The next step was to determine whether BF C-I preserves its inhibitory action on growth of another tumor. Curves showing the kinetics of growth of Lewis carcinoma are given in Fig. 3. On the 14th and 17th days of observation the volumes of the tumors in animals receiving BF C-I were significantly less than in control animals receiving physiological saline ($p = 0.05$). Thus-BF C-I has an inhibitory action not only on the ELD tumor, but also on Lewis carcinoma.

It can be postulated on the basis of these results that ELD tumor cells can secrete certain humoral factors which, when used in accordance with our schedule described above, can inhibit tumor growth. A similar inhibitory action of a larger tumor node on smaller nodes also was observed in clinical practice and, in our opinion, it is a manifestation of concomitant immunity. The creation of an experimental model of this phenomenon will enable the nature of the factors responsible for it to be studied.

REFERENCES

1. H. Friemel (ed.), Immunologische Arbeitsmethoden, Fischer, Jena (1976).
2. S. C. Bansal R. Hargreaves, and H. O. Sjögren, Int. J. Cancer, **9**, No. 1, 97 (1972).
3. P. J. Deckers, B. W. Edgerton, B. S. Thomas, et al., Cancer Res., **31**, No. 6, 734 (1971).
4. E. Gorelik, E. Segal, and M. Feldman, Int. J. Cancer, **27**, 847 (1981).
5. M. Nelson and D. S. Nelson, J. Nat. Cancer Inst., **65**, No. 4, 781 (1980).
6. M. Nelson, M. L. Booth, and D. S. Nelson, Aust. J. Exp. Biol. Med. Sci., **60**, No. 5, 493 (1982).

ASCITES FLUID AND TUMOR CELL DIALYSATE AS MODELS OF GROWTH OF EHRLICH'S CARCINOMA AND TERATOMA T-36

F. V. Donenko, A. O. Kabieva, and L. V. Moroz

UDC 616-006.6-092.4-02:615.366.381-008.8

KEY WORDS: ascites fluid; Ehrlich's carcinoma; acceleration of tumor growth

Growth of spontaneous or transplanted tumors evokes an immune response of the affected animals. However, despite the development of an immune response, as a rule the tumor continues to grow [2, 4]. Tumor growth against the background of development of an immune response can evidently be explained by protection of the tumor cells against the host's immune system. It has been shown that protection of this kind can take place with the aid of high-molecular-weight proteins (mol. wt. over 100 kilodaltons) IgG-2 antibodies or antigen-antibody complex [8]. Investigations in vitro also have shown that blood serum of cancer patients and animals with progressively growing tumors specifically inhibits the cytotoxicity of lymphocytes against tumor cells [5-7]. However, the role of tumor humoral factors in the development of a malignant process in the body is difficult to assess, for no suitable techniques have been developed with which to study this phenomenon in vivo.

The aim of this investigation was to study the effect of ascites fluid and cell dialysate of Ehrlich's carcinoma on tumor growth in vivo.

Department for the Study of New Antitumor Drugs, All-Union Oncologic Scientific Center, Russian Academy of Medical Sciences, Moscow. Kazakh Research Institute of Oncology and Radiology, Alma-Ata.

(Presented by Academician of the Russian Academy of Medical Sciences N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 8, pp. 193-195, August, 1992. Original article submitted April 27, 1991.

