ON THE PROBLEM OF THE EXOGENOUS ORIGIN OF THE TUMOR ANTIGEN OF SARCOMA M-1

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Received August 22, 1956. Presented by Active Member of the Academy of Medical Sciences of the USSR N. N. Zukov – Veretennikov)

The origin of specific antigenic substances in tumors, which are not transferred by filtrates, has not been discovered. It is not known whether these substances originate endogenously or whether, as many investigators suppose, they are substances of exogenic origin, i.e., originating exogenously to the organism.

Since one of the signs of the exogenic origin of the antigen is its ability to cause an immunological reaction in the organism in response to its parenteral administration, it would be possible to prove the exogenous origin of neoplastic antigen by discovering specific antineoplastic antibodies in the organism during the development of the neoplasm.

Many authors [1, 4, 6, 7, 8, 9, 11, 12, 13] found antibodies in animals with experimental neoplasms, which were not transferred by filtrates. However, in the majority of works, the complement fixation reaction was positive not only with neoplastic antigen, but also with the antigens from normal organs. In addition, investigation of the sera of healthy animals showed that they occasionally give a positive complement fixation reaction with neoplastic antigens. Therefore, the problem of the specifity of the antibodies which were found has also not been clarified.

The attempts of other investigators [2, 10] to discover complement-fixing antibodies in animals with developing neoplasms proved unsuccessful.

The aim of the present work was the comparative study of the sera of rats before infection with sarcoma M-1 and at various lengths of time after infection in order to discover the specific immunological changes occurring during the development of the neoplasm.

EXPERIMENTAL METHOD

Blood for the investigation was taken from the tail of the rats, by notching it and sucking the blood with vacuum, or from their heart before infection with the neoplasm and at various lengths of time after infection.

The serum which was obtained was heated for 30 minutes at 56° and kept in soldered ampules in the refrigerator. The sera of a single rat, taken at different periods, were tested in a single experiment in dilutions of 1:10, 1:20, 1:40, 1:80 by the complement fixation reaction.

The antigens were prepared from tissues of sarcoma M-1, liver and spleen of the rats by the usual method with physiological solution [5].

Before testing the rat sera for the presence of antibodies, the working dose and the specifity of the antigens were tested with the specific immune sera of rabbits (antineoplastic, antihepatic and antisplenic sera).

The rat sera, taken various lengths of time after infection with sarcoma M-1 (on the 12th, 14th, 15th, 18th, 20th, 24th, and 28th days), were tested by the complement fixation reaction by two parallel methods: the classical and the long cold method. In both cases the volume was 2.5 cc.

EXPERIMENTAL RESULTS

When the sera were investigated, the complement fixation reaction by the prolonged cold method gave a considerably greater number of sera than when testing was by the classical method*.

TABLE 1

Investigation of the Complement Fixation Reaction by Various Methods of the Sera of Rats after Infection with Sarcoma M-1

Method of investigation	Number of sera investig.	Complement fixation reaction		
		positive	negative	anticomplementary properties
Classical (at 37°)	38	2	32	4
Long (at 4°C)	46	20	22	4

Of 20 sera which reacted positively, 4 sera gave complement fixation best with rat liver antigen; 11 sera fixed the complement equally well with all the antigens investigated (M-1, liver, spleen) and 5 sera reacted predominalty with neoplastic antigen, reacting more weakly with other antigens.

Can it be assumed that the positive reaction which was obtained in our investigations occurs as a result of the appearance of antibodies in the rats during the development of the neoplasm?

In order to clarify this problem, the complement fixing properties of 39 healthy rat sera were studied. Of them, 11 sera gave a positive complement fixation reaction. The nature of the positive reaction was the same as when the sera of rats infected with the neoplasm were studied. Five sera fixed complement in the same way in the presence of all antigens, 6 sera reacted primarily with splenic neoplastic antigens. Therefore it cannot be assumed that the complement fixation which we discovered occurs due to specific neoplastic antibodies which appear in the rat's organism during the development of the neoplasm.

In order to discover whether immunological changes occur in the serum of rats during the development of sarcoma M-1, we studied the sera of rats before infection with the neoplasm, then at various lengths of time after infection.

The sera of 45 healthy rats gave a positive complement fixation reaction with rat antigens in 26% of the cases (12 sera), while 4 sera reacted equally with neoplastic, liver, and spleen antigens, while 8 sera reacted with neoplastic antigen more evidently than with the antigens of normal organs.

The investigation of the sera of the same rats (44 rats) on the 14-17th day after infection with the neoplasm showed some increase in the percentage of positive reactions. The nature of the positive reaction was the same as that of normal sera.

Of the sera taken from the same rats (39 rats) on the 25-28th day after infection with the neoplasm, 12 sera (30%) reacted positively: 7 sera were taken from rats with well-developed neoplasms and 5 sera from rats in which the neoplasms did not develop or, having developed, underwent reabsorption (3 sera reacted in the same way with all three antigens. One gave the strongest reaction with liver antigen, eight, while reacting with all the antigens, gave best fixation with neoplastic or splenic antigens). The sera of 25 rats gave a negative reaction. Of them, neoplasms were well developed in 21 (weighing from 13 to 85 g) and 4 rats had no tumors.

* The reaction of a delay in hemolysis of 1 plus and higher when the serum was diluted 1:10 was considered a positive complement fixation reaction.

