

THE RELATIONSHIP BETWEEN THE TITER OF TUMOR ANTIBODIES AND THE CHARACTER OF THE ANTIGENIC STIMULUS

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The production of immune sera with a relatively high titer of antibodies against tumors is of great scientific and practical importance. Several investigations carried out in this direction demonstrate that the production of immune antibodies depends, on the one hand, on the species and individual characteristics of the experimental animals undergoing immunization [1, 2, 3], and on the other hand, on the character of the antigenic stimulus itself. For instance it was established by the investigations of Horn [4] that sera with a high antibody titer are obtained by immunization of rabbits with whole ascitic cells from a carcinomatous tumor, but not by saline extracts of the separate fractions of these same cells. In this connection it was shown that a serum of the first type had a comparatively high titer (1280) in the agglutination reaction, whereas sera of the second type, produced by immunization of rabbits with saline extracts of the nucleoprotein fraction of ascitic cells, were in general inactive in this reaction. It is also well known that hemolytic sera, used in the complement fixation reaction, are obtained with a high antibody titer during immunization of rabbits with whole sheep's red cells, but not with a saline extract of these cells.

TABLE 1

Scheme of Immunization of Rabbits with Saline Extracts of Subcutaneous
Forms of Tumors and Whole Ascitic Cells from the Same Tumors

Form of antigen of tumor tissue	Injections				
	1st	2nd	3rd	4th	5th
	volume of antigen injected (in ml)				
Saline extract	1.0	1.0	1.5	1.5	2.0
Whole ascitic cells	1.0	1.0	1.5	1.5	2.0

In view of these findings we decided to study the relationship between the titer of antibodies against tumors and the character of the antigenic stimulus of the same origin.

EXPERIMENTAL METHOD

In the immunization experiments 20 sexually mature male rabbits, aged from 12 to 18 months, were used. The material used for immunization was two transplanted malignant tumors: an Ehrlich's mouse adenocarcinoma and a Iosida's rat sarcoma. In the experiments one group of rabbits was immunized with saline extracts of subcutaneous forms of the tumors and another group with whole ascitic cells from the same tumors.

TABLE 2

Complement Fixation Reaction of Sera Against Ehrlich's Mouse Adenocarcinoma with Homologous Antigen

Dilution of sera	Sera obtained by immunization of rabbits															
	with saline extracts of a subcutaneous form								with whole ascitic cells							
	of Ehrlich's mouse adenocarcinoma															
	no. of sera															
	5	870	881	971	102	154	148	4510	1479	3135	1015	1516	1518	309	1473	1004
1:20	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
1:40	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
1:80	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
1:160	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
1:320	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
1:640	+	r	+	++	++	++	r	+	++	+	++	++	++	++	++	
1:1280	r	r	r	+	+	+	r	r	++	+	++	++	++	++	++	
1:2560	r	r	r	r	+	r	r	r	+	+	+	+	++	±	+	
1:5120	r	r	r	r	r	r	r	r	r	r	r	r	r	r	r	

The saline extracts of the subcutaneous forms of the tumors were prepared as follows. A subcutaneous tumor transplantate 7-10 days old was extracted from the experimental animals and ground in a mortar in sterile conditions for 10 minutes.

The ground tumor tissue was suspended in physiological saline in a proportion of 1:20, i. e. 0.5 g of tumor tissue was suspended in 10 ml of physiological saline. This suspension of tumor tissue was allowed to stand in the incubator for one hour at a temperature of 37°C, after which it was kept in the refrigerator at 4°C for 24 hours.

Before immunization the suspension of tumor tissue was centrifuged for 15 minutes at 2500 rpm.

The whole ascitic carcinoma cells were taken from the experimental animals on the 6th-8th day after inoculation, and separated from ascitic fluid by weak centrifugation for 5 minutes. Next they were washed twice with physiological saline and suspended in this solution. Before immunization the number of ascitic cells in 1 ml of the cell suspension in physiological saline was measured in a Goriaev counting chamber. In the experiments ascitic fluid which was free from blood was used, containing only ascitic carcinoma cells as its basic component.

The rabbits were immunized by five intravenous injections according to the following scheme (Table 1).

The number of ascitic cells in 1 ml of physiological saline used for immunization varied in the case of Ehrlich's mouse adenocarcinoma from 100 to 200 million, and in the case of Iosida's rat sarcoma, from 50 to 100 million. Injection of tumor antigens into the rabbits was carried out every 2 days for 3 injections, and an interval of 7 days was left between the 3rd and 4th injections. Serum was taken from the rabbits on 10th day after the last injection, and it was taken from the heart. Before the experiment was set up, the sera for testing were heated to a temperature of 56°C for 30 minutes. Next these sera were tested in the complement fixation reaction in order to determine the presence of antitumor antibodies in them, and their specificity. The antigens used in the serological reactions were saline extracts of subcutaneous forms of tumors and of tissues from the liver and spleen of the corresponding species of animal. The antigens

TABLE 3

Complement Fixation Reaction of Sera Against Iosida's Rat Sarcoma with Homologous Antigen

Dilution of sera	Sera obtained by immunization of rabbits			
	with a saline extract of a subcutaneous form		with whole ascitic cells	
	of Iosida's rat sarcoma			
	no. of sera			
	3643	3095	2906	3730
1:20	++++	++++	++++	++++
1:40	++++	++++	++++	++++
1:80	+++	++++	++++	++++
1:160	++	+++	++++	++++
1:320	+(+)	++	++++	++++
1:640	+	+(+)	++++	++++
1:1280	Γ	Γ	++++	+++
1:2560	Γ	Γ	+(+)	+

were prepared in proportions of 1:10. Before the main experiment was set up all these antigens were titrated and a working dose was estimated for them. The complement fixation reactions were performed in the usual way at a temperature of 37°C, with provision of suitable controls. In the experiment the only reactions to be considered were those in which the same results were obtained on two occasions.

