

THE PRESENCE OF A HETEROGENETIC ANTIGEN IN CROCKER'S SARCOMA

T. P. Konstantinova

D. I. Ivanovskii Institute of Virology (Director — Prof. P. N. Kosyakov),
AMN SSSR, Moscow

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Experimental data which indicate that by no means can all tissues of the human and animal organisms be cultivated on the chorioallantois of a chick embryo have recently accumulated.

The attempts of researchers to cultivate normal, embryonal, and pathologically altered tissues, such as the tissues of benign tumors, on this membrane have been in vain. Prolonged cultivation on the chorioallantois is only successful with malignant tumors [1, 5, 8, 12], but no explanation of this fact has yet been discovered. There are some reports [7, 10, 11] that heterotransplantation of tumors of connective-tissue origin can be more successful than that of epithelial tumors.

The antigenic affinity of the tumor and the membrane [5, 9, 11] and the low resistance of the chick embryo during the period in which a tumor is developing in it [4, 6] may be of some significance.

In our laboratory, prolonged cultivation has been done on the chorioallantois with Crocker's mouse sarcoma which had undergone up to 70 passages in a chick embryo without losing its blastogenic properties in relation to mice.

Research on the antigenic structure of Crocker's sarcoma has shown [2] that there is a species specific antigen found in the cells of the original tumor which is identical to mouse serum proteins and which the tumor loses during the first passage to the membrane, but that the tumor does not lose its ability to be inoculated back into mice.

The purpose of this study was to further investigate the antigenic structure of Crocker's sarcoma in comparison with the antigens of the chorioallantoic membrane in order to determine whether there is or is not an antigenic affinity between them.

METHOD

The experimental object employed was Crocker's mouse sarcoma; the antigenic properties of this object were compared with the antigenic properties of the normal chorioallantois of a 17-18 day old chick embryo and with those of other mouse tissues.

The antigens from these tissues were prepared in a sterile physiological solution by the usual method [3].

Immune sera against the normal chorioallantois of a 17-18 day old chick embryo, the tissues of mouse organs,

Crocker's sarcoma and Ehrlich's ascitic tumor were obtained from rabbits by the method used in our laboratory [3]. Immune sera against mouse blood serum were obtained from rabbits by the usual method of obtaining precipitating sera. The antigens for the immunization of the animals were prepared from native tissues.

The antigenic properties of the normal chorioallantoic membrane of a chick embryo were compared with those of the mouse tissues by the method employing the complement fixation reaction at +37 deg.

All the sera were studied before the absorption of the nonspecific, incidental antibodies from them and then, if required, after their specific absorption.

RESULTS

The comparative study of the antigenic properties of three tissues — the tissues of Crocker's sarcoma, mouse spleen and the chorioallantois of a chick embryo (Table 1) — showed that the immune sera against Crocker's sarcoma, mouse spleen and the chorioallantoic membrane, studied before their absorption, reacted as strongly with the other two antigens as with the homologous antigen.

Thus the reaction of serum No. 973, obtained against Crocker's sarcoma, with the antigen from the chick embryo's chorioallantois was so strong that, in the given experiment, it was impossible to judge the specificity of serum No. 973 even from the difference in the titers of its reaction with its own and the heterogenetic antigens. Sera No. 813 and 255, obtained against the tissues of the spleen and of the chorioallantois of an 18-day old chick embryo, acted in a similar fashion.

From the data in this table, therefore, one can conclude that all three of the experimental tissues, Crocker's sarcoma, mouse spleen and the normal chorioallantois of a chick embryo, are related to each other. In view of the fact that the experimental tissues in this case are from animals of very different species, the chick embryo and the mouse, the antigen common to the mouse tissues and the chick embryo's chorioallantoic membrane is evidently heterogenetic for the two types of animals.

