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EFFECT OF ASCITES FLUID GLOBULINS ON GROWTH OF LEUKEMIA P388/DOX AND EHRLICH'S CARCINOMA IN MICE

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KEY WORDS: ascites fluid globulins; blood serum globulins; tumor growth

The writers showed previously that ascites fluid (AF) of Ehrlich's carcinoma affects the growth of this tumor in mice [1]. This effect can be explained by the presence in AF of what have been called potentiating antibodies. However, they were described previously only for the blood serum of tumor-bearing animals, including those with Ehrlich's carcinoma. It has been shown in the latter case that antibodies isolated from the blood serum of tumor-bearing animals, can interact directly with tumor cells and cause their death [2].

The question of the ability of the globulin fraction of AF to accelerate tumor growth has not been studied The aim of the present investigation was accordingly to study the effect of the globulin fraction of AF of Ehrlich's carcinoma and of leukemia P388/DOX on the rate of tumor growth in vivo.

EXPERIMENTAL METHOD

In the experiments with Ehrlich's carcinoma male (CBA × C57BL/6)F_1 hybrid mice were used, in experiments with leukemia P388/DOX, male BDF_1 hybrid mice (DBA/2 × C57BL/6) weighing 24-26 g were used. AF was isolated from mice on the 10th day after intraperitoneal transplantation of Ehrlich's ascites carcinoma (from the Tumor Strains Bank of the All-Union Oncologic Scientific Center, Russian Academy of Medical Sciences, Moscow), and on the 8th day after intraperitoneal transplantation of leukemia P388/DOX (this strain was described previously) [4]. To remove cells and fragments of cell membranes the AF was centrifuged at 3000*g* for 15 min, then at 20,000*g* for 30 min.

The blood serum globulin fraction of animals with an intramuscularly inoculated tumor and globulin fraction of AF were obtained by precipitation of proteins with ammonium sulfate. The protein precipitate was dissolved in a volume equal to the original volume of blood serum or AF, and the ammonium sulfate was removed by dialysis. Dialysis was carried out and the specimens dissolved in 0.15 M NaCl, 0.02 M phosphate buffer, pH 7.4.

To exhaust 10 ml of the globulin fraction tumor cells were isolated from 100 ml of AF. The cells were fixed in 5% formalin (pH 2.8), then washed in physiological saline and incubated with the globulin fraction for 1 h at 37°C (pH 7.4).

To record the effects the animals received an intramuscular transplantation of $1 \cdot 10^4$ tumor cells per mouse in 0.1 ml of Hanks' solution 15 min after intraperitoneal injection of AF or globulins (Gl) of AF and blood serum Gl. Animals of the control groups were given the corresponding volumes of physiological saline. The action of AF Gl

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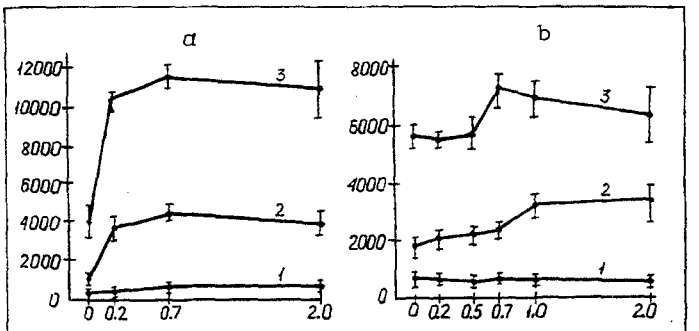

Fig. 1

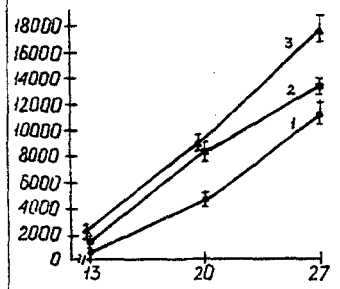

Fig. 2

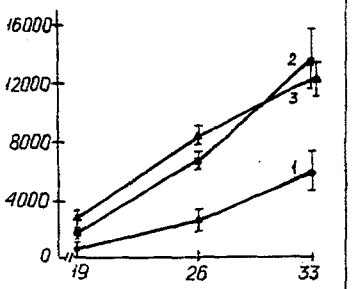

Fig. 3

Fig. 1. Effect of AF Gl on rate of growth of Ehrlich's carcinoma (a) and leukemia P388/DOX (b) in mice. Abscissa, dose of Gl (in ml/mouse); ordinate, volume of tumor (in conventional units).

