

A COMPARATIVE STUDY OF THE ANTIGENIC STRUCTURE
OF TUMOR AND HOMOLOGOUS NORMAL TISSUE
REPORT V. INVESTIGATION OF THE ANTIGENIC PROPERTIES OF GUERIN'S
CARCINOMA, NORMAL UTERUS, AND RAT ERYTHROCYTES, USING THE METHOD
OF COMPLEMENT FIXATION AND THE METHOD OF TISSUE CULTURES

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In the previous reports [2-5], with the aid of immunological reactions, the method of tissue culture, and the method of in vitro inactivation of malignant cells circulating in the blood of the animal with the tumor, it was shown that aqueous-saline extracts and desoxyribonucleoproteids, extracted from transplanted carcinoma of the uterus and normal rat uterus, differ in their antigenic properties. In connection with this, interest lay in further studying the antigenic properties of tumor tissue and the erythrocytes of animals of homologous species, since antitumor sera, when used under experimental conditions, can cause undesirable phenomena — agglutination of erythrocytes or their destruction [6].

The purpose of this work was to study the antigenic properties of tumor tissue and erythrocytes of experimental animals, and to find a means of extracting antigens from the tissue in common with antigens from erythrocytes.

EXPERIMENTAL METHOD

For a comparative study of the antigenic composition, we used tissue from rat, transplanted, uterine carcinoma (Guerin's strain), and normal rat erythrocytes. As a control, we took normal uterine and kidney tissue from the animal of the same species. The indicated subjects were subjected to immunological study, using the reaction of complement fixation (RCF) in its classical form, and employing the method of tissue culture.

Rabbits of the chinchilla family were immunized according to the schema described earlier [1], using an aqueous-saline extract of Guerin's carcinoma tissue, and normal rat erythrocytes washed three times in physiological saline. The immune sera obtained were used in the RCF. The aqueous-saline extracts of tissues from Guerin's carcinoma, normal uterus, kidney, and erythrocytes served as the antigens. In the biological experiments tissue cultures of Guerin's carcinoma and mucous membrane epithelium of normal uterus and kidney of rats were acted upon by these immune sera.

In order to remove from the aqueous-saline extract those antigens from tumor tissue in common with antigens from erythrocytes the following experiments were set up. Sera from rabbits immunized with erythrocytes were mixed with a 10% aqueous-saline extract of tissue from Guerin's carcinoma, the extract having first been centrifuged at 2500 rpm for 10 min. The mixtures were made in two proportions: 1) one volume of serum to one volume of extract (1:1); 2) four volumes of serum to one volume of extract (4:1).

This mixture, to which we added complement from guinea pig serum, was placed in a refrigerator at 4° for 24 or 48 h. The control was represented by mixtures of sera from normal rabbits and the extract, with the addition of complement. After standing in the refrigerator, two layers formed in the mixtures of immune sera with extract: a supernatant fluid and a dark-brown, flocculent precipitate, which settled into a sediment. We did not observe the same picture in the control. The supernatant fluid was then carefully removed from the test tube and used for immunization of rabbits. The remaining precipitate was diluted with a volume of normal rabbit serum equal to the super-

TABLE 1. Immunological Characterization of Antitumor and Antierythrocyte Sera, Precipitate and "Depleted" Tumor Antigens in the Reaction of Complement Fixation

Serum dilution	Sera of rabbits immunized with															
	aqueous-saline extract from Guerin's carcinoma				whole erythrocytes from normal rats				precipitate settling into a supernatant fluids, formed from the mixing of anti-sediment from the mixing of antierythrocyte sera with tumor extract in the ratio of							
									1 : 1							
									1 : 4							
Serum dilution	antigens from															
	Guerin's carcinoma	erythrocytes	uterus	kidney	Guerin's carcinoma	erythrocytes	uterus	kidney	Guerin's carcinoma	erythrocytes	uterus	kidney	Guerin's carcinoma	erythrocytes	uterus	kidney
1 : 20	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
1 : 40	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
1 : 80	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
1 : 160	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
1 : 320	+++	h	+++	h	+++	h	+++	h	+++	h	+++	h	+++	h	+++	h
1 : 640	+++	h	++	h	h	h	h	h	++	h	h	h	h	h	h	h
1 : 1280	h	h	h	h	h	h	h	h	h	h	h	h	h	h	h	h

natant fluid studied in tissue culture, and used for immunization of rabbits. A total of 14 rabbits were taken for immunization. The RCF was performed 2 or 3 times before obtaining the definitive results.

In the tissue cultures we used the method described earlier [3]. In this case we took into consideration both the total amount of growing explants and the intensity of their growth, for which we calculated an average growth coefficient. The tissues were cultivated for 3-4 days.

The components of the fluid culture medium consisted of sera from normal and immunized rabbits, complement from guinea pig serum, a 20% extract of 8-day old rat embryos, a balanced salt solution, and a solution of penicillin (100-150 units per Karrel's cup). The immune sera were diluted in the proportions of 1:2 and 1:4. Absolutely fresh rabbit sera were used in the experiment.

