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EFFECT OF ADAPTIVE TRANSFER OF SPLENOCYTES FROM TUMOR-BEARING MICE ON METASTASIS FORMATION IN THE LUNGS OF INTACT MICE

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The view that a primary tumor focus has an inhibitory action on the formation of distant metastases arose on the basis of clinical observations and experimental research [2, 5, 8, 9, 14]. In a model system developed by the writers, with transplantable mammary gland carcinoma of C3H/He mice, the inhibitory effect of the local tumor on metastasis formation in the lungs was reproduced. Surgical removal of the tumor abolished the inhibitory effect (IE) [1]. Adoptive transfer of splenocytes from tumor-bearing mice to immunodepressed recipients is known to induce changes in resistance to metastases in the recipients similar to those in the tumor-bearers [7, 10]. It appeared interesting to study the role of splenocytes in the development of IE in tumor-bearing mice using our model system.

The aim of the present investigation was to study quantitative dynamics of nucleated spleen cells in mice at different stages of growth of a syngeneic tumor and after its removal. The antimetastatic (antitumor) activity of splenocytes of tumor-bearing mice was assessed by the adoptive transfer method, by determining their ability to inhibit the formation of experimental lung metastases (ELM) in intact mice.

EXPERIMENTAL METHOD

Experiments were carried out on male C3H/f (MTV=S$^-$) mice weighing 24-26 g and on 6-8-week old mice with the nude mutation (partially inbred for the C3H/f genotype, after four back crosses to the pure line), maintained at the Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR. Transplantable mammary gland carcinoma, strain MMT1, of C3H/He mice was used. A suspension of tumor cells was prepared by a method based on preliminary trypsinization of tumor tissue [5]. Growth of the tumor was induced by injection of $2 \cdot 10^6$ MMT1 cells subcutaneously in the right subscapular region. Splenocytes were counted and transferred from the tumor-bearers on the 5th, 14th, and 25th days after subcutaneous inoculation of the cells and 14 days after removal of the tumor. ELM were induced by injection of $2 \cdot 10^5$ tumor cells into the caudal vein; metastases in the lungs were counted under the MBS-2 binocular loupe with a magnification of 8. The suspension of splenocytes was prepared by the method in [12]. The cells were washed off twice in medium 199 by centrifugation at 2000 rpm for 10 min. Each experimental animal was given an intravenous injection of $3 \cdot 10^7$ splenocytes

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TABLE 1. Changes in Weight of Spleen and Number of Nucleated Spleen Cells in Mice during Growth of MMT1 Tumor. Effect of Adoptive Transfer of Splenocytes from Tumor-Bearing Mice on Development of ELM in Recipient Mice

Serial No.	Experimental conditions	Weight of tumor, g	Weight of spleen of donor mice, mg	Number of nucleated cells in spleen of donor mice x 10^6	Number of ELM in recipient mice			MII, %
					control	experiment	P	
1	Intact mice	—	96,6±13,8	108,8±13,3	89,2±18,6	60,3±3,1	>0,05	32,3
2	Growth of tumor							
	5th day	<0,1	150,0±5,3	285,0±9,7	38,8±5,0	11,6±2,7	<0,01	70,0
3	14th day	1,8±0,7	194,5±18,0	310,7±40,0	52,6±4,8	20,3±2,9	<0,01	61,6
4	25th day	7,6±2,1	311,0±43,7	342,0±30,3	42,5±5,8	77,5±11,0	<0,05	—45,0
5	Tumor removed on 4th day							
6	no recurrence	—	119,6±6,6	168,0±44,6	37,1±4,2	41,6±16,9	>0,05	—11,0
	recurrence	0,5—1,0	214,0±51,7	212,0±39,0	37,1±4,2	14,3±3,1	<0,01	61,0
7	Nude mice							
	intact	—	115,2±38,0	94,0±31,2	28,8±6,9	8,1±3,2	<0,01	72,0
8	growth of tumor 14th day	2,0±0,4	155,2±14,3	125,0±11,9	28,8±6,9	7,5±1,5	<0,001	74,0

Legend. Significance of differences between groups relative to weight of spleen: $P_{1-2} < 0.05$, $P_{2-3} < 0.05$, $P_{3-4} < 0.05$, $P_{1-5} > 0.05$, $P_{1-6} < 05$, $P_{3-5} < 0.05$, $P_{3-6} > 0.05$, $P_{7-8} > 0.05$. Significance of differences between groups relative to number of nucleated cells in spleen: $P_{1-2} < 0.05$, $P_{2-3} > 0.05$, $P_{3-4} > 0.05$, $P_{2-4} < 0.05$, $P_{1-5} > 0.05$, $P_{1-6} < 0.05$, $P_{3-5} < 0.05$, $P_{3-6} > 0.05$, $P_{7-8} > 0.05$. In each experimental group 20 to 30 mice were used (5-8 animals to assess each parameter).

