THE PRODUCTION OF SPECIFIC SERA TO EHRLICH'S MOUSE CARCINOMA ON THE BASIS OF THE PHENOMENA OF ACQUIRED IMMUNOLOGICAL TOLERANCE

Yu. N. Mol'kov

Laboratory of Experimental Biotherapy (Head—Corresponding Member AMN SSSR M. M. Maevskii) of the Institute of Experimental and Clinical Oncology (Dir.—Corresponding Member AMN SSSR N. N. Blokhin), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR A. D. Timofeevskii)

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The problem of the production of monospecific antisera against malignant tumors has interested many researchers in recent years. Nearly all the methods described for producing monospecific sera are based on in vitro methods of absorption of nonspecific antibodies, and their results are not very satisfactory. Nevertheless, the production of a monospecific antiserum to individual species or groups of human malignant tumors would possibly be of both practical and theoretical importance, especially in the development of the immunological diagnosis of cancer.

Several authors [4, 11-13] have expressed the opinion that it is possible to obtain "pure" specific antisera by means of the method of artificial tolerance. These workers have also reported having obtained monospecific sera against certain antigens of normal and tumor tissue in animals on the basis of the phenomenon of acquired immunological tolerance.

In this communication we describe the results of experiments to produce a monospecific antiserum to Ehrlich's mouse carcinoma.

When planning this investigation, we set out from the position that animals (rabbits), when treated with foreign antigens (normal mouse tissues) in the first days of postembryonic development or in the period of embryogenesis, if given further immunization in the adult state with the tumor tissue, will not form specific antibodies to the normal tissue antigens but, at the same time, retain the power fully to form antibodies to the specific tumor antigen occurring in tumor tissue in conjunction with the normal tissue antigens.

Modern development in methods of creating immunological tolerance have encouraged the hope that it may be possible to differentiate by this means between different types of antigens and antibodies [1-3, 5, 9, 10, 14, 15].

METHOD

Investigations were carried out on female chinchilla rabbits, weighing about 3 kg and kept on an ordinary diet.

As normal tissue antigens we used a mixture of tissues, consisting of citrated whole blood, liver, spleen, and kidney, and also tissue from whole embryos of healthy adult white mongrel mice, which usually supports the ascitic strain of Ehrlich's carcinoma. This mixture of tissue antigens was prepared from organs taken from not less than ten mice.

The normal organs of the mice (liver, kidney, spleen, embryos) were ground in a sterile mortar with glass. From them we prepared a 50% suspension of the tissues in physiological saline, to which, before injection, was added citrated whole blood in a proportion of 25% of blood to the suspension of tissue cells of the organs. Whole blood from healthy white mongrel mice was collected in a 1% solution of sodium citrate in order to give a 90% suspension of whole blood in the citrate solution. After being ground, the antigen for intravenous injection into the female animals was centrifuged and the supernatant fluid was used, to which was added citrated whole blood in order to obtain a 50% suspension of citrated whole mouse blood. Altogether, each female received 15 ml of antigen intravenously (5 ml per injection). Antigen was injected on the 23rd-25th and the 27th day of embryogenesis.

The newborn rabbits usually received subcutaneous and intraperitoneal injections of a mixture of normal antigens starting on the first to the fifth day after birth, in a total dose of 1.5 ml (0.3 ml daily for 5 injections).

Antigen was injected into the embryos on the 17th day of embryogenesis. Laparotomy was performed on the females through a midline incision. Without opening the uterus, an injection of 0.2 ml of the antigen mixture was given to each embryo in the region of the spine. In

order to prevent the development of postoperative infection, 100,000 i. u. of crystalline penicillin dissolved in 5 cc of physiological saline was injected into the abdominal cavity. The operation wound was closed in layers, painted with collodion, and covered with a sterile gauze dressing. It should be mentioned that a large proportion of the embryos subsequently died and underwent absorption, and a number were born dead. Thus, it was only possible to obtain a small percentage (10-15) of living, intact young rabbits, suitable for carrying out the subsequent experiments.

