DETERMINATION BY PRECIPITATION OF SPECIFIC ANTIGENS PRODUCED BY THE ADMINISTRATION OF ORTHOAMINOAZOTOLUENE INTO THE ORGANISM

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Study of the systemic reaction to the administration of antigens formed as a result of the recombination of proteins during the pretumor period has great significance in indicating the ability of the system to hinder the beginning of the tumor process. Studies in this direction are connected with the study of the activity of cancerogenic substances by immunochemical methods, which are more sensitive than the usual biochemical methods.

In approaching the study of this problem, we first set up model experiments in order to discover how the addition of a cancerogenic group (orthoaminoazotoluene) to proteins affects their immunological properties.

We obtained combinations of human serum proteins, as well as horse and bull serum proteins with diazotized orthoaminoazotoluene. The antigenic properties of these combinations were studied by the precipitation method. It was found that artificial antigens caused the formation of precipitins of protein combined with cancerogenic substance, as well as of the original, unchanged protein. Consequently, the union of protein with the remainder of the cancerogenic substance changes its immunological properties, communicating the specificity of a cancerogenic substance to it.

Later we studied the antigenic properties of the compounds of cancerogenic substances with proteins formed in the system [3].

In order to discover the specific antigens, we used the anaphylactic method with desensitization, out by L. A. Zilber [2]. It was found that during administration to mice of a substance producing experimental hepatoma the immunological properties of the liver proteins changed during the first stages of cancerogenesis. Specific antigens provoked by the orthoaminoazotoluene were found in the liver of mice which received the cancerogenic substance along with normal kidney proteins. It is already possible to observe changes in the antigenic properties of proteins a month after the beginning of the administration of the cancerogenic substance. Thus, we established that during the experimental production of hepatoma in the liver of mice, a compound of the metabolite of the cancerogenic substance with proteins is formed [3].

Since the presence of specific antigens in the liver proteins of mice which received cancerogenic substance was first found by us (using the anaphylactic method), it was necessary to confirm this fact later using another method. We decided on the precipitation reaction since the artificial antigen which was obtained by combining the cancerogenic substance with protein, was tested by this same method. The correlation of the immunological

peculiarities of antigen extracted from liver with the synthetic antigen, regarding the structure of which some data are available, can give additional information about the nature and properties of the antigen formed in vivo.

The present work is devoted to the study by the precipitation method of the specific antigens which are formed in the liver of mice receiving orthoaminoazotoluene with their food.

Mice of the strains C_3HA and A each received daily 0.1 ml of 1% solution of orthoaminoazotoluene in sunflower seed oil. The control animals received the same amount of oil.

Various lengths of time after the beginning of the experiment (30, 45, and 50 days) a group of mice (15-20 animals) in the experiment and controls were beheaded and the proteins were extracted from the liver tissues.

The liver tissue of these mice was minced in a Lyatop meatchopper and extracted for 2 hours with distilled water at pH 7.6. The extraction took place during ice cooling and mechanical mixing. Then the extract was separated by centrifuging from the sediment and used to immunize rabbits and to set up the precipitation reaction. The antigen solutions were preserved with formalin and kept in a refrigerator.

In order to immunize the rabbits, each animal received an injection of 1 ml of 1% solution of antigen in the vein of the ear twice with a 6-day interval, then after an 8-day interval a threefold amount of the same solution was administered intraperitoneally. After 14 days blood was taken from the vein of the ear in order to obtain serum for use in the precipitation reaction.

The reaction was set up as follows. 0.2 ml of immune serum was measured into a test tube and .3 ml of various dilutions of antigens was added. The liver proteins of mice receiving orthoaminoazotoluene and of control animals which received oil were used as the antigens under study. Next the test tubes were placed in a thermostat at 37° for 2 hours, after which the results of the reaction were determined. We judged the intensity of the reaction by the amount of sediment which formed as well as by the greatest dilution at which cloudiness could be observed. In some cases in order to make the results more exact, the test tubes were left in the refrigerator overnight and were examined again in the morning.

