METHODS

DETECTION OF CELLS PRODUCING ANTIBODIES TO O-ANTIGEN OF S. typhi BY THE METHOD OF LOCAL PASSIVE HEMOLYSIS IN AGAR

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V. V. Solov'ev, L. N. Fontalin, L. A. Pevnitskii, and N. A. Kraskina

Department of General Immunology and Oncology (Head, Active Member of the Academy of Medical Sciences of the USSR Prof. L. A. Zil'ber), N. F. Gamaleya Institute of Epidemiology and Microbiology (Director, Active Member of the Academy of Medical Sciences of the USSR Prof. O. V. Baroyan) Academy of Medical Sciences of the USSR, Moscow, and the Department of Immunology (Head, Prof. M. P. Pokrovskaya), Moscow Institute of Epidemiology and Microbiology (Director, Prof. I. I. Shatrov)
(Presented by Active Member of the Academy of Medical Sciences of the USSR L. A. Zil'ber)
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During the past two years the reaction of local hemolysis (Jerne's reaction) has been widely used abroad in various immunomorphological investigations. However, the possibilities of using this reaction are limited since only erythrocytes can be used as an antigen.

Even in the first work where the method of local hemolysis was described [4], the authors indicated that by using a "covering" of erythrocytes by various antigens, this technique can be employed also for other antigen systems. This is possible if the antigen adsorbed on the erythrocytes, or combined with them by a chemical bond, interacts with homologous antibodies, as in the passive hemagglutination reaction, and upon addition of complement, lysis of erythrocytes occurs (reaction of passive hemolysis) [7, 8].

However, in the literature there are only sporadic reports on such modifications of Jerne's method, and the results obtained are quite indefinite [2, 3]. This is probably because the reaction of passive hemolysis is accomplished not in just any antigen-antibody system and requires special conditions.

We attempted to use the Jerne reaction to determine cells producing antibody against O-antigen of S. typhi. This antigen, just as other complete and polysaccharide antigens, is adsorbed on native sheep erythrocytes, in contrast with protein antigens [6, 7, etc.], and the reaction of passive hemagglutination caused by the addition of antibodies against O-antigen can be transformed to passive hemolysis [7, 8].

The reaction of passive hemolysis with erythrocytes loaded with O-antigen and S. typhi was at first reproduced in test tube experiments. The antisera was obtained by immunization of animals (rats, rabbits) with the endotoxin of strain S. typhi No. O-901 obtained by Boivin's method. For sensitization of the erythrocytes we used the same endotoxin treated with 0.02 N solution of NaOH for 18 h at 37° [5, 8]. To the triply washed sheep erythrocytes we added a solution of O-antigen of S. typhi in a concentration of 0.2 mg/ml at a rate of 20 ml of solution per ml of erythrocyte precipitate. After 2 h incubation at 37° the antigen-loaded erythrocytes were thrice washed free of their excess and a 0.5% suspension prepared from them. To the test tubes each containing 0.5 ml of immune serum in various dilutions we added 0.1 ml of complement of guinea pig in a dilution of 1:10 and 0.25 ml of the suspension of erythrocytes sensitized by O-antigen. The test tubes were incubated an hour at 37°. At the same time we set up the reaction of passive hemagglutination by the usual procedure.

As a result of eight such experiments it was established that the antibodies to O-antigen S. typhi can be elicited in the passive hemolysis reaction, the sensitivity of this reaction being about the same as that of the reaction of

Presence of Cells Producing Antibody to O-Antigen of S. Typhi in Rat Spleen Four Days after Immunization

Animals	No. of rats	Number of antibody-producing cells per 1 million spleen cells	
		Incubation with erythrocytes sensitized with O-antigen	Incubation with normal sheep erythrocytes
Immunized.	11	223 ± 62	4 ± 1
Not immunized. . .	6	5 ± 2	9 ± 4

passive hemagglutination and exceeding the sensitivity of the reaction of bacterial agglutination by the factor of 10-20. This gave us grounds to assume that the passive hemolysis reaction can be used also to elicit antibody-producing cells.

Rats of the Wistar and Avgust lines were immunized once (intravenously) with O-antigen in a dose of 10 μg per animal. It was established that such immunization causes intense antibody formation in the rats beginning with the third day after injecting the antigen. The titer of O-antibodies in the serum reaches a maximum by the 6-7th day (1:5000-1:20,000 in the reaction of passive hemagglutination or passive hemolysis). Four days after immunization the animals were killed and we removed their spleen, from which we prepared a suspension of cells on Hank's or Earl's solution. We gave a detailed account of setting up Jerne's reaction in another work [1]. Here we will describe only the general course of the experiment and its modification for the system "erythrocytes loaded with O-antigen – cells producing O-antibodies."

To 10 ml of diluted 0.85% agar preliminarily washed and prepared on Hank's or Earl's solution we added, at 39-40°, 500 million O-antigen loaded sheep erythrocytes and 15 million cells of the spleen of the immunized rats. The suspension was mixed and poured into petri dishes at a rate of 2 ml as a thin layer on top of 1% solidified agar. After the agar had solidified the dishes were incubated 2 h at 37°, then on top of the agar we poured 3 ml of guinea pig or rabbit complement diluted with physiological solution, 1:5. After 30-60 min incubation at 37°, zones of hemolysis (plaques) were noted in the agar layer which, outwardly, and in size were analogous to those in setting up the Jerne reaction with hemolysin-producing spleen cells. The pattern of the plaques under the microscope was also similar.

The results of counting the number of antibody-producing cells are shown in the table.

As we see from the table the reaction was specific: an appreciable number of antibody-producing cells was detected only upon incubation of the cells of the immunized rats with erythrocytes loaded with antigen. The cells of the unimmunized animals did not give such a reaction.

The sporadic plaques obtained in the control experiments (spleen cells of O-antigen immunized rats and normal rats and sensitized or normal erythrocytes) were probably formed owing to the presence in the rats of cells producing normal antibodies to sheep erythrocytes.

Thus, the results of these experiments showed that the passive hemolysis reaction can be used to elicit and count cells producing antibodies to O-antigen of S. typhi. Our data permit the assumption that the Jerne reaction can be used to investigate cells producing antibodies to a number of complete and polysaccharide antigens.

SUMMARY

The reaction of passive agglutination with O-antigen S. typhi can be transformed by adding complement into a highly sensitive passive hemolysis test. Modification of Jerne's test with the use of erythrocytes sensitized by O-antigen enables one to detect cells producing O-antibodies in the lymphoid tissue of immunized animals. This makes it possible to extend the sphere of use of Jerne's test to a number of experimental models not connected with the production of hemolysins.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
