Immunological Response of Three Mouse Strains to Typhoid Vaccine and Vi Antigen

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Vi-agglutinin, active cutaneous anaphylaxis and protective responses (ED50) of three mouse strains (CFW, NIH, and Balb/cAnN) to acetone-inactivated typhoid vaccine and soluble Vi antigen were compared. Seven days after immunization with either typhoid vaccine or Vi antigen the three strains of mice differed with respect to Vi-antibody titers. Significant differences were observed in the protective responses. Each mouse strain was significantly better protected by the intraperitoneal than by subcutaneous route of immunization. Active cutaneous anaphylaxis was more pronounced in showing strain differences in response to Vi antigen. The serological responses to Vi antigen of the strains of mice did not correlate with their protective response.

Recent studies chiefly organized by the World Health Organization (WHO) showed that typhoid vaccines prepared differently varied in protective efficacy for man (12). Extensive laboratory assays of potency of field trial vaccines with mice varied in relative potency and in relation to human efficacy. Influencing variables were the type of vaccine (acetone-inactivated, heat-phoenol-inactivated, alcohol-treated, etc.), route of immunization [intraperitoneal (ip) or subcutaneous (sc)], menstruum of challenge vaccination (mucin or saline), and time interval between vaccination and challenge (7 or 14 days). Pittman and Bohner (12) found that the relative potency of acetone-inactivated and heat-pheno! inactivated vaccines assayed by ip vaccination and mucin challenge after a 7-day interval (IPM7), previously referred to as type N ip or sc assay, correlated well with human efficacy, whereas the relative potency assayed by sc vaccination (SCM7) did not show correlation. Melikova et al. (10) obtained similar results with use of saline suspended challenge after a 7-day interval (IPS7 versus SC57). However, IPM7 assay did not reflect the human efficacy of alcohol-treated vaccine, and the assay after a 14-day interval (IPM14) also failed for this type of vaccine (12). Joó et al. (7) observed a difference in relative potency of acetone-inactivated and heat-pheno! inactivated vaccines (K and L) with a 7- and 14-day interval between vaccination and challenge, whereas Pittman and Bohner found no significant difference. There continues to be lack of consensus on the type of mouse assay for potency of typhoid vaccine.

We are directing our studies toward finding the cause of the variable responses to different types of vaccines. Special emphasis is being placed on the influence of the Vi antigen since the most aberrant assay results have been obtained with the alcohol-treated vaccine. Felix and Pitt (3) treated Salmonella typhosa with alcohol in order to preserve Vi antigen. The early work on acetone-inactivated typhoid vaccine claimed preservation of the Vi antigen (7). However, the field trial acetone-inactivated vaccine K induced variable Vi antibody response in rabbits (12, 14) and practically none in man (1). There is no substantial evidence that Vi antigen alone can protect man. Further, since Vi antigen preparations from heterologous species of bacteria alone are capable of protecting mice (4, 5), the influence of the Vi antigen in assay of typhoid vaccines presents a formidable problem.

Previous studies have reported variable responses of different strains of mice to Vi antigen. Swiss Bagg mice produced Vi antibodies demonstrable by passive hemagglutination. Incomplete Vi antibody was evoked by purified Vi antigen in the Cinnamon mouse and complete antibody in Balb/cAnN mice (4). In a subsequent report, Gaines et al. (5) emphasized the variable response of mice to Vi antigen.

This paper presents the Vi agglutinin response of three strains of mice (CFW, NIH, and Balb/cAnN) to whole-cell vaccine or soluble Vi antigen and relates the results to protection and active cutaneous anaphylaxis (ACA) of two strains.
MATERIALS AND METHODS

Mice. Female mice, 13 to 15 g in weight, from the CFW, NIH, and Balb/cAnN colonies of the Rodent and Rabbit Production Section, Laboratory Aids Branch, National Institutes of Health (NIH), were used.

Antigens. The U.S. Standard Typhoid Vaccine lot 6A, acetone-inactivated and dried, prepared under the supervision of J. Lowenthal of Walter Reed Army Institute of Research by the method used in production of vaccine K (2), and equivalent per bacterium in mouse potency to vaccine K (Pittman, unpublished data), was used as the source of whole-cell Vi antigen. The reconstituted dried vaccine contained 1 × 10⁶ bacteria per ml.

A soluble Vi antigen was extracted from the Vi strain of Escherichia coli 5396/38 by the method of Webster et al. (15). Based on biochemical reactivity this strain may be classified as Citrobacter freundii.

