THE IMMUNOLOGICAL ACTIVITY OF ANTIBODIES, ADSORBED ON SOLID ADSORBENTS

A. K. Adamov

Public Health and Epidemiological Laboratory (Head – F. A. Mordvintsev,
Scientific Consultant – Corresponding Member AMN SSSR Prof. A. Ya. Alymov)
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp. 71-76, November, 1960
Original article submitted November 20, 1959

The properties of antibodies when adsorbed on solid adsorbents have not been adequately studied. In the accessible literature we found only isolated reports of this subject. Eisler [3], for example, showed that diphtheria and tetanus antitoxins, when adsorbed on activated carbon, lose their power to neutralize homologous toxins. According to Vekerdi [4], precipitins against horse proteins, when adsorbed on animal charcoal, talc, powdered glass, starch, erythrocytes, barium chloride, agar agar and barium sulfate, also showed no immunological activity when mixed with the homologous antigens.

In the search for methods of improving serological reactions, we studied the properties of antibodies adsorbed on solid adsorbents.

METHOD

In view of the negative results obtained by Eisler [3] and Vekerdi [4] with adsorbed antitoxins and precipitins, we carried out experiments with different antibodies and antigens. Altogether 112 experiments were conducted with 40 strains of various species of microorganisms, 2 strains of influenza viruses, complete antigens of dysentery bacteria and of the genus Salmonella, and also with haptens and type A botulinus toxin.

The antibodies were adsorbed by the following method. To 2 ml of an immune serum,* diluted 1 : 10 with physiological saline, was added 100 mg of the adsorbents to be tested, in powder form and previously sifted through a standard brass grating of 40 μ mesh (for standardization of the particle size). The sera with the adsorbents were kept for 2 hours at 37° and for 18 hours at 4-8°, and periodically stirred with a glass rod. The sera with adsorbents were then centrifuged at 2000 rpm. The translucent supernatant fluid was removed by decantation and the residue was suspended in 10 ml of physiological saline, containing 1% boric acid as preservative. The suspensions of adsorbents thus obtained contained on their surfaces particles of antibody adsorbed from the immune sera.

The study of the immunological activity of antibodies adsorbed on solid adsorbents, by various methods, has shown that the simplest and most convenient method is the agglomeration reaction on a glass slide. If a suspension of an adsorbent, containing adsorbed antibodies on its surface, is mixed with homologous antigen on a glass slide, the particles of adsorbent merge to form large agglomerates. This does not occur if adsorbents, containing adsorbed antibodies on their surface, are mixed with heterologous antigens. To perform the agglomeration reaction, on to a thoroughly degreased glass slide were placed three drops of the suspension for testing, on the particles of which the antibodies were adsorbed. To the first drop of suspension was added a drop of homologous antigen, to the second drop – a drop of heterologous antigen, and to the third drop – a drop of physiological saline. After

* Sera were used which were preserved in 1% boric acid. Sera which were preserved with other elements and sera which underwent lypophilic drying processes were not usable for adsorption experiments.

thorough mixing of each drop with a platinum loop, observations were made for a period of 5 minutes (with slight agitation of the slide) of the changes in the structure of the suspension. The reaction was regarded as specific if agglomeration took place only in the drop in which the particles of adsorbent were mixed with homologous antigen, and in the other two drops the suspension remained homogeneous. The intensity of the agglomeration reaction was denoted by + signs.

As a result of trials of suspensions of various substances possessing the property of adsorbing antibodies (A. K. Adamov [1]), it was found that only suspensions of alizarin, infusorial earth and animal charcoal were suitable for the study of the immunological activity of adsorbed antibodies. Suspensions of these adsorbents, after adsorption of antibodies, remain stable and do not give nonspecific agglomeration with physiological saline or heterologous antigens. Comparison of the sensitivity of suspensions of animal charcoal, infusorial earth and alizarin, containing adsorbed antibodies on the surface of their particles, showed that a suspension of alizarin was most active, i.e., reacted with the smallest quantity of antigen. The activity of the antibodies adsorbed on the above-mentioned adsorbents depended not only on the type of adsorbent but also on the antibody titer of the sera with which adsorption was carried out. For instance, agglutinins adsorbed on charcoal, infusorial earth and alizarin showed immunological activity if the titer of the specific immune sera from which the agglutinins were adsorbed was not less than 1 : 3200. In carrying out experiments with alizarin it must be remembered that this substance is soluble in an alkaline medium, when the suspension of alizarin becomes unstable. In order to prevent the appearance of nonspecific reactions it is essential to use antigen solutions with a pH of not higher than 7.2-7.6, so that after mixing of the antigens with the suspensions of alizarin (preserved with 1% boric acid) the pH of the medium in which the agglomeration reaction is to take place shall not be greater than 6.0.

