THE PRODUCTION OF SPECIFIC ANTISERA TO THE NUCLEOPROTEINS OF HUMAN CARCINOMA TISSUES

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The production of specific antisera to nucleoproteins is especially important in connection with obtaining antisera to carcinoma tissues both in man and animals. The difficulty of immunological investigation of desoxyribonucleoproteins (DNP) is that they are very labile compounds, and if strong chemical or physical agents are used they undergo rapid destruction, in which case their immunological properties completely disappear [2, 8, 7].

Our object in the present investigation was to show that in the production of antisera containing antibodies to desoxyribonucleoproteins, the method of preparation of the antigens is of the utmost importance.

EXPERIMENTAL METHOD

For the serological investigation of the desoxyribonucleoproteins we employed the reaction in which protein is bound on to absorbent cotton fabric [1].

The DNP was obtained by the method of Mirsky and Pollister [9] from human gastric carcinoma tissue, frozen in dry ice immediately after operation. The nitrogen -phosphorus ratio N/P* of repeatedly purified (3 to 7 times) desoxyribonucleoproteins was from 3.7 to 4.2.

The antisera were prepared jointly by the Laboratory of Immunochemistry of the Institute of Experimental Biology and the N. F. Gameleya Institute of Epidemiology and Microbiology by means of the subcutaneous immunization of 2 horses with human gastric carcinoma tissue.

As a first step, the horses were immunized with human gastric carcinoma tissue, taken from a cadaver [6]. Antigens were prepared by grinding the tissue in a mortar containing a small quantity of powdered glass in distilled water. After 24 hours the mixture was centrifuged, and sodium chloride was added to the centrifugate to give a final concentration of 0.85%. The globulins were then separated from the saline extracts with 30% saturated ammonium sulfate, and these were used as antigens for the immunization.

The sera obtained were tested for their content of antibodies to saline extracts and to globulins [6], and also for the presence of antibodies to crude DNP isolated from human gastric carcinoma tissue by means of the protein binding test on adsorbent cotton fabrics [1].

EXPERIMENTAL RESULTS

It follows from the results given in Table 1 that the quantities of protein in the test samples "A + DNP + normal horse serum" and "A + DNP + anticarcinoma horse serum" were practically identical. The absence of

* The nitrogen was determined by Conway's method, the phosphorus by the method of Fiske and Subba Row.

TABLE 1

Reaction of the DNP of Human Gastric Carcinoma Tissue with Carcinoma Antisera from Horses, Expressed as mg of Protein as Nitrogen (the sera were obtained by immunization of horses with globulins from gastric carcinoma tissue from a human cadaver)

Experiment No.	Adsorbent (A)	A + DNP	A + DNP + normal horse serum	A + DNP + horse anti-carcinoma serum
78	0.018	0.110	0.540	0.550
80	—	0.070	0.245	0.165
81	0.020	0.120	0.525	0.542
82	—	0.093	0.612	0.630
83	0.016	0.125	0.840	0.822

TABLE 2

Reaction of the DNP of Human Gastric Carcinoma Tissue with Anticarcinoma Rabbit Sera, Expressed as mg of Protein as Nitrogen (sera were obtained by immunization of rabbits with saline extracts or pulp of human gastric carcinoma tissue taken at operation).

Expt. No.	A + DNP	A + DNP + normal rabbit serum	A + DNP + serum against a saline extract of human gastric carcinoma tissue	A + DNP + serum against pulped human gastric carcinoma tissue
84	0.227	0.997	1.015	1.520
			1.050	1.670
92	0.210	0.962	0.910	1.295
			0.980	1.405
93	0.262	1.225	1.295	1.732

a specific reaction may be due to the fact that the horse antigens used for the immunization did not contain natural desoxyribonucleoproteins, on the one hand because human cadaver tissue was used for the immunization, and on the other hand because the method of preparing the antigens itself prevented the possibility of their containing any DNP.

Further study of this problem showed that immunization of animals with saline extracts of human gastric carcinoma tissue taken directly from the operating table, or with their globulin fraction, does not give rise to the formation of antibodies of desoxyribonucleoproteins. If the animals were immunized with fresh, pulped tissue, the sera then contained antibodies to DNP. These investigations were carried out on sera obtained by immunization of rabbits. The animals were divided into two groups. The first group (5 rabbits) was immunized by intravenous injection of saline extracts of human gastric carcinoma tissue taken directly from the operating table. The second group, also of 5 rabbits, was immunized by intraperitoneal injection of pulped human gastric carcinoma tissue, removed at operation. The tissue was homogenized in the cold in physiological saline, to which was added desoxyribonuclease sodium citrate, in concentration of 0.01 M [5], as an inhibitor.

Immunization of both first and second groups was carried out every fourth day for 24 days. Sera were obtained on the 8th day after the last immunizing injection of the animals.

TABLE 3

Reaction of the DNP of Human Gastric Carcinoma Tissue with Anticarcinoma Horse Sera, Expressed as mg of Protein as Nitrogen (sera were obtained by immunization of horses with homogenates of fresh human gastric carcinoma tissue removed at operation).

