ON THE ANTIGENIC STRUCTURE OF ROUS SARCOMA

Communication II. Viral and Tissue Antigens of Rous Sarcoma

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Using the reactions of complement fixation and anaphylaxis with desensitization, the presence of two specific antigens – viral and tissue – was shown in certain tumors of viral origin (for example, Shope's cancer and papilloma) [1, 4]. In our experiments [2] using the reaction of precipitation in agar, only one specific antigen was discovered in Rous chicken sarcoma. The extract of this sarcoma and its antiserum, preliminarily depleted normal chicken serum, and a mixture of extracts from organs of a healthy chicken (liver, kidney, spleen, muscle), and the sarcoma MX-659, induced by a carcinogen, formed only one line of precipitation.

It remained unclear as to which of the antigens, viral or tissue, corresponds to that line. This question was studied in the present investigation.

EXPERIMENTAL METHOD

In the experiments we used extracts, in a physiological NaCl solution, of Rous sarcoma, transplanted MX-659 sarcoma, initially induced with methylcholanthrene, from the organs of a healthy chicken (liver, kidney, spleen, lungs, muscle), normal chicken serum, and also rabbit serum immunized to Rous sarcoma. The preparation of the extracts from tumors and normal tissue and the immune serum was described by us earlier [2].

Fractionation of the Extract of Rous Sarcoma. The extract of the sarcoma was centrifuged at 30,000 rpm (60,000 g) for 1½ to 2 hours. As a result of centrifugation the fluid divided into 4 layers: the upper – a small layer of fat and lipoproteids, the middle– supernatant fluid, the lower – sediment; on the dense lower sediment was situated a layer, barely visible to the eye, of loose sediment, which, with the slightest agitation, easily dispersed. The supernatant fluid was carefully drawn off, by means of a pipette, from the middle layer, and studied in the experiments. The dense and loose sediments were carefully mixed in the remaining supernatant fluid by a rapidly turning pestle, fastened to the axle of an electromotor. Then the suspended sediment was restored to its original volume with a physiological solution of NaCl (pH 7-7.2) and again centrifuged, at the same speed as the first time, for a period of 30 minutes. The supernatant fluid was discarded, and the sediment, resuspended in the original volume of the physiological solution of NaCl, was again subjected to ultracentrifugation according to the same regime as in the second time. The centrifugation was carried out in the domestic centrifuge UTsP – 1 at 4-6°. The supernatant fluid was drawn off with a pipette; the sediment was rinsed with physiological saline and resuspended in the saline in the proportion of 1/2 to 1/8 of the original volume of the extract of Rous sarcoma.

Thus, we obtained two fractions from the extract of Rous sarcoma: 1) supernatant fluid, taken from the middle layer, and 2) dually precipitated sediment. The sarcoma extract and the fractions obtained from it were tested for carcinogenic activity by intracutaneous inoculation of chickens, 5-6 months of age, of the white leghorn family (Table 1).

TABLE 1. The Carcinogenic Activity of the Extract of Rous Sarcoma and Its Fractions

Fraction of the extract of Rous sarcoma	Final dilution of the Rous sarcoma virus						
	per se	1:10	1:100	1:250	1:500	1:1000	1:5000
Extract	+	+	+	+	+	+	–
Precipitated sediment	+	+	+	+	+	–	–
Supernatant fluid	–	–	–	–	–	–	–

Symbols: plus) Appearance of tumor; minus) absence of tumor.

The Acquisition of Purified Specific Precipitating Antibodies to Rous Sarcoma from the Original Sera. For the acquisition of purified specific precipitating antibodies we took advantage of the method devised by Z. A. Avenirovaya and G. I. Abelev for the isolation of antibodies from a precipitate formed by an organ-specific antigen from the liver of mice and its corresponding antibody, and also an antibody to the specific antigen of mouse hepatoma.

The method of obtaining purified specific precipitating antibodies consisted of the following. First the original serum to Rous sarcoma was completely depleted of its antibodies to normal chicken serum, to antigens from the organs of healthy rats, and to the sarcoma MX-659. The method of depletion of the serum of Rous sarcoma was described by us in detail in a previous work [2]. The serum, depleted to Rous sarcoma, was centrifuged at 60,000 g for 30 minutes to afford complete liberation of the normal and tumor tissues, taken for the depletion, from the intracellular components (microsomes, mitochondriae, their membranes, etc.). Along with the intracellular components, the remnants of the precipitate, formed in association with the depletion of the serum, were withdrawn in the sediment.

