EFFECT OF MOUSE INTERFERONS ON DEVELOPMENT OF EHRLICH'S ASCITES CARCINOMA

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Intraperitoneal injection of various interferons (native cultural, concentrated and partially purified cultural, and also serum) significantly inhibits the development of Ehrlich's ascites carcinoma in noninbred mice. Because of the comparatively low activity of serum interferon, the effect of normal mouse serum on the development of this tumor and its action on the effect of native cultural interferon were investigated. Normal mouse serum was shown to depress the action of cultural interferon and to accelerate the development of Ehrlich's ascites carcinoma.

KEY WORDS: interferon; Ehrlich's ascites carcinoma; normal serum.

Much evidence suggesting that interferon preparations can inhibit growth of transplantable animal tumors *in vivo* and proliferation of cells of the corresponding neoplasms *in vitro* has recently accumulated [4-6]. The use of interferon as an antitumor preparation has also been shown to be effective in clinical practice, notably for the treatment of osteosarcoma [2, 12].

The object of this investigation was to study the action of various interferon preparations on the development of Ehrlich's ascites carcinoma in mice.

EXPERIMENTAL METHOD

Ehrlich's ascites carcinoma, obtained from the Institute of Oncology, Academy of Medical Sciences of the USSR, was put through regular intraperitoneal passage in noninbred mice (weighing 20 g).

Native cultural mouse interferon was obtained in medium No. 199 on L cells by induction with Newcastle disease virus (NDV), strain H, and concentrated with partial (by 50 times as protein) purification by salting out with ammonium sulphate (the fraction obtained at 35-55% saturation). The serum interferon was induced in noninbred mice by intravenous injection of NDV. Cultural native and serum interferons were kept at pH 2.0 for 4-6 days to inactivate the inducing virus. Normal mouse serum was treated in the same way. Interferons were titrated on L-cells on the basis of inhibition of the cytopathogenic action of vesicular stomatitis virus (Indiana strain) in a dose of 100 TCD_{50}.

In the experiments, the mice received an intraperitoneal injection of Ehrlich's ascites carcinoma cells in a dose of $1 \cdot 10^6$-$2 \cdot 10^6$, which caused death of nearly all the mice in the control group by the end of the first month; these mice, moreover, died at the same time irrespective of whether or not they received repeated injections of medium No. 199. Cultural interferons (0.5 ml) and serum interferon or normal serum (0.25 ml) were injected intraperitoneally into the mice daily for 5 or 6 days starting from the day of injection of the tumor cells or the following day. The mice died with a clear clinical picture of ascites. For statistical analysis of the data [4] reciprocals of the survival rate of the mice were used (for the animals which remained alive, the reciprocal of survival was defined as 0). The mean value of these reciprocals was determined as the harmonic mean, and confidence intervals of the mean values thus obtained were calculated by Student's t-test.

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TABLE 1. Effect of Various Interferons on Development of Ehrlich's Ascites Tumor

Interferon preparation	Titer of interferon used, units/ml	No. of mice surviving on 20th day	Harmonic mean survival rate of mice, days
Control (without interferon)	—	7 (39)	20
Native cultural interferon	1 000	16 (28)	34
Concentrated cultural interferon	8 000	25 (39)	40
Serum interferon	32 000	12 (19)	38

Legend: 1) Number of mice used in experiment given in parentheses; 2) difference between survival rates of mice in control group and in all groups in which interferon was used was significant ($P<0.01$).

TABLE 2. Effect of Native Cultural Interferon, Normal Mouse Serum, and a Mixture of Both on Development of Ehrlich's Ascites Tumor

Preparation	No. of mice surviving on 23rd day	Harmonic mean survival rate of mice, days
Control	4 (35)	18
Native cultural interferon (1000 units/ml)	9 (21)	31
Normal serum	0 (20)	15
Native cultural interferon (1000 units/ml mixed with normal serum	2 (20)	19

Legend. 1) Number of mice used in experiment in parentheses; 2) difference between survival rate of mice in group in which only native interferon was given and the remaining groups was significant ($P<0.01$), difference between survival rate of mice in group in which normal serum was used and control group also was significant ($P<0.05$).

All the interferons used inhibited the development of tumor growth. The protective effect of interferon was most clearly demonstrated toward the end of the third week. By that time most mice in the control group had died, whereas in the groups of animals receiving interferon the number of mice which died did not reach 50% of those used in the experiments (Table 1; mean results of two experiments). However, towards the end of the period of observation (2.5 months) most of the mice (90%) had died in these groups also. Nearly all the mice in the control group had died at the end of the first month.

The antitumor action of interferon was evidently due to its anticellular activity. This effect of interferon is possibly connected with its antimitotic action [9]. The possibility likewise cannot be ruled out that interferon causes death of tumor cells on account of an increase in the fragility of the cell lysosomes and the escape of enzymes from them [4, 7].

Another possibility is that interferon stimulates processes of cellular immunity or inhibits changes induced by the virus in the tumor cells [3, 8].

It might be supposed that the active antitumor principle in the preparations used was interferon, for the three interferon preparations used gave virtually the same effect of tumor inhibition. As regards cultural interferon, the effect depended on the dose of interferon used. For instance, concentrated cultural interferon in four experiments had a more effective protective action than the corresponding native interferon. However, attention is drawn to the fact that serum interferon in all experiments on the animals (*in vivo*) was rather less effective than might be expected on the basis of its much higher titers obtained on titration in cell culture (*in vitro*). The results obtained suggested that the diminution of the protective effect of serum interferon was due to a component present in the serum (and absent in preparations of cultural interferon).

Accordingly it was decided to study the effect of serum obtained from normal uninfected mice on the development of Ehrlich's ascites tumor both in the absence and in the presence of interferon. The averaged results of two such experiments are given in Table 2. They show that the use of normal serum led to inhibition of the protective action of native cultural interferon and to stimulation of development of tumor growth. The reasons for the stimulation of development of Ehrlich's ascites carcinoma under the influence of normal serum from noninbred mice and the inhibition of the action of interferon by this serum are not yet clear. However, there is evidence that the inhibitory action of large doses of serum on the antiviral action of interferon *in vitro* is evidently linked with considerable intensification of intracellular protein synthesis [10, 11]. Although the mechanisms of inhibition of tumor development by interferon and, in particular, the mechanisms of proliferation of tumor cells, are still largely unknown, nevertheless it has been demonstrated that interferon can introduce significant changes into the processes of intracellular protein synthesis [3]. The possibility therefore cannot be ruled out that in this case there is antagonism between the actions of serum and interferon on one of the stages of protein synthesis. Meanwhile information has been obtained that injection of normal serum from line IC mice into BALB/C mice stimulated the development of an RC_{19} ascites tumor in the latter [5].

It has been suggested that stimulation of tumor growth may be due to hormonal factors present in the tissue extracts and sera. The possibility cannot be ruled out that injection of serum globulins may lead to depression of cellular immunity which, as we know, plays an important role in the regulation of tumor growth [1].

In the accessible literature we found no data on acceleration of the development of Ehrlich's ascites carcinoma under the influence of normal mouse serum or to show that such serum can inhibit the antitumor action of interferon.

A detailed study of the effect of serum (and of tissue extracts) on the action of interferon on tumor development and verification of the hypotheses put forward above are matters which deserve attention because the decrease in the efficacy of interferon under the influence of blood serum, as described above, may play an important role in the practical aspects of interferon therapy of neoplasms in man.

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