Experiment No.	A + DNP	A + DNP + normal horse serum	A + DNP + anticarcinoma horse serum	
102	0.122	0.350	0.584	—
103	—	0.175	0.455	0.507
109	0.070	0.262	0.453	0.385
110	0.060	0.109	0.262	0.240
113*	0.183	0.700	1.146	—
115	0.175	1.155	1.700	—
116	0.157	1.260	1.960	—

* In experiments Nos. 113, 115 and 116 a waffle-woven cloth was used instead of the ordinary cotton fabric.

TABLE 4

Reaction of the DNP of Human Gastric Carcinoma Tissue with Anticarcinoma Horse Serum and Its Fractions (reaction expressed as mg of protein as nitrogen)

Expt. No.	A	A + DNP	A + DNP + anticarcinoma horse serum	A + DNP + γ-globulin (precipitation with methanol)	A + DNP + γ-globulin (precipitation with ethanol)	A + DNP + $d_1d_2\beta$-globulins	A + DNP + albumins
111	0.023	0.245	0.857	0.760	0.735	0.560	0.402

It can be seen from Table 2 that the desoxyribonucleoproteins of human gastric carcinoma tissues do not give a specific reaction with rabbit sera produced against saline extracts of these tissues. Conversely, in tests in which the sera used had been obtained against whole homogenate of human gastric carcinoma tissue, marked specific fixation of the protein of the antisera was observed.

The immunization of animals with saline extracts (0.85% NaCl) does not give rise to the formation of antibodies to DNP because the DNP are extracted at either very high or very low ion concentration of the medium. During the preparation of antigens by saline extraction, the DNP as a rule remains in the residue, and this extract does not cause the formation of antibodies against DNP, whereas immunization of animals with carcinoma tissue homogenate does give rise to the formation of antibodies to all the constituents of the cell, including the DNP.

In 1957 we showed that immunization of rabbits with normal human stomach tissue and with human gastric carcinoma tissue, taken from the cadaver, in contrast to the fresh tissue removed at operation does not give rise to the formation of antibodies to the natural desoxyribonucleoproteins [2].

This phenomenon is evidently due to the fact that the action of the enzyme desoxyribonuclease on desoxyribonucleic acid, which is a component part of the DNP, deprives it of both its serological and immunological properties [7, 8].

On the basis of the results given above, in a combined laboratory investigation [3] horses were immunized with homogenate made entirely from fresh human gastric carcinoma tissue. Such tissue, taken at operation, was at once placed on ice. The pooled operation specimens of human gastric carcinoma tissue were homogenized on ice in physiological saline with addition of sodium citrate. The homogenate was filtered through a layer of gauze to remove large and insufficiently ground particles, and the filtrate, consisting of the entire cell contents, acted as antigen for the immunization.

The horse sera obtained were usually tested for their content of antibodies to saline extracts of carcinoma and normal tissues [3, 4], and the presence or absence of antibodies to natural desoxyribonucleoproteins was established.

As may be seen from the results shown in Table 3, the sera obtained against fresh human gastric carcinoma tissue, taken at operation, contain antibodies to desoxyribonucleoproteins. The quantity of protein in the test samples "A + DNP + anticarcinoma horse serum" was considerably higher than that found in the test samples "A + DNP + normal horse serum".

On the basis of the data in the literature and our laboratory findings, anticarcinoma horse sera were subjected to fractionation by the method of precipitation with methanol and ethanol*. Investigation of the fractions thus obtained for the presence of antibodies to both saline extracts [3, 4] and to natural desoxyribonucleoproteins showed that the main serological activity of the sera is contained in the γ-globulin fraction.

Table 4 shows the results of the serological reaction of the desoxyribonucleoproteins with anticarcinoma horse serum and with its fractions.

The results of a typical experiment, as shown in Table 4, demonstrate that almost the whole of the serological activity towards DNP of the anticarcinoma horse serum obtained against fresh human gastric carcinoma tissue, taken at operation, is contained in the γ-globulin fraction.

On the basis of the results reported in the present communication, it may be concluded that for the production of fully effective antisera containing antibodies against the DNP, it is desirable to use homogenates of fresh tissue, taken at operation, for the antigen. The antigens must be prepared in such a way that the desoxyribonucleoproteins are not destroyed, otherwise the resulting sera will not contain antibodies against these compounds.

SUMMARY

The method of preparation of the antigens is of paramount significance for the obtaining of antiserums containing the antibodies to desoxynucleoproteins (DNP). Immunization of animals with water-salt extracts of the human stomach cancer tissue (obtained at autopsy and during operations), as well as by its globulins does not cause formation of antibodies to DNP.

Antibodies of DNP may be obtained in case of immunization of animals with homogenates of fresh surgical tissues which is especially important for therapeutic serums. The main serological (DNP) activity is contained in the γ-globulin fraction of the anticancer horse serum obtained by immunization with fresh surgical human cancer tissues.

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