THE ANTIGENIC PROPERTIES OF SOME FRACTIONS OF EHRLICH'S ADENOCARCINOMA OF MICE

COMMUNICATION II. THE STUDY OF THE ANTIGENIC PROPERTIES OF TUMOR FRACTIONS BY MEANS OF THE PRECIPITATION REACTION IN JELLY AND VACCINATION EXPERIMENTS IN MICE

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In the previous communication [2] we demonstrated the presence of specific and of species-specific antigens in a saline extract and in the globulin fractions of Ehrlich's adenocarcinoma.

The object of the present investigation was to make a detailed study of the antigenic structure of Ehrlich's adenocarcinoma and of its globulin fractions by means of the precipitation reaction in jelly, and to compare the vaccinating activity of the tumor fractions by vaccination experiments in mice.

EXPERIMENTAL METHOD

The precipitation reaction in jelly was carried out by Ouchterlony's method [13]. In a series of experiments a micromodification of the method was used: the diameter of the matrices forming the wells was reduced to 4 mm, and the distance between the wells containing antigen and antiserum was 5 mm. Lines appeared after 18 hours. One Petri dish could be used for 6-8 experiments.

The results of the reaction were recorded photographically by means of a "Zenith" camera, with oblique illumination from beneath provided by a special apparatus constructed by G. I. Avdeev. Some experiments were recorded on an MF-2 microphotometer by the original method. Use of the microphotometer enables a quantitative analysis of the fine and poorly differentiated precipitation lines to be made.

To produce antisera, rabbits were immunized intravenously with a suspension of washed ascites cells in accordance with a four-week cycle program.

Fine differentiation of the antigens in the reaction was obtained by using Björklund's method of specific inhibition of precipitation [9]. On the night before the experiment antigen was poured into all the wells. The jelly was saturated to excess with antigen, and no precipitation lines were formed to this antigen.

The vaccination experiments were carried out on mongrel white mice. In view of the considerable interest in the question of postoperative immunity, developing after the removal of a tumor [10, 12], in each series of experiments we immunized one group of mice by implantation of the tumor in the tail area by Andervont's

method [8] in order to compare the efficacy of chemical vaccination with that of vaccination with the virulent tumor. A suspension of ascites cells (0.1-0.2 ml) was injected from a tuberculin syringe beneath the skin of the tail, at the junction of the lower and middle thirds. After 9-14 days, when the tumor had reached a certain size, the tail was amputated. From 19 to 28 days after amputation the infecting dose of the tumor was injected subcutaneously into mice in which no recurrence was observed, and simultaneously into the mice of the other experimental groups.

The other groups of animals were immunized with a saline extract of the tumor, with globulin I and globulin II of the tumor (obtained by salting out with 46% and 50% saturated ammonium sulfate respectively), and with globulin from mouse liver and spleen. The dose of all the antigens was estimated in terms of dry weight of the lyophilized material. Each mouse received a single subcutaneous injection of 2 mg of antigen in the left side. From 11 to 12 days after vaccination an infecting dose of 0.2 ml of ascites fluid in a 1:4 dilution was injected beneath the skin of the animal's right side. The control group of unimmunized mice received injections of the infecting dose of tumor at the same time.

On the 16th day after inoculation all the animals were sacrificed and the tumors weighed. The experimental results were treated statistically: the weight of the tumor by the Fisher-Student method, the proportion of successful implantations of the tumor by the formula of the "fourfold" table [7]. The mean weight of the tumor was calculated only for those animals in which the tumor was successfully implanted.