In one series of the experiment, antigen was injected not only into the newborn rabbit but also into the mother during the second half of embryogenesis. In doing so, we worked on the assumption that antigens, injected intravenously in large quantities into the mother, would come into contact with the embryos. Several authors [14] have shown that it is possible to obtain tolerant rabbits after the injection of antigen by this method. After the preparation of standard solutions of antigens, the total protein content was determined in milligrams per milliliter of solution by Kjeldahl's micromethod. Thus, we could calculate the quantity of protein injected into each experimental rabbit.

When they attained the age of ten weeks, the rabbits were immunized with tumor tissue. For immunization we used undiluted ascitic fluid from an Ehrlich's carcinoma. In order to obtain specific tumor antisera rapidly, we used the standard scheme of immunization that we usually employ [8].

To determine the specificity of the sera obtained, we used the hemagglutination reaction with normal mouse red cells and also with red cells from mice with the ascitic form of Ehrlich's carcinoma. As tumor antigen we used blood taken from mice with an Ehrlich's carcinoma in an advanced stage of the disease, when abundant metastases were usually present, as revealed by postmortem examination of the animal at the time of taking the blood. The presence of carcinoma antigen in the blood of these mice could always be revealed by the specific hemagglutination reaction with tumor antisera, a detailed description of which we have given previously [6, 7, 8].

As a control of the reliability of the results obtained by the hemagglutination reaction we also used the specific precipitation reaction in agar, a description of which is given in our previous communications [6, 7, 8]. As antigens for use in the precipitation reaction in agar we used: normal blood and blood from mice with an advanced stage of carcinoma, the solid tumors and ascitic fluid of Ehrlich's carcinoma, and normal mouse tissues (spleen, liver, and kidney).

RESULTS

In order to obtain specific antitumor sera against Ehrlich's mouse carcinoma on the basis of the phenomenon of an artificially created immunological tolerance, we tested a number of schemes of production of immunological tolerance in rabbits to heterogenic cell antigens.

We investigated the possibility of obtaining immunological tolerance by injection of antigen: 1) Into newborn rabbits from the first to the fifth day of life (each young rabbit received 130.5 mg of protein); 2) intravenously into the mother in the last days of embryogenesis (each female received 2325 mg of protein) followed by supplementary injection into the newborn animals (130.5 mg to each young rabbit); 3) into the embryos on the 17th day of embryogenesis (17.4 mg of protein to each embryo); 4) into the embryos (17.4 mg of protein) with a supplementary injection into the new-born animals (130.5 mg of protein).

The experimental results are shown in summarized form for the different groups in the table.

It is clear from the results of the hemagglutination reaction that a true decrease was observed in the formation of normal hemagglutinins in the experimental rabbits by comparison with the controls in the second, third, and fourth groups, and especially in the third and fourth, in which an injection of antigens of normal tissues was given into the embryos (third group) and into the embryos, followed by a supplementary injection after birth. The difference between the mean values of the titers of the hemagglutination reaction in the experimental and control animals in these groups is statistically significant. It should also be mentioned that individual tumor antisera in the test groups reacted more intensively with the blood of mice affected by carcinoma.

The sera of these groups of experimental animals thus showed a significant fall in the formation of normal hemagglutinins and a more intensive reaction of individual tumor antisera with the blood of cancerous mice than with the blood of healthy mice. Unfortunately, it was not possible to rid it completely from the formation of normal antibodies.

In regard to nearly all the groups, especially the third and fourth, an interesting phenomenon must be mentioned which was observed during immunization of the rabbits. Beneath the skin in the experimental animals, at the site of injection, the temporary development and temporary (occasionally intensive) growth of tumor tissue was observed, which did not occur in the control rabbits. This fact undoubtedly shows some degree of immunological approximation of the tissues of the experimental animals towards Ehrlich's mouse carcinoma.

