THE EFFECT OF THE DOSE OF TUMOR TISSUE ANTIGENS ON THE PRODUCTION OF SPECIFIC ANTIBODIES

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Many workers [1, 2, 5, 7] have shown that the ability of the body to produce antibodies depends not only on the individual characteristics of the animal but also to a considerable degree on the species specificity of the antigen used for immunization, on the duration of the immunization and also on the sex, age and diet of the donor animals. These observations relate mainly, however, to infectious immunology.

Workers [3, 4] who immunized animals with tissue antigens reached similar conclusions.

The question of the effect of various factors on the production of antibodies against tumors in response to the immunization of animals with tumor material has been little studied and there are only occasional references to it in the literature. We were able to find only one paper [6], in which the author reported that immunization of rabbits with large doses of a nucleoprotein fraction of tumor antigens brought about an increase in the titer of precipitins in the serum.

In view of the sparsity of research on this subject, we carried out experiments to ascertain the effect of the dose of tumor tissue antigens injected on the production of antitumor antibodies, and also to study the dynamics of antibody formation after the cessation of immunization.

EXPERIMENTAL METHOD

Experiments were carried out on guinea pigs and rabbits. The animals were immunized with saline extracts of tumor tissue from an Ehrlich's mouse adenocarcinoma (ascitic form) in accordance with the shortest scheme which we could find in the literature, on 4 occasions at intervals of 24 hours.

The first series of experiments, carried out on guinea pigs, was of a purely exploratory character. The animals were divided into 2 groups with 6 in each. The antigens were injected subcutaneously: the first group of animals received a dose of 0.2 mg (as protein), the second group a larger dose of 12 mg (as protein) per course of immunization. The protein content of the antigen was determined as nitrogen by Conway's method. On the 5th day after the last injection of antigens, and on the 6th day thereafter, blood (5 ml) was taken from the heart of the guinea pigs and serum prepared from it for serological analysis.

The second series of experiments was carried out on rabbits. The animals were subdivided into 5 groups, with 4 in each. The animals of the first group were injected intravenously with a dose of 0.2 mg per course of immunization, the second with 2 mg, the third — 12 mg, the fourth — 40 mg and the fifth — 100 mg (as protein). Before, during and after the completion of immunization, blood was taken from the rabbits and sera prepared. After immunization blood was taken every third day for a period of 2½ months.

TABLE 1

The Complement Fixation Reaction of the Guinea Pig Sera with Antigens from Tumor Tissue and from Tissue from the Spleen of a Normal Mouse

Animal No.	Wt of animal (in g)	Dilution of sera	Antigens small dose: tumor	Antigens small dose: spleen	Animal No.	Wt of animal (in g)	Dilution of sera	Antigens large dose: tumor	Antigens large dose: spleen
11	305	1 : 40	+++	++	15	260	1 : 40	++++	+(+)
		1 : 100	++	+			1 :100	++++	h
		1 : 200	+	h			1 :200	+++	h
		1 : 400	±	h			1 : 400	++	h
		1 : 800	h	h			1 :800	±	h
12	268	1 ; 40	+	+	16	270	1 : 40	++++	+++
		1 ; 100	±	±			1 : 100	+++	++
		1 : 200	h	h			1 : 200	++(+)	+
		1 : 400	h	h			1 : 400	+	h
		1 ; 800	h	h			1 : 800	h	h
13	250	1 : 40	++	+(+)	3	240	1 :40	+++(+)	+++
		1 ; 100	+	±			1 : 100	+++	++
		1 : 200	±	h			1 : 200	++	+
		1 : 400	h	h			1 : 400	±	h
		1 : 800	h	h			1 : 800	h	h
2	265	1 : 40	++	+	8	262	1 : 40	++	+(+)
		1 : 100	±	h			1 : 100	+(+)	+
		1 : 200	h	h			1 : 200	+	h
		1 : 400	h	h			1 : 400	±	h
		1 : 800	h	h			1 : 800	h	h
1	260	1 : 40	+++	+	7	268	1 : 40	+++	++
		1 : 100	++(+)	±			1 : 100	++	+
		1 : 200	++	h			1 : 200	+(+)	±
		1 : 400	±	h			1 : 400	+	h
		1 : 800	h	h			1 : 800	h	h
4	250	1 : 40	++	+	5	236	1 :40	++(+)	+
		1 : 100	+	±			1 : 100	++	h
		1 : 200	±	h			1 : 200	+	h
		1 : 400	h	h			1 : 400	h	h
		1 : 800	h	h			1 : 800	h	h

Note to Tables 1 and 2. The letter h indicates complete hemolysis. Complete hemolysis was observed in the antigen and serum controls in every case; complete inhibition of hemolysis was found in the control of the hemolytic system.

