COMPARATIVE SENSITIVITY OF CERTAIN IMMUNOLOGICAL METHODS OF DETERMINING ANTIBODIES

(UDC 576.8.097.077)

S. K. Mikhkla

Department of Microbiology (Head—Corresponding Member of the Academy of Medical Sciences of the USSR Professor V. I. Ioffe), Institute of Experimental Medicine (Director—Active Member of the Academy of Medical Sciences of the USSR Professor D. A. Biryukov) Academy of Sciences of the USSR, Leningrad

(Presented by Active Member of the Academy of Medical Sciences of the USSR D. A. Biryukov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 9, pp. 122-125, September, 1965
Original article submitted May 14, 1964

Selection of an adequate serological and immunological method is an important and frequently a deciding factor in experimental method and in determination of antigens and antibodies in clinical specimens. For this purpose it is necessary to have definite comparative data pertaining to the sensitivity of different methods and their applications for determining antigens and antibodies of different origin.

Of the data available in the literature pertaining to the comparative sensitivity of different immunological reactions, one should point out first of all those described by Grabar [6]. The author, comparing about 20 methods for determination of antibodies from the data of different investigators, concludes that the passive hemagglutination and Ovary's passive cutaneous anaphylaxis reactions are the most sensitive ones. One can arrive at the same conclusion on the basis of other reports [11, 12, 14]. Certain data about the comparative sensitivity of Boyden's passive hemagglutination reaction and the complement fixation reaction are also found in the literature of the USSR [3, 4], but no one in this country studied comparative sensitivity of passive hemagglutination and passive cutaneous anaphylaxis reactions.

The purpose of this work is to present a comparative study of sensitivity of different immunological methods for determination of antibodies: precipitation reactions in saline and in a gel, complement fixation, Boyden's passive hemagglutination and Ovary's passive cutaneous anaphylaxis.

EXPERIMENTAL

The following antigens were used: horse serum; horse serum albumin and globulin, obtained by salting out; and the polysaccharide antigen of group A streptococci, prepared according to Lancefield's procedure [7]. Rabbit, guinea pig, rat, and mouse sera immunized with suitable protein antigens or hemolytic streptococci vaccines were sources of antibodies.

Saline precipitation reaction was carried out by the usual capillary procedure; precipitation reaction in gel according to Ouchterlony's technique, as modified by L. A. Zie'ber and G. I. Abelev [1]; complement fixation reaction according to prolonged fixation at 4° [2]. These 3 serological reactions were set up according to the so-called right angle scheme, i.e., using different doses of antigens, each of which was tested with different dilutions of corresponding immune sera. All reagents were dissolved in physiological saline. The sensitivity of the reaction was determined from the maximum dilution of immune sera which gave a clearly positive reaction with any of the tested doses of antigen which was optimal for a given serum. As a positive result, complement fixation was considered complete inhibition of hemolysis using double dose of complement.

Stavitsky's modification [13] using tannin-treated human group O erythrocytes was used for passive hemagglutination reaction. The dose of antigen used for saturation of erythrocytes was the one which produced maximum agglutination titer with the tested antibody (thus, in experiments with horse serum albumin and equal volume of

TABLE 1. Comparative Sensitivity of Procedures used to Determine the Concentration of Antibodies in Immune Rabbit Sera

Antigen	Dilution of immune serum yielding a clearly positive result						
	ring precipitation	gel precipitation	complement fixation	passive skin anaphylaxis			Boyden's passive hemagglutination
				in mice	in rats	in guinea pigs	
Horse serum	32	64	400	32	64	4 000	256 000
Horse albumin	4	4	100	8	4	400	8 000
Horse globulin	2	4	100	4	—	200	4 000
Streptococcal polysaccharide	4	8	200	8	—	1 600	12 800

Note: Reaction was not carried out

albumin containing 1 mg albumin/ml was added to 2.5% of tannin-treated erythrocytes; for saturation with serum globulins the dilution containing 0.5 mg/ml was used, horse serum was added as 1:200 dilution, and Lancefield's polysaccharide antigen was added in 1:100 dilution). Guinea pigs, rats and mice were used for Ovary's passive anaphylaxis reaction [12]. The titer of the antigen was also determined; it was equal to 0.6-0.8 ml of horse serum, 4 mg of horse albumin or globulin, and 2 ml of polysaccharide antigen (according to Lancefield) per kg of animal weight.

Comparative sensitivities of different immunologic reactions for determination of antibodies in rabbit immune sera to the complete horse serum, horse albumin and globulin is presented in Table 1. From this table it is seen that the ring precipitation reaction proved to be the least sensitive, allowing determination of serum antibodies diluted 1:2-1:32. The sensitivity of gel diffusion precipitation reaction was not any higher, but this reaction allowed us to visualize qualitative characteristics of antigen–antibody reaction. Thus, it was shown that horse albumin and globulin were not single antigens, since they formed 2 lines in the gel. However, considering the relatively low sensitivity of the reaction, one should stress that there were as many precipitating lines as antigen–antibody systems, or less, as was pointed out in a number of reports [5, 8, 10].

The complement fixation reaction proved to be 20 times more sensitive than the precipitation reaction. It was shown that it was possible to detect even low antibody concentrations in mixtures by complement fixation but not by precipitation in gels.

