ANTI-ERYTHROCYTE AUTOANTIBODIES IN ANIMALS IMMUNIZED WITH HETEROGENIC SERA

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UDC 616.155.1-008.9-097.5-092.9

The object of this investigation was to study the hemagglutinating antibodies appearing in the blood of guinea pigs during their immunization with heterogenic sera.

EXPERIMENTAL METHOD

The antigen used for immunization of the animals and in the serological experiments was the native serum of a cow, of hens, and of other animals.

Guinea pigs and albino rats received intraperitoneal injections, each of 0.16-0.8 mg serum protein (in 1 ml of dilute serum), not less than 3 times at intervals of 7 days. Blood was taken from the animals on the 7th day after each injection. Before testing the sera were kept at -25°. In the serological tests a mixture of the sera of three animals immunized with one batch of antigen was first used, but later samples of serum from individual guinea pigs were tested. For the titration of the immune sera double dilutions were prepared, starting with 1:10. The heterogenic serum, after preliminary exhaustion to remove heterophilic anti-erythrocyte antibodies, was used in all the experiments in dilutions of 1:200 or 1:100, 0.1 ml of this serum being added to 0.1 ml of the immune serum; 0.2 ml of a 1% suspension of washed erythrocytes in isotonic NaCl solution was added to a mixture of the two sera. The order of mixing the reagents had no influence on the results of the test.

The complement fixation reaction was performed with two doses of the serum in the cold, with contact for 18 h, by the drop method.

EXPERIMENTAL RESULTS

The phenomenon of serum hemagglutination was observed when the highly diluted heterogenic serum and the homologous serum of the immunized animals were added to a suspension of guinea pig's erythrocytes.

In preliminary experiments hemagglutinating activity in relation to guinea pig's erythrocytes was detected in 11 of the 12 pooled sera and in 3 individual sera obtained from 51 guinea pigs immunized with antigens containing cow serum.

In the course of the study of hemagglutinins in the blood of the immunized animals during the period of immunization, a further 68 samples of serum were investigated from 15 guinea pigs, and antibodies were found in 10 of the 12 animals examined on the 7th day after the second injection of antigen. After the 3rd and subsequent injections anti-erythrocyte antibodies were found in all the guinea pigs (Fig. 1).

The erythrocytes obtained from the guinea pigs at the same time as the samples of serum were indistinguishable from the erythrocytes of the unimmunized guinea pigs in their ability to undergo agglutination. No isohemagglutination was found during work with diluted sera in any of the control experiments.

The phenomenon discovered could be regarded as a reaction of indirect agglutination of erythrocytes sensitized by the antigen, a reaction of the Sokolov [1] or Boyden [3] type, were it not for certain differences. The most important of these were the active adsorption of antigen by erythrocytes untreated with tannic acid (this is also observed, by the way, in the Sokolov reaction) and the strict species specificity of the reaction against the erythrocytes of an immunized animal.

To study the adsorption of the antigen by the erythrocytes, its concentration in the suspension was determined by titrating the supernatant after sedimentation of the erythrocytes by centrifuging. To each

Institute of Pediatrics, Academy of Medical Sciences of the USSR, Moscow (Presented by active Member of the Academy of Medical Sciences of the USSR, L. Z. Zil'ber). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 1, pp. 63-67, January, 1957. Original article submitted February 1, 1965.

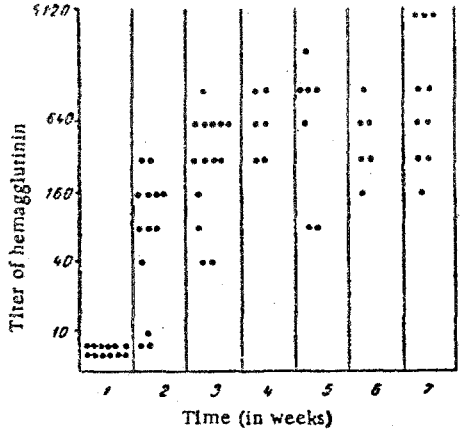

Fig. 1. Hemagglutinating activity of guinea pigs' serum during immunization. Along the axis of ordinates—reciprocals of limiting dilution of serum with which the phenomenon of hemagglutination was still observed.

