ANTIGENIC DIFFERENCES BETWEEN TUMOR TISSUES AND HOMOLOGOUS NORMAL TISSUES REVEALED BY MEANS OF NORMAL TISSUE ANTIBODIES

(UDC 616-006-018-097-07)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 6,
pp. 81-86, June, 1965
Original article submitted August 1, 1964

Certain malignant and normal tissues from man and animals differ from one another antigenically [2, 3, 5, 7, 10]. These differences may be discovered by means of the reaction of anaphylaxis with desensitization, and also by the use of heterogenic immune sera in immunological reaction.

Previous investigations by other authors and ourselves have shown that the sera of normal animals can give a positive reaction in immunological tests with saline extracts from autologous and homologous tissues [6, 8, 9, 12-15]. Such reactions are evidently due to the presence of normal tissue antibodies in these sera, although some authors deny that this is so [12, 15], for the active component in the normal sera was discovered in the albumin fraction. The view is also held that the sera contain substances of the nature of the antibodies found in globulin fractions [8, 9].

This has been shown by means of fractionation of the sera by salting out, and also by immunoelectrophoresis, and it has also been observed that normal sera react with antigens in the manner of the immunological reaction of antigen with antibody [3, 14, 15].

It was decided to investigate how normal tissue antibodies would react with saline extracts from normal and homologous tumor tissues. The study of this problem may be of importance to the elucidation of the nature of the specificity of normal tissue antibodies.

EXPERIMENTAL METHOD

The test objects chosen were the following tumor tissues and homologous normal tissues of Wistar rats: transplantable carcinoma of the uterus (Guerin's carcinoma) and uterus, transplantable hepatoma and liver, and transplantable carcinoma of the kidney (RA) and kidney. The tumors were transplanted into rats of both sexes, aged 3-6 months. The ability of the sera of normal animals to react positively with saline extracts from these tissues was studied by means of the complement fixation reaction (CFR), Ouchterlony's gel precipitation reaction (GP), and the reaction of immunoelectrophoresis (IEP). The CFR was conducted by the classical method at 37° and in the cold, and the GP by the usual method. Two types of agar were used for the reactions: from the firm of "Difco", and from China, made up in physiological saline. The IEP was carried out by the usual method [3] at room temperature and under the following conditions: voltage 75 V, potential difference 8 V/cm, current 30 mA, pH of medinal-veronal buffer 8.6, M 0.05. The agar and antigens were made up in veronal-medinal buffer at pH 8.6. The antigens were diluted in the proportion 1 : 6. The results of the reactions were read after 24-120 h.

Since the sera from normal animals reacted in the CFR in most cases and most intensively with saline extracts from kidney tissue, we decided to study how the normal sera of rats bind different fractions of this organ. The kidney tissue was differentially fractionated into nuclei, mitochondria, and microsomes with hyaloplasm. The same fractions isolated from kidney carcinoma tissue were also used in the reaction. The fractionation of the tissue was carried out in the cold by the usual technique [1, 4].

Results of CFR with Interaction between Sera of Normal Rats and Saline Extracts from Tumor Tissues and Homologous Normal Tissues

Source of saline extract	No. of sera	Intensity of reaction	Dilution of sera				Number of sera reacting in dilution of 1 : 10	
			1 : 10	1 : 20	1 : 40	1 : 80	absolute	%
Uterus	50	++	10	5	5	2	23	46
		+++	10	12	5	0		
		++++	3	3	3	0		
Carcinoma of uterus	50	++	2	2	0	0	5	10
		+++	3	2	1	0		
		++++	0	0	0	0		
Liver	30	++	12	8	0	0	18	60
		+++	6	2	0	0		
		++++	0	0	0	0		
Hepatoma	30	++	2	2	0	0	4	13,3
		+++	2	0	0	0		
		++++	0	0	0	0		
Kidney	50	++	18	21	15	4	48	96
		+++	21	17	5	0		
		++++	9	5	0	0		
Carcinoma of kidney	50	++	0	0	0	0	0	0
		+++	0	0	0	0		
		++++	0	0	0	0		
Fraction of mitochondria of kidney	25	++	2	7	8	13	23	96
		+++	4	4	12	2		
		++++	17	14	3	2		
Fraction of microsomes with hyaloplasm of kidney	25	++	6	7	6	3	15	60
		+++	6	4	0	0		
		++++	3	1	0	0		
Fraction of ground nuclei of kidney	25	++ +++ ++++	0	0	0	0	0	0
Fraction of mitochondria, microsomes, and hyaloplasm of carcinoma of kidney	25	++ +++ ++++	0	0	0	0	0	0

In the CFR and GP, the antigens used were saline extracts of the tissues and of their fractions. Since the fraction of microsomes with hyaloplasm contained less protein than the other antigens, it was concentrated by McErlean's method [11]. The fifth fraction of concentrated microsomes with hyaloplasm was used in the reactions, equal in its protein content to the remaining antigens. All the antigens taken for the immunological reactions were equal in their protein content by Kjeldahl's method.