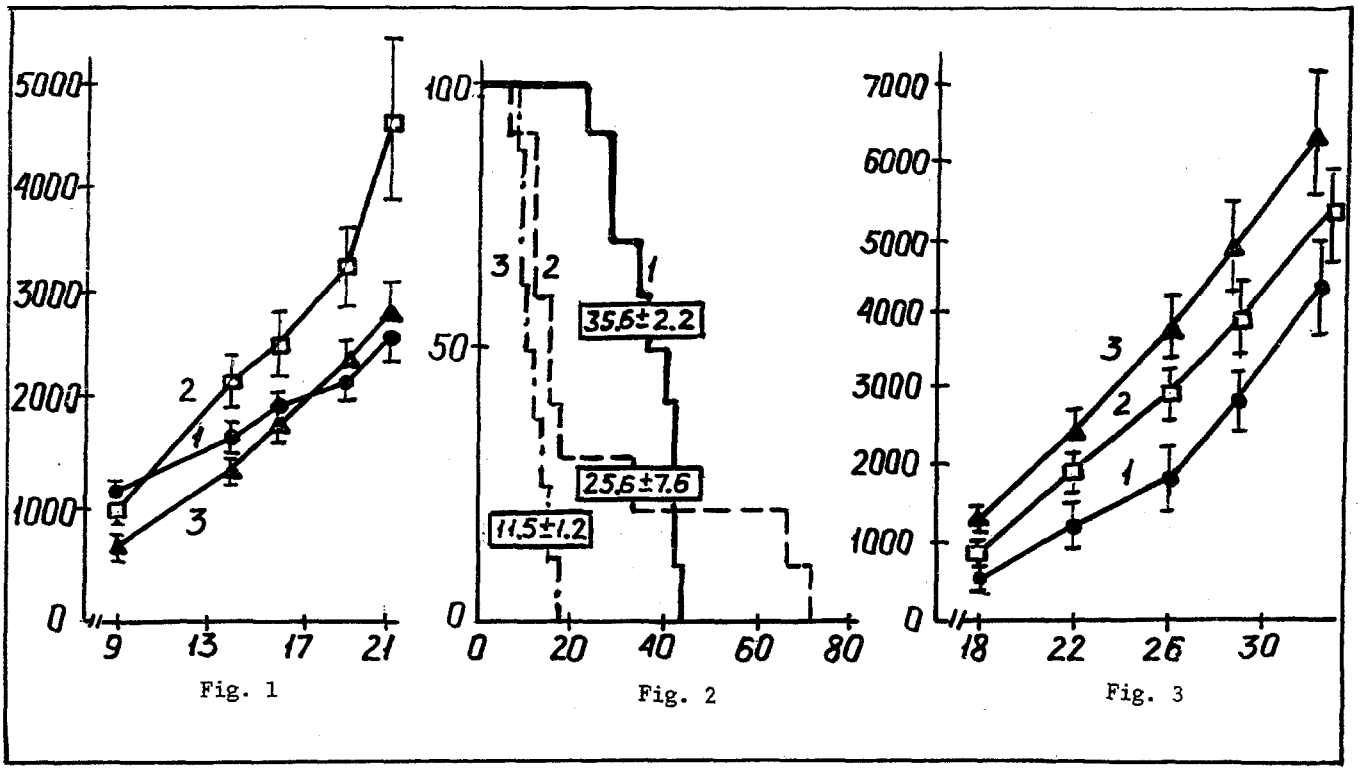

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Effect of BF C-I and C-II on growth of Ehrlich's carcinoma in mice. Abscissa, time after transplantation of tumor (in days); ordinate, volume of tumors (in c.u.). Inoculation dose $5 \cdot 10^5$ cells per mouse. 1) Control animals receiving physiological saline; 2) animals receiving BF C-I; 3) animals receiving BF C-II.

Fig. 2. Effect of biological fluids K-I and K-II on death of animals with Ehrlich's carcinoma. Abscissa, time after transplantation of tumor cells (in days); ordinate, number of surviving animals (in percent of initial number). Inoculation dose $5 \cdot 10^5$ cells per mouse. Mean life span of animals (in days) shown in boxes. 1) Control animals receiving physiological saline; 2) Animals receiving BF C-I; 3) Animals receiving BF C-II.

Fig. 3. Effect of BF C-I and T-I on growth of teratoma T-36 in mice. Abscissa, time after transplantation of tumor (in days); ordinate, volume of tumors (in c.u.). Inoculation dose $3 \cdot 10^4$ cells per mouse. 1) Control animals receiving physiological saline; 2) Animals receiving BF C-I; 3) Animals receiving BF T-I.

EXPERIMENTAL METHOD

Male (CBA × C57BL/6)F_1 hybrid mice and inbred BALB/c mice weighing 25-27 g were obtained from the "Stolbovaya" Nursery, Russian Academy of Medical Sciences. Teratoma T-36 and Ehrlich's ascites carcinoma (subline ELD, from the Tumor Strain Bank of the All-Union Oncologic Scientific Center, Russian Academy of Medical Sciences) were inoculated intramuscularly (doses given are shown in the footnotes to the Figures and Table). Biological fluids (BF) were: C-I – ascites fluid obtained from animals with Ehrlich's ascites tumor on the 10th day after intraperitoneal transplantation; C-II – the freeze-dried dialysate of Ehrlich's carcinoma cells obtained by the method described for transfer factor [1]. The pore size of the dialysis band was such that molecules with mol. wt. below 15 kilodaltons could diffuse through it. The dialysate was standardized on the basis of absorption at wavelengths of 190 and 260 nm, $\Delta A_{190\ nm} = 2.9$ relative units, $\Delta A_{260\ nm} = 3.3$ relative units. Measurements were made on a "Hitachi V-2000" spectrophotometer (Japan). BF T-I consisted of ascites fluid obtained from animals with teratoma T-36 on the 10th day after intraperitoneal transplantation. BF C-I, C-II, and T-I contained no cells and were used in a syngeneic system. BF C-II contained no proteins in concentrations detectable by Bradford's method [3]. The BF were injected intraperitoneally in a dose of 0.7 ml/mouse 15 min before transplantation of the tumor,

TABLE 1. Effect of BF C-I and C-II on Rate of Growth of Ehrlich's Carcinoma

Group of animals	Mean volume of tumor on 9th day, c.u.	Mean volume of tumor on 19th day, c.u.	Change in volume of tumor during 10 days, c.u.	Change in volume of tumor during 10 days, per cent of control
Control	1092,0±51,1	2218,4±205,8	1126,4	100
C-I	1027,8±50,6	3215,7±462,9	2187,9	195
C-II	741,1±53,7	2453,1±150,5	1712,0	153

Legend. Inoculation dose $5 \cdot 10^5$ cells per mouse.

and in a dose of 0.2 ml/mouse 24 h after transplantation of the tumor. In metastasization experiments an additional injection of 0.2 ml of the BF was given 48 h after transplantation of the tumor. Animals in the control groups were given an intraperitoneal injection of physiological saline in the same volumes. The effects were recorded as rate of growth of the tumor, the number of animals with tumors, and the mean length of survival of the animals. The volume of the tumors, in conventional units (c.u.) was calculated by the formula $V = a \times b \times c$, where a, b, and c are three mutually perpendicular diameters. Each group contained ten animals. The results were subjected to statistical analysis by the Fisher–Student method. Differences were considered significant at the $p \leq 0.05$ level. Values of $M \pm m$ are given in the table and graphs.

