ONCOLOGY

CHANGES IN THE IMMUNOBIOLOGICAL PROPERTIES OF A TUMOR AFTER PASSAGE THROUGH A CHICK EMBRYO

T. A. Pokrovskaya

From the Laboratory of Noninfectious Immunology (Head – Prof. I. N. Maiskii) of the Institute of Experimental Biology (Director – Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received April 9, 1959. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

No unanimity exists in the literature on the question of the changes in the antigenic properties of transplanted tumors after passage through a chick embryo. The findings of a number of authors [1, 2, 5, 8] testify to changes in the biological and antigenic properties of tumors when cultivated in this medium.

Other workers [2, 4, 6, 7], on the other hand, deny that such changes may be produced.

In the present research we studied the immunobiological properties of the ascitic form of Ehrlich's adenocarcinoma after passage on the chorionallantoic membrane of the chick embryo.

EXPERIMENTAL METHOD

1. Passage of the tumor was carried out on the chorionallantoic membrane of 8-9 day-old embryos. The surface of the chorionallantoic membrane was exposed in sterile conditions, and at the place where there was greatest development of the vessels, 0.1 ml of ascitic fluid was applied, diluted 1:2. The opening in the shell was closed with a glass cover slip and sealed with paraffin wax. The embryos were examined and further transplantation carried out on the 14th-16th day of incubation. Growth of the tumor cells was observed in the course of seven passages, as confirmed histologically. After the seventh passage, growth of the tumor ceased.

2. The study of the antigenic structure of the transplanted tumor was made by means of the classical complement fixation reaction. As antigens we used a nontransplanted Ehrlich's adenocarcinoma and all the tumors obtained by passage (from the first to the seventh). Immune sera (against Ehrlich's adenocarcinoma, against the chorionallantois and against tumors after 5 passages) were always used in the same titer, namely 1:320.

3. The biological properties of the transplanted tumor was studied by transplantation back into mice. In the experiments we used male white mice weighing 18-20 g. The tumor was implanted subcutaneously in the dorsal region in a dose of 0.2 ml of a 10% suspension of tumor tissue. As controls we used mice which received injections of the same dose of a nontransplanted Ehrlich's adenocarcinoma.

The day of appearance of the tumors was noted and their dimensions on the 7th, 14th, 21th and 28th day after implantation were recorded. The size of the tumor was determined from the area of an ellipse. In all, 23 series of experiments were performed on 730 mice by this method.

EXPERIMENTAL RESULTS

In order to study the antigenic properties of the tumor after passage, two sera were used: a serum against Ehrlich's adenocarcinoma and a serum against the chorionallantois of a 15-day chick embryo. The reaction of the transplanted tumor with serum against Ehrlich's adenocarcinoma showed the extent to which the original antigenic composition was retained in the cells of the heterotransplanted tumor.

TABLE 1

Immunological Analysis of an Ehrlich's Adenocarcinoma after Passage through a Chick Embryo, by Means of the Complement Fixation Reaction

Dilutions of sera	Sera																
	against Ehrlich's adenocarcinoma						against chorionallantois					against tumor after 5th passage					
	antigens																
	Ehrlich's tumor					chorion-allantois	chorion-allantois	Ehrlich's tumor					Ehrlich's tumor				chorion-allantois
	nontrans-planted	passage						non-trans-planted	passage				passage			non-trans-planted	
		I	III	V	VII				I	III	V	VII	V	III	I		
1:10	++++	+++	++	+	+	h	++++	h	h	+	+	+	++++	++	±	+	+
1:20	++++	+++	++	±	±	h	++++	h	h	±	+	+	++++	++	+	+	+
1:40	++++	++	+	h	h	h	++++	h	h	h	±	±	++++	+	h	+	+
1:80	+++	++	+	h	h	h	+++	h	h	h	h	h	++++	±	h	h	h
1:160	+++	+	h	h	h	h	+++	h	h	h	h	h	+++	h	h	h	h
1:320	++	+	h	h	h	h	++	h	h	h	h	h	++	h	h	h	h
1:640	+	h	h	h	h	h	+	h	h	h	h	h	+	h	h	h	h
Control		CHS	—	++++				CC-h		CS	—	h		CA-h			

Legend: ++++ complete inhibition of hemolysis; h, complete hemolysis; CHS, control of hemolytic system; CC, control of complement; CS, control of serum; CA, control of antigen.

TABLE 2

Incidence of Successful Retransplantation of the Tumor in Mice

passage	Experiment: number of animals	Experiment: mean area of tumor (in cm^2) 7th day	14th day	21st day	28th day	Control: number of animals	Control: mean area of tumor (in cm^2) 7th day	14th day	21st day	28th day
1st	50	0,06	1,06	2,64	3,55	50	0,06	1 06	2,57	3,6
2nd	50	0,023	0,4	1,19	2,4	50	0,014	0,73	1,75	3,04
3rd	50	0,018	0,18	0,6	1,51	50	0,069	0,64	2,05	3,51
4th	50	0,026	0,13	0,43	0,97	50	0,04	0,55	1,74	2,64
5th	30	No growth of tumor				30	0,07	1,01	2,15	3,01

The reaction with the serum against the chorionallantois enabled it to be judged whether the changes in the antigenic properties of the tumor was dependent on the presence of the tissues of the new host therein.

When studying the activity of the antigens obtained we took into consideration the limiting dilutions of the sera in which fixation to the extent of one plus sign could be observed. This was made easier by the fact that the sera which we used were all of the same titer, 1:820.