Fig. 2. Effect of exhaustion of AF Gl by preliminary incubation with cells of an Ehrlich's carcinoma on its growth. Abscissa, time after inoculation of tumor (in days); ordinate, volume of tumor (in conventional units). 1) Animals receiving 0.5 ml physiological saline before inoculation of Ehrlich's carcinoma cells; 2) animals receiving AF Gl 15 min before transplantation of Ehrlich's carcinoma; 3) animals receiving exhausted AF Gl 15 min before transplantation of Ehrlich's carcinoma.

Fig. 3. Effect of AF Gl and blood serum Gl of animals with tumor on rate of growth of Ehrlich's carcinoma. Abscissa, time after inoculation of tumor (in days); ordinate, volume of tumor (in conventional units). 1) Animals receiving 0.5 ml physiological saline 15 min before inoculation of Ehrlich's carcinoma; 2) animals receiving blood serum Gl from animals with tumor 15 min before inoculation of Ehrlich's carcinoma; 3) animals receiving AF Gl 15 min before inoculation.

and blood serum Gl was studied in a syngeneic system. The volume of the tumor, in conventional units, was determined by the formula $a \times b \times c$, where a, b, and c are three mutually perpendicular diameters.

The results were subjected to statistical analysis by the Fisher–Student method. Differences were taken to be significant at the $p < 0.05$ level.

EXPERIMENTAL RESULTS

Intraperitoneal injection of AF (0.7 ml) into mice with Ehrlich's carcinoma significantly shortened the animals' lifespan from 86.8 to 61.8 days. Injection of AF Gl (0.7 ml) into animals with Ehrlich's carcinoma increased the rate of growth of the tumor, as shown by a significant shortening of the lifespan of the animals compared both with the control group, receiving physiological saline (from 86.8 to 49.5 days), and with animals receiving AF.

In the next stage of the investigation it was necessary to determine optimal quantities of Gl causing acceleration of tumor growth. The results showing the effect of AF Gl on growth of Ehrlich's carcinoma are presented in Fig. 1a. The quantity of AF Gl changed from 0.2 to 2 ml/mouse, and corresponded in the volume injected to the volume of AF from which these Gl had been obtained. Significant differences in volumes of the tumors on the 20th and 27th days of observation were observed between animals receiving physiological saline and those receiving AF Gl when AF Gl were injected in a volume of over 0.2 ml. This stimulating effect was maintained within the dose range from 0.2 to 2 ml.

Results indicating the effect of different quantities of AF Gl on the rate of growth of leukemia P388/DOX, inoculated intramuscularly, are shown in Fig. 1b. Clearly, on the 10th, 13th, and 17th days of observation significant differences in tumor volume in animals receiving only physiological saline and Gl took place only after injection of Gl in a dose of more than 0.7 ml/mouse. After injection of Gl in a volume of 2 ml/mouse, marked inhibition of tumor growth was observed in 10-20% of animals compared with other animals of the same group. Thus the volume of AF Gl required to obtain acceleration of growth of the P388/DOX tumor was ≈3.5 times greater than the volume of AF Gl required to accelerate growth of Ehrlich's carcinoma.

The writers showed previously that serum Gl of animals with a tumor can interact with cells of that tumor. As a result of this interaction, removal of Gl from the blood serum was observed and the serum lost its ability to stimulate tumor growth [3, 5]. In the next stage of the investigation an attempt was made to remove AF Gl by sorption on Ehrlich's carcinoma cells. Cells were taken for the experiment from a volume of AF 100 times greater than the volume of exhausted AF Gl. As will be clear from Fig. 2, after incubation of AF Gl with Ehrlich's carcinoma cells no decrease in their activity was observed. The rate of tumor growth in animals receiving AF Gl and exhausted AF Gl was significantly higher than the rate of tumor growth in animals receiving physiological saline.