Since the immunological properties of the adsorbed antibodies were demonstrated most actively when alizarin was used as adsorbent, in the subsequent experiments only this substance was used. In the experiments we tested the immunological activity of different species of antibodies, adsorbed on suspensions of alizarin, from the following immune sera:

dysentery agglutinating serum (Flexner), titer	1 : 3200
paratyphoid B agglutinating serum, titer	1 : 3200
tularemia agglutinating serum, titer	1 : 1500
precipitating serum against serum protein of cattle, titer	1 : 10,000
precipitating serum against human serum protein, titer	1 : 10,000
influenza type A, titer of complement-fixing antibodies	1 : 640
influenza type B, titer of complement-fixing antibodies	1 : 640
"Diaferm 3" type A botulinus antitoxic serum, containing	50,000 units/ml
"Diaferm 3" type B botulinus antitoxic serum, containing	50,000 units /ml

All the sera were preserved with 1% boric acid. During investigation of the immunological activity of the antibodies we determined the minimal quantities of antigens producing an agglomeration reaction.

RESULTS

The results of the study of the immunological activity of the antibodies adsorbed on alizarin particles are shown in the table.

It will be seen from the Table that agglutinins, when adsorbed on alizarin, gave an agglomeration reaction with the homologous bacterial cells and complete antigens, but did not give an agglomeration reaction with haptens. It must be pointed out that intensive agglomeration reactions took place with complete antigens, extracted with trichloroacetic acid. The agglomeration reaction took place with complete antigens obtained by means of enzymic digestion only in the presence of high concentrations of antigen (1 : 500-1 : 1000). Precipitins against human and animal serum proteins, when adsorbed on alizarin, did not give an agglomeration reaction with homologous protein antigens (when working with human and animal blood sera as antigens, in order to prevent nonspecific reactions 1% boric acid must be added to them). The results of our experiments with precipitins against human and animal proteins were in full agreement with those obtained by Vekerdi [4]. Complement-fixing virus antibodies and botulinus antitoxins, when adsorbed on alizarin, gave agglomeration reactions with homologous antigens.

It can be seen from the table that antibodies, adsorbed on alizarin, reacted with antigens in small

Immunological Activity of Antibodies Adsorbed on Particles of Alizarin

Antigen	Type of antigen	Minimal quantity of antigen producing an agglomeration reaction	Type of antibodies adsorbed on alizarin								
			Agglutinins			Precipitins		Complement-fixing		Antitoxins	
			Parathyphoid B	Flexner dysentery	Tularemia	against human serum protein	against serum protein of cattle	against influenza virus A	against influenza virus B	type A botulinus	type B botulinus
Bacterial cells	Paratyphoid B	25 M bacterial cells/ml	+	–	–	–	–	–	–	–	–
	Flexner dysentery	The same	–	+	–	–	–	–	–	–	–
	Tularemia ***	100 M bacterial cells/ml	–	–	+	–	–	–	–	–	–
Complete* antigens	Paratyphoid B	1 : 1,600,000	+	–	–	–	–	–	–	–	–
	Flexner dysentery	1 : 800,000	–	+	–	–	–	–	–	–	–
Haptens**	Paratyphoid B	–	–	–	–	–	–	–	–	–	–
	Flexner dysentery	–	–	–	–	–	–	–	–	–	–
Protein	Human blood serum	– –	–	–	–	–	–	–	–	–	–
	Ox blood serium	– –	–	–	–	–	–	–	–	–	–
Viruses	Culture of influenza virus A ****	Not determined	–	–	–	–	–	+	–	–	–
	Culture of influenza virus B ****	Not determined	–	–	–	–	–	–	+	–	–
Toxins	Type A botulinus toxin	22,5000 lethal doses for white mice	–	–	–	–	–	–	–	+	–

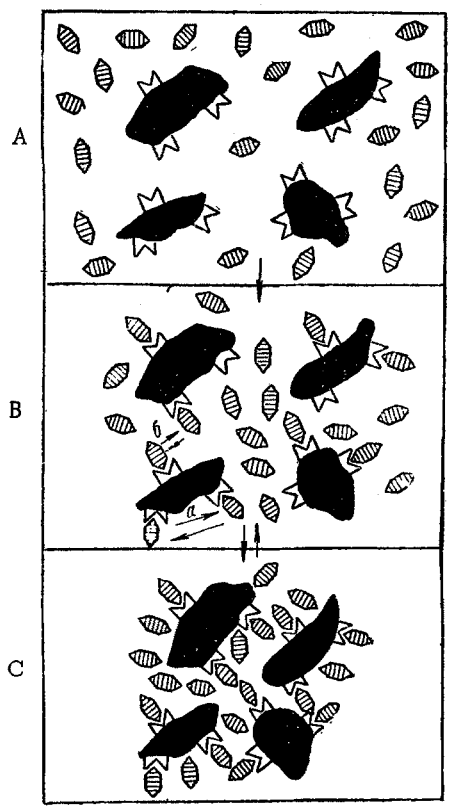
Note. The plus sign means that this antigen gives an agglomeration reaction; the minus sign means that this antigen does not give an agglomeration reaction.

* Complete antigens were extracted with trichloroacetic acid.