Serological methods. A suspension of acetone-treated bacteria of the Ty 2 strain prepared by the method of Scholten et al. (13) was used for bacterial agglutinin titrations of Vi antibody. The antigen was O inagglutinable. The test was incubated at 37 C for 2 hr and read immediately. The indirect hemagglutination test for Vi antibody was performed as described by Landy and Lamb (9). Buffered saline solution (20 ml of Fisher Buffer Solution Concentrate (pH 7.0) per liter of 0.15 m NaCl solution) was used as diluent in the assay for complete antibody, and 15% bovine serum albumin solution was employed in tests for incomplete antibody (4, 5). The sera were inactivated at 56 C and absorbed with normal human erythrocytes before assay.

Active cutaneous anaphylaxis. ACA procedure was performed by the method of Ovary (11). Mice were immunized by one sc, ip, or intravenous injection of 0.25 ml of typhoid vaccine and 10.0 μg of Vi. Challenges were administered at 7 days postimmunization, by intradermal injection of each antigen, and reactions were read after 30 min. For controls, saline was injected intradermally into sensitized animals and the challenge dose of antigen (Table 4) was injected intradermally into nonsensitized animals. Reactions were graded by the method of Ovary (11).

Mouse protection tests. Active mouse protection tests of vaccine 6A and Vi antigen were performed by the type N ip (IPM7) and type N sc (SCM7) tests described by Pittman and Bohner (12). For each group, 16 mice were injected with a 0.5-ml volume, either ip or sc, with four-fivefold dilutions of the appropriate antigen and challenged 7 days later with approximately 1,000 bacteria of the Ty 2 strain of S. typhosa suspended in 5% mucin. The vaccine doses ranged from 1 × 10⁻⁴ to 12.5 × 10⁻⁴ ml and the Vi antigen doses ranged from 0.08 to 10 μg. Three replicate tests were performed and survivals of the respective doses were pooled for calculation of the eD₅₀ by the Wilson-Worcester method (16) by using the three doses which best bracketed the 50% end point.

RESULTS

Agglutinin response. Table 1 shows the agglutinin response of the three strains of mice at 7 days after a single ip or sc injection of vaccine 6A or soluble Vi in 3 or 4 replicate tests. The route of injection did not significantly affect the response. Hemagglutinin responses of the three mouse strains to vaccine 6A were similar but were not similar to Vi antigen; the NIH and Balb/cAnN strains produced like titers of Vi antibody but CFW mice produced no detectable hemagglutinins. In contrast, bacterial agglutinin titers induced by vaccine 6A were lower than the hemagglutinin titers induced and were detected only

### Table 1. Antibody response of mice to typhoid vaccine 6A and Vi antigen in replicate tests

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Immunizing antigen</th>
<th>Route of injection</th>
<th>Agglutination titer[^a]</th>
<th>Erythrocytes</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFW</td>
<td>6A</td>
<td>ip, sc</td>
<td>60, 60, 60</td>
<td>10, 10, 10</td>
<td></td>
</tr>
<tr>
<td>NIH</td>
<td>6A</td>
<td>ip, sc</td>
<td>60, 60, 60</td>
<td>10, 10, 10</td>
<td></td>
</tr>
<tr>
<td>Balb/cAnN</td>
<td>6A</td>
<td>ip, sc</td>
<td>60, 60, 60</td>
<td>0, 0, 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vi</td>
<td>ip, sc</td>
<td>60, 60, 60, 240 (85)</td>
<td>0, 0, 0, 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vi</td>
<td>sc</td>
<td>60, 60, 60, 120 (71)</td>
<td>0, 0, 0, 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vi</td>
<td>ip, sc</td>
<td>15, 60, 60, 60 (42)</td>
<td>0, 0, 0, 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vi</td>
<td>sc</td>
<td>30, 30, 30, 30</td>
<td>0, 0, 0, 0</td>
<td></td>
</tr>
</tbody>
</table>

[^a]: A single dose 25 × 10⁴ bacteria of vaccine 6A or 10 μg of Vi antigen.
[^b]: Values are expressed as reciprocal serum dilutions of pooled sera from 10 mice per test. Three tests were performed with CFW and Balb/cAnN mice and four tests with NIH mice. Mice were bled seven days after antigen injection.

[^c]: Values in parentheses are geometric means of four tests.
in the sera of the CFW and NIH mice, but no bacterial agglutinins were induced by Vi antigen in any mouse strain. CFW mice were the poorest responders to the soluble Vi antigen; no antibody was detectable by either agglutination test. Assays of all sera failed to demonstrate incomplete Vi antibody.

