Effect of Vaccine, Route, and Schedule on Antibody Response of Rabbits to Pasteurella tularensis

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The response of the rabbit to viable or killed whole-cell Pasteurella tularensis vaccines was studied. The most practical preparation for the production of anti-P. tularensis antibodies was viable organisms of the live vaccine strain (LVS). The intravenous route of administration proved superior to either the subcutaneous or intradermal routes, and incorporation of LVS into Freund's adjuvants did not result in increased levels of antibody. Short-term hyperimmunization, three injections at weekly intervals, constituted the most efficient method for increasing levels of the antibodies.

The rabbit is commonly used for the production of antisera against a wide variety of antigens (3). There is, however, little information concerning the antibody response of this animal to Pasteurella tularensis (2, 4, 6). The objectives of this study were to determine optimal procedures for antibody production and to study the response of the rabbit to viable or killed whole-cell P. tularensis vaccines.

MATERIALS AND METHODS

Rabbits. New Zealand white rabbits, weighing between 1.8 and 2.5 kg, were used throughout the investigation. Except where noted, all experimental groups contained five animals.

Vaccines and vaccination techniques. P. tularensis was grown in a modified casein hydrolysate medium (MCPH) similar to that described by Mills et al. (Bacteriol. Proc., p. 37, 1949). Numbers of viable cells were estimated by plating appropriate dilutions on glucose-cysteine-blood-agar (6).

The vaccine strain (LVS) described by Eigelsbach and Downs (5) was used to produce viable vaccine. The usual preparation was a saline dilution of an MCPH culture containing 10⁶ or 10⁷ viable organisms per ml. In one experiment, equal portions of LVS in saline were mixed with either Freund's complete or incomplete adjuvant (BBL) to give a final concentration of 10⁶ viable organisms per ml.

Strain SCHU S4, a fully virulent North American strain, was employed as a killed (phenol-Merthiolate) or viable vaccine; the killed preparation contained 10⁹ organisms per ml. To infect rabbits intravenously (iv), 1,000 viable cells were used; after clinical symptoms appeared (3 to 4 days), the animals were treated with streptomycin (6).

Serological techniques. Trial bleedings were made from the marginal ear vein, and animals were exsanguinated by cardiac puncture. Three serological tests were performed on sera. Bacterial agglutination tests were performed with formalinized SCHU S4 cells as antigen (1). Passive hemagglutination tests were done with polysaccharide-treated sheep erythrocytes (9). Precipitins were assayed by double diffusion in agar by use of the technique and strain SCHU S4 antigen (sonic lysate) described by Tulis and Eigelsbach (Bacteriol. Proc., p. 138, 1961). Precipitates were allowed to develop for 21 days at 37 C in a moist atmosphere.

RESULTS

Primary and secondary antibody responses to three vaccines. The agglutinin responses of three groups of 10 rabbits, each inoculated iv with 10⁹ killed strain SCHU S4, 10⁶ viable strain LVS, or 10⁹ viable strain SCHU S4, respectively, are presented in Table 1. During the primary response the highest peak titer was obtained with viable strain SCHU S4; killed strain SCHU S4 induced the poorest response. The peak titers were compared by use of Student's t test, and the difference between titers produced by viable SCHU S4 and killed strain SCHU S4 was the only significant one (P = 0.05).

Rabbits receiving viable strain SCHU S4 maintained significantly higher titers on the 21st and 28th days than those given LVS; at these times, animals inoculated with killed SCHU S4 exhibited the lowest titers. Twenty-eight days after vaccination, the killed SCHU S4 and LVS groups were revaccinated with 10⁹ cells of the appropriate vaccine by the iv route. The animals in the viable SCHU S4 group were not revaccinated, because only three animals survived the infection despite streptomycin therapy. Both revaccinated groups of rabbits had elevated
agglutinin titers 7 days after revaccination, and maximal titers were significantly higher \((P = 0.05)\) than the declining titers observed during the late primary response \((28\) days). However, when the maximal secondary and primary response titers were compared, there were no significant differences in the values. At the 42nd, 49th, and 56th days, the titers of the rabbits revaccinated with viable LVS and the titers of the surviving animals undergoing a prolonged primary response to viable SCHU S4 were similar.