** Haptens were extracted by the method of A.T. Kravchenko and M. I. Sokolov (1946).

*** Vaccine strain.

**** Cultures of influenza viruses in the chick embryo.



Scheme of the agglomeration reaction of alizarin suspension antibodies. A) Appearance of the suspension antibodies in the first moment after mixing with homologous antigens; B) adsorption of antigens on alizarin suspension antibodies; C) agglomerate of alizarin suspension antibodies and homologous antigens.

concentrations (25M bacterial cells/ml, complete antigens in dilutions of 1 : 800,000 - 1 : 1,600,000), and may be used for the rapid detection of pathogenic microorganisms, complete bacterial antigens and viruses.

These experimental results thus show that the adsorption of agglutinins, antitoxins and virus complement-fixing antibodies on solid adsorbents is not accompanied by their destruction. The above-mentioned antibodies, adsorbed on solid adsorbents, retain their specific activity and are capable of giving immunological reactions with homologous antigens, which are shown in the form of agglomeration of the particles of the adsorbent. With regard to the immunological activity of precipitins against human and animal serum proteins, adsorbed on solid adsorbents, it is too early yet to form definite conclusions. The absence of an agglomeration reaction between precipitins adsorbed on solid adsorbents and homologous protein antigens gives no grounds for the assertion that the adsorption of these antibodies is accompanied by their inactivation, since immunological reactions between the above-mentioned components may not be accompanied by agglomeration of the particles of the adsorbents. It is also evident that the immunological reaction taking place between antibodies, adsorbed on solid adsorbents, and haptens is not accompanied by agglomeration of the particles of the adsorbent.

The study of the properties of suspensions of adsorbents containing adsorbed antibodies on the surface of their particles showed that, as a result of adsorption of the antibodies on the particles of the adsorbent, new compounds are formed, which combine the properties of the suspensions of adsorbents and of the serum antibodies. We therefore suggest that suspensions of adsorbents containing antibodies on their particles be called "suspension antibodies," and that the reactions between the suspension antibodies and the homologous antigens be called "suspension antibody agglomeration reactions."

The mechanisms of the suspension antibody agglomeration reaction is not yet sufficiently clear. Like other serological reactions, it does not take place in the absence of electrolytes. When the processes taking place in the agglomeration reaction between typhoid suspension agglutinins and typhoid bacilli were observed under the phase-contrast microscope, it was found that in the first moment after mixing the components the bacilli were adsorbed on the particles of the alizarin-suspended agglutinin, and the particles of suspension agglutinin, together with their adsorbed bacilli, then collected together into agglomerates. The combination of antigen with antibodies, adsorbed on alizarin, apparently takes place at the moment of adsorption of the antigen on the alizarin particles. Agglomerates of alizarin suspension antibodies are readily broken up by agitation; when the agitation ceases the alizarin suspension antibodies again form agglomerates. On the basis of our observations the mechanisms of the agglomeration reaction of alizarin suspension antibodies may be illustrated in the form of the following scheme (see Figure).

It will be seen that after mixing of the alizarin suspension antibodies, adsorption of microorganisms takes place on the surface of the alizarin particles (B). The antigens adsorbed on the particles of alizarin suspension antibodies are combined at the moment of adsorption with antibodies, are sensitized and acquire the property of agglutination with each other. The forces a, however, attracting one molecule of antigen to another on the surface

of a single alizarin particle, cannot overcome the forces of adsorption keeping these antigens on the surface of the alizarin particle. For this reason the suspension of alizarin particles, containing sensitized antigens on their surface, becomes unstable. Equilibrium in the system alizarin suspension antibodies – homologous antigens is achieved as a result of the agglutination of the antigens adsorbed on different particles of the alizarin suspension antibodies. The reaction between the sensitized antigens, adsorbed on different particles of the suspension antibodies (forces b), causes agglomeration of the particles of the suspension antibodies (c). Forces a and b are identical in nature – they are the forces which cause agglutination of antigens in the ordinary serological reactions.

There is no doubt that an important part in the agglomeration reaction of alizarin suspension antibodies is played by the electrical changes of the reacting particles and by several other factors. The description of the mechanism of the agglomeration reaction which has been given is therefore purely schematic and does not claim to provide an exhaustive explanation of this complex serological reaction.

SUMMARY

Agglutinins, virus complement-fixing antibodies and antitoxins retain their immunological activity after adsorption on solid adsorbents (alizarin etc.). Precipitins against human and animal serum protein, adsorbed on solid adsorbents do not give agglomeration reactions with homologous antigens. Antibodies, adsorbed on solid adsorbents react even with a small amount of antigen and may be used for quick detection of microorganisms and viruses. A term "suspension antibodies" is suggested for antibodies, adsorbed on solid adsorbents, and "suspension antibodies of agglomeration reactions" – for reactions between suspension antibodies and homologous antigens. The mechanism of this reaction is described.

LITERATURE CITED

1. A. K Adamov, Byull. Éksp. Biol. Med. 50, 8 (1960).*
2. A. T. Kravchenko and M. I. Sokolov, Zhur. Mikrobiol., Épidemiol. i Immunobiol., 12, 10 (1946).
3. M. Eisler, Biochem. 135, 416 (1923).
4. L. Vekerdi, Acta physiol. Acad. Sc. hung 8, 91 (1955).

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