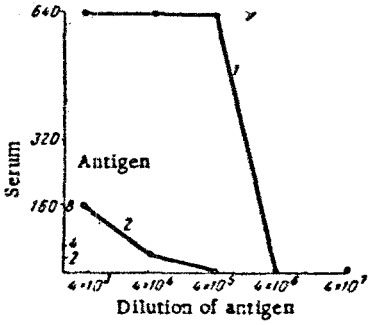

Fig. 2. Elution of serum antigen by washing "sensitized" erythrocytes. 1) hemagglutinating activity of serum against "sensitized" erythrocytes during repeated washing with physiological saline. Each observation corresponds to successive washing in 10 times its volume of physiological saline; 2) titer of antigen in the liquid phase of a suspension of erythrocytes used for determining the hemagglutinating activity of a serum. Along the axis of abscissas—reciprocal of the calculated dilution of the antigen in the erythrocyte suspension during washing.

dilution of antigen, immune serum in a dilution of 1:100 (4–8 hemagglutinating units) and fresh erythrocytes were added. A decrease in the concentration of antigen, the presence of which was essential for the agglutination described, was regularly observed in the diluted cow's serum treated with erythrocytes of healthy guinea pigs. The adsorption was increased with an increase in the concentration of the erythrocyte suspension. However, a 40% suspension did not extract more than 70% of the antigen from the dilute serum, if the adsorption took place at room temperature or at 4°.

The bond between the antigen and the erythrocytes was unstable. The adsorbed antigen was completely removed from the erythrocytes by repeated (from 4 to 7 times) washing of the erythrocytes in a large volume of isotonic sodium chloride solution. During the washing of the erythrocytes a suspension of "sensitized" erythrocytes could be obtained, and no antigen was found in its liquid phase (Fig. 2). This suspension could be used to detect the hemagglutinating activity of a serum without the addition of dilute cow's serum to it.

The washed erythrocytes, completely free from antigen, did not lose the property of readsorbing antigen from the medium. The agglutination titer of these erythrocytes corresponded to the agglutination titer of the untreated erythrocytes.

The concentration of antigen in the medium had no significant effect on the hemagglutinating activity of the serum within a wide range: from 400 to 2 mg%, expressed as total serum protein. A further decrease in the concentration of antigen led to the appearance of a zone of depression of the reaction in low dilutions of the immune serum.

The second and most important difference between the described reaction and the well known methods of indirect hemagglutination was the precise species specificity of agglutination of the erythrocytes. In the present experiments the immune sera of the guinea pigs agglutinated only the erythrocytes of these animals. Erythrocytes of sheep, hens, rabbits, rats, and man, in the presence of cow's serum, were not agglutinated by the "anti-cow" serum of the guinea pigs. In albino rats immunized by the same antigens, hemagglutinins against their own erythrocytes were seen irregularly.

Besides species-specificity, strict specificity of the reaction against the heterogenic antigen used for immunization of the animals was also observed. The erythrocytes of the guinea pigs were agglutinated by "anti-cow" serum only in the presence of cow's serum but not of other sera. The results of preliminary experiments in which guinea pigs were immunized with other heterogenic sera showed that the hemagglutinins thus formed corresponded to the species of the heterogenic serum. (Table 1).

When the titers of hemagglutinating activity of the serum were compared with the level of antiserum complement-fixing antibodies, no direct relationship was found between them (Table 2).

