Agar Plaque Formation by Mouse Spleen Cells in Response to Vaccination with Vi Antigen and Typhoid Vaccines

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Vi-containing *Salmonella typhosa* organisms were resistant to killing by immune spleen cells in the presence of complement even when the cells were producing Vi antibody.

Landy et al. (4) modified the Jerne plaque technique (3) for the study of antibody response of living cells to bacterial antigens by using erythrocytes coated with somatic polysaccharides as the indicator of plaque formation, and Schwartz and Braun (7) modified the technique for use with living gram-negative bacteria which are killed in the presence of complement. Erythrocytes are not used as an indicator system in the latter technique. This paper reports the plaque formation by spleen cells from mice immunized with *Salmonella typhosa* vaccines and purified Vi antigens with the use of both of the modified techniques.

NIH female mice, 13 to 15 g, obtained from the Rodent and Rabbit Production Section, Laboratory Aids Branch, National Institutes of Health, were injected intravenously (iv), intraperitoneally (ip), or subcutaneously (sc) with 2.5×10^8 bacteria of the acetone-inactivated and dried, U.S. Standard Typhoid Vaccine lot 6A made from Vi-positive strain *S. typhosa* Ty 2 or an acetone-inactivated and dried vaccine made from Vi-negative strain *S. typhosa* 0901 in this laboratory, or 10.0 μ g of soluble Vi antigen prepared by the method of Webster et al. (8). For growth of *S. typhosa*, the plating media of Schwartz and Braun (7) was modified to include 2% Trypticase. Seven-day postimmunization mice were killed, and spleens were excised. Spleen cells from non-immunized mice and immunized cell suspensions without complement were included as controls.

Table 1 summarizes the results obtained by iv injection. Similar results were obtained by sc and ip immunization. Spleen cells of mice immunized with either vaccine 6A or Vi antigen, when plated with Vi-coated erythrocytes (Vi-rbc), formed hemolytic plaques; spleen cells of mice immunized with vaccine 0901, when plated with Vi-rbc, did

not form plaques. Hence the reaction was Vi-antibody dependent and also complement-dependent. Control plates without complement showed no plaques. When spleen cells of mice immunized with any one of the three antigens were plated with living *S. typhosa* Ty 2, they formed no plaques. The spleen cells of mice immunized with the two bacterial vaccines, when plated with 0901 bacteria, formed lytic plaques in the layers of confluent growth; cells from Vi-antigen immunized mice formed no lytic plaques with 0901 bacteria. It appears that the specificity of the reactions with 0901 bacteria may be O-antibody-complement dependent.

The results indicate that this bacterial lytic-plaque system is complement- and Vi antigen-dependent, the same as in the test tube bactericidal test. Muschel and co-workers (5, 6) described variations between Vi-positive and -negative strains of *S. typhosa* to the bactericidal activity of sera. The resistance of some strains appeared to be related to the O-inagglutinability or Vi content of the organisms. Bactericidal antibody titrations in this laboratory with Ty 2 and 0901 strains (*unpublished data*) confirm the antigen dependence of the reaction.

The resistance of strain Ty 2 to bactericidal action is consistent with the concept that the greater virulence of Vi-positive *S. typhosa* as compared to Vi-negative bacteria is due to a protective effect of Vi antigen which renders the organism less susceptible to bactericidal activity. Ineffectiveness of bactericidal activity was not due to the absence of Vi antibody. In the present experiments, the presence of Vi-specific antibody was shown by lysis of Vi-rbc when plated with spleen cells from mice vaccinated with vaccine 6A or Vi antigen and complement and, in an

TABLE 1. *Plaque formation of spleen cells from immunized NIH mice tested against homologous and heterologous antigens*

Spleen cells from mice immunized with	Plaque formation against antigen plated with spleen cells and complement ^a		
	Vi-coated erythrocytes	<i>S. typhosa</i> Ty 2	<i>S. typhosa</i> 0901
Vi antigen	50, 28, 21, 43, 39, 32, (36) ^b	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Vaccine 6A (<i>S. typhosa</i> Ty 2)	23, 11, 35, 17, 23, 38 (25)	0, 0, 0, 0, 0, 0	5, 17, 28, 11, 39, 41 (24)
Vaccine 0901 (<i>S. typhosa</i> 0901)	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	33, 19, 56, 21 63, 37 (38)

^a Values are the number of plaques (PFC per 10⁶ spleen cells) minus the background count.
^b Arithmetic means are expressed parenthetically.

earlier study, by production of Vi-hemagglutinins in NIH mice after immunization with vaccine 6A or Vi antigen (1, 2).
Vi-containing *S. typhosa* organisms were resistant to killing by immune spleen cells in the presence of complement even when the cells were producing Vi antibody.

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