ONCOLOGY

SPECIFIC ANTIGENS FROM TUMORS AFTER PROLONGED CULTIVATION ON THE CHORIOALLANTOIC MEMBRANE OF THE CHICK EMBRYO

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Research by many workers has shown that tumors of man and animals possess a complex antigenic constitution. Besides the antigens common to both normal and tumor cells [5, 6, 7, 10], the latter also possess antigens specific to tumors [1, 2, 8, 9]. Insufficient attention has been paid to the study of changes in the antigenic composition of tumors during heterotransplantation; for this reason we still have no information on the fate of individual antigens composing the tumor cell when the tumor is grafted into an animal of a different species. The solution of this problem is important for the establishment of the relationship between the blastomogenic activity and the various antigens present as components of the tumor.

We have shown that not all tumor antigens are directly related to its blastomogenic properties [4]. The problem of the role of other antigens could only be solved experimentally, and was the object of the present investigation.

METHOD

Crocker's sarcoma of mice and M-1 sarcoma of rats were cultivated on the chorioallantoic membrane of the developing chick embryo and fully preserved their blastomogenic properties in relation to their original hosts. Back transplantation of the tumors was successful in 100% of cases

We obtained immune sera in rabbits against Crocker's mouse sarcoma and its heterotransplants on the membrane, and also against M-1 sarcoma of rats and organs of normal rats, by a method previously described by us [7]. Immune sera were also obtained in White Leghorn fowls against tissues of Crocker's sarcoma and its first transplantation on the chorioallantoic membrane. Immunization was carried out as follows: on the first, third and sixth days the fowls were injected with 1 ml of a saline extract of the tissue intravenously, and thereafter three times with 2 ml of the extract intraperitoneally, at intervals of four days. Blood samples were taken from an incision of the comb on the seventh day after the last injection of antigen. To obtain sera, the fowls were exsanguinated on the 9th-10th day.

In order to detect the specific tumor antigen in M-1 sarcoma and its heterotransplantates, the immune rabbit sera were tested by the complement fixation reaction at + 37°. To study the tumor antigen in Crocker's sarcoma, however, it was impossible to use this reaction, for during immunization of rabbits with an extract of Crocker's sarcoma antibodies were produced not only to the tumor antigen but also to the heterogenic antigen common to the chorioallantoic membrane of the chick embryo; as we have shown, this antigen is a constituent of the Crocker's sarcoma cell [3]. For this reason, in the investigation of the sera before absorption by this means, it was impossible to differentiate between tumor and membrane. It was also impossible to free the immune sera against Crocker's sarcoma from antibodies against the membrane by means of the absorption reaction, because these antibodies did not arise nonspecifically in the antitumor sera, but were true, immune antibodies.

In the study of the tumor antigen of Crocker's sarcoma, we therefore investigated the sera without specific absorption, and by the precipitation reaction.

The antigens used in the reactions were saline extracts of the tissues to be tested. The antigens for the precipitation reaction were prepared in a ratio of tissue to physiological saline of 1 : 20. In addition, these antigens were centrifuged for 30 minutes at 10,000-13,000 rpm. The precipitation reaction was read at 20 minutes.

RESULTS

In Table 1 we give the results of a comparative study of the antigenic properties of a Crocker's mouse sarcoma and of its heterotransplants on the chorioallantoic membrane.

It can be seen from Table 1 that the sera of fowls immunized with a saline extract from the first transplantation of the Crocker's tumor (Nos. B-7210, B-7214, and B-3269) reacted from only with antigens from tumors grown on the membrane, but also with antigens from mouse tissues, and completely failed to react with antigens from normal chorioallantoic membrane. This indicated an antigenic difference between the cultivated tumor and the membrane, and also that the antigen used for the immunization, i.e., the first transplantation of Crocker's tumor on the chorioallantoic membrane of the chick embryo, was foreign in relation to the fowl.

