THE IMMUNOLOGICAL PROPERTIES OF CERTAIN FRACTIONS OF EHRLICH'S ADENOCARCINOMA OF MICE

COMMUNICATION I. THE STUDY OF THE ANTIGENIC COMPOSITION OF TUMOR FRACTIONS BY THE METHOD OF ANAPHYLAXIS WITH DESENSITIZATION

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The study of the specific antigens and their location in the tumor cell is of great importance in the diagnosis of tumors and vaccination against them. Research in the last few years [1-4] suggests that serological activity is present in the globulin, microsomatic and other fractions of the tumor. The vaccinal activity of the serologically active fractions has, however, received very little study. In connection with the isolation of "protective antigens" - globulin fractions of the microorganisms of anthrax and plague [5, 6, 8, 9], which possess high vaccinal activity - interest in chemical vaccination has increased considerably.

The aim of the present research was to compare the serological and vaccinal activity of different tumor fractions, some of which were obtained by methods used to isolate the "protective antigens" of microorganisms. In this communication we give the results of a study of the antigenic composition of the tumor fractions.

EXPERIMENTAL METHOD

As an experimental model we used a transplanted strain of Ehrlich's mouse adenocarcinoma.

Fractionation of the tumor material. Fresh, washed ascitic cells were homogenized in a cold 0.14 M solution of NaCl. After centrifugation at 2500 rpm for 15 minutes, the fractions precipitated at 33, 46 and 50% saturation were successively salted out with ammonium sulfate at +4°. The last fractions, precipitated at 46 and 50% saturation, were called globulin fractions I and II respectively. The protective antigen of the microorganism of anthrax was also obtained from a filtrate of a culture at 46 and 50% saturation with ammonium sulfate [6, 8].

Fraction III was isolated from the tumor extract in 0.14 M NaCl by means of precipitation with potassium aluminum alum, in accordance with the technique used [8] for isolation of the anthrax protective antigen. A 10% solution of potassium aluminum alum was added to the saline extract to give a final concentration of 0.1%; 10% acetic acid was added to bring the pH to 5.9. The precipitate which formed overnight at +4° was collected by centrifugation, dissolved in cold 0.2 M citric acid and precipitated with 2.5% trichloroacetic acid. The fraction III thus obtained was called the alum precipitate.

Tumor homogenate in cold 0.14 M NaCl solution with 0.01 M sodium citrate was twice centrifuged (8000 rpm) for 20 minutes at +4°. The precipitate was homogenized in an equal volume of 2 M NaCl. The extraction took place while the mixture was allowed to stand overnight at +4°. After centrifugation (8000 rpm) for an hour at +4°, the supernatant fluid was poured into cold distilled water to give a final concentration of 0.14 M NaCl. The threads thus formed were transferred to a 1 M solution of NaCl. Fraction IV was given the name of nucleoprotein.

The residue remaining after extraction with 1 M NaCl was extracted with 0.25% NaOH overnight at +4°. After centrifugation, the supernatant fluid was acidified with dilute acetic acid to pH = 5.9. The precipitate forming overnight at +4° was separated by centrifugation. Fraction V was called alkaline extract.

Fractions I, II, III and IV were dialyzed against physiological saline. All the fractions were lyophilized and kept in sealed ampoules at +4°.

Serological activity of the fractions. In order to detect the specific and species antigens in the fractions obtained, we used the reaction of anaphylaxis with desensitization. In the first series we studied the content of specific antigen in the fractions.

Guinea pigs weighing 220-250 g were sensitized subcutaneously with washed ascitic cells in a dose of 5.2 mg of protein (according to Kjeldahl's method) per animal. Three weeks after sensitization, intraperitoneal desensitization was carried out with 1 ml of normal antigen (a mixture of mouse serum and a saline extract of

the skin of the abdomen of a female mouse in proportions of 1:3) in a dose of 20 mg dry weight. Next day, after testing for completeness of desensitization to normal antigen, as an assaulting antigen solutions of fractions I, II and V of the tumor were injected. All the antigens were assayed according to dry weight of the lyophilized preparation, and injected into the heart.

EXPERIMENTAL RESULTS

The results obtained are shown in Table 1.

The sensitized guinea pigs responded by an anaphylactic reaction to the injection of globulin fractions I and II. This suggests the presence in the globulin fractions of a specific tumor antigen, absent from normal tissues. Injection of the alkaline extract of the tumor (fraction V) did not elicit a response reaction. It is evident that treatment with alkali appreciably lowered the antigenic properties of the fraction thus obtained.

