DETERMINATION OF THE SEROLOGICAL ACTIVITY OF THE DESOXYRIBONUCLEOPROTEINS (DNP) OF HUMAN GASTRIC CARCINOMA TISSUES AFTER ADSORPTION ON COTTON FABRIC

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V. S. Gostev and D. G. Grigor'ian [1, 2, 3] devised a new method of serological study of the desoxyribonucleoproteins (DNP), based on the specific fixation of the proteins of antisera on cotton fabric adsorbents soaked with DNP.

In the present investigation our aim was to ascertain the possibility of studying the serological activity of DNP in another reaction – in the quantitative complement fixation reaction.

The CFR method of Heidelberger [6] and of A. P. Konikov [4] was used by V. S. Gostev and N. A. Shagunova [2, 5] with a 50% titer, and modified to study the azoproteins fixed on paper. We utilized this method for investigating the serological activity of desoxyribonucleoproteins adsorbed on cotton fabric.

Preparation of test antigens of DNP. Nuclear DNP were obtained from material removed at operation – tissue of human gastric carcinoma – by the method described in the paper by V. S. Gostev and D. G. Grigor'ian [1].

Preparation of antisera. Sera were obtained by the intraperitoneal immunization of rabbits with suspensions of both human gastric carcinoma tissues and human thyroid gland. For immunization of the animals only tissues removed at operation were used. It was shown in our laboratory [6] that the immunization of animals with postmortem material does not lead to the formation of antibodies to crude DNP on account of the fact that DNP is present in postmortem tissue in a state of destruction. The animals were injected on alternate days with increasing doses of protein which were estimated as nitrogen. Exsanguination of the rabbits was carried out on the 8th and 12th days after the last immunization, from the auricular vein. The sera were heated for 30 minutes at 56°C and stored at 2-4°C. Boric acid was used as a preservative.

Quantitative CFR at a 50% titer with adsorbents impregnated with DNP from human gastric carcinoma tissue. The protein nitrogen of the antigen was estimated by Conway's method, and a red cell suspension for the hemolytic system was standardized on the electrophotocolorimeter. The complement was titrated as a first stage and for the reaction an excessive number of units of complement was always taken in order to ensure that it was not all entirely fixed in either the control or the experimental tests. The complement which remained unfixed was titrated, and by the difference in the titers of the control and experimental reactions, the degree of fixation in units of complement was established. A unit of complement was taken to be that quantity of it (in a volume of 0.5 ml) which, after keeping in an incubator at 37°C, brings about 50% hemolysis of red cells in 0.5 ml of hemolytic system.

The present reaction, like the classical form, is composed of two stages. The first stage – fixation of complement by a specific antigen – antibody complex – takes place in the cold. A cotton fabric test antigen (see preparation of test antigen) was placed in a tube in 0.3 ml of physiological saline, and to this was added

Quantitative CFR at a Titer of 50% with DNP from Human Gastric Carcinoma Tissue, Adsorbed on Cotton Fabric

	Antigen	Antiserum to human gastric carcinoma (slurry of surgical tissue)		Antiserum to human thyroid gland (slurry of surgical tissue)		Serum of an unimmunized rabbit		Control of antigen	
	Units of complement	free	fixed	free	fixed	free	fixed	free	fixed
Experiment	DNP from human gastric carcinoma	–	31	12.8	18.2	31	–	31	–
Control	Clean cotton fabric	31	–	31	–	31	–	31	–
Control	Control of serum	31	–	31	–	31	–	31	–
Control	Control of complement	31	–	–	–	–	–	–	–

0.2 ml of antiserum (dilution 1:10) and 0.1 ml of complement, either whole or diluted, depending on the strength of the complement. The volume of the reacting mixture in the first stage of the reaction was 0.6 ml. The experiment was accompanied by suitable controls of the antigens, the antiserum, and the complement.

