THE QUANTITATIVE CHARACTERISTICS OF ANTI-TUMOR γ-GLOBULINS OF NARROW SPECIFICITY

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Antisera of narrow specificity are of interest from both the theoretical and practical points of view. A number of investigations have been concerned with the development of methods for isolating specific antibodies from antisera which are polyspecific. Particular attention has been paid, in this regard, to the work with tumor antigens [2, 3, 6].

Highly specific anti-tumor antisera will contribute greatly toward a solution of the problems of specific immunoprophylaxis, therapy and diagnosis of neoplastic disease [1].

In our previous reports [4, 5] results were presented on the conditions for obtaining anti-tumor antisera of narrow specificity by means of specific adsorption. To adsorb out antibody against normal tissue we used the antigens of the homologous normal organ adsorbed onto chromatography paper in contrast to the aforementioned work where dermatol-treated [6] and formalinized tissue [2, 3] were used as the immunosorbents.

At the present time it can be considered conclusively demonstrated that it is possible to obtain anti-tumor antisera of narrow specificity. However there are no data in the literature which would indicate the quantitative characteristics of the activity of antisera obtained by specific adsorption. The present report has as its aim to present results of experiments pertaining to this problem.

METHODS

Horse γ-globulin fractions from anti-tumor antisera (series No. 1, 2, 3, 4, 5, 6) were used for this investigation.

Anti-tumor antisera were obtained by Prof. V. S. Gostev, Yu. V. Zikov, and A. K. Saakov by immunizing horses with homogenates of surgical specimens from human stomach cancers in the presence of DNA depolymerase inhibitor.*

γ-Globulin fractions** were isolated from the horse antisera to free the antisera of extraneous material [4].

Homogeneity of the obtained fraction was verified by paper electrophoresis. The figure illustrated the electrophoretic pattern of the whole horse antiserum and the γ-globulin fraction derived from it.

It was necessary to characterize the activity of the given preparations of anti-tumor γ-globulins destined for clinical use, by definite standardized titers.

In this regard we worked out a method [4] of expressing serological activity of the anti-tumor γ-globulin in units related to the neoplastic and normal human tissue. A unit of serological activity was taken as the minimal amount of the anti-tumor preparation (in milligrams of protein nitrogen) reacting with the corresponding test-antigen, adsorbed onto paper, in the quantitative complement fixation reaction (CFR) by 50% end-point titration. The number of anti-tumor units (ATU) and units against normal tissue (ANU) were calculated on the basis of 1 ml of the preparation under investigation. For this purpose the total number of milligrams of protein in 1 ml of a preparation was divided by the number of milligrams of protein which corresponded to one unit of activity.

*Immunization of horses and sterile preparation of the sera were carried out in the N. F. Gamalei Institute of Epidemiology and Microbiology under the guidance of G. E. Ryabkov.

**Fractionation of the antisera was carried out in the I. I. Mechnikov Institute's γ-globulin laboratory under the direction of N. A. Ponomarevaya.

Using the indicated method of expressing the serological activity we were able to characterize quantitatively anti-tumor preparations of series No. 1, 2, 3, 4, 5, 6.

RESULTS

Data of the analysis are given in Table 1.

From the data presented in Table 1 it is evident that the γ-globulins under investigation had a polyspecific character but in the majority of instances a significant predominance of anti-tumor units was noted (series No. 2, 3, 5, 6). γ-Globulins with high serological activity against normal tissue, such as No. 4, were not permitted to be used

