THE FORMATION OF IMMUNE ANTIBODIES IN RABBITS DURING THE GROWTH OF A BROWN-PEARCE TUMOR

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The research of A. A. Bogomolets and his co-workers [1] revealed the important role of the reticulo-endothelial system in the resistance of the body to malignant disease.

One of the indices of the state of the defensive forces of the body is its power of formation of antibodies.

The findings in relation to the study of immunogenesis in the presence of malignant neoplasms are very contradictory. I. Kirichinskaya and M. Sirotina [2] studied the sera of rabbits with an implanted Brown-Pearce tumor, immunized with sheep's red cells, and found a lower titer of hemolysins by comparison with the sera of immunized normal rabbits. Certain authors have conducted a clinical investigation of immunogenesis in patients with malignant tumors. As antigens for immunization, bacteria, viruses and toxins were used. Various methods were used, and the titers of the variety of heterophilic antibodies – complement-fixing, agglutinins and precipitins – were determined. The investigations of Parfent'ev, Clifton and Duran-Reynals [7], and Southam, Goldsmith and Burchenal [8] showed that in patients with malignant neoplasms, production of antibodies was feebler than in a group of persons not suffering from tumors. On the other hand, Logan [6], Larson and Tomlinson [4] and Leskowitz, Phillipino et al. [5] found no difference in antibody formation between cancer patients and healthy persons. In some cases the authors did not take into consideration the character of the antigenic stimulus, and immunized with a mixture of different antigens, and so on, which is very important in immunogenesis [3]. The discrepancies in the findings on this problem led to the necessity for further research.

The aim of our work was to study immunogenesis in rabbits with implanted Brown-Pearce tumors by comparison with that in healthy animals, when immunized with a heterogeneous malignant antigen – the ascitic form of Ehrlich's adenocarcinoma of mice, and a homologous antigen – Brown-Pearce tumor.

EXPERIMENTAL METHOD

In the first series of experiments 16 male rabbits of the chinchilla variety were divided into 2 equal groups. The eight animals of the first group were given intratesticular inoculations of a 20% suspension of Brown-Pearce tumor cells. The second group, consisting of 8 healthy rabbits, were used as controls. Sere were obtained from all the rabbits and the preliminary background titer was determined in the complement fixation reaction with antigens from ascitic cells of Ehrlich's adenocarcinoma, Brown-Pearce tumor and the liver and spleen of normal mice and rabbits. On the 7th day after inoculation, when development of the carcinoma could be detected clinically, immunization of the first and second (control) groups began. As antigen we used a saline extract of ascitic cells of Ehrlich's adenocarcinoma in a dilution of 1:20. Immunization was carried out by injection of 1 cm^3 of antigen intravenously on 3 successive days.

The short cycle of immunization was due to the fact that the animals might die from the carcinoma before the immune sera had been obtained from them. On the 7th day, after the completion of immunization, blood was taken from the rabbits, and subsequently blood was taken every 5 days. In the sera which we obtained we determined the antibody titer in the complement fixation reaction at 37° and observed its change with time. In the animals dying with signs of malignant cachexia, we studied the macroscopic picture, post mortem.

In the second series of experiments we used 12 rabbits, 6 in each group. The conditions of the experiment were analogous to those described, except that immunization was done with a saline extract of Brown-Pearce tumor in a dilution of 1:20, i.e., with a homologous antigen.

EXPERIMENTAL RESULTS

In the table are given the results of the first series of experiments. Here are shown the antibody titers of the immune sera of rabbits with carcinoma and of healthy rabbits, as revealed by the complement fixation reaction with an identical antigen, with a saline extract of ascitic cells of Ehrlich's adenocarcinoma in mice. The antibody titers were studied on the 17th, 22nd, 27th and 32nd days after implantation of the tumor. The serum dilutions used were from 1:50 to 1:400. As an additional control, in the complement fixation reaction, in addition to antigen against which the immune sera were obtained, we used additional antigens prepared from tissues of normal organs of mice — the spleen and liver — and also antigen from Brown-Pearce tumor tissue. In the table, however, for the purpose of simplification, we do not give the results of these control experiments. In the right hand column of the table are indicated the time of death of the animals suffering from carcinoma.

As can be seen from the table, the titers of immune antibodies in the group of rabbits suffering from carcinoma were mainly much lower than those in the control group, consisting of healthy, immune animals. A reaction of + + + and + + + + was observed in 4 of the 8 rabbits with carcinoma, most often in a dilution of 1:50. The exception was rabbit No. 2204, which, in spite of its rapid death (on the 28th day), showed a quite high antibody titer, giving fixation of + + + in dilution of 1:200.