For the acquisition of the specific precipitate to the antisarcoma serum, depleted in the same manner, we added the following fraction from the extract of Rous sarcoma – supernatant fluid, following ultracentrifugation, practically devoid of corpuscular, biologically active virus. To 1 ml of depleted antisarcoma serum 0.2-0.3 ml of this fraction was required. The mixture, consisting of depleted antisarcoma serum and the virus-free fraction of Rous sarcoma, was incubated for $1\frac{1}{2}$ hours at 37° and 16-18 hours at 4°. The resulting specific precipitate was divided by centrifugation at 4,000 g for 10-15 minutes. The supernatant fluid, i.e., antisarcoma serum, was neutralized with both normal and tumor (sarcoma MX-659 and Rous sarcoma) tissues, studied for the reaction of precipitation in agar, and also investigated for viral-neutralizing activity (Table 2, IB). The specific precipitate was washed three times with large volumes of a physiological solution of NaCl (pH 6.6-6.8) using centrifugation at 4,000 g for 5-10 minutes.

Following the washing of the precipitate with physiological saline the latter was discarded, and a phosphate-citrate buffer (pH 3.2) was added to the precipitate in the proportion 1/8 in relation to the original volume of antisarcoma serum. In addition, a considerable portion (up to 15-20%) of the specific precipitating antibodies was separated, which yielded one zone of precipitation with the antigens of Rous sarcoma and did not react with the antigens from the organs of healthy chicken and from sarcoma MX-659. This precipitate was eliminated by centrifugation, and the supernatant fluid, in which the separated specific precipitating antibodies were present, was carefully neutralized with dry sodium carbonate (Na_2CO_3) to a pH of 7-7.2. Subsequently, for brevity in the presentation, we will call the eluted specific precipitating antibodies the eluates against Rous sarcoma. The latter were tested for virus-neutralizing activity and by the reaction of precipitation in agar. In all we obtained 9 different samples of the eluviate against Rous sarcoma from two rabbit antisarcoma sera (No. 865 and 912, and mixtures of them). The eluates against Rous sarcoma obtained from the original serum No. 865 were the most active when tested by the reaction of precipitation in agar.

EXPERIMENTAL RESULTS

The results of the 2 separate experiments, carried out for the purpose of testing the fractions isolated from the extract of Rous sarcoma, are presented in Table 1. As can be seen, the sediment from the Rous sarcoma obtained after its two-fold rinsing in physiological saline by means of centrifugation at 60,000 g gave rise to tumors in chicken in a titer of 1 : 500. The supernatant fluid formed following ultracentrifugation did not bring about the appearance of tumors in the chickens. Thus, we obtained a fraction with the corpuscular, biologically active virus of Rous sarcoma, and a fraction which did not contain it.

As has already been shown [2], the sera against Rous sarcoma, depleted with normal chicken serum and a mixture of the extracts from the organs of healthy

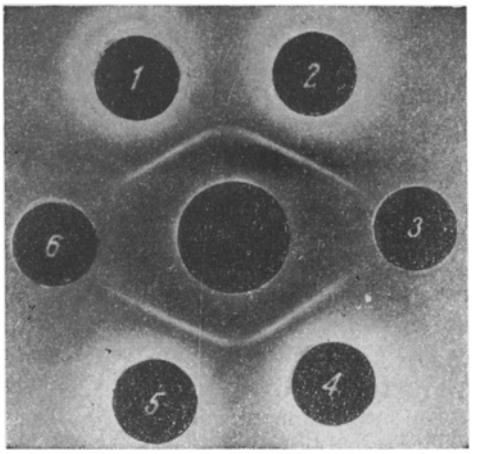

Fig. 1. Results of the reaction of precipitation in agar. Depleted antisarcoma serum No. 865 (in the center). Depletion was carried out with normal chicken serum and a mixture of the extracts from the normal organs of a chicken (liver, kidney, spleen, muscle) and sarcoma MX-659. 1,4) Antigens from the extract of Rous sarcoma; 2,5) antigens from the supernatant fluid, not containing corpuscular, biologically active virus; 3,6) antigens from the doubly precipitated sediment which was centrifuged at 60,000 g.

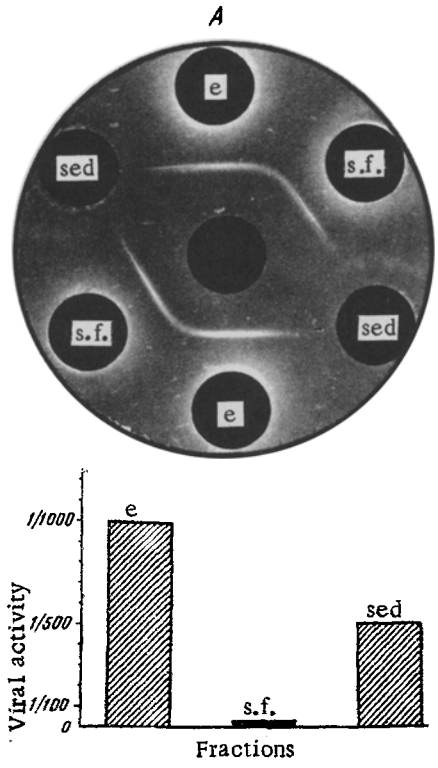

Fig. 2. Distribution of the precipitating antigen and the Rous sarcoma virus. A) Reaction of precipitation in agar (in the center—purified, specific precipitating antibodies to Rous sarcoma); e) Rous sarcoma extract; s.f.) supernatant fluid of Rous sarcoma (60,000 g); sed) doubly precipitated sediment of Rous Sarcoma (60,000 g).