The immunological analysis on the transplanted tumor is shown in Table 1. It will be seen from Table 1 that after the 1st passage, the tumor gave a reaction of + in a dilution of 1:160 with serum against Ehrlich's adenocarcinoma. In the course of passage, however, the antigenic activity of the tumor fell slightly: after the 7th passage, the tumor reacted + with this serum only in a dilution of 1:10. In the reaction with serum against the chorionallantois, tumours after the 5th and 7th passages reacted + only in a dilution of 1:20. The nontransplanted tumor and tumors after the 1st to 4th passages did not react with this serum.

In the course of repeated passages on the chorionallantoic membrane, the Ehrlich's adenocarcinoma thus gradually lost the power to react with serum against this tumor.

At the same time, the absence of reaction with serum against the chorinallantois confirmed that the change in the original antigenic activity of the tumor in the complement fixation reaction was not connected with the presence of chorionallantoic tissue in the tumor.

Changes in the antigenic properties during passage were also confirmed by the complement fixation reaction with serum against the tumor after the 5th passage. (Because of the absence of large amounts of the transplanted tumor, it was possible to obtain serum against the tumor after only the 5th passage).

The results of this reaction are also shown in Table 1.

Serum against the tumor after the 5th passage reacted ++ in a dilution of 1:320 with homologous antigen.

Tumors after the 1st and 3rd passage reacted rather differently with this serum: the 3rd passage tumor reacted + in a dilution of 1:40, and the 1st passage tumor only in a dilution of 1:20.

As pointed out above, the study of the biological properties of the transplanted tumor was carried out by transplantation of the tumor back into mice. Table 2 gives in summarized form the results of all these experiments.

As is apparent from Table 2, in the course of repeated passage, the tumor gradually lost its ability to be transplanted into mice. Whereas in the first four passages the rats of successful transplantation of the tumor was still 100%, in the 6th passage it was zero.

The rate of successful transplantation of the tumor after the 5th passage into mice will be discussed below.

In the process of passage the tempo of growth of the tumor is modified as well as the rate of its successful transplantation, as may be seen from a comparative analysis of the dimensions of the tumor size at different

periods. The dimensions of the 1st passage tumor were in no way different from those of the control tumors. Starting with the 2nd passage, however, the tempo of growth of the tumor was slightly retarded.

The tumors appeared at the same time as those in the control animals, but then they grew more slowly; on the 28th day, the tumor in the experimental group had attained dimensions of 2.4 cm^2, whereas the area of the control tumors was 3.4 cm^2. Similar results were obtained from a study of the dimensions of the tumors after the 3rd and 4th passages.

Statistical treatment of the results showed that the difference between the dimensions of the tumors after the 3rd and 4th passages and those of the control tumors was significant, $P < 0.01$.

A somewhat different picture was obtained from the study of the rate of successful transplantation of the tumor after the 5th passage.

In the experimental group, the tumors appeared 7-8 days later than in the control group; on the 19th-26th day they attained an average diameter of 0.2-0.5 cm and then regressed for 1 to $2^1/_2$ months. The tumor after the 5th passage was transplanted into 135 animals, in 83% of which it was absorbed and in 8.8% it grew progressively and led to death of the animals. In 8.2% of mice transplantation of the tumor after the 5th passage was inconclusive.

The tempo of growth of the tumor after the 5th passage was retarded still further. Whereas the area of the tumors after the 3rd and 4th passages reached 0.18 and 0.13 cm^2 on the 14th day, the area of the tumor after the 5th passage did not exceed 0.11 cm^2. Retardation of the tempo of growth of the tumor after the 5th passage was also indicated by their later appearance by comparison with the tumors in the control group.

In the course of passage of an Ehrlich's adenocarcinoma on the chorionallantois of the chick embryo, changes were observed in the initial antigenic activity of the tumor and the rate of its successful transplantation in mice was diminished.

The results obtained show that during passage of the tumor on the chorionallantois of the chick embryo, a change takes place in its antigenic properties. This change in the antigenic properties coincides with changes in its biological properties.

SUMMARY

The author studied the antigenic and the biological properties of Ehrlich's ascitic adenocarcinoma grown on the chorionallantois of chick embryo. In the process of cultivation there was noted a change of the initial antigenic activity in complement fixation reaction and decrease of its ability to grow on mice. It is of interest to note that the changes of the antigenic properties of the cultivated tumor coincided with the change of its biological properties.

LITERATURE CITED

[1] V. A. Anan'ev and V. R. Obukhova, Zhur. Mikrobiol., Épidemiol. i Immunobiol., No. 11, 48 (1955).

[2] T. P. Konstantinova and Z. I. Rovnova, Byull. Éksptl. Biol. i Med., 45, No. 4, 112 (1958).*

[3] A. S. Kriviskii and L. F. Larionov, Arkh. Patol., 12, 3, 52 (1950).

[4] B. S. Ruchkovskii, Med. Zhur., 24, No. 6, 34 (1954).

[5] A. K. Shubladze, Zhur. Mikrobiol., Épidemiol. i Immunobiol., No. 11, 36 (1955).

[6] C. P. Dagg, D. A. Karnofsky and H. W. Toolah et al., Proc. Soc. Exper Biol and Med., 1954, v. 87, N. 1, p. 223.

[7] L. Korngold and R. Lipari, Cancer Res., 1955, v. 15, N. 3, p. 159.

[8] E. A. Mirand and J. G. Hoffman., Proc. Soc. Exper. Biol and Med., 1955, v. 88, N. 4, p. 656.

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