A STUDY OF THE COMPARATIVE EFFECTIVENESS OF INOCULATING RABBITS WITH LIVE OR KILLED BROWN-PEARCE TUMORS

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The literature indicates that the best antitumor immunity results from spontaneous tumor regression while the use of killed tissue as an immunizing agent seems relatively ineffective [2, 3, 7]. However, some authors have again raised the issue of the effectiveness of vaccinating against transplantable tumors by means of tumor material devoid of living cells [1, 6, 8]. Realizing the need of further studies in this field [4] and taking into account the fact that there has been so far no actual comparison of the comparative effectiveness of antitumor immunization using killed and living tumor tissues, we determined to make such an investigation employing on rabbits the transplantable Brown-Pearce carcinoma.

EXPERIMENTAL METHODS

Living tumors were inoculated under the skin of the ears of the rabbits [5]. Killed tumor tissues were extracted and similarly injected. Two series of experiments were run.

In the first series a comparison was made between the effectiveness of living tumor cells and saline extracts of tumor cells. In these experiments 23 male chinchilla strain rabbits weighing on the average 3.3 kg were used. 8 rabbits of the first group received an inoculation of the tumor under the skin of the right ear. The injection consisted of 0.2 cc of 40% by weight of a fresh tumor tissue suspension in physiological saline. A definite tumor growth was noted in 5 of the rabbits; a weak growth was noted in 2, while in one rabbit there was no take. By the 20th day the tumor had regressed in all the rabbits.

The 7 rabbits of the second group were inoculated with saline tumor extracts. The frozen tumor tissue was placed in physiological saline 1 : 9 and macerated in a blender for 5 minutes. The obtained material was cold-extracted and centrifuged for 20 minutes at 3000 rpm. The supernatant liquid was examined microscopically and found to have no undamaged tumor cells so that it could be used for the injections. The protein content of this supernatant fluid had its nitrogen content determined using the method of Conway. This extract was injected subcutaneously into the rabbits every 3 days for a total of 5 times. 20 days following the last injection, a sixth injection was given. Altogether, each rabbit received about 12 cc of the extract with a total protein content of about 34.45 mg. The 8 rabbits of the third group were used as controls. 21-26 days after the tumors of the first group had absorbed and 17 days after the last extract injection to the animals of the second group, all the rabbits received testicular injections of the tumor (25 % by weight of a fresh tumor extract suspended in physiological saline, the dose being 1 cc). Tumor development was followed for 75 days at which time all the surviving rabbits were subjected to an autopsy. The effectiveness of the preventive inoculations was judged by the degree of tumor take and extent of its development.

Taking into consideration the fact that a tumor extract prepared with normal rabbit serum has a definite immunizing effect [1], in our second series of experiments we compared the effectiveness of immunizing with

tumor tissues themselves as against tumor extracts prepared with normal rabbit serum. In these experiments we employed 20 male rabbits averaging 2.7 kg in weight. The 6 rabbits of the first group received under the skin of the right ear 40 % by weight of a physiological saline emulsion of tumor tissue, the dose being 0.25 cc. In all the rabbits the tumor grew and then regressed completely by the 20th-23rd day. The 7 rabbits of the second group were inoculated with the cell-free extract from frozen tumor tissue prepared with whole normal rabbit serum. The tumor tissue was added to the serum in a ratio of 1 : 4 and run in the blender for 5 minutes. After cold extraction the emulsion was centrifuged for 40 minutes at 3000 rpm. A direct determination of the tumor protein present in such an extract is impossible because of the large quantities of serum proteins. Indirectly, the amount could be judged on the basis of the fact that tumor extracts made under analogous conditions in physiological saline contained 8.75 mg of protein per 1 cc. The extract was given to the rabbits every 4 days for 4 times, the additional inoculation being made 20 days afterwards. Altogether the rabbits received 14.5 cc of the extract. The 7 remaining rabbits served as controls.

To determine the extent of the obtained immunity, in the first group 15-20 days after the regression of the tumor and, in the second group, 21 days after the 5th injection of the extract the animals were given an intratesticular tumor inoculation (25 % by weight of a suspension of fresh tumor tissue in physiological saline, the dose being 1 cc). The results of the immunization and its effectiveness were judged by the rate of tumor growth and the pathological study of the autopsied animals.

