HIGH-POLYMER XENOGENEIC RNA AS A STIMULATOR OF ANTITUMOR IMMUNITY

V. P. Nikolin, V. P. Tomsons, K. N. Verevkina, and Academician D. K. Belyaev

UDC 616-006-097.3-02:615.276.4:547.963.32

KEY WORDS: Krebs-2 tumor; xenogeneic RNA; antitumor immunity.

The writers previously [2] obtained evidence of enhancement of the immunogenic properties of syngeneic transplanted tumors after their formation in vitro by a preparation of xenogeneic liver RNA. Intraperitoneal injection of this RNA, in conjunction with preliminary immunization with tumor cells, preincubated with the same RNA preparation, significantly increased the resistance of C3H/He mice, with a high predisposition to cancer, to the development of spontaneous tumors [3].

The object of the present investigation was to study with which RNA fraction the increase in antitumor resistance under the influence of the RNA preparation from the tumor is associated.

EXPERIMENTAL METHOD

Freeze-dried RNA preparations from bovine liver, obtained under laboratory conditions or during experimental production conditions in the Special Design and Technical Bureau for Biologically Active Substances (Novosibirsk), were used in the experiments. In both cases the RNA was isolated by Kirby's phenolic method [7]. High-polymer RNA was obtained from the total preparation by triple reprecipitation with 1M NaCl. The extinction (E) of the total preparation at 260 nm was 150-180 optical units, and that of the high-polymer preparations was 220-240 optical units. The ratio E_{260}/E_{280} and E_{260}/E_{230} for the total RNA was 1.58-1.88 and 1.60-1.75 respectively, and for high-polymer RNA it was 1.97-2.43 and 2.06-2.59 respectively. To characterize the degree of polymerization of the RNA preparations gel-chromatography on Sepharose 4B was used. The RNA was passed through the column (1 × 25 cm) with Sepharose 4B, equilibrated with 0.1M NaCl in 0.005M Tris-HCl buffer, pH 7.5. Elution was carried out with the same buffer at the rate of 12 ml/h. The chromatographic profile of the RNA isolated under the different conditions is illustrated in Fig. 1.

To obtain an RNA digest, pancreatic ribonuclease (EC 2.7.7.16) from the Leningrad Meat Combine was used. The RNA was hydrolyzed in 0.1M Tris-HCl buffer, pH 7.4, for 15-17 h at 37°C. The substrate/enzyme ratio was 100:1 w/w. In some cases further purification of the RNA preparation was carried out with dimethyl sulfoxide and Cetavlon [9].

Experiments were carried out on CC57BR mice. Cells of a Krebs-2 ascites tumor were separated from ascites fluid by centrifugation at 2500 rpm for 5 min, and resuspended in physiological saline; the RNA preparation was added (1-5 mg/ml) to the cell suspension, which was incubated at 37°C for 2.5-3 h. A control suspension was incubated without RNA or with an enzymic digest of RNA in the same concentration. After incubation the cells were washed twice with physiological saline to remove RNA. Mice for immunization were given a subcutaneous injection of $5 \cdot 10^6$ tumor cells incubated with RNA (or without RNA) in 0.05 ml physiological saline into the tail, and when nodules began to appear at the site of injection of the tumor suspension, part of the tail was amputated. Immunization was repeated 1 month later. Eight days after immunization the animals were inoculated intraperitoneally with Krebs-2 ascites tumor in a dose of $5 \cdot 10^6$ to $1 \cdot 10^7$ tumor cells. Injections of the RNA preparation began 24 h after inoculation of the tumor. The RNA was injected intraperitoneally (in a dose of 5 mg of total RNA or 2 mg of the high-polymer fraction) once a day for 5 days. On the 8th or 11th day after inoculation the mice were killed and the quantity of ascites fluid determined in the experimental and control groups. Statistical analysis of the data was carried out by Student's t-test.

Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk.
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 8, pp. 199-201, August, 1980. Original article submitted May 4, 1979.

0007-4888/80/9008-1106$07.50

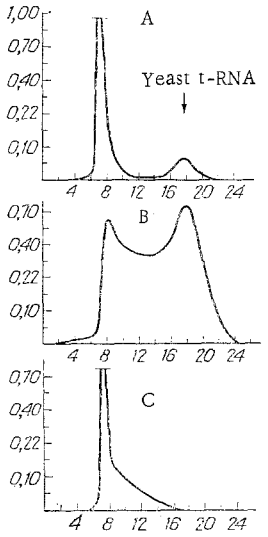

Fig. 1

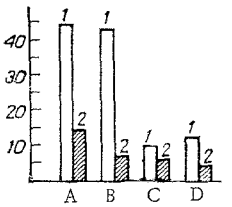

Fig. 2

Fig. 1. Chromatographic profile of RNA isolated under laboratory (A) and semiproduction (B) conditions and of RNA precipitated by 1M NaCl (C). Abscissa, Nos. of fractions; ordinate, optical density at 254 nm.

