Role of Serum Humoral Factors in the Development of Metastases and Relapses of Ehrlich's Carcinoma in Mice

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Induction of metastases in a guest mouse in parabiotic pair of a mouse with removed tumor and a mouse with secondary transplant is demonstrated in male hybrids (CBA× C57Bl/6) F_1. The minimum dose of transplanted tumor cells inducing the growth of a secondary transplant in parabiont mice is decreased. Surgical removal of primary tumor is believed to lead to the appearance of humoral factors regulating the development of tumor process.

Key Words: *humoral factors; metastases; relapsing tumors*

Development of metastases after surgical removal of the primary tumor is a phenomenon well known in oncology [5-7]. The problem is often aggravated by the absence of drugs effective against tumors of a given localization or by subsequent development of cytostatic resistance in cells which were initially sensitive to them [4]. We believe that regulation of tumor development is a probable approach to the treatment of such patients.

Our previous studies demonstrated that removal of primary Ehrlich's carcinoma did not prolong the life span of operated animals in comparison with animals in which the tumor was not removed; treated animals died from relapses and metastases at the same times as untreated mice [1], i.e., the therapeutic effect in this case did not depend on the number of tumor cells removed. This conclusion disagrees with the modern strategy of cancer treatment: maximum elimination of tumor cells from the organism. Presumably, the tumor-bearing organism regulates the growth of individual tumor cells left after removal of the tumor by accelerating the development of relapses and metastases. It may release certain factors regulating tumor growth.

Our purpose was to confirm the existence of humoral factors in the tumor-bearing organism which can accelerate tumor growth after removal of the primary tumor node.

MATERIALS AND METHODS

Experiments were carried out on male hybrid mice (CBA×C57Bl/6) F_1 aged 2-3 months. Ehrlich's carcinoma (Oncology Research Center) was transplanted into the hip muscle in a dose of 10^6 cells in 0.2 ml medium 199. The tumor was removed under thiopental anesthesia on day 30 after transplantation. The formation of parabionts was carried out 7 days after the primary tumor node had been removed. Parabionts were formed by layer-by-layer connection of the skin and muscles to form a common abdominal cavity.

RESULTS

The parabiont model was used to establish the regulation by the tumor-bearing organism of metastasizing and relapsing after removal of the primary

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TABLE 1. Induction of Metastases in Parabiont Mice by Humoral Factors of Tumor Host with Removed Primary Tumor (*M±m*)

Parabiont pair	Number of metastases	
	host mouse	guest mouse
1	0	0
2	6±2	0
3	7±2	8±2
4	0	0
5	0	0

tumor [8]. This model is used because an experiment directly demonstrating the effect of humoral factors on the growth of the secondary inoculum of tumor cells in the primary tumor host is impossible. The secondary inoculum does not grow in animals with the primary tumor. This phenomenon was described in 1906 and denoted as concomitant immunity [2].

The following parabiotic pairs were created: 1) mouse with tumor (host) and intact mouse (guest); 2) mouse with removed tumor (host) and intact mouse (guest); 3) mouse with removed tumor (host) and mouse with secondary transplant 6 h after the operation (guest); 4) intact mouse (host) and mouse with secondary transplant (guest); 5) mouse with tumor (host) and mouse with secondary transplant (guest). Table 1 shows that only in the third pair did the guest mouse develop metastases.

The results are illustrated by Figure 1.

One more proof of the effect of humoral factors on the development of tumor process is a drastic (20 times) fall of the minimum count of transplanted tumor cells inducing the growth of secondary transplant in parabiont mice (Table 2). Primary signs of the tumor manifest themselves almost a month after inoculation of the secondary transplant.

Thus, we have good grounds to suggest that surgical removal of primary tumor leads to the release of humoral factors regulating the development of tumor process. Two different phenomena developed under the effect of humoral factors: metastasizing in the guest mouse in the parabiotic pair mouse with removed tumor and mouse with secondary transplant and a decrease in the minimum dose of transplanted tumor cells. These results are in line with the data of other scientists demonstrating that removal of one tumor node stimulates proliferation of tumor cells in another node as early as 18 h after the operation [3]. However, the effects of humoral factors on the parameters described in this paper could not be recorded in model experiments, because two inoculums were transplanted simultaneously in order to rule out the effect of concomitant immunity.

Our experiments explain the previous failure of surgical removal of the tumor in mice with Ehrlich's carcinoma to prolong the mean life span of operated animals in comparison with the nonoperated ones [1]. We believe that humoral factors released in the tumor host after removal of primary tumor play a certain role in the development of relapses and metastases of the tumor after surgical treatment. We think

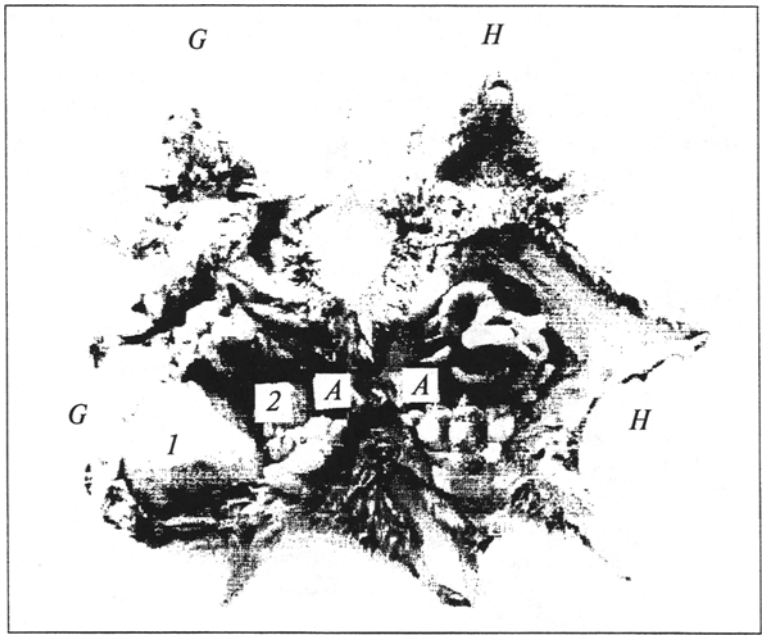

Fig. 1. Induction of metastases of Ehrlich's carcinoma in parabiont mice. *H*) mouse with removed tumor (host mouse); *G*) mouse with secondary transplant (guest mouse); *1*) secondary transplant; *2*) metastasis between the abdominal cavities of animals; *A*) anastomosis.

TABLE 2. Effect of Humoral Factors on the Minimum Dose of Transplanted Tumor Cells of Secondary Graft in Parabiont Mice

Parabiont pairs*	Number of transplanted cells	Number of mice with tumor/total number of mice	Days of tumor registration
1	1×10^4	3/3	7
2		3/3	7
1	1×10^3	1/3	15
2		3/3	10-15
1	1×10^2	0/3	—
2		3/3	20-30
1	0.5×10^2	0/3	—
2		3/3	20-35

Note. *The first parabiont pair: intact mouse/intact mouse; the second parabiont pair: mouse with removed tumor/intact mouse.

that this phenomenon is interesting for both experimental and clinical oncology and requires further investigation.

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