A COMPARATIVE STUDY OF THE SEROLOGICAL PROPERTIES OF FRACTIONS OF A CANCER ANTISERUM FROM HORSES

N. A. Nazarenko and V. S. Gostev

From the Laboratory of Immunochemistry (Head - Prof. V. S. Gostev) of the Institute of Experimental Biology (Director - Prof. I. N. Maiskii) of the AMN SSSR, Moscow

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It is now an accepted fact that antibodies are firmly combined with those serum proteins which have a high molecular weight, a large particle size, and a low electrical mobility, i.e. with the γ-globulins.

It is impossible to separate antibodies from the serum γ-globulins by precipitation [2, 11, 12, 14], ultrafiltration [10, 13, 17], ultracentrifugation [8, 15] or electrophoresis [16].

The proof of the γ-globulin nature of antibody has led to the use of the γ-globulin fraction of the serum instead of whole serum as a therapeutic preparation.

N. V. Kholchev and L. I. Kolesnikova [6], for instance, developed a commercial method of obtaining an antimeasles γ-globulin by precipitating it with alcohol in the cold. The use of this preparation showed that 1.5-3.0 ml of it was equivalent in its effect to 30-60 ml of the whole serum.

At the I. I. Mechnikov Moscow Institute of Vaccines and Sera a method has been developed for the production on a commercial scale of 15 types of therapeutic antisera against various infectious diseases. These preparations do not produce in animals any of the temperature reactions or sensitization phenomena usually observed after administration of whole serum [4].

Undoubted interest is being shown in the fractionation of cytotoxic sera, with the removal of ballast proteins from the sera and separation of the fraction bearing the cytotoxic properties in the form of a homogeneous preparation of maximum purity.

The association between the cytotoxin and the globulins has now been proved. It has been shown, for example, that the serologically active fraction of the antireticuloendothelial cytotoxic serum (ACS) obtained from rabbits was the β-globulin, and of the ACS obtained from horses it was the γ-globulin [5].

By far the majority of authors believe that the antibodies of the rabbit serum are combined with the γ-globulin fraction of the antiserum. This was very convincingly shown by Tiselius and Kabat [16], by means of a combination of electrophoretic and serological analysis of the serum of hyperimmunized rabbits.

At the present time, however, many workers are increasingly suggesting the possible combination of antibodies with the other fractions, and in particular, with the β-globulin fraction. The problem of which fraction of the cancer antiserum obtained from the horse has the serological activity concentrated in it has received little study.

In pilot experiments by our laboratory colleague Yu. V. Zykov (1957), it was shown that the γ-globulin of a cancer antiserum from the horse contained the main serological antibody activity.

TABLE 1

The Classic CFT with Fractions of Cancer Antiserum No. 2 from Horses (experiment dated November 10, 1957)

Serum fraction	Antigens	Intensity of reaction in relation to quantity of protein (in mg) taken for experiment									Antigen control
		2,6	1,3	0,65	0,32	0,26	0,13	0,065	0,032	0,016	
γ-globulins	Tissue from carcinoma of the stomach	—	—	—	—	++++	++++	+++	+	h	h
	Normal stomach tissue	—	—	—	—	+	h	h	h	h	h
	Spleen tissue	—	—	—	—	+	h	h	h	h	h
γ-globulin control		—	—	—	—	h	h	h	h	h	h
α_1-α_2- and β-globulins	Tissue from carcinoma of the stomach	2+	+	h	h	h	h	h	h	h	h
	Normal stomach tissue	+	h	h	h	h	h	h	h	h	h
	Spleen tissue	±	h	h	h	h	h	h	h	h	h
α_1-α_2- and β-globulin control		h	h	h	h	h	h	h	h	h	h
Albumins	Tissue from carcinoma of the stomach	h	h	h	h	h	h	h	h	h	h
	Normal stomach tissue	h	h	h	h	h	h	h	h	h	h
	Spleen tissue	h	h	h	h	h	h	h	h	h	h
Albumin control		h	h	h	h	h	h	h	h	h	h

Note: – indicates anticomplementary effect of γ-globulins; h) hemolysis.