Fig. 2. Effect of preparations of native (A) and partially degraded (C) RNA and of their high-polymer fractions (B and D respectively) on immunogenic activity of Krebs-2 tumor cells. 1) Immunization with tumor cells treated with RNA; 2) immunization with untreated tumor cells. All mice after inoculation of the tumor received RNA intraperitoneally. Ordinate, inhibition of tumor growth (in %).

EXPERIMENTAL RESULTS

The results showing the immunostimulant effect of total RNA preparations are summarized in Table 1. They show that injection of the RNA preparation after inoculation of the Krebs-2 tumor into unimmunized mice had practically no effect on the development of this tumor. Immunization against this tumor itself, under the experimental conditions used, had little or no effect (Table 1, series I and III). Incubation of the tumor cells with RNA did not potentiate their immunizing ability (series I and III). However, a combination of immunization with tumor cells treated with RNA and subsequent injections of RNA after inoculation of the tumor, led to significant inhibition of tumor growth. If in this variant of the experiment cells not treated with RNA were used for immunization, the effect was much weaker. The most marked increase in antitumor resistance after exposure of the tumor to the RNA preparation was thus observed in cases when the animals were sensitized beforehand with tumor material treated with the same preparation. Results similar in principle were obtained by the writers previously using other tumor models [2, 3].

The preparation continued to remain active after additional purification with dimethyl sulfoxide and Cetavlon (Table 1, series IV). The purified preparation, added to a culture of mammary gland tumor cells in concentrations of between 6 and 24 mg/ml, had no appreciable cytopathogenic effect during an exposure of 24 h. This is evidence that the effect observed was not the result of the toxic action of the RNA preparation on the tumor cells, but was evidently due to the immunologic response of the host animal to the tumor. In our view, based on findings obtained previously [2] and also on data in the literature, indicating the ability of exogenous RNA to induce synthesis of proteins or antigens in tumor and normal cells not characteristic of those cells [1, 4-6, 8, 10], the biological effect of RNA in this case can evidently be reduced to a change in the antigenic specificity of the tumor cells as a result of the appearance of new antigenic determinants on their surface, capable of inducing an immunologic response of the host.

TABLE 1. Effect of Immunization and Treatment with RNA Preparations on Development of Krebs-2 Ascites Tumor

Frequency of immunization	Series	Number of mice	Immunization with	Treatment with	Mean weight of ascites fluid, g (M ± m)	Per cent inhibition of tumor	P
Once	I	30	—	PS	11,3±1,07	—	—
		25	—	RNA	9,6±0,98	15,0	—
		28	TC	PS	11,0±0,91	—	—
		14	TC + RNA	PS	9,7±1,10	14,2	—
		14	TC	RNA	9,8±1,00	13,3	—
		30	TC + RNA	RNA	5,3±0,69	53,1	<0,001
	II	62	—	PS	7,6±0,26	—	—
		40	TC	RNA	6,9±0,25	9,2	—
		22	TC	ED	6,4±0,44	15,8	<0,05
		40	TC + RNA	RNA	4,3±0,31	43,4	<0,001
		22	TC + ED	ED	7,3±0,35	3,9	—
Twice	III	25	—	PS	6,4±0,38	—	—
		16	TC	PS	4,9±0,60	23,4	<0,05
		16	TC + RNA	PS	5,0±0,71	21,9	—
		17	—	RNA	6,0±0,82	6,3	—
		10	TC	RNA	2,8±0,43	56,3	<0,001
		15	TC + RNA	RNA	1,0±0,30	84,4	<0,001
	III	16	—	PS	7,6±0,35	—	—
		20	TC	RNA	4,1±0,48	46,1	<0,001
		20	TC + RNA	RNA	0,9±0,17	88,2	<0,001

Legend. TC) Krebs-2 tumor cells; TC +RNA) tumor cells incubated with RNA; ED) enzymic digest of RNA; PS) physiological saline. In the experiments of series IV the RNA preparation was used after additional purification with dimethyl sulfoxide and Cetavlon.