At postmortem examination metastases were found in the spinal cord with resulting paralysis, whereas the parenchymatous organs were free from metastases. This may probably explain the comparatively high antibody titer in the sera, in spite of the animal's rapid death. The four remaining rabbits of this group, even in dilution of 1:50, fixed complement only to the extent of + and + +. In the control group the immune sera reacted to + + + and + + + + in dilution of 1:200 and in some cases of 1:400.

Thus a fall was clearly seen in the power of antibody formation to this particular antigen by rabbits suffering from carcinoma. We were unable to establish any regular relationship in the trend of antibody formation at the different stages of the malignant disease, since the antibody titers were roughly the same over the period of 20 days during which blood was taken.

In the second series of experiments immunization was carried out with a saline extract of a Brown-Pearce tumor. In order to determine the titers of immune antibodies lower dilutions of sera were used — from 1:20 to 1:160.

Furthermore, as an additional control in this series we used an immune goat serum to Brown-Pearce tumor, and also sera of rabbits inoculated with Brown-Pearce tumors at the same times but not subjected to immunization with a saline extract of this tumor.

The complement fixation reaction was performed both with antigen from the Brown-Pearce tumor and with antigens from normal tissues — spleen and liver of rabbits.

In the experimental and control groups, immunization with homologous tumor antigen did not produce antibody formation. The exceptions were 3 rabbits (Nos. 459, 606 and 893), in which after the formation of metastases (in two — metastases in the eyes, in the third — in the internal organs) the malignant process terminated spontaneously and the tumor was absorbed. In these animals antibodies were detected in dilutions of 1:20 to 1:80 to the extent of + + + and + + + +. We did not, however, attribute the presence of these antibodies to injected antigen, but we regarded their appearance as due to the absorption of the tumor.

The investigations carried out demonstrated that the formation of immune antibodies in rabbits in response to the injection of heterologous and homologous tumor antigens depended not only on the state of the animal but also on the character of the antigenic stimulus.

Titers of Complement-Fixing Antibodies in the Blood of Rabbits with Carcinoma and Healthy Rabbits after Their Immunization with Heterologous Antigen

Group of rabbits	Rabbit No.	Day after inoculation on which blood was taken: 17th — dilutions of sera 1:50	1:100	1:200	1:400	22nd — 1:50	1:100	1:200	1:400	27th — 1:50	1:100	1:200	1:400	32nd — 1:50	1:100	1:200	1:400	Day of death of rabbit
With implanted Brown-Pearce tumor	2603	++	+	—	—	++	+	—	—	++	+	—	—	++	+	—	—	40-th
	1061	+++	++	+	—	+++	++	+	—	+++	++	+	—	++	+	—	—	35-th
	3780	++++	+++	+	—	+++	++	+	—	++	+	—	—	+	—	—	—	34-th
	1883	+++	++	+	—	++	+	—	—	++	+	—	—					29-th
	2204	++++	+++	+++	++	++++	+++	++	++	++++	+++	++	—					28-th
	3368	++	+	—	—	+++	++	+	—									23-rd
	2045	++	+	—	—	++	+	—	—									23-rd
	3612	+	—	—	—													20-th
Control	3121	++++	++++	+++	++	++++	++++	+++	++	++++	+++	+++	++	++++	+++	+++	++	
	3535	++++	++++	+++	++	++++	+++	++	++	++++	++++	+++	++	++++	+++	+++	++	Rabbits survived
	2537	++++	++++	+++	++	++++	++++	+++	+++	++++	++++	+++	++	++++	+++	++	+	
	3482	++++	++++	+++	++	++++	++++	+++	++	++++	++++	+++	++	++++	++++	+++	++	
	2862	++++	++++	+++	+	++++	+++	++	+	++++	+++	++	+	++++	++++	+++	++	
	2445	++++	+++	+++	++	++++	+++	++	+	++++	+++	++	++	++++	+++	++	++	
	3811	++++	+++	+++	++	++++	++++	+++	++	++++	++++	+++	++	++++	+++	++	+	
	3315	++++	++++	+++	+++	++++	++++	+++	+++	++++	++++	+++	++	++++	++++	+++	+++	

At the same time these results showed that immunogenesis in rabbits suffering from carcinoma was considerably suppressed by comparison with healthy animals.

SUMMARY

Experiments were conducted on healthy rabbits and on those suffering from cancer. The author undertook a comparative study of the antibody production resulting from administration of heterologous antigen (water-salt extract of the ascitic cells of the Ehrlich adenocarcinoma),and of homologous antigen (water-salt extract of the Brown-Pearce tumor). In immunization with a heterologous tumor antigen immunogenesis was depressed in the affected animals in comparison with the control ones. As to immunization with a homologous tumor antigen, it caused no antibody formation either in the experimental or in the control group of animals.

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*Original Russian pagination. See C.B. Translation.