The sera of the experimental and control rabbits of all the groups were also tested by the specific precipitation reaction in agar, to test the validity of the results obtained, and identical results were found. The results of the precipitation reaction in agar showed that the tumor antisera obtained from the experimental rabbits were actually more specific than the sera from the control rabbits. The majority of antisera from the third and

TABLE

Mean Titers of the Hemagglutination Reaction in the Experimental and Control Rabbits in the Different Groups of Experiments

Experimental group	Serial No. of rabbit	Titer of natural hemagglutinins		Titer of immune hemagglutinins	
		with blood of healthy mice	with blood of cancerous mice	with blood of healthy mice	with blood of cancerous mice
1st experimental	1	–[1]	–	64[2]	64
	2	–	–	32	64
	3	–	–	64	64
	4	–	–	128	128
1st control	5	–	–	(P>0.06) 128	128
	6	–	–	256	256
	7	–	–	128	128
	8	–	–	128	128
2nd experimental	1	–	–	128	128
	2	–	–	64	64
	3	–	–	64	128
	4	–	–	32	128
	5	–	–	64	64
	6	–	–	64	128
	7	–	–	128	128
	8	–	–	(P≥0.001) 64	128
2nd control	9	–	–	128	256
	10	–	–	256	256
	11	–	–	256	256
	12	–	–	128	128
	13	–	–	1024	1024
	14	–	–	128	128
3rd experimental	1	2	2	32	64
	2	–	–	32	32
	3	8	8	64	64
	4	–	–	16	32
3rd control	5	–	–	(P>0.000) 128	256
	6	–	–	128	128
	7	–	–	128	128
	8	–	–	256	256
4th experimental	1	–	–	8	64
	2	–	–	128	128
	3	–	–	64	64
	4	8	8	64	64
	5	32	32	64	64
	6	–	–	64	64
	7	–	–	64	128
	8	–	–	32	32
4th control	9	–	–	(P<0.01) 256	256
	10	–	–	128	128
	11	–	–	1024	1024
	12	–	–	256	512
	13	–	–	256	512
	14	–	–	512	512
	15	–	–	128	128
	16	–	–	512	512

[1] Absence of hemagglutination reaction

[2] Final dilution of sera, where a hemagglutination reaction is still present.

fourth experimental groups, and also individual antisera from the second group did not react with normal antigens (liver, kidney, spleen, blood, and embryos) even in the initial dilutions, whereas they continued to react with tumor antigen. The tumor antisera of the control rabbits reacted to an equal degree with both normal and tumor antigens.

In the second, third, and fourth groups of experimental rabbits it was thus possible to attain only a slight degree of immunological approximation towards the heterologous mouse antigen. This immunological approximation was incomplete and showed itself only as a slight fall in the formation of normal antibodies and the more intensive growth of Ehrlich's carcinoma tissue, injected subcutaneously during immunization, in the experimental rabbits than in the controls.

In conclusion it should be pointed out that the antisera which we obtained on the basis of the phenomenon of acquired immunological tolerance were, in our opinion, more specific than those obtained by the ordinary method of immunization of the control rabbits.

SUMMARY

The author attempted to obtain specific antitumor sera on the basis of the phenomenon of an artificially created immunological tolerance. It was shown that only an insignificant degree of immunological approximation can be attained in rabbits with respect to heterologous mice antigens by administering the latter to the embryos, especially when this is supplemented by a subsequent inoculation at an early period of extra-uterine life. This immunological approximation is incomplete and is manifested only by an insignificant reduction in the formation of normal antibodies, inddequate for obtaining "pure" monospecific and antitumor sera to Ehrlich's mouse carcinoma. The antisera to the Ehrlich's carcinoma obtained on the basis of acquired immunological tolerance were more specific than those derived by the usual method of immunization in control rabbits.

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