In spite of the considerable reaction of the sera against the mouse tissues with the heterogenetic antigen from the chick embryo's chorioallantois and of the serum

TABLE 1. The Antigenic Affinity Between the Tissues of a Chick Embryo's Chorioallantoic Membrane and the Tissues of Crocker's Sarcoma and Mouse Spleen

Serum No.	Serum dilution	Absorbent	Results of complement fixation reaction with antigens from the tissues of:		
			Crocker's sarcoma	spleen	chorioallantois
973 Against Crocker's sarcoma	1:40	—	++++	+++	++++
	1:80		++++	++(+)	++++
	1:160		++++	+(+)	++++
	1:320		+(+)	±	±
813 against spleen	1:40	—	++++	++++	++++
	1:80		++++	++++	++++
	1:160		++++	++++	++++
	1:320		++++	++++	+++
255 against membrane	1:40	—	++++	++++	++++
	1:80		++++	++++	++++
	1:160		++++	++++	++++
	1:320		++++	++(+)	++++
973	1:40	Chorioallantoic membrane	++++	±	—
	1:80		++	—	—
	1:160		—	—	—
	1:320		—	—	—
813	1:40	Crocker's sarcoma	+++	++++	+
	1:80		+	++++	—
	1:160		—	++++	—
	1:320		—	++++	—
255	1:40	Crocker's sarcoma	+	±	++++
	1:80		—	—	+++(+)
	1:160		—	—	±
	1:320		—	—	—

Key: +++, ++(+), ++, ++(+), ++, +(+) show different degrees of a positive complement fixation reaction; ± shows a doubtful reaction, - shows a negative reaction.

against the chorioallantois with the antigens from the mouse tissues, these sera could be subjected to specific absorption, and the incidental, nonspecific antibodies could be removed from them.

Table 1 also gives typical records of the experiments with the absorption of these sera by formalin-treated tissues in order to remove nonspecific antibodies. It is evident from this table that when the immune serum against Crocker's mouse tumor was absorbed by the tissue of a chick embryo's chorioallantoic membrane, the antibodies to the membrane were completely eliminated, but the serum in this case kept its ability to react mainly with the antigen against which it was obtained.

Absorption of the serum against the chorioallantoic membrane by Crocker's tumor in the same way eliminated nonspecific antibodies to the mouse tissues, but left the antibodies to the antigens from the chorioallantoic membrane.

Therefore, the heterogenetic antigen contained in the tissues of mouse spleen and Crocker's sarcoma as well as in the tissue of a chick embryo's chorioallantois is an incidental antigen, accessory to the specific antigens contained in these tissues.

The presence of the heterogenetic antigen in the tissues of mouse spleen and Crocker's sarcoma made it necessary to investigate the question of whether a heterogenetic antigen common to the chorioallantois of a chick embryo exists in other mouse organs.

Table 2 presents experimental records obtained in the investigation of serum No. 254, obtained against the normal chorioallantois of a chick embryo, and the antigens from the tissues of mouse organs in the complement fixation reaction. This table also includes the records from the investigation of immune sera against the antigens from the mouse tissues with water-salt extracts of the normal chorioallantois of a chick embryo. In this case, all the sera were studied before absorption.

The data in Table 2 show that the immune sera obtained against mouse liver and brain and those against mouse blood serum and the cells of Ehrlich's ascitic adenocarcinoma did not react with the antigen from a chick embryo's normal chorioallantoic membrane. In the same way, the serum against the chorioallantois did not react with the antigens from the above tissues.

One can therefore conclude that mouse blood serum, liver and brain, as well as the cells of Ehrlich's ascitic

TABLE 2. The Lack of Antigenic Affinity Between the Tissues of a Chick Embryo's Chorioallantois and Certain Mouse Tissues