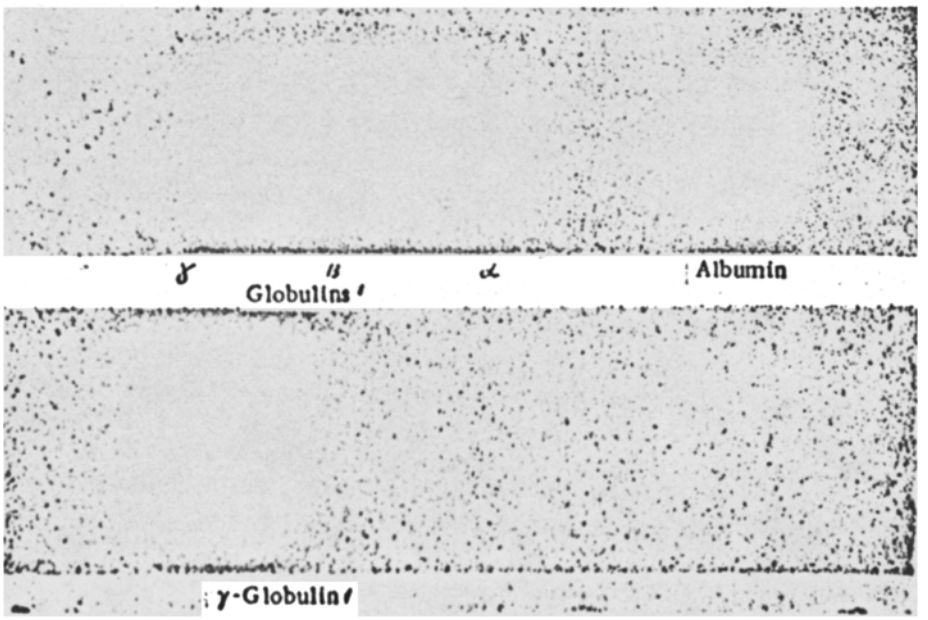
TABLE 1. Quantitative Characteristics of Anti-Tumor γ-Globulins in Units of Activity

Series number of anti-tumor γ-globulin	Number of units of activity	
	Anti-tumor tissue from human stomach (ATU/ml).	Anti-normal tissue from human stomach (ANU/ml)
1	136,0	75.6
2	133.0	13.3
3	134.9	67.2
4	563.2	563.2
5	135.1	71.0
6	85.2	26.8

TABLE 2. Activity of Anti-Tumor Preparations Expressed in Units

Number of the preparation	Number of units (in ATU per ml)				The decrease in anti-tumor units as a result of adsorption (in ATU/ml)	
	In the original preparation		In the adsorbed preparation			
3	134.9	67.2	81.2	—	53.7	67.2
4	563.2	563.0	80.4	—	482.8	563.2
5	135.0	71.0	57.0	—	78.0	71.0
6	85.2	26.8	22.6	—	62.6	26.8

clinically. The polyspecificity of the anti-tumor γ-globulin bore witness to the polyspecificity of the antigen used for immunization. Tumor tissue, apart from its specific tumor antigens, possesses a complex of antigens whose properties correspond to those of normal tissue antigens so that immunization of the animals brings about the formation of both specific anti-tumor antibodies and antibodies to normal tissue, the activities of which are reflected in the data given in Table 1.



The electrophoretic pattern of equine anti-tumor antiserum No. 2 and the γ-globulin fraction obtained from it — series No. 1.

We performed a series of experiments designed to separate out the nontumor antibodies from the antitumor antisera by means of adsorbed antigens.

The method of adsorbing nontumor antibodies by antigen adsorbents has been previously published by us [5, 7].

Series No. 3, 4, 5, 6 were the antitumor antisera used for adsorption.

As a result of innumerable experiments of adsorbing the nontumor antibodies we succeeded in obtaining anti-tumor γ-globulin of narrow specificity, i.e. γ-globulin which displayed activity only against tumor tissue from human stomach and none against normal stomach tissue.

The results of a standard protocol of the experiments on adsorption of anti-tumor γ-globulins series No. 3, 4, 5, 6 are given in units of activity in relation to neoplastic and normal human stomach tissue. The quantitative characteristics of the aforementioned γ-globulins before and after adsorption are presented in the form of a summary table (Table 2).

The analysis graphically demonstrates that the process of adsorbing nontumor antibodies causes a simultaneous fall in the titers of antibody to the normal and neoplastic tissue both derived from human stomach. Thus, for example, the activity of preparation series No. 3 has 134.9 ATU per ml and 67.2 ANU per ml; after removal of the nontumor antibody, the activity with regard to tumor tissue falls to 81.2 ATU per ml. An analogous situation is observed with the other preparations No. 4, 5, 6.