The passive cutaneous anaphylaxis in mice and rats was no more sensitive than the precipitation reaction. We also carried out experiments after injecting mice with pertussis vaccine, since 20 treated animals are markedly more sensitive to anaphylaxis than are the normal animals. However, regardless of a 20-fold increase of sensitivity to histamine, the intensity of passive cutaneous anaphylaxis remained unchanged. This corresponded to the available data [9]. The use of guinea pigs allowed us to increase the sensitivity of this reaction 50- to 100-fold.

The passive hemagglutination reaction was the most sensitive, allowing a disclosure of a minimal concentration of antibodies. This reaction proved to be 40-80 times as sensitive as the complement fixation reaction with pure protein antigens, and 500 times as sensitive with horse serum antigen.

In a comparative study of antibodies from different animals (mice, rats and guinea pigs) to horse protein (Table 2), we also observed interaction between the sensitivity of precipitation reaction, complement fixation and passive Boyden's hemagglutination. However, the sensitivity of passive cutaneous anaphylaxis depended upon the animal species used for antibody production. It was shown that the sensitivity of reaction in experiments with guinea pigs and rats using mouse antibodies is no higher than that of the precipitation reaction. At the same time, in

TABLE 2. Comparative Sensitivity of Procedures used to Determine Antibodies to Horse Albumin in Immune Sera of Different Species of Animals

Source of antigen	Dilution of immune serum at which there is a definite positive reaction						
	ring precipitation	gel precipitation	complement fixation	passive cutaneous anaphylaxis in mice	passive cutaneous anaphylaxis in rats	passive cutaneous anaphylaxis in guinea pigs	Boyden's passive hemagglutination
Mouse	8	64	160	640	40	40	25 600
Rat	32	64	80	20	2	—	200 000
Guinea pig	2	8	40	—	—	320	40 000

Note: Negative result.

experiments with homologous animals (mice) the reaction was 20 times more sensitive. The opposite response was observed when studying guinea pig antibodies, i.e., the sensitivity of their response was 50-100 times the sensitivity of the precipitation reaction, while it was impossible to establish the reaction in mice and rats. The rat immune serum differed from the other in that the use of homologous animals for anitgen–antibody reaction did not yield greatest sensitivity. Thus, the passive cutaneous rat anaphylaxis was only possible when using 1:2 dilution of rat immune serum, while a similar reaction in mice was 10 times as sensitive. It was not possible to obtain this reaction in guinea pigs. The difference in the sensitivity of a reaction in studying immune sera of different animals species is explained, apparently, by the difference in ability of experimental animals' tissue to fix antibodies.

It should be stressed that in the present study Boyden's passive hemagglutination proved to be the most sensitive reaction. Therefore, this reaction is superior to other immunological reactions with protein antigens and independent of the animal species used for antibody production. When compared to the passive cutaneous anaphylaxis, it is simpler to prepare and it does not require experimental animals.

In experiments with the polysaccharide antigen (streptococcal polysaccharide) and the corresponding immune rabbit serum, similar comparative relation of the reactions was observed as in determination of protein antigens in rabbit sera (see Table 1). Addition of this antigen to tannin-treated erythrocytes allowed for study of antisera in 1:12,800 dilution of immune sera. Replacing the tannin-treated erythrocytes with the native sheep erythrocytes for addition of polysaccharide antigen also allowed for establishment of a passive hemagglutination response, but with a low titer (1:80). However, it should be pointed out that the use of phenol for removal of protein in preparation of the antigen, according to Lancefield's technique, led to cessation of specific agglutination of tannin-treated erythrocytes. At the same time, this preparation retained the ability to participate in reactions. Studies in this direction are continuing. At present, the passive cutaneous anaphylaxis in guinea pigs is the most sensitive reaction for disclosing antibodies to the polysaccharide antigen.

LITERATURE CITED

1. L. A. Zil'ber and G. I. Abelev, Virology and Immunology of Cancer, Moscow (1962), p. 309.
2. V. I. Ioffe and K. M. Rosental', Zh. Mikrobiol., 12 (1943), p. 65.
3. V. I. Ioffe and P. V. Osipova, In the book: The Annual Report of the Institute of Experimental Medicine of the Academy of Medical Sciences of the USSR for 1960, Leningrad (1961), p. 297.
4. V. Ya. Fel', Zh. Mikrobiol., 12 (1959), p. 22.
5. G. N. Chistovich, Zh. Mikrobiol., 11 (1955), p. 56.
6. P. Grabar, In the book: Atti del VI Congresso Internazionale di Microbiologia. Roma, 2 (1953), p. 169.
7. R. C. Lancefield, J. exp. Med., 47 (1928), p. 91.
8. J. Munoz and E. L. Becker, J. Immunol., 65 (1950), p. 47.
9. J. Munoz and R. L Anacker, Ibid., 83 (1959), p. 640.
10. J. Oudin, Meth. med. Res., 5 (1952), p. 335.
11. Z. Ovary and M. Briot, Ann. Inst. Pasteur, 81 (1951), p. 670.
12. Z. Ovary, Progr. Allergy, 5 (1958), p. 459.
13. A. B. Stavitsky, J. Immunol., 72 (1954), p. 360.
14. Idem, Ibid., p. 368.