The data which were obtained are shown in the Table, in which the results of 4 experiments are presented. New antigens were prepared for each experiment from the liver tissue of the group of mice receiving orthoaminoazotoluene and of the control group. Three to six rabbits were immunized in each experiment: some of the animals with liver proteins of the mice which received orthoaminoazotoluene, some with the liver proteins of the control mice. The precipitation raction was set up with the immune sera of all the rabbits. Each serum was tested with two antigens: experimental and control. As is evident from the Table, all the rabbits proved to be well immunized. The zone of maximum precipitation for the experimental and control antigen is found within the limits of the same concentrations of test antigens. This permits comparison of the two antigens.

On examining the Table, the fact that the immune sera of rabbits, prepared with antigens from the livers of mice receiving orthoaminoazotoluene, reacted well with these antigens (rabbits No. 1, 2, 3, 7,8, 9, 12, 13, 15, and 16) stands out. The same sera reacted very weakly with the antigens obtained from the liver of control mice.

In one experiment (No. 3) the sera of rabbits immunized with the liver proteins of mice receiving cancerogenic substance reacted in the same way with the liver proteins of the experimental, as with those of the control animals (rabbits Nos. 7, 8, 9) at first. Since these results did not correlate with the data obtained in other experiments, we retested the sera of the same rabbits, changing the concentration of test-antigens. A number of dilutions from 1:220 to 1:440 were tested. At these dilutions the difference in the reaction of the sera of rabbits No. 7 and 8 to various antigens was very marked. The sera of these rabbits reacted more strongly to antigen from the livers of mice receiving orthoaminoazotoluene than to control antigens. This confirms the results obtained in other experiments.

It should be noted that in dilutions of from 1:55 to 1:220, both of the antigens under investigation reacted very slightly with the serum of rabbit No. 8. These antigens formed a considerably greater amount of precipitate at a lower concentration (dilution from 1:220 to 1:440) with the same serum.

This can apparently be explained by the appearance of the zone of maximum precipitation at such concentrations, which is masked in the preceding case.

Precipitation of the Sera of Rabbits Immunized with the Liver Proteins of Experimental and Control Mice, by the Corresponding Antigens

Rabbit No.	Antigen used to immunize rabbit and the preparation number	Antigen used in precipitation reaction and the preparation number	Antigen dilution used in the precipitation reaction									
			1:57	1:114	1:228	1:456	1:912	1:1 824	1:3 648	1:7 296	1:14 592	1:29 184
	Liver proteins of mice receiving	Liver proteins of mice receiving										
1	orthoaminoazotoluene 174	orthoaminoazotoluene 174	+++·	++++	+++	—	—	—	—	—	—	—
1	same	oil 175	±	±	±	+	±	—	—	—	—	—
2	» »	orthoaminoazotoluene 174	—	—	—	—	—	+	+++	—	—	—
2	» »	oil 175	±	±	±	+	+	±	—	—	—	—
3	» »	orthoaminoazotoluene 174	+	++++	+	±	—	—	—	—	—	—
3	» »	oil 175	±	±	—	—	—	—	—	—	—	—
4	oil 175	orthoaminoazotoluene 174	±	++++	+++	±	±	±	—	±	—	—
4	same	oil 175	++++	++++	+	+	+	±	±	—	—	—
5	» »	orthoaminoazotoluene 174	++++	++++	±	±	±	±	±	±	—	—
5	» »	oil 175	++++	++++	±	±	±	±	±	±	—	—
6	» »	orthoaminoazotoluene 174	+	+++	+	±	±	±	±	±	—	—
6	» »	oil 175	±	+	+	±	±	±	—	—	—	—
7	orthoaminoazotoluene 185	orthoaminoazotoluene 185	1 : 55	1 : 110	1 : 220	1 : 440	1 : 880	1:1 760	1 : 3 520	1:7 040	1:14 080	1:28 160
7	same	oil 186	++++	++++	+++	+++	+	—	—	—	—	—
8	» «	orthoaminoazotoluene 185	++++	++++	+++	+++	+	—	—	—	—	—
8	» «	oil 186	±	±	+	+	—	—	—	—	—	—
9	» »	orthoaminoazotoluene 185	±	±	±	±	—	—	—	—	—	—
9	» »	oil 186	±	±	+	±	—	—	—	—	—	—
10	oil 186	orthoaminoazotoluene 185	±	±	±	+	±	±	—	—	—	—
10	same	oil 186	±	±	±	+	+	±	—	±	—	—
11	» »	orthoaminoazotoluene 185	+	+	+	+	++	+	+	+	+	—
11	» »	oil 186	+	+	+	+	++	+	+	+	+	+
7	orthoaminoazotoluene 185	orthoaminoazotoluene 185	1 : 220	1 : 240	1 : 260	1 : 280	1 : 300	1 : 320	1 : 340	1 : 360	1 : 400	1 : 440
7	same	oil 186	++++	++++	++++	++++	++++	++++	+++	+++	+++	+
8	» »	orthoaminoazotoluene 185	++++	++++	++++	+++	+++	+++	++	+	+	+
8	» »	oil 186	+	++++	+++	+++	+++	+++	+++	+++	+++	+
9	» »	orthoaminoazotoluene 185	+	+++	+++	+++	+	+	+	+	+	+
9	» »	oil 186	±	+	+	+	+	+	+	+	+	+
			±	+	+	+	+	+	+	+	+	+

