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Cure of Mice with Advanced Ovarian Adenocarcinoma CaO1 by the Serum Blood Proteins

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After removal of the primary tumor node, tumor-specific activity appears in the serum that blocks tumor growth in mice. This activity is observed at the time interval when activity of the tumor growth-stimulating factor is not determined. Administration of blood serum (0.1 ml) from mice with removed tumor to mice with CaO1 adenocarcinoma for 14 days led first to a stop of its growth, and then to tumor regression. The animals cured of adenocarcinoma lived for at least one year without signs of relapse. The cured animals did not develop resistance to repeated tumor transplantation. Repeated transplantation led to the growth of the new tumor. No cellular immune response was observed on histological slides of the regressing tumor. It was concluded that a serum factor is required for the growth of a tumor in the body and the state of the serum with blocked activity of this tumor-stimulating factor can be used for tumor treatment in oncology patients. This is the first result in the syngeneic system, when the tumor was cured by syngeneic serum proteins.

Key Words: *tumor cure; blood serum proteins; removal of primary tumor*

It was shown that the phenomenon of accelerated tumor growth is based on the appearance of serum tumor-stimulating factor (STSF) [10]. In modern medical practice, there is only a cytoreductive approach to the treatment of tumors. At the same time, removal of these tumor cells leads to the appearance of tumor-specific serum factor in the blood that promoted the growth of the remaining tumor. Moreover, acceleration of the tumor growth, according to our data, depends on the volume of the removed tumor and can reach a 10-fold increase in the number of mitoses [4].

However, high activity of STSF was recorded by researchers only within 24 h after the removal of the tumor [10]. This time is obviously insufficient for recovery of the removed volume of the tumor. It is logical to assume that STSP is constantly produced by the tumor-bearing host and is constantly absorbed by the tumor, but after the removal of the tumor node, STSP utilization decreases, and its blood concentration increases until feedback activation, which blocks the STSF production. Taking into account the duration of the cell cycle of in mice, blockade of STSF production can be expected in 5 h after elimination of tumor cells. Thus, we are dealing not with acceleration, but with a short-term compensatory blockage of the tumor growth.

Our aim was to document the effect of blocking of the tumor growth by mouse serum obtained 24 h after removal of the primary tumor node. Mouse ovarian adenocarcinoma CaO1 was used as the experimental model; this tumor described by us earlier has common antigenic determinants with mucin of human ovarian carcinoma CA125 [3].

MATERIALS AND METHODS

The study was performed on male CBA mice aged 2-3 months with subcutaneously transplanted adenocarcinoma CaO1 (Cryopreservation bank of biomaterials of the N. N. Blokhin National Medical Research Center of Oncology; transplantation dose 10^6 cells in

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0.2 ml RPMI-1640 medium). The animals received water and standard food *ad libitum*. All experiments were carried out in accordance with the legal requirements for animal experiments in the Russian Federation and with the official permission of the Research Institute for Experimental Diagnostics and Therapy of Tumors, N. N. Blokhin National Research Center of Oncology. Adenocarcinoma CaO1 is a very aggressive tumor: no spontaneous regression was never recorded when working with this tumor [3].

The tumor was removed and the serum was prepared from animal blood by the methods published earlier [10]. The obtained sera were aliquoted (0.6 ml per test tube) and stored at -20°C. We used 3 groups of blood sera: from healthy mice, from tumor-bearing mice, and from mice with removed tumor 24 h after removal.

After tumor transplantation, the animals were divided into 4 groups (6 mice per group). The serum was daily administered to animals of groups 1-3 intraperitoneally in a volume of 0.1 ml on days 10-24 after tumor transplantation. Group 1 animals received serum from mice without tumors, group 2 mice received serum from mice with adenocarcinoma (tumor volume 10^3 arb. units), and group 3 mice received serum from mice with removed adenocarcinoma. Group 4 (control) received no injections. The tumor size was measured on days 10, 17, 24, and 27 days after tumor transplantation.

The tumor volume was calculated by the formula $V=ab^2$, where a and b are the maximum and minimum tumor size (mm), and expressed in arb. units.

Histological examination of the tumors was performed by phase contrast microscopy; the sections were photographed using the AxioVision 4 system (Carl Zeiss). Samples from groups 2 and 3 were taken for histological examination, when the tumor volume in group 3 decreased by 2 times.

