THE REACTION OF COMPLEMENT FIXATION AS A METHOD FOR SEROLOGICALLY CHARACTERIZING ANTITISSUE SERA

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At the present time, it has been established that there are a large number of antigens in the tissue cells of the animal organism (species specific, organ specific, etc.), inherent to the individual [1-12]. In particular, the tissue of human malignant tumor contains, besides antigens of the normal organs, an additional specific cancer antigen. Thus, upon immunization of animals (rabbits, goats, horses, etc.) with tissue from human cancer, these animals develop antibodies against all the antigens, including ones against the cancer tissue. We attempted to bring about depletion of non-cancer antibodies in vivo, in order to obtain finely specific anti-cancer sera in the organism of the animals. Working with these antitissue sera, we used the reaction of complement fixation (CFR), in order to investigate the dynamics of antibody titer rise during the process of immunization of the animal, and also to record the results of the non-cancer antibody depletion at individual stages of this process.

In this work we have described the method procedures which we used in setting up the CFRs. In our experiments, over a long period of time, we studied blood samples of a single animal many times. In order to obtain comparable results, it was necessary to use standard test antigens for the CFR. The antigens selected were water-saline extracts of the tissues from normal organs and human malignant tumor. The indicated tissues were pulverized in a homogenizer, combined with distilled water (10 ml of water/gram of tissue), and placed in a refrigerator for one night. On the following day, the aqueous extract was separated from the settled tissue fragments by 10-15 min of centrifugation at 1000 rpm. Then, sodium chloride was added to make 0.85%, and the material was bottled in ampoules. The standardicity of the test antigens employed was achieved by three-fold freezing and thawing of the prepared extracts, with subsequent maintenance of the material in the frozen state within sealed ampoules. After this treatment, the activity of the test antigens does not change for up to 3 months. Before determining the working dose, or using the test antigen in the basic experiment, it was thawed, centrifuged or filtered, and, in the necessary amount, added to the reaction mixture. The working doses of the test antigens were established by the widely accepted method, and complement, diluted 1:10, was added with a surplus of 25% over the anticomplement activity of the antigen.

In the majority of cases, antitissue sera obtained from the immunization of rabbits and, especially, horses, heifers, and donkeys (for the latter, the complement was diluted 1:5) possess high anticomplement activity. Thus, a surplus of 25-50% added to the titrated dose of pure complement, diluted 1:10-1:5, is insufficient, and in the control sera of the basic experiment with CFR hemolysis is not observed.

In our investigations, the dose of complement for the basic experiment was determined by its titration in the presence of 0.5 ml of the starting dilution of the serum under study. For the working dose of complement in the basic experiment we took the amount of complement causing hemolysis of sheep erythrocytes in the presence of the subject antitissue serum plus an additional 25% over the anticomplement activity of the antigen.

In this work, we studied rabbit cytotoxic sera by the method of CFR. The animals were immunized with an aqueous-saline extract of fresh-frozen tissue from human stomach cancer. The antigen was injected intraperitoneally three days in a row (one cycle of immunization), with intervals between the cycles of 4 days (3 cycles), and then for the last two cycles (4th and 5th) the immunization was carried out intravenously. The amount of antigen injected was measured according to the protein contained, which was determined by the method of Conway.

TABLE 1. Depletion of Non-Cancer Antibodies After Intravenous Injection of an Extract of Tissue from Normal Human Stomach (CFR Method)

Dilution of serum No. 3597	Blood samples (time after experiment) and dose of complement (in ml)															
	before depletion; 0.18				1 hour; 0.21				2 hours; 0.21				4 hours; 0.18				24 hours; 0.18			
	Test antigens															
	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp
1:10	++++	++++	++++	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h
1:20	++++	++++	++++	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h
1:40	++++	++++	++++	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h
1:80	++++	++++	++++	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h
1:160	++++	++++	++++	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h
1:320	++++	++++	++++	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h	++++	++++	h h

Note. The reactions were set up with corresponding controls for the serum, antigens, complement and hemolytic system. Symbols: CS) cancer of the stomach; NS) normal stomach; NSp) normal spleen; h) hemolysis.

Prior to immunization, after the 3rd and 5th immunizations, and subsequently twice a week, blood samples were taken from the rabbits and the dynamics of antibody formation were studied.

By the method of CFR it was established that the rise in cancer and non-cancer antibodies is not uniform in time. In other words, in the process of immunization there are moments when the titer of antibody in the blood of the animal against cancer tissue is the same, lower, or higher than the titer of antibody against the tissue of the normal organ equivalent to the tumor under study, or against the tissue of normal human spleen.

In the second portion of the work, we carried out depletion of the non-cancer antibodies in the organism of the immune animal, controlling the process by investigating the antibody titer in the blood of the experimental animal, using the method of CFR.

As in the first part of the work, the rabbits were immunized with the aqueous-saline extract of human stomach cancer (the amount of antigen injected represented 140 mg of protein, determined by nitrogen). Immunization of the animals was carried out under serological control, i.e., the titer of antibody was ascertained before immunization, after the 2nd cycle, and at the completion of the immunization.