An attempt was made to exhaust the antiserum antibodies by precipitation, mixing the immune serum with cow's serum. From 4 to 5 times in succession, at intervals of 40 min, antigen diluted 1:16 was added to 1 portion of the immune serum, diluted initially 1:2 with salt solution. Addition of the 1st and 2nd

TABLE 1. Specificity of the Hemagglutination Reaction against the Heterogenic Component of the Complex Antigen

Antigen used for immunization of animals	No. of tested guinea pigs' sera	Titer of hemagglutinins of immune sera in the presence of different serum antigens			
		cow's	hen's	rabbit's	human
Cow's serum	859	320	<10	<10	<10
	742	640	<10	<10	<10
	866	1280	<10	<10	<10
	890	640	<10	<10	<10
	892	320	<10	<10	<10
Hen's serum	77	<10	32	<10	<10
	78	<10	16	<10	<10
Rabbit's serum	1023	<10	<10	160	<10
	1024	<10	<10	80	<10
	1025	<10	<10	40	<10

TABLE 2. Hemagglutinating Activity of Serum of Guinea Pigs Immunized with Cow's Serum, Compared with Complement-Fixing Antibodies

Serum No	Content of cow's serum in antigen used for immunization (in mg% of total serum protein)	Titer of antibodies	
		hemagglutinins	antiserum complement-fixing antibodies
709	1.0	20	<10
792		320	<10
841		160	<10
861		320	80
863		640	40
866		1280	<10
890	0.5	640	<10
891		40	<10
892		320	<10
614	0.2	640	40
617		40	<10
859		320	40
742		640	20
839		20	<10

TABLE 3. Exhaustion of Immune Serum by "Sensitized" Erythrocytes

Number of repetitions of experiments in which serum was treated with erythrocytes	Titer of hemagglutinating serum		
	before treatment	after treatment	
		with normal erythrocytes	with sensitized erythrocytes
1	320	320	20
2		240	<10
3		240	<10

portions of antigen led to the formation of a precipitate, which was removed each time by centrifuging. The supernatant after each extraction of the precipitate was titrated to determine the hemagglutinating activity of the exhausted mixture. The dilutions of antigen and serum in the control series corresponded to those in the experimental. In the control the reagents were mixed in each dilution immediately before addition of the erythrocytes. Extraction of the antiserum antibodies into the precipitate did not cause a reduction in the hemagglutinating activity of the serum. Hence, the agglutination of the erythrocytes described above evidently was not associated with the presence of antibodies against heterogenic protein in the immune sera.

The species specificity of the reaction against the erythrocytes of the guinea pig, together with the negative results of the attempt to extract the hemagglutinins with heterogenic antigen used for immunization of the animals, demonstrated the presence of specific anti-erythrocyte antibodies in the serum of the guinea pigs, the specificity of which was determined equally by the antigenic structure of the erythrocyte and of the heterogenic antigen or hapten. The presence of antibodies against such a complex antigen was confirmed by direct experiments in which the sera were exhausted with "sensitized" erythrocytes (Table 3).

After treatment of the immune serum with a 25% suspension of "sensitized" erythrocytes, the titer of the hemagglutinating activity was reduced 16 times. Repeated treatment of the serum led to its complete exhaustion. In the control experiments, treatment of the serum with normal erythrocytes produced no appreciable decrease in its activity.

The bispecificity of the detected antibodies places them in the same category as the antibodies sensitizing human skin [4], or other antibodies of the reagin type found in animals [2, 5]. The appearance of hemagglutinins in the blood can only be explained by autoimmunization of the guinea pigs by a complex antigen formed in the body by adsorption of heterogenic antigen by the erythrocytes. Such autoantigens and the corresponding antibodies may perhaps play an essential role in allergic reactions.

LITERATURE CITED

1. M. I. Sokolov, Zh. Mikrobiol., Nos., 4-5, 57, (1945).
2. B. Benaceraff and E. Kabat., J. Immunol., 20 (1931), p. 20.
3. S. V. Boyden, J. Exp. Med., 20 (1951), p. 81.
4. A. F. Coca and E. F. Grove, Ibid., 10 (1925), p. 455.
5. W. B. Sherman and A. E. O. Menzel, J. Exp. Med., 92 (1950), p. 192.