All the sera so obtained were subjected to serological examination by the classical complement fixation reaction method at 37° after preliminary estimation of the working dose of complement and antigens.

TABLE 2

The Complement Fixation Reaction of the Rabbit Sera with Antigens from Tumor Tissue and from Tissue from the Spleen of a Normal Mouse

Dilution of sera	No. of animal	Antigens dose 0.2 mg course: tumor	Antigens dose 0.2 mg course: spleen	No. of animal	Antigens dose 2 mg course: tumor	Antigens dose 2 mg course: spleen	No. of animal	Antigens dose 12 mg course: tumor	Antigens dose 12 mg course: spleen	No. of animal	Antigens dose 40 mg course: tumor	Antigens dose 40 mg course: spleen	No. of animal	Antigens dose 100 mg course: tumor	Antigens dose 100 mg course: spleen
1 : 20	3007	++++	+++	2448	++++	++++	2428	++++	++++	3305	++++	++++	2305	+++	+++
1 : 40		+++	++		++++	++++		+++	+++		++	+++		++	++
1 : 100		++	+		++++	+++		+++(+)	++		++(+)	+		+	-
1 : 200		+	h		+++(+)	++		+++	+(+)		++	±		±	h
1 : 400		h	h		+++	+		++	h		±	h		h	h
1 : 800		h	h		++	h		+	h		h	h		h	h
1 : 20	2282	++++	++++	1138	++++	++++	2395	++++	++++	3992	++++	++++	2597	++	++
1 : 40		+++(+)	++++		++++	+++		++++	+++		+++	++++		±	±
1 : 100		+++	+++		+++(+)	+		++++	++		++	++			h
1 : 200		+(+)	++		+++	±		++++	+		+	±			h
1 : 400		±	+		++	h		+++	h		h	h			h
1 : 800		h	h		±	h		++	h		h	h			h
1 : 20	2772	++++	++++	2652	++++	++++	2484	++++	++++	2648	++++	++++	1602	++	+++
1 : 40		+++	+++		++++	+++(+)		++++	+++		+++(+)	+++(+)		+	++
1 : 100		++	+(+)		++++	+++		+++(+)	+(+)		++	++		h	+
1 : 200		+	+		++++	+(+)		+++	h		+	±		h	h
1 : 400		h	h		+++	±		++	h		h	h		h	h
1 : 800		h	h		++	h		+(+)	h		h	h		h	h
1 : 20	2255	++++	++++	3162	++++	++++	2014	++++	++++	3659	++++	++++	2562	++++	++++
1 : 40		+++(+)	++(+)		++++	+++		++++	++++		+++	++++		+++	+++
1 : 100		+++	+(+)		++++	++		+++	+++		++(+)	+++		+(+)	++
1 : 200		++	±		+++	+		++(+)	+(+)		+	+(+)		±	+
1 : 400		h	h		++	±		+(+)	±		h	h		h	h
1 : 800		h	h		+(+)	h		±	h		h	h		h	h