chickens and from sarcoma MX-659, reacted only with the extract of Rous sarcoma. Testing these sera with fractions obtained from the extract of Rous sarcoma showed that a specific precipitation zone was noted with both the Rous sarcoma extract and the supernatant fluid following high-speed centrifigation (60,000 g for $1\frac{1}{2}$ to 2 hours). In contrast to this, the doubly precipitated sediment did not react with the depleted antisarcoma serum in the same manner (Fig. 1). That picture was presented by eluates to Rous sarcoma (Fig. 2.). Following the removal of the specific precipitating antibodies from the depleted antisarcoma serum, the latter no longer reacted with the Rous sarcoma extract and its fractions.

On the basis of the data presented it would be possible to infer that the specific zone of precipitation is caused either by the dissolved viral antigen or by the tissue-specific protein arising from the reaction between the virus and the tissue of the chicken.

Simultaneously with the reaction of precipitation in agar, the fractionated sera against Rous sarcoma were tested by the neutralization reaction. The results from one of the 5 experiments are presented in Table 2. The serum against Rous sarcoma (No. 865), depleted with normal chicken serum and with a mixture of extracts from the organs of a healthy chicken and sarcoma MX-659 (see Table 2, IA), and that same serum (see Table 2, IB) from which the precipitating antibodies against Rous sarcoma were also removed, neutralized the virus in the same manner as the original antisarcoma serum (see Table 2, I), lowering the titer of the virus by 50 times. In the other experiments the titer of the virus was lowered from 40-100 times. The eluted precipitating antibodies against Rous sarcoma (see Table 2, IC) did not possess virus-neutralizing activity, which affords us a basis for thinking not of the dissolved viral antigen, but of the tissue-specific protein.

Thus, the serum against Rous sarcoma contains 2 types of specific antibodies: first, virus-neutralizing, and second, precipitating a specific tissue antigen of Rous sarcoma.

A specific tissue antigen of Rous sarcoma was discovered, in our experiments, which yielded a zone of precipitation with the original antisarcoma sera, the eluates against Rous sarcoma, and the sera against Rous sarcoma, preliminarily depleted with normal chicken serum and with mixtures of the extracts from the organs of a healthy chicken and sarcoma MX-659. On the basis of the data presented it is difficult to judge the nature of the specific antigen demonstrated by us, inasmuch as it could be either a specific tumor protein which arises

TABLE 2. Neutralization of the Virus of Rous Sarcoma by Antiserum Depleted by Various Methods

Fraction from serum No. 865 against Rous sarcoma	Dilution of the Rous sarcoma virus						
	1:10	1:50	1:100	1:250	1:500	1:1 000	1:5 000
I	+	—	—	—	—	—	—
I A	+	—	—	—	—	—	—
I B	+	—	—	—	—	—	—
I C	+	+	+	+	+	—	—
Physiological saline	+	+	+	+	+	—	—

Symbols: plus) Appearance of tumor; minus) absence of tumor.

in the presence of viral action on the tissue of the chicken, or an antigen of mucin-like substance which is copiously produced in association with the growth of Rous sarcoma. Experiments performed with the antigen from mucin-like material showed that an antigen is contained within it which corresponds to the specific antigen of Rous' sarcoma.

In conclusion, we note that we were unsuccessful in obtaining a specific zone of precipitation for the virus antigen in the reaction of precipitation in agar, apparently due to an insufficient concentration of the virus or an insufficient amount of the virus-neutralizing antibodies. The possibility has not been excluded that further concentration of the Rous sarcoma virus and the antibodies against it might lead to the acquisition of a specific zone of precipitation for the virus antigen. Experiments elucidating the nature of the specific antigen of Rous sarcoma are continuing.

SUMMARY

The following constituents were obtained in fractionating Rous' sarcoma: 1) supernatant fluid (60,000 g; 1,5-2 hours), which did not contain corpuscular biologically active virus; 2) doubly reprecipitated precipitate, provoking tumors in chickens in the 1 : 500 titre. Purified specific precipitating antibodies were isolated from the precipitate formed by the specific Rous sarcoma antigen (supernatant fluid). Testing of these antibodies in reaction of precipitation in agar has demonstrated that they react with the supernatant fluid in the same way as with the Rous sarcoma extract and do not react with the doubly reprecipitated precipitate; they did not neutralize sarcoma virus. Consequently, specific precipitating antigen of Rous sarcoma is not a virus antigen and is evidently of tissue origin. Apart from the specific precipitating antibodies, the virus neutralizing antibodies were revealed in the Rous sarcoma antiserum. These antibodies neutralized the virus at the same titre, as the initial Rous sarcoma antisera. However, they did not react in precipitation reactions in agar with any of the fractions of the Rous sarcoma tissue.

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