The passive hemagglutinin titers of two groups of rabbits for each of the three vaccines are presented in Table 2. Highest primary response titers \((1:10,240)\) were obtained from animals vaccinated with viable SCHU S4. Second highest titers \((1:5,120)\) were exhibited by animals that had received the LVS vaccine. Killed SCHU S4-vaccinated rabbits had the lowest titers. Revaccination of the animals previously given killed strain SCHU S4 or viable strain LVS induced maximal passive hemagglutinin titers 7 days after administration of the vaccines. When LVS was employed, the maximal secondary titers were fourfold to eightfold higher than during the primary response. The hemagglutinin response of rabbits surviving the viable SCHU S4 infection showed that these animals maintained relatively high primary titers \((1:5,120)\).

The agar gel precipitin response of the three groups of rabbits is presented in Table 3. During the primary response, the viable LVS and viable SCHU S4 vaccines induced similar precipitin patterns with four or five bands; the killed SCHU S4 vaccine induced a poorer response. Revaccination caused an increased response with one of the two killed SCHU S4 pooled samples; the number of precipitin bands in animals given live strain LVS was not increased over that observed during the primary response.

**Vaccination by various routes.** The bacterial agglutinin responses of rabbits inoculated with 10\(^9\) viable LVS cells via three routes are presented in Table 4. The routes employed were iv, subcutaneous \((sc)\), or intradermal \((id)\). For the id route, one group of animals was inoculated at one site and another at five sites \((each\ extremity\ and\ interscapular\ region)\). During the primary response, maximal titers of all groups occurred on the 14th day; the highest mean titer \((1:768)\) was obtained by iv vaccination. During the secondary response, the group revaccinated by the iv route had significantly higher titers than those vaccinated by other routes. In no case was the maximal titer during the secondary response significantly different from that after primary
vaccination. These findings are supported by passive hemagglutination data obtained on the sera.

**Antibody response of rabbits to viable LVS contained in Freund's adjuvants.** The primary administration of 10⁸ LVS organisms in complete or incomplete adjuvant by the sc route did not result in greater antibody production than was observed in animals that received 10⁶ LVS without adjuvant by the iv route (Table 5). Revaccination on the 28th day did not result in an appreciable increase in agglutinin titers over maximal levels observed after primary vaccination. Similar conclusions resulted from the passive hemagglutination and agar gel precipitation studies.

**Hyperimmunization with killed and viable vaccines.** The antibody response of 10 rabbits to eight weekly iv injections of 10⁸ killed SCHU S4 cells is presented in Table 6. Regardless of the assay technique employed, hyperimmunization did not result in a higher antibody response than that previously observed after a secondary response to the killed SCHU S4 vaccine. Maximal values during the hyperimmunization were comparable to those obtained after primary immunization with viable LVS.

The mean agglutinin titers and passive hemagglutinin titers of pooled sera from rabbits vaccinated iv with 10⁸ or 10⁹ viable LVS at weekly intervals for 8 successive weeks are presented in Table 6. Maximal titers by both techniques were obtained on the 21st day (three prior doses of vaccine) with sera from animals vaccinated with 10⁸ cells; the agglutinin titer was 1:1,920 and the passive hemagglutinin titer was 1:81,920. Antibody levels then decreased even though vaccination was continued. The agar gel precipitation technique revealed that the animals responded with an early and continued production of four or five precipitin bands (Fig. 1). Some groups of rabbits were extremely sensitive to this hyperimmunization technique and mortality rates as high as 80% were observed.

To alleviate the high mortality, rabbits were hyperimmunized with 10⁸ viable LVS cells at weekly intervals for 8 weeks. The antibody response to this vaccination schedule was somewhat lower than with hyperimmunization by 10⁹ cells per dose. Maximal bacterial agglutinin and hemagglutinin titers were reached by the 21st day with titers of 1:1,024 and 1:20,480, respectively. Subsequently, the titers, as assayed by both techniques, declined. The precipitin response was slower with 10⁹ than with 10⁸ cells per dose, and comparable patterns were not observed until the 35th day.