The purpose of the present research was to obtain a purified, concentrated anticancer preparation by fractionation of a cancer antiserum from horses, and to study the immunological features of the fractions obtained.

EXPERIMENTAL METHOD

Fractionation was carried out on a cancer antiserum of high specificity, obtained from horses for therapeutic purposes in our own laboratory by Prof. V. S. Gostev, Yu. V. Zykov and A. A. Saakov [3].*

The antiserum was fractionated by the method of cold precipitation with methanol [9]; three fractions were obtained: γ-globulins, α_1-, α_2- and β-globulins and albumins. The purity of the fractions was tested by paper electrophoresis. The fractions obtained were examined by the classic complement fixation test (CFT) and by the quantitative CFT to 50% hemolysis end-point, with both soluble and adsorbed [7] antigens.

The test antigens used were saline extracts of human normal and tumor tissue prepared in a 0.85% solution of NaCl in a proportion of 1:10. The adsorbed test antigens were prepared by adsorption of these extracts on chromatographic paper, by a method previously described by us [1].

* Immunization of the horses and preparation of the sera were undertaken at the Gamaleya Institute of Epidemiology and Microbiology, under the direction of G. E. Ryabkov and A. V. Beilinson.

TABLE 2

Quantitative CFT to 50% Hemolysis End-Point with Cancer Antiserum from the Horse and with Its Fractions
(test antigen – saline extract of tissue)

Test antigens	Antigen dilutions	Serum No. 2 dated November 10, 1957, original		γ-Globulins		α_1-α_2- and β-globulins		Albumin		Antigen controls	
		units of complement									
		free	fixed	free	fixed	free	fixed	free	fixed	free	fixed
Human gastric carcinoma tissue	1:10	—	43,7	—	43,7	3,6	40,1	43,7	—	43,7	—
	$1:10^2$	—	43,7	—	43,7	4,6	39,1	43,7	—	43,7	—
	$1:10^3$	4,6	39,1	—	43,7	43,7	—	43,7	—	43,7	—
	$1:10^4$	4,8	38,9	4,1	39,6	43,7	—	43,7	—	43,7	—
Normal human stomach tissue	1:10	—	43,7	—	43,7	43,7	—	43,7	—	43,7	—
	$1:10^2$	3,4	40,3	—	43,7	43,7	—	43,7	—	43,7	—
	$1:10^3$	4,8	38,9	3,7	40,0	43,7	—	43,7	—	43,7	—
	$1:10^4$	43,7	—	4,5	39,2	43,7	—	43,7	—	43,7	—
Human spleen tissue	1:10	3,4	40,3	3,4	40,3	43,7	—	43,7	—	43,7	—
	$1:10^2$	3	40,7	3,4	40,3	43,7	—	43,7	—	43,7	—
	$1:10^3$	43,7	—	3,7	40,0	43,7	—	43,7	—	43,7	—
	$1:10^4$	43,7	—	4,6	39,1	43,7	—	43,7	—	43,7	—
Serum controls	1:10	43,7	—	43,7	—	43,7	—	43,7	—	43,7	—
Complement control	Undiluted	43,7	—	—	—	—	—	—	—	—	—

TABLE 3

Quantitative CFT to 50% Hemolysis End-Point with Fractions of Cancer Antiserum from the Horse (antigen – saline extract adsorbed on paper)

Test antigens	γ-Globulins		α_1-α_2- and β-globulins		Albumins		Antigen control	
	units of complement							
	free	fixed	free	fixed	free	fixed	free	fixed
Gastric carcinoma tissue	—	47,5	3,5	44,0	47,5	—	47,5	—
Normal stomach tissue	—	47,5	3,1	44,4	47,5	—	47,5	—
Serum control	47,5	—	47,5	—	47,5	—	47,5	—
Complement control	—	—	—	—	—	—	—	—