EXPERIMENTAL RESULTS

I. Immunological Investigations. Table 1 gives the immunological characterization of the antitumor and anti-erythrocyte sera, as well as the sera of rabbits immunized with the precipitate and the supernatant fluids. The antitumor sera reacted with homologous antigen in a dilution of 1:640 by +++, with the antigen from normal uterus - approximately in the same dilution and with the antigens from the kidney and erythrocytes - in a dilution of 1:160 by +++ and +++. The sera of the rabbits immunized with whole erythrocytes reacted in high dilutions with homologous antigen (in a dilution of 1:1280 by ++++), and more weakly with antigen from tumor tissue (in a dilution of 1:640 by +), and reacted in the same manner with antigens from normal uterine and kidney tissue. The sera of rabbits immunized with the precipitate that settled into a sediment upon mixing of antierythrocyte serum and aqueous-saline extract of tumor tissue reacted with the antigen from Guerin's carcinoma in a dilution of 1:320 by ++, in the same manner with antigen from normal uterus, and in a somewhat lower dilution with antigen from erythrocytes (in a dilution of 1:80 by ++). These data show that in the tissues of Guerin's carcinoma, normal uterus, kidney, and in erythrocytes there are common antigens, which, in all probability, determine their general species - specific properties.

The sera of rabbits immunized with the supernatant fluids reacted to a large degree with the antigens from tissues of Guerin's carcinoma and normal uterus, and to a considerably smaller degree with the antigen from erythrocytes. Thus, the sera of rabbits immunized with the supernatant fluids in a ratio of 1:1 reacted with the tumor antigen in a dilution of 1:640 by ++, somewhat weaker with the antigens from normal uterus (1:320 by +++), in the same manner with the antigen from the kidney, and considerably weaker with the antigen from erythrocytes (in a dilution of 1:40 by +++).

TABLE 2. The Results of Cultivating Tissues from Guerin's Carcinoma, and Normal Uterus and Kidney of Rats in Immune Sera Over a Course of 3 Days

Subject under study	No. of experiments	Guerin's carcinoma			Uterus			Kidney		
		no. of cultures in the experiment	growth	average growth coefficient	no. of cultures in the experiment	growth	average growth coefficient	no. of cultures in the experiment	growth	average growth coefficient
Sera of non-immunized rabbits	4	214	142 66.3	1.22	233	161 68.0	1.07	259	221 85.3	1.15
Sera of rabbits immunized with aqueous-saline extract of Guerin's carcinoma	4	338	1 0.2	0.02	272	4 1.5	0.27	280	9 3.2	0.11
Sera of rabbits immunized with aqueous-saline extract of Guerin's carcinoma	4	235	4 1.7	0.24	240	9 3.7	0.15	242	18 7.9	0.19

Note. In the numerator - the number of growing cultures in absolute units, in the denominator - in percents.

A more clearly defined difference in the antigenic structure was observed in the CFR of the sera from rabbits immunized with the supernatant fluids in a ratio of 1:4. In this case, the sera reacted with the antigen from Guerin's carcinoma in a dilution of 1:320 by ++, in the same dilution with the antigen from normal uterine tissue, somewhat weaker with antigen from kidney tissue (in a dilution of 1:80 by ++), and in a low dilution with the antigen from erythrocytes (in a dilution of 1:20 by +++).

These data show that by the method of "depletion" of the aqueous-saline extracts of tumor tissue by anti-erythrocyte serum it is possible, to a certain degree, to remove antigens from the tissue which are in common with the antigens from erythrocytes. In addition, the results of these CFR's testify that in tumor tissue (its aqueous-saline extract) there are at least three types of antigens: specific, which was shown in our previous reports [3-4], generally specific (inherent in tumors and erythrocytes), and tissue (common to tumor tissue and tissue of the homologous normal organ).

"Depletion" of the aqueous-saline extract of tumor tissue with antierythrocyte serum may be described in the following manner. In the antierythrocyte serum there are generally specific antibodies and antibodies pertaining to specific antigens of erythrocytes. The antibodies of the first type, binding in the aqueous-saline extract with antigen common to erythrocytes and cancer tissue (generally specific), remove it from the mixture and settle out in the form of a sediment. Along with this, other antigens remain in the supernatant fluid, which are inherent to tumor tissue: specific and tissue antigens. With immunization of the rabbits by these supernatant fluids, antibodies are developed to the specific and tissue tumor antigens, and in a lesser degree, to antigens common to erythrocytes.

II. Investigations in Tissue Cultures. Table 2 presents the total data of 4 experiments on cultivation of tissue from Guerin's carcinoma, and normal uterus and kidney of rats, over a period of 3 days.

On cultivating these tissues in the sera of normal rabbits the following picture was observed. Out of 214 explants of cancer tissue, growth occurred in 142 (66.3%); out of 233 explants of uterine tissue - 161 (68%); and out of 259 explants of kidney tissue - 221 (85.3%). These data show that the sera of normal rabbits do not exert a noticeable inhibitory action on the growth of experimental cultures.

