THE IMMUNOGENIC PROPERTIES OF EXPLANTATES OF BROWN-PEARCE RABBIT TUMORS WHEN CULTIVATED IN HUMAN SERUM

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The study of the character of growth of explantates or heterotransplantates of malignant tumors in animals of the homologous species has contributed greatly to out ideas of immunity against tumors and is, therefore, of undoubted theoretical and practical interest. The experimental treatment of this subject, however, remains incomplete. Certain aspects of the problem are debatable. Some authors [1, 5] consider that malignant tumors, after prolonged heterotransplantation or explantation, remain absolutely unchanged in immunological, biological, and cytological respects, but others, on the contrary, accept the possibility of changes in the antigenic and biological properties of such tumors [2, 4, 6]. Mollendorf [16], for example, inoculated rabbits with explantates of a Brown-Pearce carcinoma, cultivated in a homologous medium, and in a large percentage of cases observed positive results. Similar observations have been made by other authors [10, 15]. Favorite and Cheever [9], in their experiments, observed only 59% of positive inoculations of explantates of a Brown-Pearce rabbit tumor in the animals originally bearing the tumor. There are reports [6, 18] of loss of virulence and a considerable decrease in the number of successful inoculations of malignant tumors in mice after explantation of these tumors in heterogenic nutrient media.

In view of the contradictory findings on this subject, we decided to study the character of the inoculation rate and the growth in the original carriers of the primary tumor after its comparatively prolonged explantation in a heterogenic nutrient medium.

At the same time we attempted to study the reaction of the animals to the inoculated tumor explantates.

METHOD

For the investigations we used a Brown-Pearce rabbit carcinoma, taken for explantation from metastases of this tumor in the internal organs of cancerous animals. The tumor tissue was cultivated in Carrel dishes by the method which we have previously described [3]. The solid phase was chick plasma. The liquid medium consisted of 40-50% human serum, 20-25% chick or rat embryonic extract, 0.5% yeast extract, and a balanced salt solution in which, besides the other ingredients, were included glucose and ascorbic acid. Human serum was used because its action on animal tumors has been adequately studied [13, 14], and it contains a large number of antigens [11, 17]. After cultivation for a predetermined time, the test explantates were taken from the Carrel dishes in sterile conditions, cut into small pieces and made into a suspension in fresh, undiluted human serum, in a volume of 1 ml, which was injected twice into adult chinchilla rabbits, into the same testis, with an interval of 30 days between inoculations. For the first inoculation we used 60-65-day old explantates of tumor tissue and for the second, 30-day old explantates. Animals in which the inoculation of tumor tissue explantates was unsuccessful on both occasions were given an injection of untreated tumor tissue into the same testis on the 10th day, and a proportion of them received, in addition, injections of tissue into the other testis and intraperitoneally. The dose of material inoculated in these cases amounted on the average to 0.5-0.7 ml of a 10% suspension of tumor fragments in physiological saline. Sera were taken from these animals before and after inoculation of the test cultures and also after growth and regression of the untreated tumor tissue, and were tested by the complement fixation reaction for the presence of antibodies against the antigens of the tumor tissue and normal rabbit liver.

RESULTS

Inoculation of rabbits with explantates. During the cultivation of the Brown-Pearce rabbit carcinoma in this medium it was found that not all explantates showed intensive growth. In some cases growth of the test cultures was very intensive and this applied to many of the fragments (Fig. 1), but in others the cultures were in a stage of survival or had zones of growth with solitary cells migrating along the periphery (Fig. 2). Both intensively growing explantates and those in a stage of survival were used for the inoculations.

TABLE 1

Results of Double Intratesticular Inoculation of Rabbits with Explantates of a Brown-Pearce Rabbit Carcinoma, Cultivated in Human Serum

Rabbit No.	Inoculation I			Inoculation II		
	number of cultures which grew		results of inoculation	number of cultures which grew		results of inoculation
	well	badly		well	badly	
1206	2	14	Unsuccessful	4	16	Unsuccessful
1824	1	19	"	5	11	" "
993	3	12	"	6	14	" "
669	5	10	Temporary growth	10	12	" "
1721	1	11	Unsuccessful	8	16	" "
1287	3	14	Temporary growth	5	15	" "
1974	2	13	Unsuccessful	7	15	" "
2956	1	12	"	3	15	" "
1015	1	14	"	5	15	" "
1516	1	10	"	3	14	" "
2434	5	15	Temporary growth	10	15	" "
2591	3	13	" "	8	7	" "
2051	2	11	Unsuccessful	4	17	" "
2877	2	7	"	7	11	" "
3323	3	10	Temporary growth	6	14	" "
2556	4	15	" "	7	15	" "
3724	5	19	" "	7	7	" "
1819	1	17	Unsuccessful	5	15	" "
2195	1	14	"	6	11	" "
3970	4	15	Temporary growth	9	11	" "
3651	3	27	Unsuccessful	5	15	" "
3138	4	26	"	7	13	" "