Apparently the loss of anti-tumor antibody during specific adsorption of the antisera, in the form of decreased activity of the given antisera in relation to the tumor tissue, cannot be explained alone by nonspecific adsorption which accompanies the process of combining the antigen with its corresponding antibody. In the majority of instances presented in Table 2 (series No. 3, 4, 5) the number of units (against tumor and normal tissue) lost during the adsorption of the given preparation on the adsorbed antigen of normal stomach tissue were practically the same. Obviously under the given experimental conditions, there is a primarily specific adsorption. In analyzing the data it is necessary to consider that not only the tumor tissue used in the immunization has a polyspecific character; the test-antigen of tumor tissue which is customarily employed in the serological reactions (a saline-aqueous extract) also is polyspecific. Thus the test-antigen of tumor tissue can only be tentatively designated as being neoplastic. In the removal of nontumor antibody the decrease in the activity of the given antisera in regard to tumor tissue is brought about on account of the loss of nontumor antibody which, by virtue of the polyspecificity of the test-antigen, is usually considered to be truly anti-tumor antibody. In the meanwhile we cannot gainsay the presence of nonspecific adsorption. Thus in series No. 6, on account of nonspecific adsorption, only 35.8 units are lost rather than 62.6 ATU.

The quantitative characterization of the adsorbed anti-tumor antisera showed that the loss of activity of the γ-globulin due to specific loss of antibody is not reflected in the amount of protein nitrogen in the adsorbed antisera in comparison with the amount of protein in these antisera prior to adsorption.

As an example we present the results of the analysis on series No. 4 and 6 anti-tumor γ-globulins in Table 3.

TABLE 3. Amount of Protein Nitrogen (in mg) in the Volume of Antiserum Taken for Adsorption (the data of 8 experiments)

Preparation No.	Before adsorption	After adsorption
4	1.80	1.80
6	3.63	3.63

Protein nitrogen was determined by the method of Conway. Analogous experiments with γ-globulin preparations of series No. 3 and 5 supported the data presented in Table 3. Despite the fact that the serological analysis (see Table 2) demonstrated a sharp decline in the activity of the γ-globulin with specific adsorption, we could not detect changes in the protein nitrogen of the antisera employing the method of Conway.

Thus the experimental data support the contention that adsorption of antigens onto paper can be used for quantitative study of the process of specific adsorption of anti-bodies from anti-tumor antisera by which means highly specific antisera can be obtained.

The quantitative characteristics of equine γ-globulins from anti-tumor antisera expressed as units of activity (relative to neoplastic and normal human gastric tissue) before and after adsorption of nontumor antibodies showed that the activity of the γ-globulin with regard to tumor tissue is decreased, when the nontumor antibody is lost, by as many units as are lost in regard to normal tissue. This can be explained by the polyspecificity of the tumor tissue

extract which is used as a test-antigen in the reaction. In other words quantitative study of specific adsorption of antibody helps establish the real tumor specificity, differentiating it from the specificity of normal elements which are contained in the structure of neoplastic tissue. The amount of protein nitrogen in the γ-globulin preparations did not change after adsorption of nontumor antibody. This apparently is explained by the fact that the antibodies probably constitute an infinitesimally small portion of the antiserum protein and it was impossible to detect their disappearance by the method of Conway under the stated conditions of the experiment.

SUMMARY

A comparative quantitative characteristic of anticancer γ-globulins is given in activity units (with respect to the cancer and normal human tissues) with the aid of antigens sorbed on paper. As shown, in extraction of noncancer antibodies the activity of γ-globulins decreases to about the same extent with respect to the normal tissues of human stomach as to the cancer ones, which is evidently explained by the polyspecificity of the cancer tissue extracts used in the reaction at test-antigens.

There were no changes in the amount of protein (according to nitrogen determination by Conway's method) after complete extraction of the noncancer antibodies in these experimental conditions of exhaustion.

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