Protective response. The results of the protective response of each of the three strains of mice to vaccine 6A and Vi antigen are given in Tables 2 and 3, respectively. With the vaccine, each mouse strain was significantly better protected by ip than by sc immunization (Table 2). By either route, a significantly lower ED50 was obtained with NIH than with CFW mice, but the ratios of the sc and ip ED50 or relative potencies obtained with the two strains were alike, 6.5 and 6.2. With the Balb/cAnN mice, the ratio of the sc and ip ED50 was 13.0 and significantly larger than the ratios obtained with NIH or CFW mice. The ip ED50 protective response was equivalent to that of the NIH mice, 1.18, but the sc ED50 response was only 0.59 of that of the NIH mice.

With Vi antigen, only CFW and NIH mice were compared (Table 3). The Balb/cAnN mice were not included because their Vi agglutinin response was similar to that of the NIH mice. The results were in the same order as those obtained with the vaccine. With each mouse strain, the ip ED50 was significantly lower than the sc ED50. The NIH mice responded significantly better than the CFW mice; the ip and sc ED50 of the NIH mice were about 33% of the respective CFW values and lower than those obtained with vaccine 6A. This suggests that NIH mice were more responsive to Vi antigen than were CFW mice. The relative potencies obtained by the ip versus sc injection were lower with the Vi antigen (4.5, NIH and 5.4,

### Table 2. Comparison of protective response of three mouse strains to typhoid vaccine 6A

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Vaccination route</th>
<th>ED50 (μg)</th>
<th>Ratio sc ED50/ip ED50</th>
<th>Relative mouse response (NIH = 1.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFW</td>
<td>ip</td>
<td>4.4</td>
<td>6.2</td>
<td>0.59c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.33–8.27)</td>
<td>(2.9–13.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sc</td>
<td>27.4</td>
<td></td>
<td>0.62c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(17.54–42.70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIH</td>
<td>ip</td>
<td>2.6</td>
<td>6.5</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.03–6.55)</td>
<td>(2.5–16.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sc</td>
<td>16.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(13.98–20.45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balb/cAnN</td>
<td>ip</td>
<td>2.2</td>
<td>13.0b</td>
<td>0.59c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.34–3.61)</td>
<td>(5.7–29.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sc</td>
<td>28.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14.92–55.39)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data were calculated from combined survivals of three replicate tests (total, 48 mice per dose of vaccine). Values are expressed as ED50 × 10−4 ml. Ranges are expressed parenthetically.

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Vaccination route</th>
<th>ED50 (μg)</th>
<th>Ratio sc ED50/ip ED50</th>
<th>Relative mouse response (NIH = 1.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFW</td>
<td>ip</td>
<td>1.52</td>
<td>5.4b</td>
<td>0.36b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.90–2.58)</td>
<td>(2.3–12.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sc</td>
<td>8.23</td>
<td></td>
<td>0.30b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.16–16.30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIH</td>
<td>ip</td>
<td>0.54</td>
<td>4.5b</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.36–0.79)</td>
<td>(2.2–9.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sc</td>
<td>2.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.50–3.87)</td>
<td></td>
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</tr>
</tbody>
</table>

* Data were calculated from combined survivals of three replicate tests (total, 48 mice per dose of antigen).

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Vaccination route</th>
<th>ED50 (μg)</th>
<th>Ratio sc ED50/ip ED50</th>
<th>Relative mouse response (NIH = 1.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFW</td>
<td>ip</td>
<td>1.52</td>
<td>5.4b</td>
<td>0.36b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.90–2.58)</td>
<td>(2.3–12.7)</td>
<td></td>
</tr>
<tr>
<td>NIH</td>
<td>ip</td>
<td>0.54</td>
<td>4.5b</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.36–0.79)</td>
<td>(2.2–9.4)</td>
<td></td>
</tr>
</tbody>
</table>

* Data were calculated from combined survivals of three replicate tests (total, 48 mice per dose of antigen).

* Significantly different from 1.0 at the 95% confidence level.
CFW) than with the vaccine (6.5, NIH and 6.2, CFW) but the differences were not significant. These differences were not significantly different from the relative ip-sc potency of vaccine K observed by Pittman and Bohner (12) with the use of CFW mice in a larger number of tests. In spite of the difference in the Vi agglutinin response of the two mouse strains, the relative potency obtained with Vi antigen by the two routes was the same between the mouse strains and the same as observed with the vaccine. Hence, the presence of Vi agglutinins did not appear to affect the protective response. The fact that the NIH mouse injected with soluble Vi antigen produced Vi hemagglutinins, whereas the CFW mouse did not, and the fact that the NIH mouse was protected with a lower amount of vaccine or antigen than the CFW mouse indicate that the NIH mouse is a better responder.

