

IMMUNIZATION OF MICE WITH A TUMOR GROWN ON THE CHORIOALLANTOIC MEMBRANE OF THE CHICK EMBRYO

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Many workers have shown that the most stable immunity to a tumor can be attained only after a certain degree of growth of the tumor, followed by its absorption [1, 2, 3]. A great variety of methods has been used in order to produce experimental absorption of the tumor: intradermal injections, interference with the blood supply of the tumor, the injection of chemicals into it, and so on. Nevertheless, these methods of immunization against tumors have not always been successful, for instead of the desired result of absorption of the tumor, its progressive growth has been observed [1, 6, 7].

In the present research, in order to produce immunity to a tumor, we decided to use an Ehrlich's carcinoma grown on the chorioallantoic membrane of the chick embryo. No similar work has been reported in the literature.

When we studied the biological properties of a tumor cultivated in the chick embryo, we observed [5] that in the course of passage the tumor acquires the property of undergoing absorption when reinoculated in the original animals in 80% of cases. This property was found at the fifth passage of the tumor in the embryo. We therefore thought that, after passage in this way, such tumors could be used for immunization.

METHOD AND RESULTS

For immunization we used male white rats weighing 24-25 g. Altogether four groups of mice were immunized; two were controls. Each group consisted of 112 animals. The first group included mice in which a tumor, inoculated after the fifth passage in the chick embryo, was undergoing absorption. The animals of the second group were immunized with tumor material after the fifth passage in the chick embryo and treated with 0.2% formalin solution. The third group consisted of mice immunized with Ehrlich's carcinoma, not cultivated in the chick embryo, treated with the same formalin solution. The animals of the fourth group were immunized with tissue from the chorioallantoic membrane of a 14-day old chick embryo.

Immunization was carried out by the subcutaneous injection of a 10% tumor suspension in the dorsal region. The dose injected was estimated as protein, which was determined by Conway's method. Each animal received 3-4 mg of protein in 0.2 ml of 10% tumor suspension.

From 10 to 15 days after immunization (in the first group—after absorption of the tumor) all the mice received a second inoculation of the ascitic form of the tumor intraperitoneally in a minimum inoculating dose. This dose was preliminarily titrated in mice; in our experiments the dose was 0.2 ml of ascites fluid diluted 1:1000. For the reinoculation we used as a rule a slightly larger dose of tumor in order to obtain a 100% take.

After reinoculation, we noted the time taken for an obvious ascitic tumor to develop, the character of its subsequent growth and the time of death of the animals.

Immunization of Mice with Tumor After Passage in the Chick Embryo

Group of experiments	Material used for immunization	Time of appearance of visible ascites (in days)	Time (in days) of death of mice	
			after reinoculation	after appearance of ascites
First	Inoculation of tumor after 5th passage with subsequent absorption . . .	18-21	31-36	15
Second	Tumor after 5th passage, treated with 0.2% formalin	8-11	18-23	11
Third	Tumor, not subjected to passage, treated with 0.2% formalin	8-11	17-23	11
Fourth	Tissue of chorioallantoic membrane of 14-day old chick embryo	10-11	18-24	11
Fifth	Material control, inoculation of minimum dose of ascites fluid . . .	8-11	18-24	12
Sixth	Technical control, inoculation of usual doses of ascites fluid	5	14-17	10

Note. Mean times of death of the mice are shown in the table.

The rate of growth of the ascitic tumor was also judged by the change in the circumference of the abdomen of the mice and by their increase in weight. By this means the changes in tumor growth could be detected sooner. In order to determine the significance of the results obtained, a statistical analysis was made of the survival rate of the mice of the experimental and control groups by the Fisher-Student method. Altogether we carried out 4 series of experiments on 672 mice.

The method which we used enabled us not only to study the immunizing properties of the Ehrlich's tumor after the fifth passage in the chick embryo (first group of experiments), but also to compare the immunizing properties of the "living" and the formalinized tumor tissue (second and third groups of experiments). We could, moreover, examine the immunizing properties of tumor tissues, both subjected to passage (as in the second group of experiments) and not (third group of experiments), and treated with formalin.

Immunization with tissue of the chorioallantoic membrane was undertaken in order to exclude its immunizing influence after absorption of the tumor after the fifth passage. The possibility of the production of relative immunity to a tumor after immunization with embryonic tissues is well known from the literature [4].

When giving the injection to detect the presence of immunity, we considered it essential to use minimum inoculating doses. The use of large doses may lead to the suppression of an immunity the degree of which is unknown.

The results of the experiments are shown in the table. In it are recorded the times of appearance of visible ascites fluid, of death of the mice after the inoculation to detect immunity, and of death of the mice after the appearance of visible ascites fluid.

In the first experimental group ascites fluid was found on the 18th-21st day after inoculation, on the average 10 days later than in the mice of the control group. Somewhat different results were obtained during the study of the times of appearance of ascites fluid in the mice of the other experimental groups.

The times of appearance of ascites fluid in the animals of all these groups were the same, and they coincided with the moment of formation of visible ascites fluid in the control mice. For example, in mice immunized with formalin-treated tissue from a tumor not subjected to passage, ascites fluid appeared on the 8th-11th day, i. e., at the same time as in the control animals.

Thus in mice immunized with the tissues of a tumor after passage, the appearance of ascites fluid was later than in the control animals. The fact that the latent period of development of the tumor in these mice was prolonged cannot be explained by weakening of the virulence of the strain, for growth of the ascitic tumor was observed as usual in the control animals; appearance of the tumor on the fifth day and death of the mice on the 14th-17th day after inoculation of the tumor.

Consequently, the prolongation of the latent period of development of the ascitic form of the tumor is the result of immunization of the mice with tissues of the tumor after passage.

Similar results were also obtained from the study of the time of death of the animals. The mice of the first experimental group died on the 31st-36th day after reinoculation of the tumor, i. e., 12 days later than the controls. The control animals died on the 18th-24th day after inoculation. At these times death of the animals in the remaining experimental groups was also observed.

Thus the study of the time of death of the mice also revealed a lengthening of their survival period in the principal experimental group, not only by comparison with the control animals, but also with the animals of the other experimental groups. After statistical analysis of the figures showing the time of death of the mice in all the experimental groups and the controls, only the figures of the survival of the mice in the first experimental group were significant ($P = 0.00001$). By studying the time of death of the mice after the appearance of ascites fluid it was impossible to find any significant difference between the experimental and control figures. The mice of the first experimental group died on the 15th day after the development of ascites fluid. The control animals and the mice of the remaining experimental groups died slightly earlier—on the 11th-12th day.

The study of the immunizing properties of an Ehrlich's tumor after the fifth passage in the chick embryo thus showed that a relative vaccinating effect could be obtained, as shown by the prolongation of the survival of the experimental animals and by the slower rate of growth of a reinoculated tumor. This is confirmed by an increase in the latent period of development of ascites in the experimental mice by comparison with the controls. The experiments also showed that positive results may only be obtained from immunization in the case of adsorption of the implanted tumor.

SUMMARY

The author studied the immunizing properties of Ehrlich's carcinoma after the 5th passage in the chorio-allantoic membrane of a chick embryo. As a result of such immunization the life-span of experimental animals may be prolonged and the growth rate of a reinoculated tumor changed. Positive results of this vaccination were noted only after the resolution of the tumor used for immunization.

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