ONCOLOGY

THE ANTIGENIC PROPERTIES OF TUMORS WHEN CULTIVATED ON THE CHORIOALLANTOIC MEMBRANE OF THE CHICK EMBRYO

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Work has been reported recently pointing out the possibility of prolonged cultivation of tumors on the chorioallantoic membrane of the chick embryo. In the Institute of Virology of the Acad. Med. Sci. USSR, in Prof. A.K. Shubladze's laboratory a Crocker's sarcoma was cultivated for a long time by T.M. Maevskaia. To date this tumor has already undergone over 100 passages, and still retains its tumor-forming properties towards mice.

However, the nature of the tumor-forming agent, which survives for such a long time in heterotransplantation, has not yet been elucidated. Neither is it known whether the tumor loses any of its properties during cultivation, or retains them fully, despite the prolonged development in a foreign environment.

A comparative study of the antigenic properties of the original and transplanted tumors makes possible a detailed immunological analysis of the changes in the antigenic structure of the tumors when cultivated on the chorioallantoic membrane of the chick embryo.

There is no unanimity in the literature on the question of the preservation by heterotransplanted tumors of their antigenic properties. Some authors for instance [11, 12, 13, 17] consider that the antigenic properties of heterotransplanted tumors are preserved, while others [1, 5, 16, 18] come to the conclusion that the antigenic properties of the tumors undergo variation during the process of heterotransplantation and that the tumors lose the antigenic properties of the primary host and acquire the properties of the new host. However, these authors have not undertaken the task of differentiating the antigens of the tumor cell or of ascertaining which of these antigens are changed in the process of cultivation.

It has recently been shown that human tumor cells have a complicated antigenic structure. Besides the antigens present in the normal human cell – group-specific, type-specific and Rh [6, 7] – tumor cells are characterized by the presence of specific tumor antigens [2, 4, 15]. In human tumor cells [9], as in the cells of normal tissues [liver, 8], the existence of proteins identical with those of the serum has been proved.

The antigen structure of tumors in animals has been less fully studied. As in the case of human tumors, animal tumors are known to contain specific tumor antigens [3, 14].

The aim of the present investigation was to study the species-specific antigens of tumors in animals and also to make a comparative analysis of the species-specific antigenic properties of tissue of Crocker's mouse sarcoma before and during cultivation on the chorioallantoic membrane of the chick embryo.

EXPERIMENTAL METHOD

For the immunological investigation we used tissue of an ascitic form of Ehrlich's adenocarcinoma, Crocker's sarcoma and M-1 sarcoma, and liver tissue of mice and rats. Heterotransplants of Crocker's sarcoma on the

chorioallantoic membrane of the chick embryo were also studied.

The investigation of the species-specific antigen in the cells of the chorioallantoic Crocker's tumor was mainly carried out from material from the first passage, obtained as follows. The Crocker's tumor, freshly excised from a mouse, was cut into pieces 2 mm in diameter under sterile conditions and, after treatment for 30 minutes with penicillin and streptomycin was transplanted onto the chorioallantoic membrane of a nine to ten day chick embryo close to the blood vessels or else directly over their ramification. In 372 embryos surviving the experiments tumors developed in 337, or 90.6%. On the 18th-19th day of incubation of the embryo (at 37°C) the cultivated tumor was excised from the chorioallantoic membrane, carefully freed from traces of the latter, and weighed. In one experiment we usually used tumor from 15 to 17 embryos, amounting to 3.5-4 g.

After each passage the biological properties of the tumor and its ability to grow in the body of a mouse were tested. For this purpose tumors after the first or second passage were transplanted back into mice; the tumors became established and grew in all mice inoculated. In addition, to the first passage of Crocker's tumor onto the chorioallantoic membrane of the chick embryo, we also tested tumors after the 2nd, 3rd, 6th and 14th passages.*

For investigation of the species-specific antigens, precipitating sera from rabbits against mouse and rat protein were obtained. Rabbits were immunized with the sera of these animals according to the usually accepted method of preparation of precipitating sera.

For the work we used precipitating sera with an antibody titer not less than 1:10,000.

For detection of species-specific serum proteins in the cells of the animal tissues for examination we used the method introduced by P.N. Kosiakov for the detection of species-specific antigenic substances in the cells of human tissues and tumors [8, 9].

Tumor and liver tissue (3.5-4 g), washed free from erythrocytes, was finely chopped up in a mortar for ½-1 minute in order to obtain cells (ascitic cells of the Ehrlich's adenocarcinoma were used in the experiment without any special preliminary preparation). The tumor and liver cells so obtained were washed in 10% saccharose solution (pH = 7.2) or else in physiological saline until the washings were free from serum protein. Examination of the final washings with saccharose solution by the precipitation reaction was carried out after dialysis for 16 hours against physiological saline. In order to completely remove the serum proteins from the surface of the cells of the tumors and tissues, washing five to seven times was required.