The second stage of the reaction consisted of back-titration of the complement remaining unfixed. For this purpose, on another day from each experimental and control tube definite doses of the reacting mixture (from 0.01 to 0.1 ml) were made up to a volume of 0.5 ml with physiological saline, and an equal volume of hemolytic system was added. The tubes were incubated at 37°C for 30 minutes. Later, after cooling and centrifugation of the tubes the coefficient of extinction of the supernatant fluid was determined by the electrophotocolorimeter. The percentage of hemolysis was determined from the calibration curve expressing the relationship between the percentage hemolysis and the coefficient of extinction.

In order to establish the content of unfixed complement in units in the sample under examination, doses of the reacting mixture were chosen so that the percentage of hemolysis lay between limits of 20 and 80. It was possible only comparatively rarely to determine the dose of complement giving 50% hemolysis. In such cases 50% hemolysis was determined according to the dose of complement causing partial hemolysis by means of Krogh's formula:

$$\lg x_2 = \lg x_1 - n \lg \frac{y}{100-y},$$

where: x_2 is the amount of complement causing 50% hemolysis; x_1 the amount of complement taken for the experiment; n the coefficient under these particular experimental conditions, 0.2; and y is the percentage hemolysis observed in the experiment.

Starting from a titer of 50%, the number of units of complement present in the test sample was calculated.

In order to obtain the value of the specific fixation it is necessary to subtract the number of units of free complement in the experiment not from the total number of units of complement taken in the experiment, but from the number of free units in the control if this is smaller than the total number on account of the presence of anticomplement. For this subtraction, the control is taken in which the anticomplementary properties are more strongly expressed. Where the anticomplement content of the antigen or antiserum is high, it is advisable to increase the dose of complement slightly, although very large doses reduce the accuracy of the reaction.

Figures from a typical record of an experiment, obtained during a serological study of the DNP adsorbed on cotton fabric, using the method as described above, are given in the Table.

EXPERIMENTAL RESULTS

As seen from the Table, the DNP of human gastric carcinoma tissue shows a high serological activity, specifically fixing complement in the presence of antiserum. DNP from human gastric carcinona tissue, when combined with antiserum to human thyroid gland, fixes only 18.2 units of complement out of 31, whereas the same DNP with antiserum to human gastric carcinoma tissue fixes the entire stock of free units of complement taken in the experiment.

Thus DNP, fixed on cotton fabric adsorbents, are serologically active and may be studied by means of the quantitative complement fixation reaction at a titer of 50%.

SUMMARY

The author investigated the quantitative complement fixation test with desoxyribonucleoproteins adsorbed on cotton fabric. The serological activity of desoxyribonucleoproteins and the possibility of their examination by this method was demonstrated.

LITERATURE CITED

[1] V. S. Gostev and D. G. Grigor'ian, Biull. Eksptl. Biol. i Med. 45, 1, 122-125 (1958).*

[2] V. S. Gostev, D. G. Grigor'ian, N. A. Shagunova and N. M. Teplova, Annotations of Scientific Papers of the AMN SSSR for 1955, v. 1, 239-241, Moscow (1956).* *

[3] D. G. Grigor'ian, Proceedings of a Scientific Conference on Methods in the Immunology of Malignant Neoplasms, 17, Moscow (1956).* *

[4] A. P. Konikov, Zhur. Mikrobiol. Epidemiol. i Immunobiol., 1, 57-66 (1953).

[5] N. A. Shagunova, Proceedings of a Scientific Conference on Methods in the Immunology of Malignant Neoplasms, 16, Moscow (1956).* *

[6] M. Mayer, A. Osler, O. Bier and M. Heidelberger, J. Immunol. v. 59, 195-206 (1948).

[7] M. Mayer, B. Ealton and M. Heidelberger, J. Immunol. v. 53, 31-35 (1946).

[8] A. Mirsky and A. Pollister, J. Gen. Physiol. v. 30, 117-143 (1946).

* See English translation.

** In Russian.