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Immunoglobulins of the Tumor-Bearing Host as Potential Regulators of the Tumor Recurrence Rate and Metastasis Development: a Study on the Model of Ehrlich Carcinoma

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On the model of Ehrlich carcinoma transplanted in mice it is shown that following the removal of the primary tumor the organism of operated mice produces factors enhancing the rate of relapses and metastasis. The tumor cells are shown to fix on their surface immunoglobulins capable of enhancing tumor development in the mice. The serum-derived tumor-enhancing immunoglobulins undergo active pinocytosis by the tumor cells. The fall in the level of these immunoglobulins is accompanied by a reduction of the rate of tumor growth.

Key Words: *Ehrlich carcinoma; recurrence; metastasis; immunoglobulins; mechanism of action*

Earlier we showed on the model of Ehrlich carcinoma that the surgical removal of the primary tumor fails to prolong the survival of mice. The operated animals died from relapses or metastases within the same time range as unoperated ones [1]. However, the unoperated mice did not develop metastases, their death being caused by the growth of the primary tumor. Thus, in this particular case the massive elimination of tumor cells resulted in no therapeutic effect. This conclusion is in conflict with the modern strategy of treatment of malignant diseases, which is based on the maximal removal of tumor cells from the organism. We speculated that the tumor-bearing organism can itself direct the growth of the solitary tumor cells left in the organism after the operation by boosting the development of relapses and metastases, thus canceling out the therapeutic effect of the operation. It follows from this assumption that the organism of a tumor-bearing individual produces certain substances capable of governing tumor growth. Although this assumption contradicts the prevailing view of autonomy of tumor development, it still represents a logical consequence of our earlier results.

In this report we have made an attempt to prove the existence of factors governing tumor growth in mice having undergone the surgical re-

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moval of a tumor, and to identify the nature of these factors.

MATERIALS AND METHODS

Two- to three-month old (CBA×C57Bl) F_1 male mice were used in the experiments. The animals were purchased from the Stolbovaya Animal Breeding Center, Russian Academy of Medical Sciences, Moscow Region. The mice received standard laboratory feed and water *ad libitum*. The Ehrlich carcinoma strain was obtained from the Department of Experimental Models, Cancer Research Center. Tumor volume was estimated according to the formula: $V=0.4ab^2$, where a and b are the greatest and smallest tumor diameters, respectively. The animals were inoculated intraperitoneally (i.p.) with 1 mln tumor cells suspended in 0.2 ml of medium 199.

The mean survival time was recorded as usual [5].

The surgical removal of the tumor was performed under thiopental anesthesia 29-30 days after transplantation. The mean tumor diameter at that time was equal to or more than 25 mm. The F_1 parabionts were obtained by joining the peritoneal cavities of animals 29-30 days after transplantation. The operation was performed under thiopental anesthesia, and in some cases was done simultaneously with tumor eradication. Each group included 3 pairs of parabionts.

The serum globulin fraction from animals with an intramuscularly grafted tumor and the globulin fraction of the ascitic fluid (AF) was obtained by sedimentation with ammonium sulfate. Sediments were dialysed and diluted using medium containing 0.15 M NaCl and 0.02 M phosphate buffered solution, pH 7.4.

Electrophoretic monitoring of immunoglobulins was performed as described [4].

Absorption of the globulin fraction was conducted as following: cells harvested from 100 ml of AF were fixed with 5% Formalin (pH 2.8), washed with saline, and incubated with 10 ml of the globulin fraction for 1 h at 37°C (pH 7.4).

For a study of the effect of different factors on tumor growth, animals received ascitic fluid i.p. and 15 min later were injected im with $1\times \times 10^4$ tumor cells in 0.1 ml of Hanks solution. Control animals received saline instead of AF. All the experiments were carried out in a syngeneic system.

Tumor cells (10^6 in 0.1 ml medium 199) were injected i.m. into the right hind paw. In the experiments on the effect of AF on tumor growth, the tumor cell dose inoculated was equal to 10^3 cells.

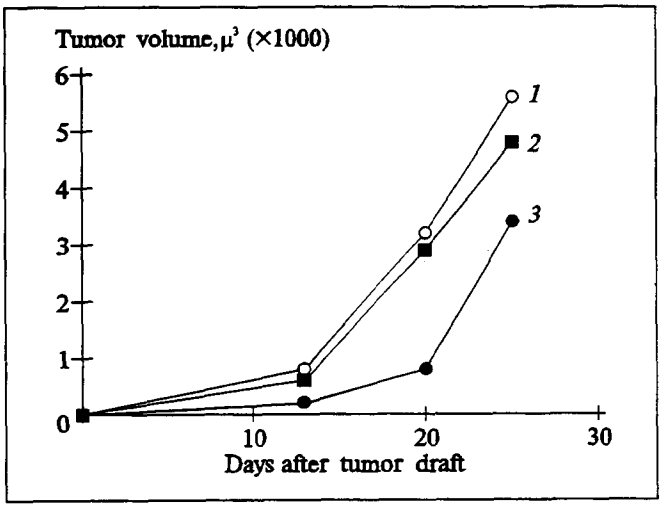

Fig. 1. Effect of globulin fraction of acidic eluate on tumor growth. *1*) mice which received a globulin acidic eluate from the cells of Ehrlich carcinoma; *2*) mice which received globulins from AF; *3*) mice which received saline.

