A MODIFICATION OF THE LOCAL PASSIVE HEMOLYSIS IN GEL TECHNIQUE FOR DETECTING CELLS PRODUCING ANTIBODIES AGAINST Vi ANTIGEN OF Salmonella typhi

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UDC 612.017.1.014.2 : 612.118.221.3

A modification of the local hemolysis in gel technique is suggested for the detection of antibody synthesis by lymphocytes of mice immunized with the Vi antigen of Salmonella typhi. A method of sensitizing erythrocytes with Vi antigen for subsequent use in Jerne's reaction has been developed. The dynamics of the primary immune response of mice to Vi antigen as reflected in the number of plaques was investigated.

The local hemolysis test, which was suggested for the quantitative estimation of antibody-synthesizing cells, is widely used nowadays in immunological research when studying cell populations.

Many attempts have been made to make this method more universal and suitable for different antigens, not only for foreign erythrocytes as in the original variant [5].

Different modifications of the local passive hemolysis in gel tests have been developed for detection of cells synthesizing antibodies against certain proteins [2, 6, 10], synthetic polypeptides [11], haptens [8], and bacterial polysaccharides. Erythrocytes loaded with lipopolysaccharide antigens of Escherichia coli [9], Salmonella enteritidis [7], Salmonella newport [4], and O-antigen of Salmonella typhi [3] were tested in the hemolytic plaque method.

To detect antibodies against Vi antigen of S. typhi, the passive hemagglutination test was developed [1]. However, the human erythrocytes sensitized with Vi antigen used in the investigation cited have proved unsuitable in the author's hands for the local passive hemolysis test.

The optimal method of obtaining indicator cells sensitive to passive hemolysis by the action of specific Vi-antibodies and complement was therefore sought.

A preparation of Vi antigen obtained by a modification of Webster's method at the Moscow Research Institute of Epidemiology and Microbiology was used.*

The blood of a sheep taken with sterile precautions was preserved in Alsever's solution at 4°C; the necessary number of erythrocytes for sensitization was washed with physiological saline in a centrifuge 4 times for 5 min at 600 g.

The sheep erythrocytes were sensitized with Vi antigen as follows. To 9.5 ml physiological saline containing 5 μg Vi antigen, 0.5 ml of the residue of erythrocytes, washed to remove the preservative, was added. The mixture was kept for 1 h at 37°C and shaken every 15 min. At the end of the incubation

* The author is grateful to A. P. Alliluev for providing the dried preparation of Vi antigen.

Laboratory of Immunological Tolerance, Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 12, pp. 103-104, December, 1971. Original article submitted May 31, 1971.

TABLE 1. Dynamics of Immune Response of Mice to Soluble Vi Antigen of S. typhi

Day after injection of antigen	No. of mice	Number of antibody-forming cells per spleen ($M_{geom} \pm m$)
3rd	12	19,680 (13,430-28,840)
4th	12	117,800 (80,910-171,400)
5th	12	11,070 (7015-17,460)
6th	12	5649 (2704-11,800)

time the erythrocytes were washed 10 times with physiological saline, resuspended in 2 ml physiological saline, and counted in a Goryaev's chamber.

To perform Jerne's test, sensitized erythrocytes were added to agarose gel, melted and cooled to 45°C, in the proportion of 100 million cells/ml.

Suspensions of spleen cells from immune animals were examined by the passive local hemolysis test.

Noninbred and inbred mice were immunized by a single injection of 0.2 ml of the Vi antigen solution in a concentration of 5 μg/ml into the caudal vein. On the day of the experiment the animals were killed with chloroform and the spleen minced in Hanks' medium in a pestle-type homogenizer. The number of nucleated cells was counted in the filtered suspension.

The technique of Jerne's test is fully described elsewhere [2]; only the special features of the present modification will be discussed.

Agarose gel containing sensitized erythrocytes, in a volume of 1.5 ml, was poured into test tubes immersed in a water bath at 45°C. The suspension of spleen cells (0.04-0.3 ml) was then added, and the contents of the tubes were mixed and poured out on previously prepared Petri dishes and spread evenly over the whole surface of the bottom layer. Before application of the top layer, the dishes should be heated in an incubator at 37°C. After the gel had solidified, the dishes were incubated for 2 h at 37°C, 3 ml complement solution was added to each, and incubation was continued for a further hour at 37°C. The number of plaques formed was then counted and the number per million nucleated cells or per whole spleen calculated.

Complement was obtained from guinea pig serum frozen at −20°C on the day after taking the blood. On the day of the experiment the serum was thawed and diluted 1 : 10.

It must be noted that for working with this system agarose must be used as the gel and freshly frozen guinea pig serum as the source of complement. Washed Difco agar, commercial lyophilized complement, and rabbit complement all proved unsuitable in these experiments.

By the use of this method to investigate suspensions of spleen cells taken at various times after injection of antigen the dynamics of the immune response of the mice could be determined from the number of plaques (Table 1).

The results of these experiments show that during the first 4 days there is a rapid increase in the number of antibody-forming cells in the spleen. After reaching a maximum on the 4th day, the immune response falls sharply; on the 5th day the number of antibody-forming cells is approximately one order of magnitude lower.

In the control series (mice not receiving antigen) the number of plaques is the same whether the test is carried out with native or with sensitized erythrocytes. The specificity of the test is also confirmed by delay in plaque formation on the addition of 1 μg Vi antigen to the top layer of gel.

The method of local hemolysis can thus be adapted for soluble Vi antigen of S. typhi.

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