A different picture was observed with cultivation of the tissues in the sera of rabbits immunized with aqueous-saline extract of tissue from Guerin's carcinoma and with erythrocytes. Thus, out of 235 explants of cancer tissue, cultured in antierythrocyte serum, growth occurred in only 4 cultures (1.7%), with an average growth coefficient of 0.24 (in the control the growth coefficient was equal to 1.22). Out of 240 explants of uterine tissue, with explantation to these sera, growth occurred in 9 cultures (3.7%), with an average growth coefficient of 0.15 (in the control, 1.07), and out of 242 explants of kidney tissue - 18 cultures grew (7.9%), with an average growth coefficient of 0.19 (in the control, 1.15).

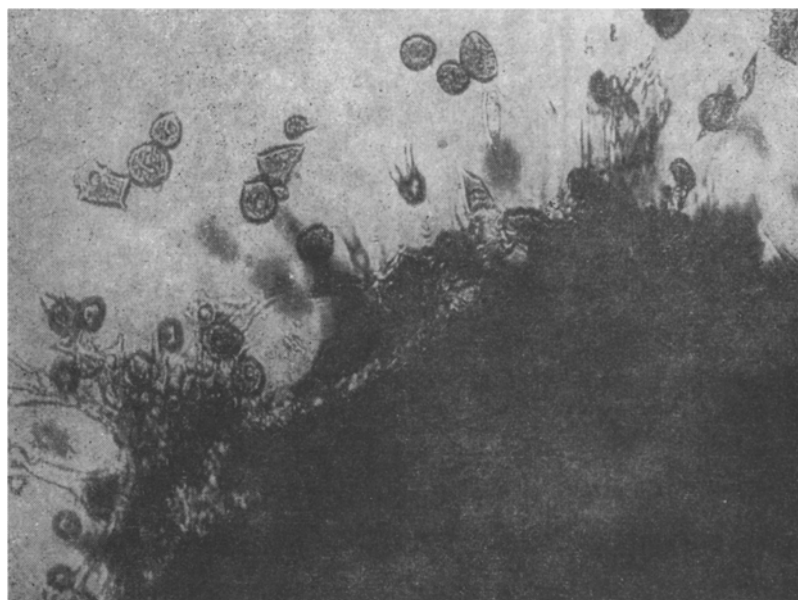


Fig. 1. Three-day old culture of tissue from Guerin's carcinoma, explanted to the serum of a rabbit immunized with whole rat erythrocytes. Magnification 258X.



Fig. 2. Living 3-day culture of tissue from Guerin's carcinoma, explanted in the serum of a normal rabbit. Magnification 258X.

It must be noted that antierythrocyte sera exert not only an inhibitory action on the growth of experimental cultures, but also cause destructive changes in the cells of the growth zone. Thus, Fig. 1 shows a 3-day culture of tissue from Guerin's carcinoma, explanted in antierythrocyte serum; the zone of growth in this case consists of single, migrating, altered cells of small size, with round form and coarsely granular protoplasm. The control (Fig. 2) yielded a living 3-day culture of Guerin's carcinoma, growing in the serum of a normal rabbit. The growth zone in this case presented another picture. It consisted of intensely multiplying cells of varying form and dimensions.

On cultivation of these tissue in antitumor sera an analogous picture was observed. In this case, growth was depressed in the experimental cultures of both homologous tissue and the tissues of the uterus and kidney. These data show that in the tissues of Guerin's carcinoma, normal uterus, and kidney, and in erythrocytes, there are common antigens, since the antibodies formed against these antigens exert an inhibitory influence on all the experimental cultures.

Thus, with the aid of CFR and the method of tissue cultures, it was possible to demonstrate that there are antigenic properties in common between tissues of Guerin's carcinoma, normal uterus, and kidney, and the erythrocytes of rats. In all probability, these common properties are determined by a generally specific antigen, which, to a certain degree, can be removed from the aqueous-saline extract of tumor tissue by the addition of antierythrocyte rabbit serum and guinea pig serum complement. In such a "depleted" extract there remain antigens that are in common with the antigens in the tissues of the homologous normal organ.

SUMMARY

Sera of rabbits, immunized with water-salt extract from the tissue of Guerin's carcinoma and normal rat erythrocytes, react with the antigens of the tumor tissue, the uterus; kidney, and erythrocytes. This points to the presence of a common antigen in these tissues and erythrocytes. It is possible to extract (to a considerable extent) antigens common to the erythrocyte antigens from the water-salt extract of tumor tissue by means of antierythrocytic serum in the presence of the guinea pig serum complement. Antigens, common to the tissue of a homologous normal organ (uterus), are retained in such an exhausted extract. These data show that at least two types of antigens are present in the tumor tissue (along with other antigens) i.e., species and tissue ones. The commonness of antigenic properties of Guerin's carcinoma, normal uterus, kidney tissue, and erythrocytes were also demonstrated by the tissue culture method.

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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.