After washing the cells were finely ground with a pestle or crushed with glass and extracted with physiological saline for 20 minutes. The cell extract was centrifuged for 30 minutes at the rate of 10,000 revolutions per minute and then tested by the precipitation reaction. A clear ring at the place of contact of serum and antigen was expressed by the sign +, an indistinct ring by ± and no reaction by −. The presence of protein in the washings and in the cell extracts was detected by the ring precipitation test with 20% sulfosalicylic acid.

EXPERIMENTAL RESULTS

The results of the investigation of the species-specific serum protein in tumor and rat and mouse liver tissue cells are given in Table 1.

It can be seen from Table 1 that the first washings of tumor and liver tissue cells contained a certain amount of serum proteins. In the second and third washings (not shown in the table) the amount of serum proteins detected was very small or else none was present. As a rule the 5th, 6th, and 7th washings were free from serum proteins, demonstrating the complete removal of contaminating serum proteins from the tumor and liver cells as a result of washing.

Investigation of the extracts obtained from the ground-up tumor and liver cells for the presence of species-specific serum proteins showed that on maceration of these cells the extract is found to contain proteins identical with serum proteins and reacting with the corresponding precipitating sera.

The absence of serum proteins from the final washings and their appearance in the extract from the macer-

*Material from 2nd, 3rd, 6th and 14th passages of Crocker's tumor for this investigation was kindly made available to us by T.M. Maevskaia, to whom we should like to record our thanks.

TABLE 1

The Presence of Species-Specific Antigens, Identical with Serum Protein, in the Cells of Tumors and of Liver Tissue of Mice and Rats

Antigen tested	Precipitation reaction set up with	Results of the precipitation reaction with various dilutions of antigen								
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Ehrlich's adenocarcinoma										
1st cell washings	Precipitating serum to mouse protein	+	+	+	+	+	±	−	·	·	·
5th cell washings	Ditto	−	−	−	−	·	·	·	·	·	·
Extract of cells after maceration	"	·	+	+	+	+	±	−	·	·	·
Ditto	Sulfosalicylic acid	·	+	+	+	+	+	+	+	+	±
Crocker's sarcoma										
1st cell washings	Precipitating serum to mouse protein	·	·	·	+	+	±	−	−	·	·
6th cell washings	Ditto	−	−	−	−	·	·	·	·	·	·
Extract of cells after maceration	"	+	+	±	−	−	·	·	·	·	·
Ditto	Sulfosalicylic acid	+	+	+	+	+	±	±	−	·	·
Mouse liver										
1st cell washings	Precipitating serum to mouse protein	·	+	+	+	+	−	−	·	·	·
6th cell washings	Ditto	−	−	·	·	·	·	·	·	·	·
Extract of cells after maceration	"	+	+	+	+	−	−	·	·	·	·
Ditto	Sulfosalicylic acid	+	+	+	+	+	+	+	−	−	−
M-1 sarcoma										
2nd cell washings	Precipitating serum to mouse protein	+	+	+	±	−	−	·	·	·	·
7th cell washings	Ditto	+	−	−	−		·	·	·	·	·
Extract of cells after maceration	"	+	+	+	+	+	+	−	−	·	·
Ditto	Sulfosalicylic acid	+	+	+	+	+	+	+	+	+	+
Rat liver										
1st cell washings	Precipitating serum to mouse protein	+	+	±	−	·	·	·	·	·	·
5th cell washings	Ditto	−	−	·	·	·	·	·	·	·	·
Extract of cells after maceration	"	+	+	+	+	±	−	·	·	·	·
Ditto	Sulfosalicylic acid	+	+	+	+	+	+	+	+	+	−

Note: A dot in the table means that no test was performed with this dilution.

TABLE 2

Comparative Investigation of Species-Specific Antigens in the Tissues of Crocker's Mouse Sarcoma Before and After Transplantation onto the Chorioallantoic Membrane of the Chick Embryo

Antigen tested	Precipitation reaction set up with	Results of the precipitation reaction with various dilutions of antigen						
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Crocker's sarcoma									
4th cell washings	Precipitating serum to mouse protein	–	–	–	·	·	·	·	·
	Ditto	+	+	–	–	·	·	·	·
Extract of cells after maceration	Sulfosalicylic acid	+	+	+	+	±	±	–	–
Ditto									
1st Transplantation of tumor									
3rd cell washings	Precipitating serum to mouse protein	·	–	–	–	–	·	·	·
Ditto	Sulfosalicylic acid	±	–	–	–	–	–	–	–
Extract of cells after maceration	Precipitating serum to mouse protein	–	–	–	–	–	–	–	·
Ditto	Sulfosalicylic acid	+	+	+	+	+	+	+	+

ated cells shows that these proteins are derived from the cells.