Serum No.	Results of reaction with antigens		Serum No.	Serum dilution	Results of reaction with antigens		Serum No.	Serum dilution	Results of reaction with antigens		Serum No.	Serum dilution	Results of reaction with antigens	
	embryo membrane	mouse liver			embryo membrane	mouse brain			embryo membrane	mouse serum			embryo membrane	Ehrlich's carcinoma
254 against mem- brane tissue	1:40	—	254 against mem- brane tissue	1:40	+++	—	254 against mem- brane tissue	1:40	+++	—	254 against mem- brane tissue	1:40	+++	—
	1:80	+++		1:80	+++	—		1:80	+++	—		1:80	+++	—
	1:160	+++		1:160	+++	—		1:160	+++	—		1:160	+++	—
	1:320	+++		1:320	+++	—		1:320	+++	—		1:320	+++	—
225 against liver tissue	1:40	+++	612 against brain tissue	1:40	—	+++	248 against mouse serum	1:40	—	+++	893 against Ehr- lich's carci- noma	1:40	—	+++
	1:80	+++		1:80	—	+++		1:80	—	+++		1:80	—	+++
	1:160	+++		1:160	—	+(+)		1:160	—	+++		1:160	—	+++
	1:320	±		1:320	—	—		1:320	—	+++		1:320	—	+++

Note: Symbols mean the same as in Table 1.

carcinoma, do not have the heterogenetic antigen common to the chorioallantois of a chick embryo. The nature of this antigen and its relation to the heterogenetic Forssman's antigens is not yet clear.

As to the two experimental mouse tumors, Ehrlich's ascitic carcinoma does not contain a heterogenetic antigen identical to the antigen found in the chick embryo's chorioallantoic membrane, but Crocker's sarcoma does

contain such an antigen. It is known from the literary data [1, 10] that cultivation of Ehrlich's carcinoma on the chorioallantois of a chick embryo is less successful than cultivation of Crocker's sarcoma. One can propose that the presence of a common heterogenetic antigen in Crocker's sarcoma and the chorioallantois of a chick embryo is one of the factors aiding the successful prolonged cultivation of this tumor after heterotransplantation on the chorioallantois of a chick embryo.

SUMMARY

The author investigated the antigenic properties of Crocker's sarcoma in mice and of the chorioallantoic membrane of a chick embryo. A common heterogenic antigen was soon to be present in the chorioallantoic membrane of a chick embryo in the Crocker's mouse sarcoma and in the mouse spleen. The tissues of mouse liver and brain and the mouse blood serum, as well as the cells of Ehrlich's ascitic adenocarcinoma do not contain this antigen.

It may be suggested that the affinity of the tumor and the membrane with respect to the heterogenetic antigen is one of the factors aiding successful prolonged cultivation of Crocker's tumor on the chorioallantoic membrane of a chick embryo without loss of its blastomogenic properties in mice.

LITERATURE CITED

- [1] V. A. Anan'ev and V. R. Obukhova, Byull. Eksptl. Biol. i Med. 3, 62 (1955).
- [2] T. P. Konstantinova and Z. I. Rovnova, Byull. Eksptl. Biol. i Med. 4, 112 (1958).
- [3] P. N. Kosyakov, V. S. Korosteleva and N. I. Kuznetsova, Byull. Eksptl. Biol. i Med. 9, 63-65 (1955).
- [4] B. S. Ruchkovskii, Vrachebnoe Delo 10, 1035 (1956).
- [5] D. V. Serpukhovitin, Problems of Oncology [in Russian] Trudy Akad. Med. Nauk SSSR 5, 123 (1952).
- [6] V. Z. Bisceglie, Z. Krebsforsch 40, 122 (1934).
- [7] C. P. Dagg, D. A. Karnofsky and J. Roddy, Proc. Am. Asspc. Cancer Research 2, 11 (1955).
- [8] W. G. Heim and A. M. Schlechtman, J. Nat. Cancer Inst. 15, 1313 (1955).
- [9] E. W. Hurst, B. Cooke, and G. C. McLennan, Austral J. Exper. Biol. Med. 17, 215 (1959).
- [10] D. A. Karnofsky, P. A. Patterson and L. P. Ridgway, Cancer Research 10, 228 (1950).
- [11] N. Kaufman, T. D. Kinney, and R. J. Mason, et. al., Am. J. Path. 32, 271 (1956).
- [12] E. A. Mirand and J. G. Hoffman, Proc. Soc. Exper. Biol. Med. 88, 656-660 (1955).