RESULTS

To study the effect of humoral factors from the mice with resected tumors (below referred to as host animals) on tumor growth in the syngeneic mice (recipients), we used the conventional parabiotic model (see Materials and Methods). Earlier it was shown that Ehrlich carcinoma gives rise to metastasis only in mice with a surgically removed primary tumor nodule. This fact made it easier to record the effect of humoral factors. It was shown that metastases did not develop in the parabiotic pairs: tumor-bearing recipient - tumor-bearing or tumor-free host. However, metastatic involvement of mesenteric lymph nodes was observed in tumor-bearing recipients when the host had previously underwent tumor removal.

Figure 1 presents the case of a parabiotic pair: host mouse with previously removed tumor and recipient mouse with transplanted tumor. The metastatic involvement of the mesenteric lymph nodes can be seen.

Thus, there are humoral factors capable of enhancing the growth of disseminated tumor cells left in the organism following surgery. These factors appear to augment the growth of tumor cells in a selective way, i.e., no enhanced proliferation in other (normal) tissues can be detected. The factors appear only in the tumor-bearing organism.

In our opinion, the last two facts make it plausible to assume that the humoral factors described are immunoglobulins. Such an assumption implies that the tumor cells become coated with immunoglobulins that can enhance tumor growth in the organism.

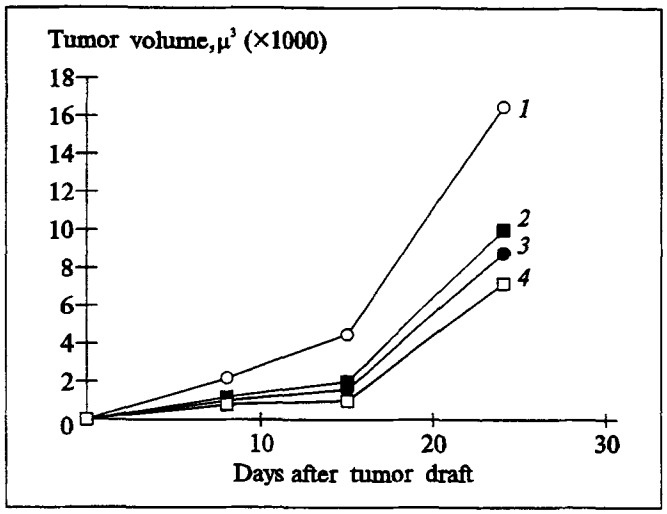

Fig. 2. Effect of intact and absorbed AF on the growth of Ehrlich carcinoma in mice. *1*) mice treated with AF preabsorbed with tumor cells at 4°C; *2*) mice treated with AF preabsorbed with tumor cells at 37°C; *3*) mice treated with AF preabsorbed with tumor cells having unblocked antigenic determinants; *4*) mice treated with saline.

As is seen in Fig. 1, the product(s) eluted from the Ehrlich carcinoma cells under acidic conditions accelerate tumor development. According to the molecular weight and electrophoretic mobility, the products must be immunoglobulins.

The results obtained enable us to explain the lack of serum and/or AF-derived tumor-enhancing factor to be absorbed by the tumor cells [1]. Apparently, all antigenic determinants on the surface of the tumor cells are already blocked *in vivo* by the corresponding immunoglobulins that are abundant in the tumor-bearing organism. Thus, the sorption of immunoglobulins from the serum and/or AF requires a prior unblocking of the antigenic determinants on the tumor cells. Therefore, in order to be able to absorb the biological fluid-derived tumor-enhancing factors, the tumor cells used should bear "unblocked" antigenic determinants. Re-expression can be achieved by the acidic elution or by preincubation of the tumor cells *in vitro* at 37°C in the presence of AF. In the latter case the absorption of the antibodies can proceed via their active pinocytosis by the tumor cells.

After incubation of AF with tumor cells at 4°C the tumor-enhancing activity of the AF remained at the initial level. On the other hand, when the cells with unblocked antigenic determinants were used for absorption, or the process was performed with living tumor cells at 37°C, the subsequent AF activity was reliably reduced (Fig. 2, *3* and *4*). The survival time of animals which received AF exhausted by preincubation with tumor cells bearing the unblocked antigenic determinants or with live tumor cells at 37°C reliably increased, attaining 58.0±0.9 and 56.3±0.6 days, respectively. Meanwhile, the survival time of animals given AF exhausted with tumor cells at 4°C or untreated AF was 41.3±0.8 and 39.7±0.7 days, respectively ($p<0.05$ vis-a-vis the two above-mentioned groups). The survival time of mice receiving saline (control) was 61.3±0.9 days.

From our point of view, the results provide evidence that: a) immunoglobulins in fact block (cover) antigenic determinants on the tumor cell surface; and b) these immunoglobulins undergo pinocytosis by the tumor cells, the latter process reducing the tumor-enhancing AF activity and strengthening the resistance components of the organism.

Taking into consideration the failure of the operation to have a survival-prolonging effect and the active consumption of AF imunoglobulins by the tumor cells (in just two hours), one may conclude that it is this particular protein fraction which is specifically responsible for regulating the growth of relapses and metastases. Such an active consumption of specific proteins by the tumor cells may be explained by assuming that these proteins serve as a source of protein nutrition for the actively recurring tumor cells. Moreover, taking into account that the removal of a primary tumor leads to an explosion of metastasis, we may state that the eradication of a primary tumor nodule is followed by a fall in the consumption of immunoglobulins specifically protecting (nourishing) the remaining tumor cells (due to the overall diminishment of tumor cell number in the host).

Thus, in our model system immunoglobulins play a regulatory role, fully controlling both the relapse and metastasis development. The fact that within the globulin fraction it is precisely the immunoglobulins which influence the specific defense of the tumor cells and that immunoglobulins may lose their organism-protecting function in the syngeneic system has been described earlier [2,3].

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