in 0.3 ml of medium 199, 1-1.5 h before injection of the tumor cells. Control mice received an intravenous injection of tumor cells only. The tumor was removed on the 14th day after subcutaneous inoculation (mean weight of tumor 1.8 g). The operation was done under ether anesthesia. The magnitude of the inhibitory effect of the splenocytes on ELM formation in intact mice was determined as the metastasization inhibition index (MII), calculated by the equation MII = $(a - b)/b \times 100\%$, where a is the number of ELM in mice of the control group and b the number of ELM in mice of the experimental group. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTSL RESULTS

During growth of the syngeneic MMT1 tumor in the mice an increase was observed both in the weight of the spleen and in the number of nucleated cells in it (Table 1). These changes were significant as early as 5 days after inoculation of the tumor cells. With an increase in weight of the tumor a progressive increase was observed in the weight of the spleen in the mice (on the 14th and 25th days) with no significant changes in the number of nucleated cells in it, i.e., in the later stages of tumor growth changes in the weight of the spleen were not parallel to changes in the number of splenocytes in the mice. The weight of the spleen and the number of splenocytes fell virtually to normal 2 weeks after surgical removal of the tumor from the mice. If growth of the tumor recurred in the animals after the operation, both the weight of the spleen and the number of splenocytes in it in this case were significantly higher than the corresponding normal values. Growth of the MMT1 tumor in nude mice (by the 14th day after inoculation of the tumor cells) caused no significant increase in the weight of the spleen or in the number of nucleated cells in it.

Transfer of splenocytes from normal mice caused no significant change in the number of ELM appearing in the recipients. Transfer of splenocytes from mice bearing tumors for 2 and 14 days (weight of tumor not more than 2.5 g) caused inhibition of ELM formation in the recipient mice (MII was 70 and 61%, respectively). Conversely, the same dose of splenocytes from mice bearing tumors for 25 days (mean weight of tumor 7.8 g) stimulated metastasization in the recipients. For instance, the number of metastases in the lungs of the experimental animals was significantly greater than in the control. The result of transfer of splenocytes from mice undergoing the operation to intact mice depended on the presence or absence of recurrence of tumor growth in the donors. Transfer of splenocytes from mice with recurrence of the tumor inhibited ELM development, whereas transfer of splenocytes from donors without recurrence of the tumor did not affect the number of ELM formed in recipient mice. As a result of transfer of splenocytes from nude mice bearing tumors for 14 days, just as from intact nude mice, the magnitude of IE which developed in the recipients was similar (MII 72-74%). The degree of the

inhibitory action of the splenocytes from mice with the nude mutation on development of metastases in the lungs of intact mice was thus not connected with growth of the tumor in the donor mice.

On analysis of these data special attention must be paid to the fact that transfer of splenocytes from tumor-bearing mice (depending on the stage of tumor growth) may have both an inhibitory and a stimulating effect on the number of ELM formed in the recipients. This is in agreement with previous results from a study of the dynamics of IE development in mice during tumor growth [1]. It was shown that in the early stages of development of an MMTl tumor (7-15 days) in mice the inhibitory action of the tumor on metastases was clearly exhibited, but by the 20th day of tumor growth this effect had virtually disappeared. By transfer of splenocytes it is thus possible to reproduce the effect in recipient mice that is observed in the donors at the time of removal of the splenocytes. The development of IE in tumor-bearing mice is evidently connected with enlargement of their pool of splenocytes with antitumor activity. Experiments with removal of tumors also indicate that the presence of a tumor focus is essential for initiation and maintenance of proliferative activity of splenocytes responsible for the development of IE in mice.

During tumor growth (from the 14th to the 25th day) the number of splenocytes in the spleen of the mice showed no significant change. Changes observed in their functional activity, expressed as a switch from ability to inhibit to ability to stimulate metastasis formation, may be connected with qualitative changes in the composition of the spleen cells [4, 13]. It has been shown, for example, that accumulation of macrophagal-lymphocytic complexes in the mouse spleen during growth of a solid tumor in the animal leads to intensification of the suppressor activity of the lymphocytes [4].

During growth of the MMTl tumor (toward the 14th day) in nude mice no significant change took place in the number of splenocytes in the spleen. Transfer of splenocytes from nude mice in our experiments, irrespective of the presence or absence of a tumor in them, had an equal effect of inhibition of metastasis formation in the lungs in the recipients. This fact, on the one hand, is evidence of the presence of initial enhanced antitumor activity of the splenocytes of the mice and, on the other hand, it indicates that tumor growth in nude mice does not lead to any change (increase) in the antitumor activity of the splenocytes. The high natural antitumor activity of the splenocytes of nude mice can be explained by an increase in the number of natural (normal) killer cells (NK cells) [6, 3]. It can be postulated that the development of IE in mice with a defect of the T cell system of immunity and in immunologically normal mice is controlled by different mechanisms.

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