Active cutaneous anaphylaxis. NIH mice, 7 days postimmunization, displayed ACA when challenged with either vaccine 6A or Vi antigen (Table 4). There was no significant difference in the reactions in NIH mice vaccinated by different routes and challenged by the two antigens. There were differences in degree of reactivity between individual animals within a treatment group. In contrast, CFW mice immunized and tested in the same fashion did not respond with an immediate type hypersensitivity reaction. All control sites were negative in immunized animals challenged with saline and in nonimmunized mice challenged with the test antigens.

The difference in sensitization of the mice correlated with their difference in hemagglutinin response to Vi antigen. Whether there is a direct relation remains to be determined.

**DISCUSSION**

In the laboratory potency assays of typhoid vaccines employed in controlled field trials, considerable variation was noted between laboratories in the relative protective ratios of vaccines K (acetone inactivated) and L (heat-phenol inactivated; 14). Correlation with human efficacy for most typhoid vaccines was obtained when ip immunization and mucin challenge were employed, but the results by this method with alcohol-treated vaccine reflected a poorer relationship to efficacy in man (12). As part of an attempt to identify possible causes for these inconsistencies, the response of three strains of mice to Vi antigen was investigated.

The three strains of mice responded differently in production of Vi antibody after injection of acetone-inactivated vaccine or purified Vi antigen. However, the differences in agglutinating antibody response or ACA were not reflected by differences in protective response to challenge with *S. typhosa*. CFW mice, which failed to develop hemagglutinating antibody or show ACA, exhibited the lowest protective response, but the ratios of sc to ip ED50 of both vaccine and Vi antigen were the same as with NIH mice. With NIH and Balb/cAnN mice the response to ip injection was similar in antibody and protective activity but their protective responses to sc injection differed significantly. The lower response was in the Balb/cAnN mice.

In this connection, it is interesting that the different routes of immunization did not result in significant differences in Vi antibody titers after a single injection of antigen. This appears to indicate that factors other than humoral Vi antibody must be involved in the level of protection against challenge. Perhaps some type of cellular response is initiated within the peritoneal cavity to a greater extent by ip than by sc vaccination. The concept of an important cellular response is strengthened by the appearance of protection in CFW mice in the absence of demonstrable humoral antibody after immunization with purified Vi antigen.

Further strain variation was apparent from the ACA experiments in which CFW mice failed to display this phenomenon.

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**Table 4. ACA in mice immunized and challenged with typhoid vaccine 6A and Vi antigen**

<table>
<thead>
<tr>
<th>Immunizing antigen</th>
<th>Vi Challenge</th>
<th>6A Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ip</td>
<td>sc</td>
</tr>
<tr>
<td>Typhoid vaccine 6A</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>1+</td>
</tr>
<tr>
<td>Vi</td>
<td>± 2</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>Tr</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>± 3</td>
</tr>
</tbody>
</table>

* NIH and CFW mice were tested 7 days postimmunization. Values shown are for NIH mice and expressed for individual mice. With CFW mice, no reaction occurred. ACA reactions were rated as follows: 0 = no reaction, tr = trace reaction, ± = less than 4 mm, 1+ = 4-6 mm, 2+ = 6-10 mm, and 4+ = >10 mm.
* Immunizing doses were 0.25 ml of typhoid vaccine 6A (10⁹ organisms per ml) or 10 μg of Vi antigen.
* Mice were challenged by intradermal injection of 0.01 ml of typhoid vaccine 6A or 2 μg of Vi antigen.
The significance of Vi antigen in the immunization of man against typhoid fever is yet to be ascertained. Studies by others (4, 5) with various mouse strains showed the ability of Vi antigen to protect this species against challenge with S. typhosa. However, Benenson (1) noted that Vi response to vaccines employed in field trials could not be correlated with efficacy in man, and Hornick et al. (6) found no correlation between Vi antibody titers and immunity to induced typhoid in a human volunteer population.

The present paper (i) provides additional evidence of the variable response of mice strains to acetone-inactivated typhoid vaccine and soluble Vi antigen, (ii) confirms the previous findings in this laboratory by Pittman and Bohner (12) on the relative protective response of CFW mice to ip and sc routes of immunization, and (iii) points up the significant difference in the relative response of Balb/cAnN mice. The differences observed in the WHO study (14) which ranged from 1.18 to 15.3 could have been due to the inherent capacity of the mouse strains used. The study has failed to provide an explanation for the lack of correlation between sc and ip injection of acetone- and alcohol-inactivated vaccine (12). The antibody response of the different strains to Vi antigen did not correlate with their protective response. Although this study was not concerned with classification of immunoglobulins, it would be of interest to determine if different classes were produced by the mice in response to whole-cell vaccine versus soluble Vi antigen. The antibody response of rabbits to Vi antigen is presented in an accompanying paper.

LITERATURE CITED