TABLE 1. Comparative Study of the Antigenic Properties of Crocker's Sarcoma and of its Heterotransplants on the Chorioallantoic Membrane of the Chick Embryo

Serum no.	Animal from which serum was obtained	Results of precipitation reaction with tissue antigens from: Crocker's sarcoma	mouse liver	mouse spleen	normal chorio-allantoic membrane	Crocker's sarcoma after transplantation: once	10 times	11 times	22 times	23 times	24 times	26 times	28 times	mouse serum
Not numbered, nonimmune	Fowl	—	—	—	—	—	—	—						—
B-7210, against 1st transplantation	»	+	+	+	—	+								—
B-7214, against 1st transplantation	»	+	+	+	—	+								—
B-3269, against 1st transplantation	»	+	+	+	—	+	+	+						—
B-0921, against Crocker's sarcoma	»	+	+	+	—	+								—
B-0997 " "	»	+	+	+	—	+								—
B-0923 " "	»	+	+	+	—	+	+	+						—
691	Rabbit	+	+	+	—				+	+	+	+	+	+
248, against mouse serum proteins	»	+	+	+	—	—	—	—	—	—	—	—	—	+
254, against normal membrane	»	—	—	—	+		—	—						—
255, against normal membrane	»	—	—	—	+				—	—	+	+	+	—

Sera against the original Crocker's tumor, also obtained in fowls (Nos. B-0921, B-0997, and B-0923) and rabbits (No. 691), reacted with mouse tissue antigens and with antigens from the allantoic tumors, and thus indicated an antigenic relationship between them.

Immune sera against Crocker's tumor and the first transplantation of the tumor on the membrane were not specific antitumor sera, for they reacted with normal mouse tissues; consequently, the antigen which we discovered in the chorioallantoic tumors could not be described as a specific tumor antigen. This cytoplasmic antigen bore the species properties of the mouse, although it was not identical with mouse serum proteins, for the precipitating serum No. 248, reacting with a species-specific antigen identical with the serum proteins, did not react with antigens from transplanted tumors, although it did react with a species-specific antigen in extracts obtained from mouse tissues.

Antisera against the normal chorioallantoic membrane of the developing chick embryo reacted only with antigens from the membrane and with antigens from a few transplanted tumors, which could be accounted for by the presence of membrane in the allantoic tumors in the capacity of stroma.

The serum of a nonimmune fowl, taken as a control, did not give a positive precipitation reaction with any of the antigens mentioned, and it thus followed that normal fowl serum did not contain antibodies to these tissues.

It could thus be shown by means of the sera investigated that Crocker's sarcoma retains the antigens inherent in the original tumor even after prolonged cultivation on the chorioallantoic membrane of the chick embryo. This common factor is independent of the presence of a heterogenic antigen, and also of a species-specific antigen identical with the serum proteins, in the original tumor and especially in the tumors grown on the chorioallantoic membrane. This species-specific antigen, as we have shown earlier [4], is lost by the tumor during heterotransplantation.

The results of the study of the specific tumor antigen in the M-1 sarcoma of the rat are shown in Table 2.

It can be seen from the results in Table 2 that immune sera against M-1 tumors (Nos. 169 and 773) were specific antitumor sera, and reacted only with M-1 rat sarcoma and with its heterotransplants on the chorioallantoic membrane of the chick embryo, while failing to react with normal rat tissues and normal chorioallantoic membrane. The sera thus detected a specific tumor antigen in heterotransplants of the tumors on the membrane and demonstrated the absence of an antigenic relationship between the M-1 sarcoma and the chorioallantoic membrane. Serum No. 145 against M-1 sarcoma was less specific, but it too did not react with antigen from the membrane, and it reacted more strongly with tumor antigens than with antigens from normal rat tissues. Sera against normal organs of the rat reacted neither with M-1 sarcoma nor with its heterotransplants, but reacted specifically with antigens from the particular organ against which they were obtained. Immune serum No. 254 against normal chorioallantoic membrane detected the presence of