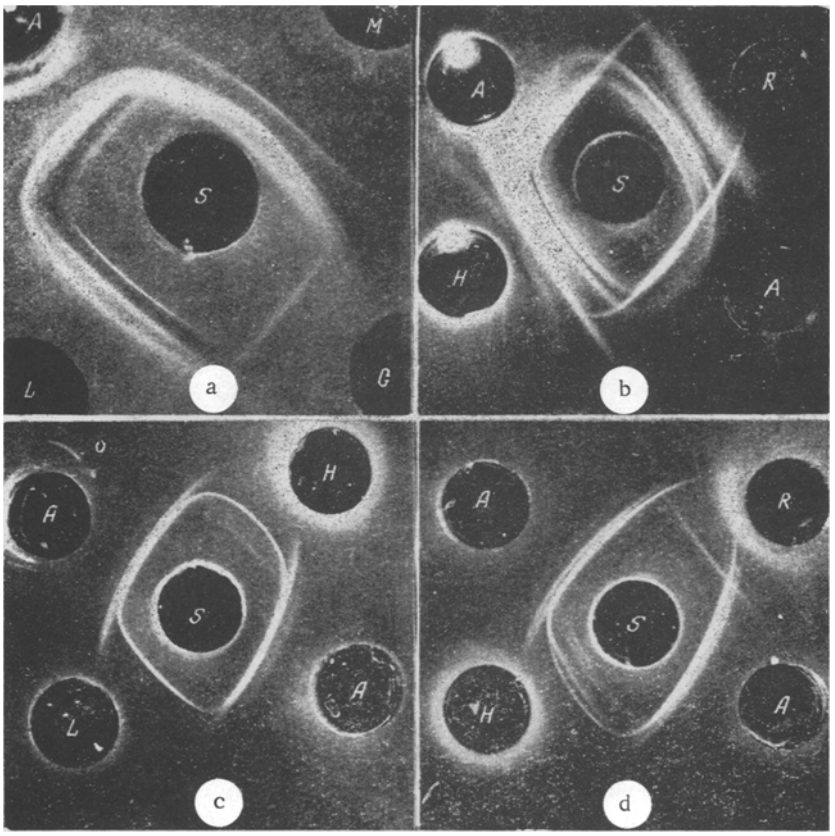

Fig. 1. Detection of specific and normal tissue and species antigens of Ehrlich's adenocarcinoma. a, b) Common antigenic behavior of tumor antigens, serum and spleen, liver and kidney of a normal mouse; c, d) detection of specific and normal tissue antigens of the tumor (adsorption by normal mouse serum in jelly). S) Antiserum to whole cells of Ehrlich's ascites adenocarcinoma; A) tumor; G) tumor globulin; R) kidney; M) mouse serum; H) liver; L) mouse spleen.

EXPERIMENTAL RESULTS

The antigens of the saline extract of the tumor were studied first. The antiserum to the tumor (Fig. 1, a, b) gave a series of common lines between the saline extract of the tumor and the antigens of normal organs: saline extracts of spleen, liver and kidney, and mouse serum. This suggests that the tumor contained a series of antigens common to the antigens of normal organs of mice.

After carrying out specific inhibition of precipitation with mouse serum, we found that most of the common lines disappeared. We called these disappearing antigens, serologically identical with serum proteins, conditionally species antigens. The antigens detected after specific inhibition of precipitation with mouse serum we called tissue antigens.