A blood sample was preliminarily taken from the immune animal (3 ml), and then extract of tissue from normal human stomach was injected intravenously very slowly in a single dose of 1-5 ml (or 12-20 mg of protein), or plasma, or whole human blood of a different group (1-3 ml). As a rule, the animal did not die from this dose, but endured the injection of antigen with difficulty.

Blood samples were taken from the rabbits 1, 2, 4, and 24 hours after the intravenous injection of antigen.

Table 1 shows that the maximum lowering of the antibody titer occurred at the 2nd hour after initiation of the experiment, and the lowering of the titer of non-cancer antibodies was only 2 times greater than for the cancer antibodies. For example, before depletion the titer of antibody against cancer tissue was +++ in a dilution of 1:160, and after 2 h it became ++ in a dilution of 1:80. The titer of antibody against normal stomach and spleen was ++ in a dilution of 1:80, and after 2 h the titer of antibody against stomach tissue was ++ in a dilution of 1:20. In this case, the depletion of antibody against splenic tissue was somewhat greater, but this was an exceptional case; as a rule, these antibodies were more difficult to deplete. Restoration of the antibody titer to the starting level occurred on the 2nd day.

Analogous results were obtained in those experiments where depletion was carried out by a single intravenous injection of aqueous-saline extract from normal splenic tissue (3-4 ml or 17-22 mg of protein).

TABLE 2. Fluctuation in the Antibody Titer After Two-Fold Depletion (CFR Method)

Dilution of serum No. 1968	Blood samples (time after experiment) and dose of complement (in ml)														
	before depletion; 0.14			1 hour; 0.17			2 hours; 0.2			4 hours; 0.2			24 hours; 0.175		
	Test antigens														
	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp
1 : 10	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1 : 20	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1 : 40	++++	+++	++++	++++	±	+++	+	±	++	++	+	+	++	+	±
1 : 80	++	h	h	±	h	h	h	h	h	h	h	h	++	h	±
1 : 160	+				h	h									

Symbols: CS) cancer of the stomach; NS) normal stomach; NSp) normal spleen; h) hemolysis.

TABLE 3. Results of Investigating Blood Samples from Rabbits Nos. 3971 and 3184 That Died After the Intravenous Injection of Antigen (CFR Method)

Dilution of the rabbit sera	Rabbit No. 3971												Rabbit No. 3184					
	blood samples (time after experiment) and dose or complement (in ml)																	
	before depletion; 0.32			1 hour; 0.34			2 hours; 0.34			4 hours; 0.32			before depletion; 0.3			15 minutes; 0.3		
	Test antigens																	
	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp	CS	NS	NSp
1 : 10	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1 : 20	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1 : 40	++	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
1 : 80	+	+	±	±	h	h	+	h	h	+	h	h	++	h	++	±	h	++
1 : 160		h			h	h		h	h		h	h		h	h		h	h

We observed a reduction in the titer of antibody against cancer and against normal tissues of human organs, but the antibodies against normal stomach tissue were depleted to a greater degree than the antibodies against cancer tissue or splenic tissue.

Having obtained this result, we decided to alter the set-up of the experiment somewhat: first, we decreased the amount of antigen injected during immunization to 40 mg of protein, and second, depletion was carried out with a single injection of plasma or whole human blood (up to 3 ml) into the vein of the immune animal.

As a result of this depletion, we obtained a minimal (by one dilution) lowering of the titer of all antibodies, and in individual cases, a slightly stronger depletion against stomach tissue. Thus, we decided to perform a second injection of blood (2 ml) 2 h after the first. As can be seen from Table 2, two-fold injection of "antigen" (sample, taken after 4 h) did not lead to a better result. It should be noted that the rabbits almost did not react at all to the second injection of antigen.

It is interesting that even in those cases where the animal died as a result of the intravenous injection of antigen (immediately, or after 2-4 h), the serum did not show a large depletion of non-cancer antibodies in comparison with the previous sample. Thus, for example, rabbit No. 3971 (Table 3) died 4 h after injection with 5 ml of extract from normal stomach tissue (20 mg of protein), but the titer of antibody against the normal organs and the cancer tissue was almost unchanged. In rabbit No. 3184, death occurred 15 min after the intravenous injection of 2 ml of human blood.

As can be seen from Table 3, even in this case no changes in the titer of non-cancer antibody occurred.

On the basis of investigations carried out on 58 rabbits, we have concluded that the method of CFR is completely worthwhile in investigating the dynamics of antibody concentrations during experiments with antitissue sera, under conditions of standardized tissue test antigens and the obligatory titration of complement in the presence of the sera under study.

We did not succeed in appreciably depleting non-cancer antibodies in vivo with a single injection into the immune animals of extract of normal human stomach or splenic tissue (up to 20-22 mg of protein), or with single or two-fold injections of plasma or whole human blood (up to 3-4 ml).

SUMMARY

Experiments concerned with antitissular sera staged on 58 rabbits revealed the opportunity of obtaining comparable data in dynamic investigations of the antibody content. Conditions for the preparation and standardization of tissue antigens and complement titration are presented. A single injection of an extract of normal human organ tissues to immune animals does not yield an effective in vivo exhaustion of antibodies. A single or two-fold injection of blood plasma or whole blood acts in the same way.

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