TABLE 2

Complement Fixation Reaction (in a Cold Atmosphere) of Rat Sera Before Infection and After Infection With Sarcoma M-1 With Rat Antigens

Serum dilution	Before infection, antigens: M-1	liver	spleen	Control serum	On the 14-17th day, antigens: M-1	liver	spleen	Control serum	On the 25-28th day, antigens: M-1	liver	spleen	Control serum	Notes
10	+	+(+)	+	±	++++	++++	++++	±	+++	+++	+++	—	Neoplasm
20	±	±	±	±	++++	++++	++++	—	++	++	++	—	in ovary
40	—	—	—	—	++++	++++	++++	—	±	±	±	—	
80	—	—	—	—	++++	++++	++++	—	—	—	—	—	
10	±	±	±	±	—	—	—	—	++++	+++	+++(+)	+	Subcuta-
20	±	±	±	±	—	—	—	—	++++	++	+++(+)	±	neous
40	±	±	±	±	—	—	—	—	++++	+(+)	++	—	Tumor
80	±	±	±	±	—	—	—	—	+++(+)	±	±	—	
10	+(+)	+(+)	+	+	—	—	—	—	++++	++++	++++	++++	The same
20	+(+)	+	±	±	—	—	—	—	++++	++++	++++	++(+)	
40	+	+	±	±	—	—	—	—	++++	++++	++++	±	
80	+	+	±	±	—	—	—	—	+++	+(+)	+(+)	±	
10	—	—	—	—	—	—	—	—	—	—	—	—	The same
20	—	—	—	—	—	—	—	—	—	—	—	—	
40	—	—	—	—	—	—	—	—	—	—	—	—	
80	—	—	—	—	—	—	—	—	—	—	—	—	
10	+++(+)	+++(+)	+++(+)	+++	+	+	+	—	—	—	—	—	The same
20	++	+(+)	+(+)	+(+)	+	+	+	—	—	—	—	—	
40	++	±	±	±	+	±	±	—	—	—	—	—	
80	±	—	—	—	+	—	—	—	—	—	—	—	
10	+++(+)	++	++	+(+)	++	+	±	±	—	—	—	—	The same
20	++(+)	+	+	±	++	+	—	—	—	—	—	—	
40	+(+)	+	±	±	±	±	—	—	—	—	—	—	
80	+(+)	±	±	—	±	—	—	—	—	—	—	—	

TABLE 3

Investigation of the Sera of Neoplastic RSK Rats at a Cold Temperature Before and after Absorption

Rat serum	Dilution of serum	Before absorption: antigens: M-1	Before absorption: antigens: Liver	Before absorption: antigens: spleen	Before absorption: Control serum	Absorbed by	After absorption: antigens: M-1	After absorption: antigens: Liver	After absorption: antigens: spleen	After absorption: Control serum
Rat No. 2 Ovarian tumor on 14th day after infection	10	++++	++++	++++	±	Formalin-treated splenic tissue, + alcohol-treated liver tissue	++(+)	++(+)	+++	++
	20	++++	++++	++++	–		++	++	±	++
	40	++++	++++	++++	–		+	±	–	–
	80	++	++	++	–		±	–	–	–
The same on the 25th day after infection	10	+++	+++	++	–	Formalin-treated splenic tissue	++	++	+(+)	–
	20	++	++	++	–		±	±	±	–
	40	±	±	±	–		–	–	–	–
	80	–	–	–	–		–	–	–	–
Rat No. 6 Ovarian tumor on 14th day after infection	10	++(+)	++++	–	–	Alcohol-treated liver tissue	++	–	–	–
	20	++(+)	++++	–	–		++(+)	–	–	–
	40	+++	++++	–	–		+	–	–	–
	80	++	++	–	–		–	–	–	–
The same on the 25th day after infection	10					Formalin-treated splenic tissue, + alcohol-treated liver tissue	±	–	–	–
	20						–	–	–	–
	40						–	–	–	–
	80						–	–	–	–

Thus, it was not possible to observe any relationship between neoplastic development and the rats' ability to participate in the complement fixation reaction with rat antigens.

When an attempt was made to trace how the properties of the sera of individual rats changed during the neoplastic development, it was found that as the neoplasm developed in some rats, the serum acquired the ability to give a complement fixation reaction with rat antigens (Table 2), while in others, on the contrary, it lost this ability, and in the majority of the rats the serum both before infection with the neoplasm and during its development gave a negative reaction.

Thus, study of the complement fixation reaction of rat sera before infection with the neoplasm and after it by the long cold method did not allow establishment of the appearance of specific antibodies in the rat sera during the process of neoplastic development.

Proof of the specifity of the antibodies in the rat sera by the absorption method was also unsuccessful (preliminary data).

For the absorption experiments, sera which gave a positive complement fixation reaction with rat antigens were taken. Absorption was carried out by splenic and hepatic tissues preserved in 5% formalin and alcohol [5].

From the data presented in Table 3 it is apparent that, during the absorption of the sera, a regular decrease occurs in the antibody titer with respect to all antigens with which the serum reacted before absorption, or they completely disappear.

The data which were obtained indicate that the complement fixation which we discovered is, apparently, nonspecific.

Thus, our investigation did not give convincing data to indicate that specific antineoplastic antibodies appear in the sera of rats during development of sarcoma M-1 in them.

SUMMARY

The immunological properties of the serum of normal rats and of rats infected with sarcoma M-1 were compared at different lengths of time after infection. The prolonged cold complement fixation test was used.

No convincing evidence proving the appearance of specific tumor antibodies in the serum of rats inoculated with sarcoma M-1 was observed.

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* In Russian.