It can be postulated that AF Gl and blood serum Gl from tumor-bearing animals differ in their action on tumor cells, for no interaction of AF Gl with Ehrlich's carcinoma cells was found (in the absence of a change in effect). It was therefore necessary to compare the action of AF Gl and blood serum Gl of tumor-bearing animals on the rate of tumor growth. As Fig. 3 shows, injection of identical volumes of serum Gl and AF Gl caused a similar effect, expressed as virtually equal acceleration of growth of Ehrlich's carcinoma compared with the rate of tumor growth in animals receiving physiological saline.

Thus AF Gl contain proteins capable of causing acceleration of tumor growth and shortening the lifespan of tumor-bearing animals. Activity of Gl isolated from ascites fluid of Ehrlich's carcinoma was higher than activity of Gl isolated from AF of leukemia P388/DOX, possibly due to the ability of Ehrlich's carcinoma to grow in all existing strains of mice, whereas leukemia P388/DOX will grow only on the DBA/2 strain and its hybrids. No decrease in activity of AF Gl could be recorded after their incubation with tumor cells, possibly due: a) to blockade of the antibodies by the antigenic determinants of the tumor; b) to blockade of antigens of tumor cells by antibodies; c) to the fact that the effect of acceleration of tumor growth by Gl is unconnected with antibodies.

To study the mechanism of acceleration of tumor growth by AF Gl further research is necessary. An essential factor is that AF Gl and blood serum Gl of animals with a tumor possess equal activity under the conditions described, thus simplifying the obtaining of the necessary quantities of Gl for investigation.

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EFFECT OF CLOVER EXTRACT ON PROLIFERATION OF HUMAN AND MURINE LYMPHOCYTES IN VITRO

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KEY WORDS: clover extract; lymphocytes; proliferation

Synthetic and semisynthetic flavonoids dibenz-γ-pyrone derivatives are currently regarded as promising antitumor preparations whose action is associated both with a direct cytotoxic effect of tumor cells [5, 6] and with modification of the biological response, namely with stimulation of activity of natural killer lymphocytes in the absence and in the presence of interleukin-2 [4, 6, 7, 10]. Various species of clover are sources of natural flavonoids, and they accumulate the highest concentration of these biologically active substances in the flowering phase [2].

The aim of this investigation was to study the effect of an extract of biologically active substances isolated from red clover (*Trifolium pratense* L.) on the proliferative activity of normal human peripheral blood lymphocytes and mouse spleen cells.

EXPERIMENTAL METHOD

Extracts of biologically active substances of *T. pratense,* including total flavonoids, were obtained by treating the raw material with 80% ethanol, followed by heating on a waterbath for 5 min [1]. The alcoholic solution was subjected to freeze-drying. The extracts were obtained from plants in the flowering period (extract 1) and in the budding phase (extract 2). Healthy human peripheral blood mononuclears (MON) were isolated by the method in [3]. For this purpose, after centrifugation for 30 min at 450g and at room temperature, the cells which were concentrated above the Ficoll-Verografin layer with density of 1.077 g/cm^3 were harvested, washed twice, and suspended in medium RPMI 1640, enriched with 5% fetal calf serum (FCS). To obtain splenocytes of BALB/c mice (aged 3-4 months, weight 20-25 g, bred at the "Stolbovaya" Nursery, Moscow Region), the animals were killed by cervical dislocation, the spleens were removed, and a suspension of single cells was prepared in a Potter homogenizer in medium RPMI 1640 with 5% FCS. The human or murine cells ($5 \cdot 10^4$-$1 \cdot 10^5$) were cultured in 0.2 ml medium RPMI 1640 with 5% heat (56°C)-inactivated FCS, $2 \cdot 10^{-3}$ M L-glutamine, 5 mM HEPES ("Flow Laboratories"), $3 \cdot 10^{-5}$ M 2-mercaptoethanol ("Merck"), and 40 μg/ml of gentamicin in 96-well round-bottomed plates for 3, 4, and 5 days at 37°C and in a humid atmosphere containing 5% CO_2. The concentrations of clover extract were 0.04-2.5%. DNA synthesis was determined by measuring incorporation of ^{3}H-thymidine, added in a dose of 0.5 μCi to each

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