EXPERIMENTAL RESULTS

Table 1 shows the results of a typical classic CFT with the fractions of the cancer antiserum from horses – γ-globulins, α_1-, α_2- and β-globulins and albumins. The quantity of the fractions taken for the experiment was estimated in accordance with their protein content. As test antigens we used saline extracts of tissue from a carcinoma of the stomach, of normal stomach tissue and of spleen tissue from human subjects. Complete hemolysis was observed in the control experiments to check the antigens, the fractions and the complement. In view of the high anticomplementary effect of the γ-globulin, the test was only valid starting with the samples containing 260 γ of protein.

The serological activity of cancer antiserum from the horse, as determined by the classic CFT, was concentrated mainly in the γ-globulin fraction; the α_1-, α_2- and β-globulin fractions were considerably less active. No antibodies were found in the albumins.

Analagous results were also obtained by another method – the quantitative CFT to a 50% hemolysis end-point with soluble antigens. The test antigens (saline extracts of tissue from carcinoma of the stomach, normal stomach tissue and spleen tissue from human subjects) were used in dilutions of 1:10, 1:10^2, 1:10^3 and 1:10^4. The antiserum and its fractions were used in a dilution of 1:10.

As shown in Table 2, the original serum reacts with human gastric carcinoma tissue in an antigen dilution of 1:10^4, fixing 38.9 units of complement of a total of 43.7 units taken in the experiment; with normal stomach tissue the reaction was weaker: in an antigen dilution of 10^4 there was no reaction, and with spleen tissue there was no reaction at an antigen dilution of 1:10^3.

The serological activity of the γ-globulin fraction was significantly higher than that of the original serum. The number of units of complement fixed by antigens from gastric carcinoma tissue, from normal stomach and spleen tissue in a dilution of 1:10^4 was greater than with the original serum. The serological activity of the α_1-, α_2- and β-globulin fractions was sharply reduced. The reaction took place only at the first two antigen dilutions of human gastric carcinoma tissue. No antibodies could be found in the albumins.

The γ-globulin fraction, therefore, is a fraction of high serological activity, in which is concentrated almost all the antibodies which are specific toward cancer and normal tissues in man. So far as the α_1-, α_2- and β-globulin fractions were concerned, they were active only in relation to human gastric carcinoma tissue. The albumins were serologically inactive.

Analagous results were obtained by the CFT to a 50% hemolysis end-point. For this test, 20 mg of adsorbed test antigens was usually taken (human gastric carcinoma tissue and normal human stomach tissue).

The antiserum fractions used in the tests were diluted 1:10.

It can be seen from the results shown in Table 3 that the γ-globulin fraction of the cancer antiserum from the horse also revealed the highest serological activity in the reaction with adsorbed test antigens, by specifically

fixing complement. Gastric carcinoma tissue, like normal stomach tissue, fixed all 47.5 units of complement used in the test; a somewhat weaker reaction was given with these antigens by the α_1-, α_2- and β-globulin fractions. The albumins were serologically inactive.

It follows from the experimental findings that the antibodies in the cancer antiserum of the horse were concentrated mainly in the γ-globulin fraction. So far as the albumin fraction is concerned, from the immunological point of view this could be regarded as ballast protein.

Hence in the preparation of a concentrated, purified anticancer preparation, the albumins should be removed.

On the basis of our findings, under the direction of N. A. Ponomareva in the laboratory of the I. I. Mechnikov Institute, an anticancer preparation was produced by alcohol precipitation from a large volume of cancer antiserum of high specificity, and consisted of the γ-globulin fraction of cancer antiserum from the horse.

The serological activity of the anticancer preparation was expressed in units of activity towards cancerous and normal human tissues.

