PROPHYLAXIS OF TUMORS INDUCED BY CARCINOGENS IN INBRED MICE

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Antitumor vaccines were obtained with the aid of a filtrate of the culture fluid after growth of a culture of Bacillus mesentericus AB-56 from the cells of a sarcoma K-239 induced by methylcholanthrene and of an undifferentiated sarcoma Rt induced by an extract of the plant Rubia tinctorum. Immunization of inbred CBA mice and CBA × C57BL/j hybrids with the vaccines led to resistance to subsequent inoculation with the same tumors in 84-100% of cases. The effectiveness of vaccination depended on the time elapsing since the vaccine was prepared and on the quantity of vaccine material taken for testing postvaccinal immunity.

KEY WORDS: sarcoma of mice; antitumor vaccine; prophylaxis of tumors.

Previous investigations [1-5] showed that the use of tumor cells treated with a filtrate of culture fluid of Bacillus mesentericus AB-56 is effective for the prophylaxis and treatment of noninbred albino mice inoculated with tumors after prolonged passage (Ehrlich's carcinoma, sarcoma 37).

The object of this investigation was to study whether antitumor resistance can be produced in inbred animals against inoculation with tumors induced by carcinogens.

EXPERIMENTAL METHOD

Vaccines with a marked prophylactic action were obtained from the tissues of sarcoma K-239 and sarcoma Rt with the aid of a filtrate of the culture fluid of Bacillus mesentericus AB-56. The method of obtaining the antitumor vaccine was described previously [1-5] and consists essentially as follows: the residue of thrice-washed tumor cells is diluted with active filtrate of the culture fluid of B. mesentericus AB-56 to a concentration of 10-12 million cells/ml. The resulting suspension is kept for 2 h at 37°C. Viable cells are demonstrated by vital staining. Only those vaccines that contain no living cells are used for immunization. The other experimental conditions are shown in Table 1, which summarizes the results of immunization of inbred CBA and hybrid CBA × C57BL/j mice.

EXPERIMENTAL RESULTS

As Table 1 shows, when injected intraperitoneally the vaccines induced resistance in the mice to subsequent inoculation with the corresponding tumors: sarcoma K-239 after 6 or 7 passages in mice, and sarcoma Rt obtained by the writers [6] by injecting an extract from the plant Rubia tinctorum into mice, which had undergone 3 or 4 passages only at the time of these experiments.

The effectiveness of immunologic protection depended on the time of keeping of the vaccines. For instance, a freshly prepared vaccine from cells of sarcoma K-239, if injected 3 times in a dose of 0.6 ml on the 15th and 28th days after immunization produced resistance in 100% of the animals, at a time when the mean survival rate of the mice in the control group was 5.4%.

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TABLE 1. Immunization of Mice with Vaccines from Tumor Cells of Sarcomas K-239 and Rt

Preparation of immunization	Time of keeping preparation (in days)	Number of injections of preparation	Dose (in ml)	Mice	No. of tumor cells taken for testing immunity (in millions)	Number of days after end of immunization	Number of mice		
							total in group	without tumors	
								absolute	% (M±m)
Vaccine from cells of sarcoma K-239	5—17	5	1	CBA x C57BL/j hybrids	100	17	16	10	62,5± 12,5
The same	5—17	5	1	The same	1000	17	12	2	16,6± 11,2
" "	3—20	6	1,2	" "	100	15	24	16	66,6±9,8
" "	3—20	6	1,2	" "	1000	15	26	10	40± 9,8
" "	1	3	0,6	" "	100	15	20	20	100± 0
" "	1	3	0,6	" "	1000	15	24	19	80± 8,3
" "	1	3	0,6	" "	100	28	29	29	100± 0
Vaccine from cells of sarcoma Rt	1—8	3	0,6	CBA	15	12	19	16	84± 8,6
The same	4—10	3	0,6	"	15	14	14	9	64± 13,3
Filtrate of culture fluid of B. mesentericus AB-56	1—10	3	0,6	"	15	12—14	22	3	13,5± 7,4
	1—5—17	3—5	0,6—1	CBA x C57BL/j hybrids	100—1000	17—28	34	4	11,8± 5,6
0.85% NaCl solution	1—17	3—6	0,6—1,2	The same	100—1000	15—28	93	5	5,4± 2,3
The same	1—10	3	0,6	CBA	15	12—14	23	1	4,3± 4,3

If the keeping time of the vaccine was increased from 3 to 20 days the level of protection fell to 62.5-66.6%. The same dependence on keeping time was found if the dose of tumor cells given to test immunity was increased tenfold. In these experiments the survival rate varied between 80 and 16%.

No tumors appeared in the immune mice for 7-15 months (period of observation). The animals in which the vaccine did not prevent development of a tumor lived longer than the controls (56 and 30 days respectively); tumors appeared in them appreciably later and they grew much more slowly. The difference in the survival period thus obtained, when subjected to statistical analysis [9], was highly significant ($t = 12.5$; $P < 0.05\%$). Consequently, immunization of inbred mice with killed antitumor vaccines obtained with the aid of a filtrate of the culture fluid of B. mesentericus AB-56, results in a high proportion of cases in adequately strong and prolonged immunity against tumors induced by carcinogens.

The results of this investigation confirm those of many other workers [7, 8, 10-12] who showed that, in principle, active immunization of inbred mice and rats against spontaneous tumors and tumors induced by carcinogens is possible. However, by contrast with these investigations just cited, in which as a rule the vaccines used consisted only of tumor cells attenuated in some way, in the present experiments the operations contained no living tumor cells, thus ruling out the possibility of transplantation.

The high effectiveness and complete harmlessness of vaccines obtained by treating the appropriate tumor cells with products of microbial origin thus provide a basis for further experimental investigations aimed at studying the possibility of using these autovaccines in oncologic practice.

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