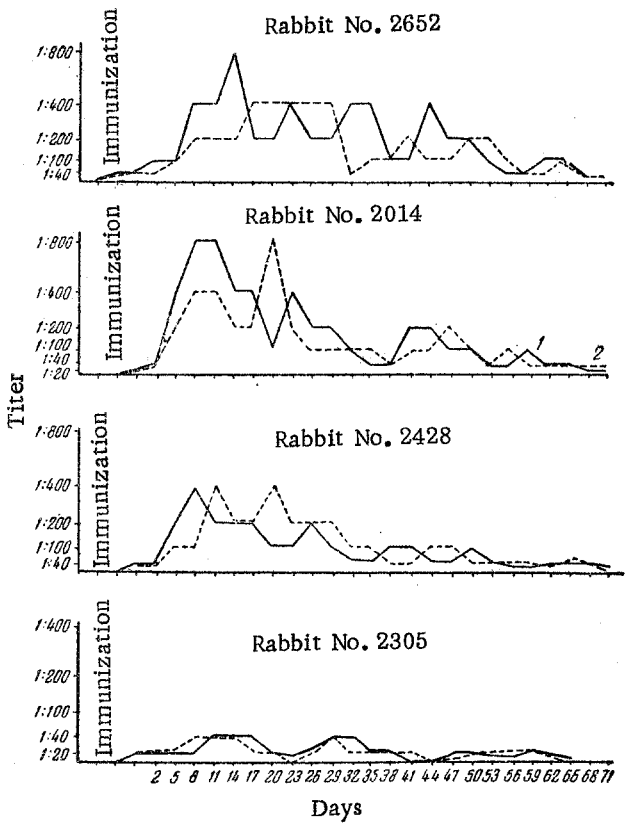
EXPERIMENTAL RESULTS

The results of the first series of experiments are given in Table 1, from which it can be seen that the titer of antitumor antibodies in the sera of guinea pigs immunized with relatively small doses of antigens varied from 1:40 ++ to 1:100 ++. The titer of antitumor antibodies in the sera of guinea pigs which received injections of larger amounts of antigens was considerably higher, and varied from 1:200 ++ 1:400 ++.

With the relatively larger dose of antigen which we used in the immunization of the guinea pigs, the production of antitumor antibodies thus was enhanced. The complement fixation reactions illustrated in Table 1 were carried out with sera obtained on the 9th day after the last injection of antigen. As a result of the study of the trend of antibody production in the guinea pigs it was found that the highest titer of antitumor antibodies was observed between the 15th and 20th days after the last injection of antigens.

In order to determine with greater accuracy the optimal dose of antigens, in the second series of experiments the rabbits were immunized with five different doses.

In Table 2 are shown the results of titration of antisera obtained on the 9th day after the conclusion of immunization (second series of experiments). The titer of antitumor antibodies in the sera of the animals immunized with antigens in a dose of 0.2 mg (as protein) per course of immunization was very low (1:100 ++ or 1:200 ++). In the groups of animals receiving injections of antigens in a dose of 2 and 12 mg per course, a much higher titer of antitumor antibodies was observed than in the sera of the first group of animals, varying from 1:100 ++ to 1:800 ++. When the dose of antigen injected was increased to 40 mg per course, the titer of antitumor antibodies fell perceptibly and hardly reached 1:200 ++. The titer of antitumor antibodies was even lower in the sera of the animals immunized with a dose of 100 mg of antigens per course; in only one rabbit (No. 2562) did it reach 1:100 ++, and in the rest it varied between limits of 1:20 ++ and 1:40 ++.



Trend of antibody formation in rabbits after immunization with antigens from tissue of an Ehrlich's mouse tumor. 1) Complement fixation reaction with antigens from tumor tissue; 2) complement fixation reaction with antigens from spleen tissue.

It was thus demonstrated in the second series of experiments that the dose of tumor antigens injected played a definite role in the formation of specific antibodies. It must be pointed out that a direct relationship between the production of antitumor antibodies and the dose of antigens injected clearly existed up to a certain moment, after which an increase in the dose of antigens did not lead to an increase in the titer of specific antibodies.

These observations were in agreement with the findings of various authors both in infectious immunology and during immunization of animals with tissue antigens.

We also studied the trend of antitumor antibody formation in rabbits after immunization.

The figure illustrates the most characteristic curves showing the trend of antibody formation in rabbits after immunization with antigens from tissue of an Ehrlich's mouse tumor. The apices of the angles on the curves designate readings of the complement fixation reaction with an intensity of ++.

As may be seen from the figure, antibody formation took place in a fluctuating manner. Antitumor or antispleen antibodies predominated in the serum. This fluctuating antibody formation was observed in the sera of the animals of all the groups. It is necessary to stress that each animal reacted in its own way to the injection of antigens. During immunization of rabbits with the optimal

doses of antigen in accordance with our scheme, this fluctuating trend was more pronounced and was apparent for a longer time (animals Nos. 2014 and 2652).

It follows from the results described that the optimal doses in the immunization of rabbits with antigens from tumor tissue (Ehrlich's ascitic strain) varied from 2 to 12 mg (as protein) per course. It is also necessary to point out that by constant serological control of the course of antibody formation it is possible to obtain sera with a considerable preponderance of antitumor antibodies.

SUMMARY

The antigen dosage of the Ehrlich's ascitic tumor tissue employed in immunization of rabbits has a considerable effect on the titer of specific antibodies. However, the antitumor antibody production depends directly on the dosage of the antigens only when administered up to a certain moment following which an increase of the dose does not promote the rise of the specific antibody

After cessation of immunization the antibody production has an undulating character. At one time it is the antitumor antibodies that prevail in the serum, while at the other, the antisplenic antibodies. By a constant serological control of dynamics of the antibody production it is possible to obtain a serum with a considerable prevalence of the antitumor antibodies.

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