In our opinion the unit of serological activity should be taken as the minimum quantity of the anticancer preparation (in mg of protein as nitrogen) reacting with the corresponding test antigen, adsorbed on paper, in the CFT to 50% hemolysis end-point.

Sorbed antigens in the dry form retain their original serological activity for many months, which ensures standard conditions of determination of the activity of the preparations. This is also facilitated by the conditions of performance of the quantitative CFT to 50% hemolysis end-point.

We have determined the number of anticancer units (ACU) and antinormal tissue units (ANU) calculated per ml of the preparation. For this purpose the preparation was titrated in successive dilutions to estimate the minimum quantity of protein taking part in the reaction with the corresponding adsorbed test antigens. This minimum quantity of protein in the preparation was taken as 1 unit of serological activity. In order to determine the number of units of activity in a given preparation, the total number of mg of protein per ml of the preparation must be divided by the number of mg of protein equivalent to one unit of activity.

Example. Number of mg of protein in the original preparation — 80
1 ACU — 0.6 mg
1 ANU — 6.0 mg
80: 0.6 — 133.3 ACU
80: 6.0 — 13.3 ANU

SUMMARY

As a result of the comparative study of the serological properties of the various fractions of the anticancer serum of the horse by different methods, it has thus been shown that the serological activity is concentrated mainly in the γ-globulin fraction and, to some extent, is present also in the α_1-, α_2 and β-globulin fractions, and this must be taken into consideration during the production of purified, concentrated preparations. The method of determination of the serological activity of an anticancer preparation which we have developed expresses this as units of activity towards malignant and normal human tissues.

LITERATURE CITED

[1] V. S. Gostev and N. A. Shagunova, Byull. Éksptl. Biol. i Med. 10, 121-125 (1957).*

[2] S. K. Dzerzhgovskii and S. N. Predtechenskii, Arkh. Biol. Nauk SSSR. 14, 1-2, 163-185 (1908).

[3] Yu. V. Zykov, Proceedings of a Conference of Young Scientists of the Institute of Experimental Biology of the AMN SSSR, pp. 9-10, Moscow, 1958.**

[4] N. A. Ponomareva, G. I. Babaeva and M. N. Durasova, Theses Commemorating the 35th Anniversary of the Mechnikov Moscow Institute of Vaccines and Sera, 1955.*

[5] Yu. V. Rapp, Cytotoxins in Modern Medicine, 270-274, Kiev, 1956.**

*Original Russian pagination. See C. B. Translation.

**In Russian.

[6] N. V Kholchev and L I Kolesnikova, Zhur. Mikrobiol., Épidemiol. i Immunobiol., No. 5, 6-12 (1947).

[7] N. A. Shagunova, Byull. Éksptl. Biol. i Med. 3, 122-127 (1958).*

[8] J. Biscoe, F. Herivick and R. Wyckoff, Science 83, 602-603 (1936).

[9] J. M. Dubert, P. Slizewicz, P. Rebeyrotte and M. Machebocuf, Ann Inst. Pastur. 84, 370-375 (1953).

[10] W. Elford, P. Grabov and W. Fischer, Bioch. Z. 30, 92-99 (1936).

[11] L. Felton, J. Imm. 21, 357-373 (1931).

[12] L. Felton, J. Imm. 22, 453-467 (1932).

[13] K. Goodner, F. Horsfall and I. Bauer, Proc. Soc. Exp. Biol. and Med. 34, 617 (1936).

[14] P. Murdick and S. Cohen, J. Imm. 29, 205-208 (1935).

[15] E. Smith and D. Brown, J. Biol. Chem. 183, 1, 241-249 (1950).

[16] A. Tiselius and E. Kabat, J. Exper. Med. 69, 119-131 (1939).

[17] S. Went and L. Sarkady, Z. Imfrschg. 91, 157 (1937).

*Original Russian pagination. See C. B. Translation.