series of experiments BM and PE cells, injected before the tumor cells, effectively cleared the lungs of tumor cells in the course of 5-8 days after injection (these results are not given).

It was interesting to study whether spontaneous metastases of a tumor can be inhibited by PE, BM, and SC cells in the course of its growth. For this purpose cells of strain KhÉTR-MLN-8 were transplated subcutaneously into Syrian hamsters and, during the first 16 days after the appearance of palpable tumor nodules the animals were given five intraorbital injections (at intervals of 2-5 days) of PE, BM, or SC cells in doses of between 1×10^7 and 6×10^7 cells per injection. Tumor-bearing animals into which these cells were not in-

TABLE 4. Inhibition of Spontaneous Pulmonary Metastases of Subcutaneous KhÉTR-MLN-8 Tumor (during its growth) by Normal Allogeneic BM, PE, and SC cells

Injection of effector cells		Spontaneous metastases in lungs	
type of cells	total* number of cells injected into animal ($\times 10^7$)	number of animals with metastases	number of metastases in separate animals
Control of frequency of spontaneous metastases	—	17 (n=17)	4, 5, 8, 22, 25, 37, 41, 47, 54, 55, 60, 63, 64, 72, 78, 112, 141†
PE	10,3	6 (n=10)	0, 0, 0, 0, 2, 6. 9, 19, 23, 84
BM	24,7	3 (n=10)	0, 0, 0, 0, 0, 7, 10 25
SC	16,7	7 (n=8)	0, 1, 3, 3, 22, 32, 115, 116

*All types of effector cells were injected 5 times (at interval of 2-5 days) in first 16 days after appearance of a palpable tumor. Animals were autopsied 20 days after last injection of effector cells.

† In the group of control animals in this experiment metastases were found in the kidney (in two cases), in the liver (in two), and in the retroperitoneal lymph nodes (in one).

jected served as the control. The number of spontaneous metastases in the lungs and other organs of the experimental and control animals was counted 36 days after the appearance of palpable tumors. The results of this experiment (Table 4) show that injection of nonactivated normal BM and PE cells and, to a much lesser degree, of SC, into the blood stream inhibits the development of spontaneous metastases in the lungs and other organs of animals bearing a subcutaneous tumor.

The mechanism of inhibition of spontaneous and experimental metastases in lung tissue by means of normal allogeneic BM and PE cells is not clear. The most likely explanation was a direct cytotoxic action of the injected cells (macrophages in particular) on tumor cells. However, this explanation is evidently insufficient or incorrect, for injection of effector cells in some animals was unsuccessful and a high frequency of pulmonary metastases was observed. This state of affairs and the wide variations usually observed in the frequency of lung metastases in individual control animals (including syngeneic), inoculated with an equal dose of tumor cells, may perhaps indicate significant individual differences in the level of natural resistance to the tumor in normal animals in the population and that the mechanism of the inhibitory action of BM and PE effector cells on metastases is inductive in character.

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