Table (continued)

Rabbit No.	Antigen used to immunize rabbit and the preparation number	Antigen used precipitation reaction and the preparation number	Antigen dilution used in the precipitation reaction									
			1:57	1:114	1:228	1:456	1:912	1:1 824	1:3 648	1:7 296	1:14 592	1:29 184
	Liver proteins of mice receiving	Liver proteins of mice receiving										
12	orthoaminoazotoluene 188	orthoaminoazotoluene 188	1 : 66	1 : 133	1:266	1:532	1:1 064	1:2 128	1:4 256	1:8 512	1:17 024	1:34 048
			+	+	+	+	+++	+	—	—	—	—
12	same	oil 189	+	+	±	±	—	—	—	—	—	—
13	»	orthoaminoazotoluene 188	+	+	+++	+++	+	±	—	—	—	—
13	»	oil 189	+	+	+	+	+	±	—	—	—	—
14	oil 189	orthoaminoazotoluene 188	+	+++	+++	+++	+	±	±	—	—	—
14	same	oil 189	+	+++	++	+++	+	+	±	—	—	—
15	orthoaminoazotoluene 172	orthoaminoazotoluene 182	1 : 60	1 : 121	1:243	1:487	1:974	1:1 948	1:3 897	1:7 795	1:15 590	1:31 180
15	same		+	+++	—	—	—	—	—	—	—	—
15	»	oil 173	±	+	—	—	—	—	—	—	—	—
16	»	orthoaminoazotoluene 172	±	±	±	+	++++	+++	+++	+++	++	+
16	»	oil 173	+	+	+		±	++	+++	+++	++	+
17	oil 173	orthoaminoazotoluene 172	+	+	+++	++++	±		+	—	—	—
17	same	oil 173	+	+++	+++	++++	+++	+++	+++	+++	—	—
18	»	orthoaminoazotoluene 172	±	++++	+	+	+	±	—	—	—	—
18	»	oil 173	+	++++	+++	+++	+	+	+	+	+	—
19	»	orthoaminoazotoluene 172	±	±	±	±	+	+	+	+	+	—
19	»	oil 173	±	+	+	+	+	+	+	+	+	—

Thus, as shown on the Table, the antibodies formed in the systems of rabbits injected with the liver proteins of mice receiving cancerogenic substance react strongly with the latter proteins and weakly with the liver proteins of control animals. The one rabbit No. 9 formed an exception whose serum reacted in the same way with the experimental and control proteins.