The bacterial agglutinin response of rabbits hyperimmunized with three doses of 10⁸ viable LVS cells administered iv at weekly intervals was studied. Termination of the hyperimmunization after 3 weeks did not result in data appreciably different from those with hyperimmunization continued for 8 weeks.

**DISCUSSION**

These experiments demonstrated that the most practical whole-cell preparation for the production of anti-<i>P. tularensis</i> antibodies in rabbits was viable strain LVS. The initiation of an overt infection with virulent SCHU S4 organisms followed by eradicative streptomycin therapy induced a slightly better response, but this technique is certainly more cumbersome and hazard-
TABLE 6. Antibody response of rabbits after iv hyperimmunization with viable or killed P. tularensis

<table>
<thead>
<tr>
<th>Vaccine dose</th>
<th>Serological technique</th>
<th>Reciprocal titer on indicated day</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>7</td>
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<tr>
<td>10⁰ Viable LVS</td>
<td>Bacterial agglutination</td>
<td>272</td>
</tr>
<tr>
<td></td>
<td>Passive hemagglutination</td>
<td>2,560</td>
</tr>
<tr>
<td>10⁰ Viable LVS</td>
<td>Bacterial agglutination</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>Passive hemagglutination</td>
<td>2,560</td>
</tr>
<tr>
<td>10⁰ Killed SCHU S4</td>
<td>Bacterial agglutination</td>
<td>224</td>
</tr>
<tr>
<td></td>
<td>Passive hemagglutination</td>
<td>2,560</td>
</tr>
</tbody>
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* Injections at eight weekly intervals.
* Mean values.
* Pooled samples.

Fig. 1. Precipitin response of rabbits hyperimmunized with viable P. tularensis LVS. Ag, antigen wells. Numbered wells are the antiserum wells, and the numbers indicate the days after initiation of immunization.

The antibody response of rabbits after inoculation with viable LVS by one of three routes showed that the iv route resulted in the best response, but the reason for the clear superiority of this route is not known. This finding is similar to that of Snyder et al. (8), who studied the antibody responses of rabbits after injection of viable Mycobacterium tuberculosis. Undoubtedly, there is widespread dissemination of the viable organisms and their antigenic products to most, if not all, of the lymphoid tissues after iv administration, and this could account for the better response. The similarity in responses between the animals vaccinated at either one id or five id sites indicates that a subdivision of the initial antigenic mass into five equal portions and the involvement of five rather than one draining lymphoid network are insufficient to induce a better response than that following iv administration. The pathogenesis of the LVS “vaccination infection” for the rabbit has not been studied; possibly, when the organisms are administered by either the sc or id route, the organisms and their antigenic components of importance remain localized.

The failure of eight weekly iv injections of killed SCHU S4 vaccine to induce better antibody responses than one injection of viable LVS or secondary vaccination with killed SCHU S4 is interesting because hyperimmunization is commonly used for the production of high-titered antisera against a variety of antigens (3). In contrast, short-term hyperimmunization of rabbits with viable LVS constituted the most practical method for increasing the levels of anti-P. tularensis antibodies. The viable LVS schedule could be terminated after three injections at weekly intervals, because the titers subsequently declined in spite of a continuing antigenic challenge. The decline in antibody levels despite repeated vac-
Antibody response of rabbits to P. tularensis
cination has also been observed in chickens inoculated with killed or viable SCHU S4 organisms (unpublished data).

In general, the results obtained with the killed strain SCHU S4 antigen compare favorably with those reported by Carlisle et al. (2). Maximal levels of antibody in the rabbits were achieved 1 week after both primary and secondary vaccination; however, these investigators obtained somewhat higher antibody titers. This discrepancy most likely resulted from the use of an estimated 700-fold more bacteria for the primary response and 100-fold more for the secondary response (7).

These studies have shown that the rabbit can be used for the production of high-titered anti-P. tularensis antiserum and that the optimal method of vaccination is the iv administration of viable LVS cells at three weekly intervals.

Acknowledgments

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Literature Cited