EXPERIMENTAL RESULTS

The results of a study of the effect of BF C-I and C-II on growth of Ehrlich's carcinoma are given in Fig. 1. After injection of BF C-I the mean volume of the tumor on the 9th day of transplantation was the same as in the control group. However, after the 14th day of observation a significant increase in the rate of growth of the tumor was observed in the animals receiving BF C-I compared with the control. Injection of BF C-II caused a significant decrease in the initial volume of the tumor on the 9th day of observation compared with the control animals, whereas on the following days more rapid growth of the tumors also was observed and the difference in their mean volumes in animals receiving BF C-II and physiological saline disappeared.

To assess quantitatively the increase in the rate of tumor growth in response to injection of BF C-I and C-II, data on the change in volume of the tumors in the animals during 10 days of observation – from the 9th through the 19th days after transplantation of the tumor – are given in Table 1.

After injection of BF C-I the rate of growth of the tumor reached 195%, compared with 153% of the rate of growth of the tumor in the control after injection of BF C-II. It can be tentatively suggested that acceleration of tumor growth of this kind is connected with protection of the tumor cells by BF C-I and C-II.

The protective action of BF C-I also was manifested as a sharp decrease in the minimal inoculation dose of Ehrlich's ascites tumor cells. For instance, after intramuscular inoculation of $2 \cdot 10^4$ cells into BALB/c mice in the course of two months of observation of tumor developed in 60% of the animals receiving BF C-I, whereas in the control group no tumors developed. Injection of BF C-II caused the development of tumors in 20% of cases during the above-mentioned period of time.

During the biological experiments it was noted that injection of BF C-I and C-II after 24 and 48 h additionally after transplantation of the tumor caused death of some animals during the 1.5-3 weeks after intramuscular inoculation of the cells, whereas in the control group death of the animals was observed after 5-6 weeks, when a solid tumor had developed (Fig. 2). Only injection of BF according to a similar schedule without inoculation of tumor cells did not cause death of the animals. It was postulated that more rapid death of the animals may be connected with the development of metastases of single tumor cells, against the background of their protection by BF. At autopsy on animals which died, enlargement of the subcutaneous lymph nodes was found. Intraperitoneal transplantation of these lymph nodes, taken from mice shortly before death, into intact animals led to the development of ascites and to death of the mice.

Incidentally, the protective action of BF C-I is not specific relative to Ehrlich's carcinoma cells, but holds good also for teratoma T-36 cells. Kinetic curves of growth of teratoma T-36 are shown in Fig. 3. Clearly the volume of the tumor in animals receiving BF C-I and T-I was significantly larger than in the control animals from the 18th

through the 19th day of observation. However, no significant differences were observed in the time course of tumor growth between animals receiving BF C-I and those receiving T-I.

The experiments thus showed that BF C-I, C-II and T-I can protect tumor cells in vivo. This protection by the action of BF is manifested as an increase in the rate of growth of the tumor, its more intensive metastasization, and reduction of the minimal inoculation dose of Ehrlich's carcinoma cells. The protective action of BF C-I is not specific relative to Ehrlich's carcinoma cells. It can be tentatively suggested that this effect of BF C-I and C-II is associated with substances with mol. wt. of under 15 kilodaltons. It is important to note that these substances were obtained from destroyed tumor cells. In our opinion, a clinical situation in which after cytotoxic action (chemotherapy, radiotherapy) induction of tumor growth and/or generalization of a malignant process is observed in vivo, can be simulated with the aid of humoral tumor factors. The proposed test system may be used to study the effect of humoral tumor factors on tumor growth in vivo.

REFERENCES

1. H. Friemel (ed.), Immunologisches Arbeitsmethoden, Fischer, Jena (1976).
2. G. Barski and J. K. Youn, J. Nat. Cancer Inst., **43**, No. 1, 111 (1969).
3. M. M. Bradford, Analyt. Biochem., **72**, No. 2, 248 (1976).
4. P. J. Deckers, R. C. Davis, G. A. Parker, et al., Cancer Res., **33**, No. 1, 33 (1973).
5. I. Hellström, H. O. Sjögren, G. Warner, et al., Int. J. Cancer, **7**, No. 2, 226 (1971).
6. G. H. Heppner, Int. J. Cancer, **4**, No. 5, 608 (1969).
7. H. O. Sjögren, I. Hellström, S. C. Bansal, et al., Proc. Nat. Acad. Sci., USA, **68**, No. 6, 1372 (1971).
8. M. Takasugi and W. H. Hildemann, J. Nat. Cancer Inst., **43**, No. 4, 843 (1969).