TABLE 2. Specific Tumor Antigen in Heterotransplants of M-1 Sarcoma

Serum no.	Dilution of serum	Results of complement fixation reaction with tissue antigens from													
		M-1) sarcoma	rat liver	rat spleen	normal chorio-allantoic membrane	M-1 sarcoma, after transplantation									
						once	twice	3 times	4 times	5 times	6 times	7 times	8 times	9 times	10 times
169, against M-1 sarcoma	1:60	++++*	—	—	—	++++	++++	++++		++++	+++	++++	++++	++++	+++
	1:80	+++	—	—	—	+++	+++	+++		+++	++	+++	+++	+++	+++
	1:100	+++	—	—	—	++	+++	+++		+++	++	+++	++	+++	++
	1:120	+++	—	—	—	+	+++	+++		+++	++	+++	++	+++	+
773, against M-1 sarcoma	1:20	++++	+	—	—	++++	++++	++++	++++	+++	++++				
	1:40	++++	—	—	—	++	+++	++++	+++	+++	++++				
	1:80	+++	—	—	—	++	++	++	++	+++	++				
	1:160	+++	—	—	—	+	+	—	—	++	++				
145, against M-1 sarcoma	1:60	++++	+++	++	—	++++	++++	+++	++++	++++	+++	++++	+++	++++	++++
	1:80	++++	+++	++	—	++++	+++	+++	++++	+++	++	++++	++	++++	++++
	1:100	++++	++	+	—	++++	+++	++	++++	+++	++	++++	++	++++	+++
	1:120	++++	++	—	—	++++	++	+	++++	+++	++	++++	++	++++	+++
1058, against rat liver	1:40	—	++++	—	—	—	—			—	—	—	—	—	—
	1:80	—	++++	—	—	—	—			—	—	—	—	—	—
	1:160	—	++++	—	—	—	—			—	—	—	—	—	—
	1:320	—	++	—	—	—	—			—	—	—	—	—	—
128, against rat spleen	1:40	—	—	++++	—	—	—			—	—	—	—	—	—
	1:80	—	—	+++	—	—	—			—	—	—	—	—	—
	1:160	—	—	++	—	—	—			—	—	—	—	—	—
	1:320	—	—	+	—	—	—			—	—	—	—	—	—
254, against normal chorio-allantoic membrane	1:40	—	—	—	++++					++	++	++++	++	+++	++++
	1:80	—	—	—	++++					++	++	++++	++	+++	++++
	1:160	—	—	—	++++					++	++	+++	+	++	+++
	1:320	—	—	—	++++					+	+	+	+	+	+++

* The complement fixation reaction was assessed as ++++ if complete fixation of complement too place: as +++, ++ and + if different degrees of hemolysis were observed, and by a minus sign if hemolysis was complete.

the antigen of the chorioallantoic membrane of the chick embryo in the tumor heterotransplants, which evidently indicated the inclusion of membrane into the stroma of the allantoic tumors.

It can thus be seen from these cross reactions (see Table 2) that heterotransplants of M-1 sarcoma, like the original M-1 sarcoma, possess a specific tumor antigen, differing from the antigens of normal rat tissues and of normal chorioallantoic membrane.

Our results thus showed that Crocker's sarcoma of mice possesses, in addition to the two antigens previously described, a third or cytoplasmic antigen, bearing the species properties of mice, which is evidently a tumor antigen that is preserved in spite of prolonged heterotransplantation of the tumors in a foreign environment.

The species-specific antigen identical with mouse serum proteins disappeared after only the first transplantation of the tumor on the chorioallantoic membrane, although the tumor did not lose its blastomogenic properties in relation to the original host. The heterogenic antigen, common to Crocker's sarcoma and the chorioallantoic membrane, was characteristic of normal tissues (mouse spleen, chorioallantoic membrane) besides the tumor, but is absent from tumors such as Ehrlich's adenocarcionoma [3] and M-1 sarcoma of rats, and it cannot therefore be responsible for the blastomogenic properties of tumor tissue.

The specific tumor antigen, preserved in heterotransplants of M-1 sarcoma of rats, and the third, cytoplasmic antigen, bearing the species properties of mice, which is evidently a tumor antigen also, were detected in heterotransplants of tumors which had retained their properties of producing tumors in the original hosts.

These facts give grounds for attributing a definite connection with the tumor-producing properties of the tumors to this biochemical antigenic complex.

SUMMARY

The authors studied the specific tumor antigen in Crocker's sarcoma of mice and M-1 sarcoma of rats as well as in heterotransplants of these tumors on the chorioallantoic membrane of the developing chick embryo. It was shown that this antigen was preserved in the heterotransplants of these tumors. The possible connection of preservation of the specific tumor antigen with the retention of the blastomogenic properties of the heterotransplants with regard to the initial hosts of the tumor is discussed.

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