EXPERIMENTAL RESULTS

In the immunization experiments on rabbits with antigens from tissue of an Ehrlich mouse adenocarcinoma 16 experimental animals were used, of which 9 were immunized with a saline extract of a subcutaneous form of the mouse tumor and 7 with whole ascitic cells of the same tumor. The results of these experiments are shown in Table 2.

Of the 9 rabbits immunized with a saline extract of tissue of a subcutaneous form of Ehrlich's mouse adenocarcinoma, in 4 the sera were found to have a low titer of anticarcinoma antibodies. The sera of rabbits Nos. 870 and 881 reacted with antigen from the tumor tissue in a dilution of 1:160 to ++, but sera of rabbits Nos. 102 and 4510 reacted with the same antigen in a dilution of 1:80 to ++. The serum of only one rabbit (No. 154) reacted with tumor antigen in a comparatively high titer; in a dilution of 1:1280 to +(+).

A somewhat different picture was obtained during immunization of rabbits with whole ascitic cells of Ehrlich's mouse adenocarcinoma. The sera of these rabbits reacted with tumor antigen in a comparatively high titer. The sera of rabbits Nos. 1015, 1516 and 309 reacted in dilution of 1:2560 with antigen from the tumor tissue to ++ and +++, but sera of rabbits Nos. 3135, 1518, 1473 and 1004 showed a titer of tumor antibodies corresponding to a dilution of 1:1280.

Thus sera against Ehrlich's mouse adenocarcinoma had a higher titer of tumor antibodies after immunization of rabbits with whole ascitic carcinoma cells than with saline extracts of a subcutaneous form of the tumor.

A similar picture was likewise observed after immunization of rabbits with antigens from tissue of Iosida's rat sarcoma. As may be seen from the figures given in Table 3, the sera of rabbits Nos. 3643 and 3095, obtained by immunization of experimental animals with a saline extract of tissue of a subcutaneous form of Iosida's rat sarcoma, had a comparatively low titer of tumor antibodies. These sera reacted with antigens from tumor tissue in a dilution of 1:320 to ++ and +(+), whereas the sera obtained by immunization of rabbits with whole ascitic cells of the same tumor reacted with tumor antigens in higher dilutions (1:280 to +++ and ++++).

By the example of the two inoculated malignant tumors it is clear that during immunization of rabbits the strongest antigenic stimuli are not saline extracts of the tissues of subcutaneous forms of tumors, but whole ascitic cells.

TABLE 4

Complement Fixation Reaction of Sera Against Tumor Tissues with Homologous Antigens and with Antigens from Tissues of Normal Organs of the Corresponding Species of Animal

Dilution of sera	Sera obtained by immunization of rabbits											
	with a saline extract from Ehrlich's mouse adenocarcinoma						with whole ascitic cells from Iosida's rat sarcoma					
	antigens from tissue of the						with whole ascitic cells from					
	tumor	liver	spleen	tumor	liver	spleen	tumor	liver	spleen	tumor	liver	spleen
1:20	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:40	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:80	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:160	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:320	++++	++	++++	++++	++++	++++	++	+	++	++++	++++	++++
1:640	++	r	++	++++	++++	++++	+	r	+	++++	++	++++
1:1280	+	r	r	++++	r	++++	r	r	r	++++	+	++
1:2560	r	r	r	2+	r	+	r	r	r	+	r	+

In the next experiment we tested the specificity of the antitumor sera. For this purpose we performed the complement fixation reaction simultaneously with antigens from tumor and normal tissues (liver and spleen). In Table 4 is shown a selection of results of certain of these reactions.

The experiment showed that the test sera possess a low degree of specificity since they reacted at a comparatively high titer with antigens from both tumor and normal tissues of the experimental animals. This is evidently explained by the fact that whole ascitic malignant cells include in their composition a large number of antigens common to the species, which are present in the normal organs and tissues of the particular species of animal.

SUMMARY

Serums with a comparatively high titer of immune antibodies were obtained in immunization of rabbits by ascitic cells of malignant tumors (Ehrlich's adenocarcinoma of mice and Iosida's sarcoma of rats). Serums with lower titers of immune antibodies were recovered in immunization of rabbits by the water-salt extracts of the tissue of subcutaneous forms of tumors. Serums of the first, as well as of the second type are not very specific: they react in comparatively high titers with the antigens from the tumor tissues and with those from the tissues of normal organs of the laboratory animals.

LITERATURE CITED

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