In each experiment, some of the rabbits were immunized with the liver proteins of mice receiving oil (rabbits No. 4, 5, 6, 10, 11, 14, 17, 19, and 19). The sera of these rabbits reacted in the same way with the antigens from the livers of mice receiving cancerogenic substance as with the antigens from the livers of mice receiving oil.

It should be noticed that antigens from the liver of control mice (for example, No. 175), which reacted very slightly with the sera of rabbits prepared with antigens from the livers of mice receiving a cancerogenic substance, gave larger precipitates with the sera of rabbits prepared with antigens from the liver of mice receiving oil. This indicated that the weak reaction of antigen from the liver of control mice with the serum of rabbits immunized with the proteins of mice receiving cancerogenic substance (No. 1, 2, and 3) is dependent on the peculiarities of the antibodies present in the serum, not on the low activity of the antigens from normal liver.

As follows from the Table, rabbits immunized with proteins from control mice proved to be well immunized. This also indicates that the antigens of normal mouse liver have considerable activity. Apparently, the protein composition of the liver of mice receiving orthoaminoazotoluene includes a large amount of normal liver antigens as well as specific ones. This explains the strong reaction of proteins from the liver of mice receiving a cancerogenic substance with the antibodies against the proteins of the control group of mice.

One should examine the data obtained in experiment No. 4 (rabbits No. 15, 16, 17, 18, 19). In it, strain A mice were used. Specific antigens were found in the liver of these mice, as well as of strain C_3HA mice, when cancerogenic substance was administered. The fact that such antigens were formed regardless of the mouse strain corresponds with the data of V. V. Gorodilova and L. V. Shershulskaya [1], who showed the absence of an immunogenetic difference between the antigens from the organs of mice of different strains.

However, in the experiment set up with strain A mice, some peculiarities were observed. Rabbits, immunized with liver proteins of the control group of strain A mice, reacted more intensely with these antigens than with the liver proteins of mice receiving cancerogenic substance. Maybe in this case the presence of sepcific antigens somehow inhibited the reactions of normal antigens with antibodies. This is not observed in strain C_3HA mice (rabbits No. 1-14).

Thus, the results obtained previously by the method of anaphylaxis with desensitization were confirmed by testing 19 immune sera with 8 antigens by the precipitation method. The presence of specific antigens which are formed prior to the development of a tumor in the liver proteins of mice receiving a substance which produces experimental hepatoma was confirmed.

These proteins, together with the specific antigens, are contained in the proteins of normal liver, since the rabbits immunized with them form antibodies which react with the liver antigens of mice receiving cancerogenic material. On the other hand, the precipitins formed against the liver antigens of normal mice react with the proteins in the liver of mice receiving cancerogenic material. Apparently, the specific antigens which are present in the proteins under investigation are either very active or are present in a sufficient amount to cause the formation of specific antibodies.

In one experiment (rabbits No. 15, 16, 17, 18, 19) the presence of specific antigens apparently even influenced the reaction between normal antibodies and antigens contained in the liver proteins of mice receiving orthoaminoazotoluene.

In comparing the results of the experiments described in the present communication with the data obtained earlier when testing artificial antigens, a definite similarity can be observed between the antigens which are formed in vitro and in vivo. The antigens obtained from the union of chemically pure orthoaminoazotoluene with proteins, like the antigens arising in the system of animals receiving cancerogenic material, cause the formation of precipitins in the system of rabbits. Both form antibodies – specific ones determined by the cancerogenic material, as well as nonspecific ones directed against the "original" proteins which are not joined with orthoaminoazotoluene or its derivatives.

Are the tested proteins a mixture of specific and nonspecific antigens or are they antigens which are specific for the original protein as well as for the cancerogenic combination? This problem deserves further study.

LITERATURE CITED

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