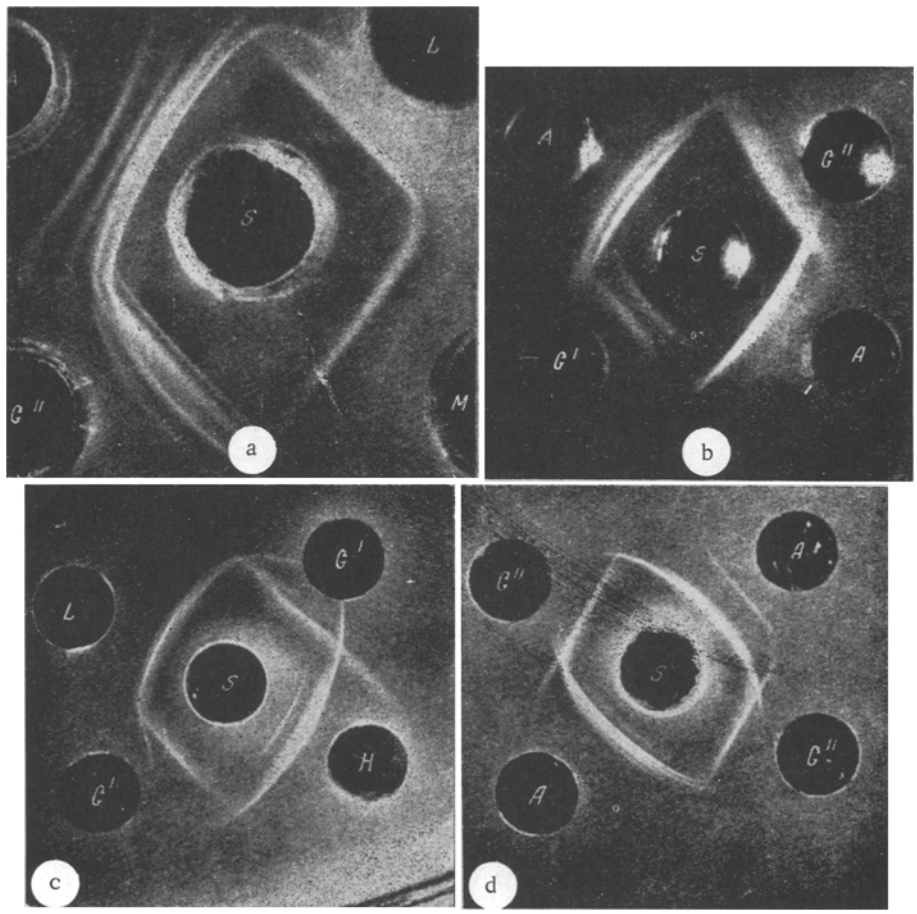

Fig. 2. Detection of tissue and species antigens of the globulin fractions of Ehrlich's adenocarcinoma. a, b) Common antigenic behavior between globulin fractions of the tumor and the saline extract of the tumor, the spleen and the serum of a normal mouse; c, d) detection of the tissue antigen of the globulin fractions of the tumor (adsorption by normal mouse serum in jelly). G', G") globulins I and II of the tumor. Remaining signs as in Fig. 1.

It is clear from Fig. 1, c, d that after saturation of the jelly with mouse serum, the saline extract gave two lines with antiserum to the tumor, suggesting the presence of two tissue antigens, differing from the serum, species proteins. One of these antigens was also present in the spleen and liver of the control mouse. The other antigen gave a clear additional line and was not present in normal organs.

Both these tumor antigens were absent from mouse kidney.

Vaccination of White Mice with Fractions of Ehrlich's Adenocarcinoma

Antigen	No. of animals	Total No. of tumors	No. of unsuccessful inoculations	Percentage of immune animals	Significance of differences between proportions of successful inoculations		Mean weight of inoculated tumors (in g)	Inhibition of growth of tumor (in %)
					χ^2 (comparison with controls)	χ^2 (comparison with vaccination in tail)		
Saline extract of tumor .	58	54	9	17	8.5	10	0.43	50
Globulin I of tumor. . . .	40	35	8	23	10	4.3	0.50	43
Globulin II of tumor . . .	43	41	9	22	12	5.3	0.55	45
Spleen	43	26	1	4	0.12	1.57	0.88	0
Globulin of liver	43	33	3	9	2.58	10	0.44	56
Vaccination in tail	72	46	22	48	28.8	–	0.27	69
Control	55	51	0	0	–	28.8	0.87	0

Note. The results of the three series of vaccination experiments are summarized in the table. The value of χ^2 was calculated from the "fourfold" formula [7]:

$$\chi^2 = \frac{(ad - bc - {}^1/_2 k)^2 \cdot k}{e \cdot f \cdot g \cdot h}$$

for $p = 0.05$, when $n = 1$, and $\chi^2 = 3.81$.

Titration experiments in jelly, using dilutions of antigen increasing in multiples of two, before and after specific inhibition of precipitation by mouse serum showed that the content of species antigens in the saline extract was twice as great as that of tissue antigens.

We next studied the antigenic structure of the globulin fractions of the tumor (globulin I and II), obtained by salting out with 46% and 50% saturated ammonium sulfate respectively [2].

Antiserum to the tumor (Fig. 2, a, b) gave a series of lines common to the globulin fractions of the tumor and saline extracts of the tumor, spleen and liver, and mouse serum. This suggests the presence of a series of common antigens in the material tested. As the experiments with specific inhibition of precipitation by mouse serum showed, this common antigenic behavior was chiefly due to the presence of serum, species antigens.

After saturation of the jelly with mouse serum, a tissue antigen was found in the globulin fractions (Fig. 2, c, d). This was serologically identical with the tissue antigen of the saline extract of the tumor and the spleen. We have not yet been able to establish to which of the two tissue antigens of the tumor that we found the tissue antigen of the globulin corresponds, nor whether it is a specific tumor antigen or a normal tissue antigen [1, 5, 11].

We thus found by means of the precipitation reaction in jelly that 1) Ehrlich's adenocarcinoma and its globulin fractions possess a number of common antigens with normal organs of the mouse, principally serum, species antigens; 2) the tumor possesses at least two tissue antigens differing from species antigens; one of them is specific for the tumor alone, the other is identical with the tissue antigens of the liver and spleen of the control mouse; 3) the globulin fractions have one tissue antigen identical with the tissue tumor antigen and the tissue antigen of mouse spleen.

The results of the three series of experiments on vaccination of white mice are shown in the table.

It will be seen from the table that the highest resistance was given by vaccination with the globulin fractions of the tumor (globulin I and globulin II)—23% and 22% of immune mice respectively—and the saline extract of the tumor—17% of immune mice. The statistical significance of the differences in the proportions of successful inoculations by comparison with the control group was very high: the values of χ^2 were 10, 12, and 8.5, respectively.