Group 3 mice were repeatedly subcutaneously transplanted with CaO1 adenocarcinoma (10^6 cells in 0.2 ml RPMI-1640 medium) 7, 30, and 180 days after the visual disappearance of the subcutaneous tumor node.

TABLE 1. Effect of Mouse Blood Sera on the Life Span of Mice with CaO1 Adenocarcinoma (*M*±*SD*)

Group	Treatment	Life span, days
1	Injection of blood serum from mice without tumor	27.8±1.2
2	Injection of blood serum from mice with transplanted CaO1	28.3±1.2
3	Injection of blood serum from mice with removed CaO1	>365
4	Control	29.3±1.3

Statistical processing of the obtained results was carried out using Microsoft Excel 2010 software. The significance of differences between the groups was determined using the Student's *t* test at $p<0.05$. The results are presented as *M*±*SD*.

RESULTS

The serum of mice without tumors and from tumor-bearing mice did not significantly change the lifespan of mice with adenocarcinoma CaO1 in comparison with the control group (Table 1): the mean lifespan of these mice did not exceed 29.3 days. At the same time, the serum obtained from mice 24 h after removal of the primary tumor significantly prolonged the lifespan of mice with tumors: the lifespan in this group was more than 365 days.

In none of the groups, serum injections over 7 days (days 10-17 after tumor transplantation) changed the rate of tumor growth (Table 2). Appreciable inhibition of tumor growth in mice receiving injection of the serum obtained in 24 h after tumor removal was revealed only at later terms of the experiment (days 24 and 27). On day 17 of the experiment, the tumor volume in this group reached its maximum value (6067 arb. units) and then decreased. In other groups, the tumor volume was approximately the same at this term (5904 and 6144 arb. units in mice receiving serum

TABLE 2. Effect of Mouse Blood Sera on the Growth of Transplanted Adenocarcinoma CaO1 (*M*±*SD*)

Group	Treatment	Tumor volume, arb. units			
		10 days	17 days	24 days	27 days
1	Injection of blood serum from mice without tumor	3190±957	5904±1770	17,820±5346	29,890±8967
2	Injection of blood serum from mice with transplanted CaO1	3094±928	6144±1843	18,176±5452	29,591±8897
3	Injection of blood serum from mice with removed CaO1	3136±940	6067±1820	5642±1692	5034±1510
4	Control	3292±987	6532±1959	18,532±5559	28,993±8697

from mice without tumors and mice with carcinoma, respectively), but continued to increase at later terms. Thus, regressions of the tumors were observed after 17 days only in the group that received blood serum from animals with removed carcinoma. Complete regression of the tumor in this group was noted after about 1 month (data are not presented). No ulceration of tumor nodes was observed during tumor regression.

Histological examination of the tumor node in mice with CaO1 adenocarcinoma receiving injections of the serum from mice with the same tumor demonstrated a typical structure of adenocarcinoma: large tumor cells with basophilic nuclei are grouped into round formations (Fig. 1, *a*, *b*). On sections of the tumor node from mice with adenocarcinoma CaO1 receiving serum obtained in 24 h after tumor removal, the typical structure of the adenocarcinoma node was absent, the tumor cells were randomly distributed (Fig. 1, *c*-*e*). At low magnification (×10), the replacement of areas of the tumor tissue with the connective tissue formations was observed (Fig. 1, *e*). However, no infiltrations of the tumors with immune cells were seen.

Thus, the preliminary assumption about the blockade of tumor growth with serum was confirmed. Hence, the tumor-bearing host constantly produces a serum factor that is necessary for the growth of the tumor. The existence of such a factor can explain the features of tumor recurrence and metastasis [1,5,6,9]. Why this factor still not identified? Previously published studies it is allow us to offer at least two explanations. First, this factor is not a new molecule, but an existing blood serum molecule with altered conformation [11]. Second, this factor does not exist. Theoretically, this possibility was discussed by us in a letter to the editor of the Science journal, if we consider blood serum as a united equilibrium chemical system [5]. Previously, we described cure of Ehrlich's ascitic carcinoma. The cure was observed in a similar situation, when an imbalance in serum proteins it was created. However, Ehrlich's carcinoma is not syngenic to linear animals [2,7]. Therefore, there are doubts whether creation of an imbalance for certain serum proteins can include a compensatory reaction of the body leading to complete regression of the tumor. Nevertheless, in this study, we observed the effect of cure the malignant tumor