The results of the first and second series of experiments are presented in Tables 1 and 2.

EXPERIMENTAL RESULTS

TABLE 1

Comparative Effectiveness of Immunization Against Brown-Pearce Tumors Using Live Tumor Subcutaneous Injections Under the Skin of the Rabbit Ear and Using Similar Injections of Saline Tumor Extracts

Method of inoculation	Total number of rabbits	Inoculability of tumor		Degree of tumor development						
				rabbits dying from tumor			rabbits sacrificed 75 days after inoculation			
		did not take	takes	number of rabbits	av. life span (days)	av. number organs affected by tumor	number of rabbits	number of rabbits free of tumor	number of rabbits with metastases	av. number organs affected by tumor
Tumor inoculated under skin of ear	8	4	4	2	43.5	4.5	6	4*	2	3.0
Subcutaneous injection of saline extract	7	—	7	4	29.2	9.0	3	1**	2	5.0
Control	8	—	8	3	35.3	7.7	5	1**	4	3.2

MARKINGS: * — tumor did not take; ** — tumor took and then regressed.

As can be seen from Table 1, inoculation with the tumor under the skin of the ear produced immunity to a second inoculation in 4 rabbits out of 8. In the remaining 4 rabbits the tumor took. Three of them were rabbits either which did not have a take from the first injection in the ear or in which it grew poorly. All the rabbits which received injection of tumor extract, just as the control rabbits, were receptive to intratesticular injections of the tumor.

All 6 rabbits of the first group in the seond series which were immunized by subcutaneous tumor injection in the ear, resisted the intratesticular secondary inoculation. Of the 7 rabbits which were injected with the tumor extract on a serum medium, the tumor failed to take in only one. The control animals presented the same

degree of inoculability. There was no difference in the extent of tumor growth between the control animals and those receiving the extract. In the majority of the animals in these groups there was progressive growth for 15-20 days followed by regression of the tumor, the rapidity of the regression being even greater in the control than in the inoculated animals.

TABLE 2

Comparative Effectiveness of Immunization Against Brown-Pearce Tumors Using Live Tumor Subcutaneous Injections Under the Skin of the Rabbit Ear and Using Similar Injections of Normal Rabbit Serum Tumor Extracts

Method of immunization	Total number of rabbits	Inoculability of tumor		Number of rabbits dying from tumor	Av. life span (days) of animals dying from tumor	Av. number organs affected by tumor	Number of rabbits with partial or total regression
		did not take	takes				
Tumor inoculated under skin of ear	6	6	—	—	—	—	—
Subcutaneous injection of extract prepared on a rabbit serum medium	7	1	6	2	24.5	11.5	4
Control	7	1	6	1	27.0	8.0	5

Later, the 6 rabbits of the first group of the second series, which had displayed complete immunity to a secondary inoculation with the Brown-Pearce tumor, were subjected within the succeeding 7 months to 3 more intratesticular tumor injections. In all these animals there were no takes. These animals were sacrificed 10 months after the beginning of the studies and pathological examination failed to uncover any traces of the tumor.

Both series of experiments demonstrate as a fact that temporary growth and recession of Brown-Pearce tumors implanted under the skin of the ear in the rabbits leads to the development and maintenance of an immunity against subsequent inoculations with this tumor. Attempts to immunize by means of saline or rabbit serum extracts of the Brown-Pearce tumor failed. It may be that our disagreement [1] on the immunizing effect of Brown-Pearce tumors' extracts prepared with normal rabbit sera may be on the basis of a difference in the method of preparing the antigen or else on some other variation in the details of the experiment.

SUMMARY

Temporary growth and the following resolution of the Brown-Pearce tumor transplanted subcutaneously into the rabbit's ear causes development of complete immunity to the secondary transplantation of this tumor into the testicle.

Vaccination by saline extract of Brown-Pearce tumor or by an extract of the tumor prepared with normal rabbit serum produces no immunity in rabbits.

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