Immunization with the globulin of normal mouse liver (specificity control) caused resistance in 9% of mice; $\chi^2 = 2.58$. Vaccination by means of implantation of the tumor in the region of the tail caused a considerable increase in the resistance of the mice to subsequent inoculation of the tumor (48% of immune mice, $\chi^2 = 28.8$).

In the control group the tumor was successfully inoculated in all the animals. The figures for the mean weight of the inoculated tumors showed basically the same relationship. Vaccination with a saline extract of the tumor inhibited development of the inoculated tumors by 50%, vaccination with globulin I—by 43%, and with globulin II of the tumor—by 45% by comparison with the controls.

Vaccination with liver globulin of the control mouse inhibited growth of the tumor by 56%. Immunization with spleen affected neither the growth nor the success of inoculation of the tumors. Vaccination in the tail inhibited growth of the developing tumors by 69%.

Analysis showed that the differences between the rate of successful inoculation and the mean weight of the tumors in the groups vaccinated with the globulin fractions and with the saline extract of the tumor were not statistically significant. The vaccinating activity of the antigens of the globulin fraction of the tumor was thus not inferior to the vaccinating activity of the original saline extract of the tumor.

The absence of vaccinating effect after inoculation of spleen was contrary to the findings of Khaletskaya [6] and other authors, possibly because we used lyophilized preparations. The high vaccinating effect of the globulin of normal liver may be due to the fact that an important part in the relative immunity observed is played by a nonspecific component, in the form of normal species and tissue antigens. The question of the role of the normal antigenic component in immunity to tumors demands further study.

The efficacy of vaccination by means of implantation of the tumor into the tail significantly exceeds the efficacy of vaccination by all the other tumor fractions (see table). This result may evidently be explained by the fact that the products of tumor metabolism entering the blood stream from this area of vaccination are more highly antigenic than the tumor fractions, which have been subjected to comparatively gross chemical treatment. These results support the view [3, 4] that a living vaccine is more effective than a chemical vaccine. We are convinced, however, that the use of milder methods of isolation of the tissue antigens, and of other schemes and doses of vaccination will greatly increase the efficacy of anti-tumor vaccination.

We thus were able to demonstrate the presence of at least two tissue tumor antigens in a saline extract of the tumor, differing from serum antigens, one of which was specific for the tumor. In the globulin fractions one tissue antigen was found. The globulin fractions were not inferior to the saline extract of the tumor in vaccinating activity. Vaccination with the living tumor was more effective than chemical vaccination with fractions of the tumor.

SUMMARY

The author studied the antigenic structure of Ehrlich's adenocarcinoma and its globulin fractions in reaction of precipitation in gel. Two tissue antigens were detected in the tumor, both differing from the species antigens; one of these tissue antigens proved to be specific. One tissue antigen was found in the globulin fractions. The latter showed pronounced immunizing activity in experiments with vaccination of mice. Inoculation into the tail with a living tumor was more effective than chemical vaccination with tumor fractions.

LITERATURE CITED

1. G. I. Abelev and V. S. Tsvetkov, Voprosy Onkol. 6, 6 (1960) p. 62.
2. I. S. Bashkaev, Byull. Éksp. Biol. i Med. 49, 3 (1960) p. 93.
3. N. N. Zhukov-Verezhnikov, I. N. Maiskii, and V. S. Gostev, in: Malignant Neoplasms [in Russian] (Moscow, 1959) p. 147.
4. A. F. Zakharov, Byull. Éksp. Biol. i Med. 45, 2 (1958) p. 105.

5. A. K. Saakov, Byull. Éksp. Biol. i Med. 32, 8 (1952) p. 61.
6. F. M. Khaletskaya, Trudy Permsk. Med. Inst. 26 (1957) p. 89.
7. A. B. Hill, Fundamentals of Medical Statistics [Russian translation] (Moscow, 1956) p. 151.
8. H. B. Andervont, Publ. Hlth. Rep. 47 (Washington, 1932) p. 1859.
9. B. Björklund, Proc. Soc. Exp. Biol. 79 (New York, 1952) p. 319.
10. H. M. Hirsh, J. J. Bittner, H. Cole, et al., Cancer Res. 18 (1958) p. 344.
11. L. Korngold and G. Van Leeuwen, Ibid. 17 (1957) p. 775.
12. C. Martinez, J. B. Aust, J. J. Bittner, et al., Ibid. 17 (1957) p. 205.
13. O. Ouchterlony, Arkif. Kemi. 26 B (1948) p. 14.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