Preliminary experiments showed that the best results were obtained in the GP with antigen in dilution of 1 : 4 and with whole serum. In the GP and IEP the sera used were obtained from animals on the day of the experiment, and these sera were tested in the CFR next day.

EXPERIMENTAL RESULTS

The results obtained in the CFR with the sera of normal rats and saline extracts from tumor tissues and homologous normal tissues are given in the table.

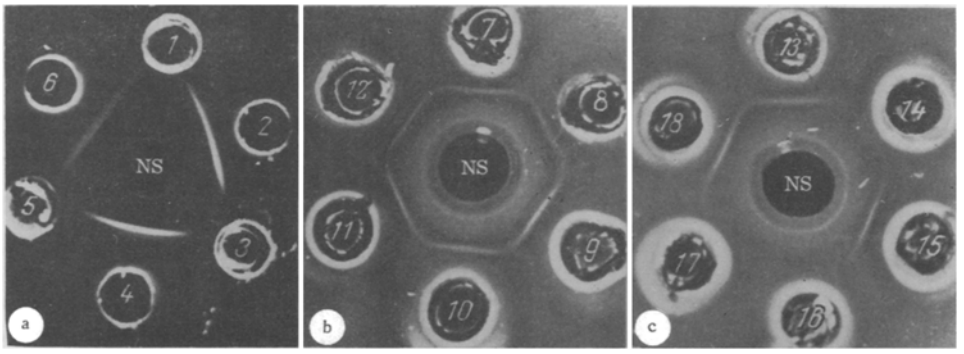

Fig. 1. Gel precipitation reaction between sera of normal rats and antigens from normal and tumor tissues. NS) Sera of normal rats; 1) antigen from kidney carcinoma tissue; 2) antigen from fraction of mitochondria of kidney; 3) antigen from fraction of mitochondria of kidney carcinoma; 4) antigen from kidney tissue; 5) antigen from fraction of microsomes with hyaloplasm from kidney carcinoma; 6) antigen from fraction of microsomes with hyaloplasm of kidney; 7, 13) antigens from autologous liver tissue; 8, 9, 10, 11, 12, 15, 18) antigens from homologous liver tissue; 14, 16, 17) antigens from hepatoma tissue.

The table shows that the sera of normal rats reacted with saline extract from tissue of the uterus in a dilution of 1 :10 in 23 of 50 cases (46%), whereas they reacted with the saline extract from the tissue of the uterine carcinoma in only 5 of 50 cases (10%). In a dilution of 1 :80, the same sera reacted with the saline extract of normal uterine tissue in 2 cases (4%), while they did not react in a single case with the saline extract of the tissue of the uterine carcinoma in the same dilution. So far as the reaction of the normal sera with saline extracts from normal liver and hepatoma tissue is concerned, here too, a similar picture was observed. Whereas the sera reacted in a dilution of 1 :20 with the saline extract from the liver tissue in 13 of 30 cases (43%), they reacted with the saline extract of the hepatoma tissue in the same dilution in only 2 of 30 cases (6.6%).

Particularly demonstrative results were obtained in the CFR with the sera of normal rats and saline extracts from the tissue of normal kidney and carcinoma of the kidney. For instance, with the saline extract from kidney tissue 48 of 50 sera (96%) reacted in a dilution of 1 :10 and only 4 (8%) in a dilution of 1 :80. Not one serum reacted with the saline extract from the kidney carcinoma tissue, even in a dilution of 1 :10. It should be noted that the majority of the sera reacting positively with saline extracts from normal tissues did not react in the same experiment with the homologous malignant tissues. For example, not one of the 18 sera reacting with the saline extract from uterine tissue did so with antigens from the tissue of the uterine carcinoma.

The investigations showed that normal tissue antibodies are capable of binding in the CFR with certain specified components of normal tissue, which either are absent from malignant tissue or are present in it in small amounts, or finally, which have modified their structure. In all cases the sera of the normal animals reacted more intensively with the fractions of the mitochondria and of the microsomes with hyaloplasm, and reacted rather less intensively with whole saline extract of normal kidney. In no case was a positive CFR observed with the fraction of kidney nuclei, washed and ground five times with powdered glass. These results suggest that the components reacting with normal tissue antibodies are mainly found in the fractions of the mitochondria and of the microsomes with hyaloplasm.

