Effectiveness of Parenteral and Oral Typhoid Vaccination in Mice Challenged with a *Salmonella typhi-Salmonella typhimurium* Hybrid


*Laboratory Center for Disease Control, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada,* and Department of Bacterial Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20022

Received for publication 13 September 1976

Live *Salmonella typhi* administered intraperitoneally, acetone-killed *S. typhi* administered intraperitoneally, and live *S. typhi* given orally, with their effectiveness decreasing in that order, protected Swiss white mice against death from challenge with a virulent *Salmonella typhimurium* hybrid expressing *S. typhi* antigens.

We reported previously (3-5) the development of an assay system for differentiating the protective activities of various typhoid vaccines. It uses Swiss Webster white mice as the test animals and mouse-virulent *Salmonella typhimurium* hybrids that express *Salmonella typhi* antigens as the challenge strains. In earlier studies with this system (4, 5), we showed that it can demonstrate differences among various kinds of typhoid vaccines with respect to their ability to confer protection against death of the animals. Our studies also provided evidence that the *Salmonella* somatic antigens are important in conferring this protection, whereas the Vi antigen was seen to play no significant role (3).

In these earlier investigations, the vaccines we examined were, in all cases, nonliving, and the immunizing doses were administered intraperitoneally (i.p.). In the present study, we have used our assay system to investigate the protective capabilities of living as well as acetone-treated (AK) *S. typhi* vaccines administered both by the oral route and i.p. We have also examined a purified *S. typhi* Vi antigen preparation administered both i.p. and orally.

The AK vaccine was prepared from *S. typhi* as described previously (4). The purified Vi antigen, prepared from *S. typhi* strain no. 59, was generously supplied by S. Marcus of the University of Utah, Utah Medical Center, Salt Lake City. Swiss Webster white mice (50 per group), HPB strain, random bred, 16 to 18 g, were inoculated i.p. with 0.5 ml of the AK vaccine (equivalent to $5 \times 10^8$ organisms) or with 0.5 ml of a live vaccine ($10^7$ organisms) prepared from a 16-h culture of *S. typhi* TY2. Vi antigen vaccine was administered in two doses of 0.5 ml (500 µg/dose), with a 1-week interval between the first and second injection. The same doses of vaccines AK, live, and Vi were administered orally to other groups of mice. Animals vaccinated with AK and Vi vaccines either i.p. or orally were challenged i.p. 2 weeks after the last vaccination, as described previously (4), with 2,500 organisms (0.5 ml) of *S. typhimurium* hybrid H42. This hybrid expresses the *S. typhi* antigens 9, 12, Vi, and d and has a mean lethal dose of less than 50 organisms. Animals injected with live vaccines either i.p. or orally were challenged after 5 weeks in the same manner. The longer interval between live immunization and challenge has been recommended by other investigators (8, 10).

From the results of these experiments, as presented in Table 1, it is apparent that the best protection against death from the hybrid challenge organism was afforded by the live and AK vaccines when administered i.p. The live vaccine was slightly better than the AK vaccine in this comparison ($P < 0.05$), a result that is in accordance with the findings of a number of investigators using various other experimental systems (1, 2, 6-9). Several workers have pointed out that the superiority of live vaccines is particularly well demonstrated when they are administered orally (2, 6, 7), and the present results would appear to bear this out; oral administration of the live cells afforded significant protection against death, whereas oral administration of the AK vaccine did not. It might be worth noting, however, that the degree of protection afforded by the live cells administered orally did not, in this system, match that afforded by the AK vaccine.
administered i.p. Whether or not a similar situation would be observed in immunizing against human typhoid fever with live, attenuated, orally administered vaccines, as opposed to killed vaccines parenterally administered, remains to be determined.

In a previous study (5), we tested purified Vi antigen prepared from Citrobacter freundii for its protective capability (administered i.p.) in this system and found it afforded no protection against death from the hybrid challenge organism. As might have been expected, the presently tested Vi antigen, prepared this time from S. typhi, also failed to protect the animals, whether administered i.p. or orally.

<table>
<thead>
<tr>
<th>Vaccinated with</th>
<th>Vaccinating dose</th>
<th>Route</th>
<th>Interval between last vaccination and challenge (weeks)</th>
<th>No. of survivors/no. injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>AK</td>
<td>5 × 10⁶ cells</td>
<td>i.p.</td>
<td>2</td>
<td>30/50⁵</td>
</tr>
<tr>
<td>AK</td>
<td>5 × 10⁶ cells</td>
<td>Oral</td>
<td>2</td>
<td>7/50</td>
</tr>
<tr>
<td>TY/2 live</td>
<td>10⁵ cells</td>
<td>i.p.</td>
<td>5</td>
<td>40/50⁴</td>
</tr>
<tr>
<td>TY/2 live</td>
<td>10⁵ cells</td>
<td>Oral</td>
<td>5</td>
<td>21/50⁴</td>
</tr>
<tr>
<td>Vi</td>
<td>2 × 500 µg</td>
<td>i.p.</td>
<td>2</td>
<td>9/50</td>
</tr>
<tr>
<td>Vi</td>
<td>2 × 500 µg</td>
<td>Oral</td>
<td>2</td>
<td>3/50</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>8/50</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly better (P < 0.005) than the controls.
* Significantly better (P < 0.01) than the controls.

**Table 1. Effectiveness of AK, live, and Vi antigen vaccines in mice challenged with S. typhimurium hybrid H42**

**LITERATURE CITED**