It is clear from the figures in Table 1 that at the first inoculation, in 14 of the 22 rabbits no growth of the test cultures took place; however, in eight rabbits, firm nodules began to be palpable in the testis on the 7th-8th day, and on the 14th-15th day they reached comparatively large dimensions, but thereafter they began to decrease in size gradually. On the 25th-30th day they had completely regressed. After the second inoculation no growth of the test cultures was generally apparent. The results of these experiments show that explantates of a Brown-Pearce rabbit carcinoma, cultivated outside the body in human serum and the other ingredients of the nutrient medium for two months, and then inoculated mixed with fresh human serum, lose their ability to be successfully inoculated back into the initial carrier of the tumor. This probably took place because we used a small number of viable cultures for the first inoculation. The results of later years, however, show that tumors may develop in animals even when inoculated with a single tumor cell [7]. Our results are in agreement with those obtained on other experimental objects [12, 6].

In subsequent experiments all the experimental rabbits received a third inoculation in the same testis, this time of untreated tumor tissue, whereupon in eight rabbits the inoculation of the Brown-Pearce carcinoma was in general unsuccessful, but in ten rabbits tumor nodules appeared in the testis on the 6th-7th day, and continued to increase in size. Later they began to grow smaller and eventually to regress completely. In one rabbit (No. 1721), a large tumor developed in the testis, and began to infiltrate along the course of the spermatic cord. On the 25th-30th day massive tumor deposits began to be palpable in the peritoneal cavity of this rabbit. Later, on the 60th-65th day, they began to decrease in size and ceased to be palpable on the 90th day. The rabbit was sacrificed on the 98th day. No metastases were found in the organs. Some of the tumor nodules, however, were found to be in a state of complete necrosis on the surface of the peritoneum and in the mesentery of the large and small intestine. In another rabbit (No. 2956), sacrificed on the 93rd day, one completely necrotic metastasis was found in the omentum. In two other rabbits (Nos. 3651 and 3138), after inoculation with untreated tumor, firm nodules began to be palpable in the testis on the 5th-7th day, and on the 12th day they reached a large size. Both rabbits, however, died from accidental infection on the 43rd and 48th days. At necropsy of rabbit No. 3138 no mestastases were found. In rabbit No. 3651, two metastases were

TABLE 2

Complement Fixation Reaction of the Sera of the Rabbits of the Experimental Group with Antigens from Brown-Pearce Carcinoma and Normal Rabbit Liver

Time of inoculation of tumor	Antigen from Brown-Pearce tumor				Antigen from normal liver tissue			
	dilution of sera							
	1:20	1:40	1:80	1:60	1:20	1:40	1:80	1:60
Rabbit No. 1015								
Before inoculation of tumor	++	+	h	h	++	++	h	h
On the 30th day—first regression	+++	++	+	h	+++	+++	++	h
On the 40th day—second regression	+++	+++	+	h	+++	+++	++	h
On the 52nd day—third regression	+++	++	h	h	++	++	h	h
Rabbit No. 1516								
Before inoculation of tumor	++	h	h	h	++	h	h	h
On the 30th day—first regression	+++	+++	++	h	+++	++	++	h
On the 40th day—second regression	+++	+++	++	h	+++	+++	++	h
On the 52nd day—third regression	++	+	h	h	+++	++	+	h
Rabbit No. 1287								
Before inoculation of tumor	h	h	h	h	h	h	h	h
On the 30th day—first regression	+	h	h	h	h	h	h	h
On the 40th day—second regression	+	h	h	h	h	h	h	h
On the 50th day—third regression	h	h	h	h	h	h	h	h
Rabbit No. 1721								
Before inoculation of tumor	++	h	h	h	++	h	h	h
On the 30th day—first regression	++	+	h	h	++	h	h	h
On the 40th day—second regression	+++	++	h	h	+++	++	h	h
On the 50th day—growth of the tumor	+	h	h	h	h	h	h	h
Rabbit No. 2956								
Before inoculation of tumor	++	±	h	h	++	+	h	h
On the 30th day—first regression	++	+	h	h	++	++	±	h
On the 40th day—second regression	+++	+++	++	h	+++	+++	++	h
On the 50th day—third regression	+++	++	+	h	+++	+++	++	h
Rabbit No. 1974								
Before inoculation of tumor	+	h	h	h	±	h	h	h
On the 30th day—first regression	++	+	h	h	++	h	h	h
On the 40th day—second regression	+++	++	±	h	+++	++	h	h
On the 50th day—third regression	+	h	h	h	+	h	h	h

