EFFECT OF EXOGENOUS POLY(A)$^+$mRNA ON ANTIGENIC PROPERTIES AND GROWTH OF KREBS-2 TUMOR CELLS

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UDC 616-006-018.1-092.4-078.33

KEY WORDS: Krebs-2 tumor; mRNA; antigenic modification

After tumor cells had been incubated with high-polymer RNA extracted from animal liver, their immunogenicity is significantly increased [3]. It was shown previously on a Krebs-2 tumor that this effect is linked with acquisition by the tumor cells of antigenic structures of the donors of the RNA, as shown by the appearance of resistance to these cells in mice preimmunized with normal tissue of the RNA donor's line, but not of a "foreign" line [4]. The mechanism of this antigenic modification of the cells is not clear. The few existing reports of the ability of RNA to induce donor antigens in normal cells assume that these antigens are translation products of messenger RNAs contained in the preparations [1, 5]. However, there is no evidence to support this hypothesis from experiments with isolated mRNAs.

The aim of this investigation was to study the effect of poly(A)$^+$mRNA and also of other functional types of RNA (rRNA and tRNA), isolated from rat liver, on the antigenic properties of tumor cells.

EXPERIMENTAL METHOD

Experiments were carried out on CBA and CC57BR mice. RNA preparations were obtained from the liver of Wistar rats: total RNA (totRNA) – by phenolic deproteinization [7], poly(A)$^+$mRNA from polysomes by affinity chromatography on poly-U-Sepharose 4B [2], and ribosomal RNA from the fraction not adsorbed on poly-U-Sepharose. Transfer RNA was generously provided by L. M. Skobel'tsina (Institute of Bioorganic Chemistry, Siberian Branch, Academy of Sciences of the USSR). Tumor cells were treated with RNA preparations and inoculated by the method described previously [4]. A suspension of Krebs-2 ascites tumor cells in Eagle's medium was incubated with RNA (or without RNA) for 1.5 h at 4°C, and then for 30 min at 37°C, and injected (in a dose of 10^5 cells) into the thigh muscle of intact mice and of mice immunized 14 days previously with Wistar rat liver homogenate. The results of inoculation were assessed 10-14 days later by noting the appearance and weight of the tumor [4]. The presence of rat antigens on the tumor cells was demonstrated by the cytotoxic test [6] with serum from CC57BR mice immunized with rat liver homogenate. Rabbit serum was used as the source of complement. The fraction of dying cells was determined with the aid of trypan blue. The tumor cells were tested immediately after incubation and after proliferation for 7-10 days in the peritoneal cavity of immunodepressed CC57BR mice. To induce immunodepression, 48 h before inoculation of the tumor cells, the mice were given an intraperitoneal injection of cyclophosphamide (200 mg/kg).

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0007-4888/92/0003-0394$12.50

TABLE 1. Effect of Immunization of CC57BR Mice by Rat Liver Homogenate on Growth of Krebs-2 Tumor Cells, Treated by RNA Preparations from Wistar Rat Liver

Exp. No.	Treatment of tumor cells	Intact mice		Immunized mice		Effect of immunization	
		Number of mice: with tumor/total	mean mass of tumor, mg	number of mice: with tumor/ total	mean mass of tumor, mg	inhi-bi-tion of tumor	
1	Control	29/29	800±45	27/27	863±45	—	—
	totRNA 2 mg/ml	30/30	753±58	23/30***	450±54***	40	<0,001
2	Control	30/30	563±41	29/30	527±34	5	—
	mRNA 8 μg/ml	28/30	527±32	23/30**	310±37**	41	<0,001
3	Control	10/10	460±55	9/10	420±62	9	—
	mRNA 8 μg/ml	10/10	420±33	5/10*	190±48**	55	<0,001
	rTNA 8 μg/ml	10/10	578±40	10/10	560±54	3	—
4	Control	10/10	690±77	10/10	580±44	16	>0,05
	mRNA 8 μg/ml	10/10	650±64	9/10	400±47**	38	<0,01
	rNA 8 μg/ml	10/10	730±73	10/10	670±63	8	—
	Poly(A) 8 μg/ml	9/10	570±68	10/10	630±93	—	—

Legend. Differences compared with mean values in control group of immunized animals statistically significant: *p < 0.05, **p < 0.01, ***p < 0.001.