All the fractions mentioned above, isolated from kidney carcinoma tissue, gave no reaction with the sera from normal animals, even in a dilution of 1 :10. This suggests that the active component present in the mitochondria and in the microsomes with the hyaloplasm of normal tissue and reacting with normal tissue antibodies is absent from malignant tissue or present in a modified form. If the component responsible for binding normal tissue antibodies were present in malignant tissue in a smaller amount than in normal, positive reactions would have been expected either with the fraction of the mitochondria or in the microsomes with hyaloplasm of the malignant tissue. This, however, was not observed in the experiments.

The gel precipitation reaction with whole saline extract from kidney tissue was carried out with 164 sera of normal rats. One line of precipitation was obtained in 128 cases (78.4%). In no case was a precipitation line obtained with whole saline extract from kidney carcinoma tissue. Likewise no precipitation line was formed with

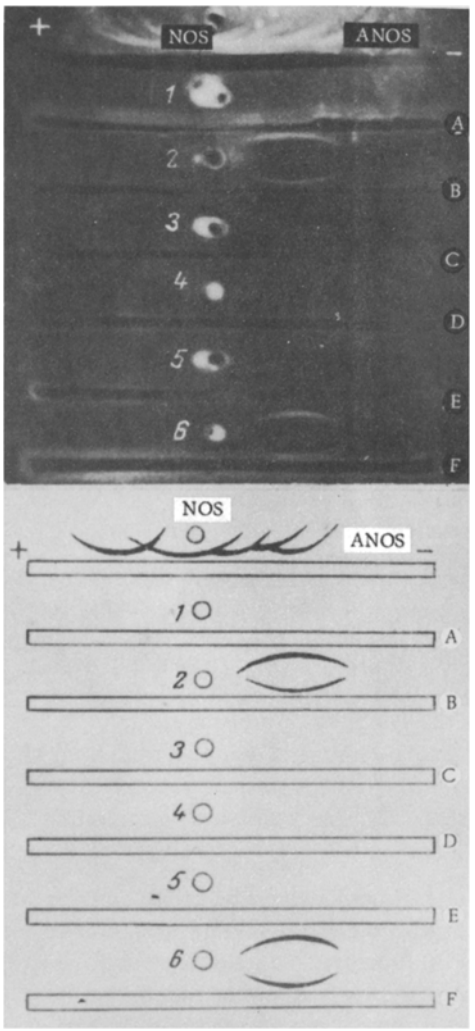

Fig. 2. Immunoelectrophoresis of sera of normal rats with antigens from autologous and homologous liver tissues. NOS) normal of serum; ANOS) antiserum against normal of serum; A, B, C, D, E, F) sera of normal rats; 1, 3, 4, 5) antigens from liver tissue; 2) antigen from liver autologous to serum A; 6) antigen from liver tissue autologous to serum E.

the fractions of ground mitochondria, microsomes with hyaloplasm, and nuclei of the kidney carcinoma tissue. Lines of precipitation were formed with the fractions of ground mitochondria and of microsomes with hyaloplasm of normal kidney

The sera of normal rats formed clear precipitation lines with the saline extract from liver tissue, but gave no lines with the saline extract from the hepatoma tissue (Fig. 1). The best results were obtained in the GP with "Difco" agar.

Since the problem of the nature of normal tissue antigens remains unsolved, an attempt was made to determine how the sera of normal rats would react in the IEP with antigens from normal tissues. Most investigators have used antigen from liver tissue for this purpose. The same antigen was used in our experiments. The results obtained showed that lines were formed in the IEP in the zone of the γ-globulins (Fig. 2). However, in the same experiment the sera of the normal rats did not always give positive results in the IEP. For instance, only 2 of the 20 sera formed lines. These results are in agreement with those obtained by other authors, who found that the sera of normal animals have active components of antibody type, contained in the γ-globulin fraction.

Hence, two immunological reactions showed that the serum of normal rats reacts differently with antigens from tumor tissues and from the homologus normal tissues. Various authors have suggested that malignant tissue contains no organ-specific antigen [16, 17]. It may be concluded from the results described above that normal tissue antibodies mainly react positively with the organ-specific antigens of normal tissues, which are absent or which exist in a modified form in the tumors which were investigated.

It has been pointed out that the positive reaction of normal human sera and antigens occurs only in the GP and IEP, and not in other reactions [15]. We were unable to detect a positive fixation of the above-mentioned components in the CFR. However, by using normal rat sera and antigens, clearly positive results were obtained in the CFR, especially with antigens from kidney and liver tissue.

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