In the second series of experiments we studied the distribution of the species antigen in the tumor fractions. Guinea pigs were sensitized subcutaneously with solutions of fractions I, III, IV and V of the tumor in a dose of 5 mg dry weight per guinea pig. Three weeks later, a solution of lyophilized mouse serum was injected as a species antigen. In order to reveal any common antigenic factor in the fraction and the initial tumor extract, a saline extract of the tumor was injected. All antigens were injected in a dose of 0.2-0.3 ml into the jugular

TABLE 1. Detection of Specific Antigen in Fractions of Ehrlich's Adenocarcinoma by the Reaction of Anaphylaxis with Desensitization

Sensitization		Second desensitization[1]		Test of complete desensitization		Assaulting injection		
ascitic cells of Ehrlich's adenocarcinoma		normal antigen				fractions of Ehrlich's adenocarcinoma		
guinea pig no.	dose of protein (mg)	dose of protein (mg)	reaction	dose of protein (mg)	reaction	antigen	dose of protein (mg)	reaction
15	5,2	—	.	12	—	Saline extract	12	++
18	5,2	—	.	9	—		8	+
57к	Control	—	.	12	—		8	—
58к	»	—	.		.		8	—
59к	»	—	.		.		8	—
2	5,2	2	+	10	—	Globulin fraction I	10	++
4	5,2	—	.	4	±		10	++
16	5,2	—	.	12	—		10	+
3	5,2	2	—	10	±		10	±
56к	Control	—	.	12	—		10	—
48к	»	—	.	10	—		10	—
17	5,2	—	.	7,5	—	Globulin fraction II	10	++
12	5,2	—	.	5	—		10	++
11	5,2	—	.	10	—		10	+
53к	Control	—	.	—	.		10	—
55к	»	—	.	—	.		10	—
55к	»	—	.	—	.		10	—
5	5,2	—	.	10	±	Alkaline extract (fraction V)	10	—
6	5,2	—	.	10	—		10	—
8	5,2	—	.	10	—		10	—
7	5,2	—	.	10	—		10	—
50к	Control	—	.	10	—		10	—
51к	»	—	.	10	—		10	—
52к	»	—	.	10	—		10	—

Legend: — no reaction; + brief scratching; ++ strong scratching, sneezing, shortness of breath, dishevelling of the fur, coughing; +++ the same but more marked, and excretion of urine and feces; ++++ convulsive jumps, fits, terminating in death of the animal; ± doubtful reaction; · no antigen injected.

[1]The first desensitization was carried out intraperitoneally the day before the experiment.

TABLE 2. Detection of Species Antigen in Fractions of Ehrlich's Adenocarcinoma by the Reaction of Anaphylaxis with Desensitization

Guinea pig no.	Sensitization	Desensitization		Test for complete desensitization		Assaulting injection	
	fractions of Ehrlich's adenocarcinoma	normal mouse serum				saline extract of Ehrlich's mouse adenocarcinoma	
	dose 5 mg dry weight	dose (mg)	reaction	dose (mg)	reaction	dose (mg)	reaction
31	Globulin fraction I	10	++++ Death	.	.	.	.
35		10	++++ Death	.	.	.	.
35		10	+++	10	±	15	++
32		5	++	10	±	15	++
33		5	++	10	—	15	+
26	Alum precipitate (fraction III)	10	++	10	—	15	+++
25		10	+	10	±	15	±
28		10	+	10	—	15	+
24		10	±	10	—	15	+
27		10	+	10	—	15	+++
21	Alkaline extract (fraction V)	20	—	30	—	16	++
20		20	+	30	±	16	+
19		20	+	30	±	16	+
23		20	—	30	—	16	+
36	Nucleoprotein (fraction IV)	5	+	15	—	15	±
37		10	+	15	—	15	±
38		10	±	15	—	15	±
40		10	—	15	—	15	±
39		10	—	15	—	15	—
41	Control	.	.	16	—	.	.
43		.	.	15	—	.	.
44		.	.	15	—	.	.

Legend as in Table 1.

vein of the animal. It can be seen from Table 2 that the majority of the sensitized guinea pigs responded by an anaphylactic reaction to the injection of normal mouse serum. The most marked reaction was observed in the guinea pigs sensitized with globulin fraction I. Animals sensitized with alum precipitate of the ascitic fluid (fraction III) reacted more weakly to the species antigen. This was evidence of the presence of a species antigen in fractions I and III. A very weak reaction was shown by guinea pigs sensitized with the nucleoprotein and alkaline extracts of the tumor (fractions IV and V).

A clear reaction to injection of the saline extract was noted in the guinea pigs sensitized with globulin fraction I and alum precipitate of ascitic fluid. An appreciable reaction was observed in the animals sensitized with alkaline extract of the tumor. This showed that in fractions I, III and V are present antigens which are different from the species antigens, probably tissue or specific antigens.

The study of the antigenic properties of the fractions obtained by means of the anaphylaxis reaction thus showed the presence of specific antigens in the globulin fractions and species antigens in the globulin and alum precipitate fractions.

The results of our experiments are confirmed by reports in the literature demonstrating the presence of specific [2, 4, 10] and normal [7] antigens in the globulin fractions of a tumor.

SUMMARY

The author obtained a number of fractions of Ehrlich's mouse adenocarcinoma by the procedure used for the iso-

lation of the protective antigens of microorganisms. The antigenic properties of the fractions were studied by means of anaphylaxis with desensitization. Specific and species antigens were revealed in the globulin fractions of the tumor.

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