found in the kidney and one in the omentum, and one of the metastases in the kidney was necrotic, whereas the other contained viable tissue.

Of the 22 experimental rabbits, 18 were thus fully immune to inoculation with untreated tumor tissue (81.8%); in two rabbits the untreated tumor showed definite growth, but thereafter it partially or completely regressed; two rabbits were doubtful, for they died from infection and, moreover, one had a metastasis consisting of viable tissue.

Subsequently nine experimental rabbits were inoculated in the other testis a second time with untreated tumor. In all cases the rabbits were immune to this inoculation too. The other eight experimental rabbits were inoculated a second time in the peritoneal cavity with untreated tumor tissue. The results of inoculation of the tumor in this case too were negative. Twenty-four rabbits were used as controls. In five of the eight rabbits which were inoculated with untreated tumor tissue (62.5%) positive results were obtained, and these rabbits died from tumors on the 28th and 38th days. In three rabbits of this group the tumor showed some degree of growth at first, and then regressed. Of the eight rabbits vaccinated twice at an interval of one month with nutrient medium alone, with explantates, six rabbits died from inoculation of untreated tumor tissue on the 24th and 40th days. Two rabbits were immune. Of the six rabbits inoculated with original tumor tissues, perliminarily

treated for one hour with fresh human serum, two died from tumors on the 22nd and 38th days. The remainder were immune to the inoculation. Finally, two rabbits which received inoculations of a minimum amount of tumor (20 fragments measuring approximately 1 mm^2) died on the 23rd and 38th day. Of the 24 control rabbits, 15 (62.5%) thus died from tumors.

The sera of the experimental and control groups of rabbits were subsequently tested for the presence of antibodies against antigens from the tumor and from normal rabbit liver.

Serological examination of the rabbits' sera. In the experimental group sera were obtained from six rabbits at the following times: a) Before inoculation of the explantates, b) after the first regression of the experimental cultures (30 days), c) after the second regression of the experimental cultures (40th day), and d) after regression of the untreated tumor tissue (50th day). In the control group sera were also obtained from six rabbits at the following times: a) Before inoculation with untreated tumor tissue and b) on the 30th day of growth or regression of the untreated tumor. These sera were tested in the complement fixation reaction with antigens from the tumor and from normal rabbit liver. The test was performed in the usual manner.

It will be seen from the results given in Table 2 that the sera of the experimental group of rabbits, taken before inoculation of the cultures, reacted feebly or not at all with tumor and liver antigens. A somewhat different picture was seen after the first and especially after the second regression of the tumors. In this case the greater part of the sera reacted with tumor and liver antigens in dilution of 1:40 or 1:80 to the degree ++. Only the serum of one rabbit (No. 1287) did not react with these antigens in any dilutions.

In the control group only one serum, taken from a rabbit after regression of the tumor, reacted with antigens from tumor and liver in dilution of 1:40 to a degree ++. The remainder of the sera did not react with these antigens in these dilutions, or they reacted like the sera taken from the rabbits before inoculation with the tumor. The results of these experiments show that explantates of a Brown-Pearce rabbit carcinoma, cultivated in human serum, possess definite immunogenic properties. It is very probable also that the appearacne of antibodies in the blood of the rabbits after regression of the explantates is also due to the components of the nutrient medium in which the experimental cultures were grown. These components were heterogenic embryonic extract, human serum, and yeast extract.

SUMMARY

The explants of a Brown-Pearce rabbit carcinoma cultured on human serum and heterogenic embryonic and yeast extract, show growth in a number of cases, when mixed with fresh undiluted human serum and transplanted intratesticularly in rabbits; later they regress.

Rabbits in which the experimental cultures have regressed twice in the same testis became immune to repeated transplantation of the untreated tumor tissue into the same testis. In these rabbits, antibodies to the tumor and normal rabbit liver are produced more intensively than in the control group.

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