TABLE 2. Growth of Tumor Cells Treated with mRNA in Intact, Immunized, and Cyclophosphamide Immunodepressed Mice

Line of mice	Preli-minary treat-ment	Inoculation	Number of mice: with tumor/total	Mean mass of tumor, mg	Inhibi-tion of tumor growth	
CC57BR	—	TC	10/10	800±83	—	—
	—	TC + mRNA (12)	10/10	530±50	34	<0,01
	—	TC + mRNA (24)	9/10	530±65	34	<0,01
	IM	TC	10/10	690±66	14	>0,05
	IM	TC + mRNA (12)	7/10*	330±78	59	<0,001
	IM	TC + mRNA (24)	9/10	390±79	51	<0,001
CBA	—	TC	16/18	744±109	—	—
	—	TC + mRNA (12)	8/18*	124±30	83	<0,001
	IM	TC	10/11	782±177	—	—
	IM	TC + mRNA (12)	5/10*	330±130	56	<0,05
	CP	TC	8/8	1237±125	—	<0,01
	CP	TC + mRNA (12)	10/10	1330±82	—	<0,01

Legend. Here and in Table 3: TC) control tumor cells, TC + mRNA) cells treated with poly(A) mRNA; IM) immunization with rat liver homogenate, CP) injection of cyclophosphamide. Concentration of mRNA (in μg/ml) shown between parentheses; *) differences from control are statistically significant.

EXPERIMENTAL RESULTS

The results of the first experiment (Table 1) demonstrate an effect reproducible in the transplantation test when the tumor cells had been treated with totRNA in a concentration of 2 mg/ml: preimmunization of the mice with rat liver homogenate, while not affecting growth of intact Krebs-2 tumor cells, induced a significant increase in resistance to the treated cells of this tumor, evidence of the appearance of "rat" antigens on them [4]. As can be seen from the results of the last three experiments, after treatment of the tumor cells with different functional types of RNA in a concentration of 8 μg/ml, this effect was reproduced only with poly(A) mRNA. As regards cells treated with rRNA, tRNA, and also with synthetic polyribonucleotide (polyadenylic acid, from "Reanal" Hungary), immunization was ineffective. If the mRNA concentration was increased by 1.5-3 times, delay of tumor growth was observed even without preliminary immunization (Table 2); in CBA mice, moreover, in which tumor growth was suppressed by 83%, immunization led to some reduction of this effect. It can be tentatively suggested that suppression of tumor growth in these cases was not due to immunologic causes. However, in animals subjected to immunodepression by cyclophosphamide, the rate of growth of the tumor, after

TABLE 3. Cytotoxic Action of Serum of Mice Immunized with Rat Liver Homogenate on Tumor Cells Treated with Rat Liver mRNA

Cells treated	Time after treatment	Cytotoxic index				
		whole serum	dilution of serum		source of serum	
			2	4	TC	TC + mRNA
Control	—	0	0	0	—	—
Treated with mRNA	3 h	0	0	0	—	—
	7 days	0,49	0,35	0,19	—	—
	10 days	0,78	0,33	0,26	—	—
Control	—	0	—	—	0	0
Treated with mRNA	7 days	0,39	—	—	0,36	0
Control	—	0,10	—	—	0	0
Treated with mRNA	7 days	0,48	—	—	0,41	0

inoculation both of cells treated with RNA and of untreated cells was the same (Table 2), evidence of the immunologic character of inhibition of growth of tumor cells modified by mRNA.

Confirmatory evidence is given by the results of the cytotoxic test with antiserum to Wistar rat antigens (Table 3). In this case the antiserum did not react with tumor cells taken soon after incubation with mRNA (absence of cytotoxic reaction in the presence of complement). However, on testing of an ascites tumor developing from mRNA-treated cells, a strong cytotoxic effect was observed, indicating expression of "rat" antigens on the tumor cells. The antiserum was inactivated by absorption with modified (but not original) tumor cells.

It can thus be concluded from these results that changes in the antigenic properties of tumor cells induced by RNA preparations are due to mRNA, which induces synthesis and expression of antigenic structures in the recipient cells similar in specificity to the donor's antigens. Investigation of the mRNA preparations which we used in a cell-free translation system showed that they are functionally active. Meanwhile, assuming that expression of new antigens is linked with translation of exogenous mRNA in the tumor cells, it is not yet clear how the phenotypic changes thus arising are preserved in subsequent cell generations.

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