Experiments with different RNA preparations showed that only nine of the 20 preparations could induce a reproducible immunologic effect as described above. The remaining preparations were ineffective. Since many of the inactive preparations were indistinguishable from active as regards the percentage of RNA which they contained, it might be supposed that the active principle in the preparation was not RNA itself, but certain impurities associated with it. Meanwhile, treatment of the active preparations with ribonuclease completely inactivated them (Table 1, series II), evidence in support of RNA and the active factor. As further investigations showed, activity of the RNA preparations depended on the quantity of high-polymer RNA fractions which they contained: an effect was observed in all cases when preparations with a high content of this fraction were used (Fig. 1A), whereas preparations in which partial depolymerization of RNA took place in the course of their isolation (Fig. 1B) were unable to potentiate the effect of immunization (Fig. 2C). The results of the experiments with high-polymer RNA fractions obtained from active preparations by precipitation with 1M NaCl showed that activity of the total RNA polymerase is associated with this fraction: removal of low-polymer RNAs did not cause any reduction in the immunologic effect (Fig. 2A, B). Meanwhile high-polymer RNA obtained in the same way from inactive preparations invariably gave negative results (Fig. 2C, D), although in its chromatographic profile it was identical with RNA from the active preparations (Fig. 1C). It can be concluded from analysis of these results that the immunologic effect was due to high-polymer RNA. This RNA may perhaps belong to a particular functional type. Tests on functionally different classes of RNA would evidently enable more definite conclusions to be drawn.

LITERATURE CITED

1. V. G. Galaktionov, in: Progress in Science and Technology. Immunology [in Russian], Vol. 7, Moscow (1978), p. 114.
2. V. P. Nikolin, Izvest. Sibirsk. Otdel. Akad. Nauk SSSR, No. 2/10, 143 (1973).
3. V. P. Nikolin, N. A. Matienko, and D. K. Belyaev, Dokl. Akad. Nauk SSSR, 255, 718 (1974).
4. S. J. Archer, Immunology, 34, 123 (1978).
5. L. Burrows, H. Muir, and J. F. Mowbray, Ann. N. Y. Acad. Sci., 129, 250 (1966).
6. R. D. Guttmann, E. D. Kraus, and M. F. Dolan, Nature, 203, 196 (1964).
7. K. S. Kirby, Biochem. J., 64, 405 (1956).
8. M. C. Niu, Science, 131, 1321 (1960).

9. R. K. Ralf and A. R. Bellamy, Biochim. Biophys. Acta, 87, 9 (1964).
10. P. Tuohimaa, S. J. Segal, and S. S. Koide, Proc. Natl. Acad. Sci. USA, 69, 2814 (1972).

SENSITIVITY OF SYRIAN HAMSTERS TO INOCULATION OF TUMOR CELLS DURING PREGNANCY AND LACTATION

E. L. Vendrov

UDC 616-006-018.1-089.843-092.9-06[168.3+618.7]-092:612.017.1

KEY WORDS: SV40 virus; tumor cells; pregnancy.

The ability of embryonic antigens to induce antitumor immunity is being widely discussed at the present time. Some workers state that immunization with embryonic antigens can be used to prevent both primary induced and transplanted tumors [4, 5]. According to some workers, repeated pregnancy during the latent period of carcinogenesis led to a decrease in the frequency of appearance of tumors in parturient females compared with virgins, and this was interpreted as proof of immunization of the mother with embryonic antigens of the fetus [6]. However, some of the published data has not been confirmed by other workers [1, 7].

The writer previously studied the effect both of artificial immunization with embryonic tissues and of single and repeated pregnancies during the latent period on the frequency of onset of primary tumors and on growth of transplantable tumors induced by SV40 virus in Syrian hamsters [1, 2]. These investigations showed that the frequency of tumors was much lower in females which became pregnant 1-5 times during the latent period of SV40 carcinogenesis than in females not becoming pregnant. However, these differences were evidently not connected with immunization of the parous females with embryonic antigens, for the frequency and times of onset of primary tumors in males were the same as in the parous females.

In the writer's previous experiments [2] immunization of inbred hamsters with embryonic hamster tissue followed by transplantation of continuous strains of syngeneic tumors led to neither inhibition nor to stimulation of tumor growth.

Meanwhile, a considerable decrease in the frequency of onset of tumors in females becoming pregnant several times during the latent period could be due both to their more effective natural immunization with embryonic antigens during pregnancy and to an increase in the level of natural resistance of these females during pregnancy or lactation.

The object of the present investigation was to study the effect of pregnancy and lactation in syngeneic hamsters on the sensitivity of females to inoculation of tumor cells.

EXPERIMENTAL METHOD

Noninbred Syrian hamsters were used. Experiments were planned so that the sensitivity of the animals to inoculation was tested 1-6 days before the first day of pregnancy or during the 1st-8th day of pregnancy. In the last series of experiments tumor cells were injected into lactating females on the 1st or 2nd day after parturition. Males inoculated simultaneously with identical doses of tumor cells served as the control. Continuous strain E-1 of hamster sarcoma, induced by SV40 virus, was used as the test tumor.

To determine sensitivity of the hamsters to inoculation of tumor cells the transplantation test was used in its most sensitive modification [3], in which each animal was inoculated with doses of test tumor differing by a factor of 5-10 (starting with about one tumor cell).

Laboratory of Immunology of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 8, pp. 201-203, August, 1980. Original article submitted December 12, 1979.

0007-4888/80/9008-1109$07.50