As already pointed out above, the cell extracts obtained were also tested with sulfosalicylic acid, whereupon it was shown (Table 1) that by maceration of the cells a large quantity of cytoplasmic protein, distinct from serum protein, enters the saline solution.

Thus the experiments which we performed showed that animal tissue and tumor cells contain species-specific antigens identical with serum proteins.

Since by transplantation of the tumors onto the chorioallantoic membrane of the chick embryo the tumor finds itself in a foreign environment, it was of great interest to find out if the species-specific serum antigen was preserved in the cells of the tumor after the first transplantation onto the chorioallantois of the chick embryo.

It can be seen from Table 2 that the final washings of the intact cells of the Crocker's tumor after first transplantation do not contain mouse serum protein, as shown by the negative reaction of the washings with precipitating serum to mouse protein. There is very little protein in the final washings as was seen in the test with sulfosalicylic acid. After maceration of the cells of the transplanted Crocker's tumor, cytoplasmic proteins appeared in the extract in large quantity and gave a clear ring of precipitate with sulfosalicylic acid in an antigen dilution of 1:128. However, missing from these proteins was a species-specific protein, identical with serum protein, which may be noticed by the absence of reaction with mouse protein antiserum.

On investigation of Crocker's sarcoma tissue after the 2nd, 3rd, 6th and 14th transplantation on the chorioallantois of the chick embryo, the results obtained were identical with those described above; species-specific serum protein was not found in these tumors after passage.

We examined the sera of rabbits immunized with Crocker's mouse tumor and also with a Crocker's tumor grown during the first transplantation on the cho-

rioallantois to see if the serum contained precipitating antibodies to species-specific mouse protein. As a control we used serum from tissue of a normal chorioallantoic membrane of an 18 to 19-day-old chick embryo. We assumed that if mouse serum protein was preserved in the transplanted Crocker's tumor, which we did not find by means of the precipitation reaction, rabbits immunized to the transplanted tumor must have produced precipitating antibodies to it. The rabbits were immunized with saline extracts of the tissues listed above according to a scheme adopted in our laboratory [10].

TABLE 3

Comparative Examination of the Sera of Rabbits Immunized to Crocker's Mouse Tumor and to the Same Tumor After First Transplantation onto the Chorioallantoic Membrane of the Chick Embryo

No. of rabbit	Antigen for immunization	Results of the precipitation reaction with various dilutions of mouse serum			
		1:20	1:40	1:80	1:160
125	Crocker's mouse sarcoma	+	+	+	+
621	" " "	+	+	+	−
250	" " "	+	+	+	+
750	" " "	±	−	−	−
896	1st transplantation of tumor onto chorioallantoic membrane	−	−	−	−
275	" " "	−	−	−	−
698	" " "	−	−	−	−
855	" " "	−	−	−	−
886	" " "	−	−	−	−
254	Normal chorioallantoic membrane	−	−	−	−

As can be seen from Table 3, precipitating antibodies to mouse protein serum were produced only by those rabbits which were immunized with a Crocker's tumor taken from a mouse. Furthermore rabbits Nos. 125 and 250 produced antibodies in quite a high titer (1:160). In not one of the rabbits immunized with a Crocker's tumor cultivated after first transplantation on a chorioallantois of a chick embryo were antibodies produced to mouse serum protein. It may be assumed that in the second group of rabbits, immunized by transplanted Crocker's tumor, that because precipitating antibodies to species-specific mouse protein were not produced, this protein was not injected into the rabbits, since it was not present in the cell extracts of the transplanted tumors.

It was thus possible to show in two mutually confirmatory experiments that species-specific serum antigen, present in Crocker's mouse sarcoma, is lost after only one transplantation of the tumor onto the chorioallantoic membrane of the chick embryo.

On cultivation of Crocker's tumor on the chorioallantoic membrane of the chick embryo the tumor loses its species-specific serum antigens after only one transplantation.

However, the loss of this antigen by the tumor transplanted onto the chorioallantois does not mean complete loss by the tumor of its species properties. It may be that the species specificity of the heterotransplanted tumor is determined by the presence of other antigens. However, the absence of the species specific serum antigen for which we tested is evidence of changes in Crocker's tumor with heterotransplantation onto the chorioallantoic membrane of the chick embryo.

The species-specific serum antigen entering into the composition of the tumor cell is evidently not important in respect to the blastomogenic properties of the tumor since, in spite of the loss of this antigen, the tumor cultivated on the chorioallantoic membrane of the chick embryo preserves the ability to be transplanted into mice and to grow.

SUMMARY

The presence of the species-specific antigen, identical to serum proteins was established in the cells of Ehrlich's ascitic cancer, Crocker's sarcoma, sarcoma M-1, as well as in the cells of the normal liver of mice and rats. When Crocker's tumor was cultivated on the chorioallantoic membrane of chick embryo the species-specific antigen could not be detected even after the first inoculation. However, the tumor retains its blastomogenic properties with regard to mice.

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