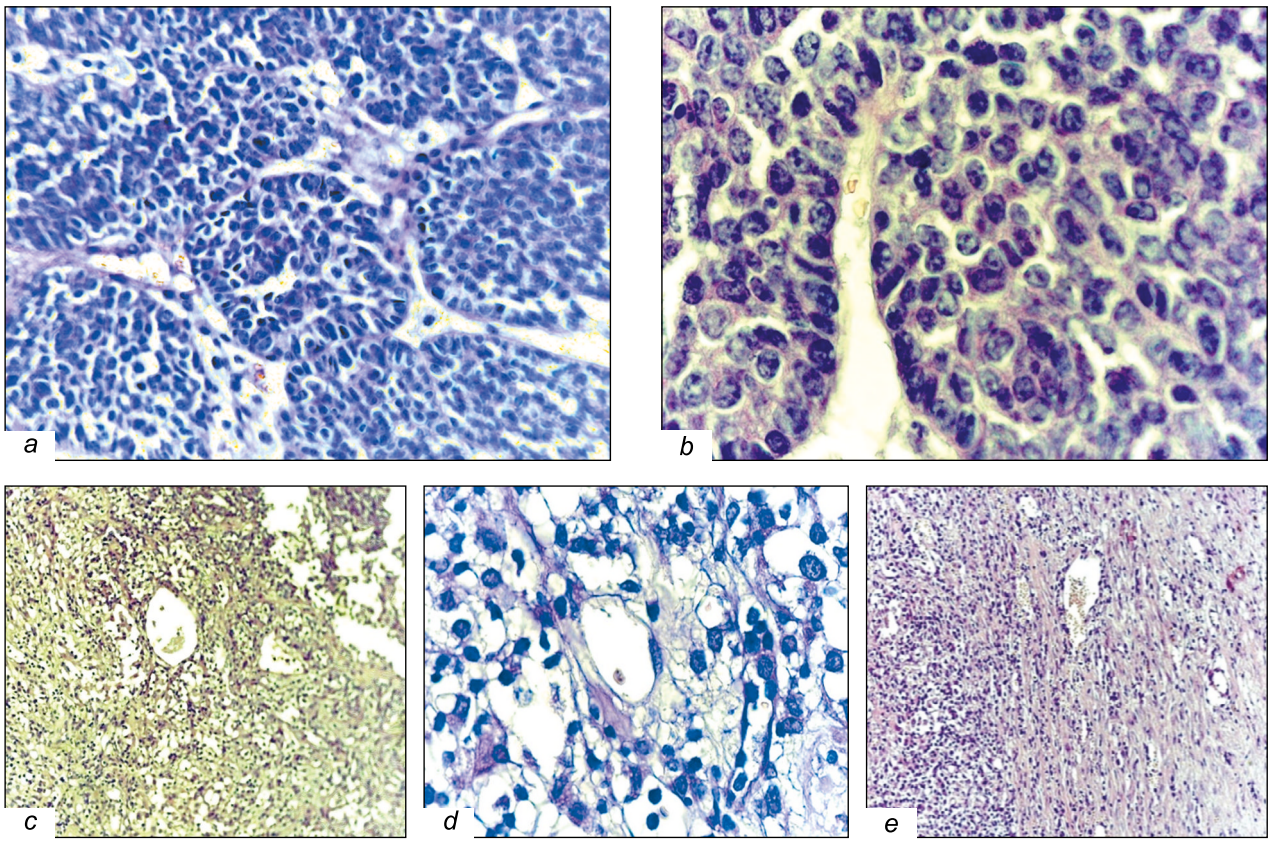

Fig. 1. Histological picture of the tumor node of mice with transplanted adenocarcinoma CaO1. *a*, *b*) Tumor node of mice treated with serum obtained from mice with the same tumor, ×40 (*a*) ×90 (*b*); *c*-*e*) tumor node of mice treated with serum obtained from mice 24 h after removal of the primary tumor, ×40 (*c*) ×90 (*d*), ×10 (*e*).

in the syngenic system. That is a block of the growth of a tumor in the tumor bearing host. This reality it is not associated with cellular immunity and with immunity in general (as we understand it now). However, this reality is in good agreement with our hypothesis about the role of protease—serpine interaction in the regulation of not only tumors, but also all body tissues [8]. It is extremely important that this it is not a cytoreductive approach, and it does not lead to the appearance of STSF in the blood serum. Therefore, no relapses or metastases are observed in cured animals. This gives us reason to be cautiously optimistic about the possible use of the effect described by us in clinical practice in the future.

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