Aerosol Immunization of Guinea Pigs with Fluid Tetanus Toxoid

HERBERT M. YAMASHIROYA, RICHARD EHRLICH, AND JOSEPHINE M. MAGIS

Life Sciences Division, Illinois Institute of Technology Research Institute, Chicago, Illinois

Received for publication 18 October 1965


The feasibility of immunization via the respiratory route was explored by using fluid tetanus toxoid administered to Hartley strain guinea pigs. Purified concentrated fluid toxoid with a potency of 2,405 Lf (limit of flocculation) units per milliliter was used. The vaccine contained no preservatives. Young adult guinea pigs in groups of eight were subjected to predetermined respiratory doses of toxoid by varying the duration of aerosol exposure. The aerosol chamber has been described previously (Miller and Ehrlich, J. Infect. Diseases 103:145, 1958). The aerosol cloud produced by a University of Chicago-type atomizer was sampled with an all-glass impinger fitted with a particle-size discrimination device, a British preimpinger. The impinger contained 10 ml of physiological saline as the collecting fluid. The collecting fluid, containing particles <5 μ, was assayed for toxoid concentration by by the flocculation test. Tetanus flocculating serum was furnished by the Division of Biological Standards, National Institutes of Health. The calculated respiratory dose was the product of the aerosol concentration of particles <5 μ, minute volume of respiration, and the duration of exposure.

The primary antibody response after inhalation of aerosolized fluid tetanus toxoid was measured by the passive hemagglutination method (Stavisky, J. Immunol. 72:360, 1954). For comparative purposes, groups of guinea pigs were inoculated subcutaneously with fluid toxoid concentrations equivalent to the calculated respiratory doses. Blood for serological analysis was obtained by cardiac puncture 3 and 5 weeks after vaccination. At the end of 6 weeks, the guinea pigs were challenged subcutaneously with 10 MLD of tetanus toxin. The animals were observed for 14 days, and the survivors were bled to determine the extent of the secondary response.

As shown in Table 1, there was only a marginal primary antibody response in the majority of the guinea pigs after inhalation of 9 Lf units of toxoid. Five of eight guinea pigs responded with a mean serum hemagglutination titer of 17 at 5 weeks after vaccination. These animals showed signs of tetanus within 10 days after challenge with toxin, and subsequently died. The three surviving guinea pigs had a mean titer of 732 prior to challenge and showed no signs of tetanus.

Inhalation of 15 Lf units of toxoid increased the titer two- to fourfold over that produced by 9 Lf at 3 and 5 weeks after vaccination. Five of seven guinea pigs showed a mean titer of 526 at 5 weeks, and were fully protected from the lethal challenge dose. One guinea pig, with a titer of only 40 at 5 weeks, survived, but showed paralysis on and after the 5th day postchallenge. The remaining guinea pig, which died, had a titer of 80 prior to challenge.

Guinea pigs vaccinated subcutaneously with 9 or 15 Lf units showed mean titers of 1,158 and 4,609, respectively, at the end of 5 weeks. Except for a slight paralysis on the 1st day after challenge in three guinea pigs receiving 9 Lf units, none of the animals showed any response to the challenge dose.

These results indicate that although serum antibody titers are lower after aerosol vaccination than after subcutaneous vaccination, definite immunity is conferred after primary inhalation of tetanus toxoid. A titer of approximately 526 or greater afforded protection against 10 MLD of tetanus toxin.

The secondary response in guinea pigs surviving the toxic challenge is of considerable interest. Although the prechallenge titers in the aerosol-vaccinated guinea pigs were much lower than in
Table 1. Primary antibody response in guinea pigs after aerosol or subcutaneous vaccination with fluid tetanus toxoid, and survival 14 days after subcutaneous challenge with tetanus toxin

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose (Lf)</th>
<th>3 weeks*</th>
<th>5 weeks*</th>
<th>Survival after challenge</th>
<th>Mean hemagglutination titer Prechallenge</th>
<th>Mean hemagglutination titer Postchallenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td>9</td>
<td>98</td>
<td>71</td>
<td>3/8†</td>
<td>732</td>
<td>5,861</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>196</td>
<td>278</td>
<td>6/7</td>
<td>342</td>
<td>512,000</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>9</td>
<td>640</td>
<td>1,158</td>
<td>6/6</td>
<td>1,158</td>
<td>1,465</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2,560</td>
<td>4,609</td>
<td>6/6</td>
<td>4,609</td>
<td>94,305</td>
</tr>
</tbody>
</table>

* Time after primary dose.
† Survivors/total.

Those vaccinated subcutaneously, the antibody response 2 weeks after challenge was greater. The titer increased eightfold in guinea pigs receiving a respiratory dose of 9 Lf, compared with less than a twofold increase in guinea pigs vaccinated subcutaneously with an equivalent dose. Guinea pigs receiving a respiratory dose of 15 Lf units showed an approximately 1,500-fold increase in titer 2 weeks after challenge. In comparison, subcutaneously vaccinated guinea pigs showed only an approximately 20-fold increase in titer.

The marked increase in titer elicited by subcutaneous challenge of aerosol-vaccinated guinea pigs probably reflects the response of multiple antibody-producing sites activated by the greater dissemination of antigen via the respiratory route. The rate of acceleration of the secondary response after subcutaneous challenge with tetanus toxin is being investigated.

This investigation was supported by the Office of the Surgeon General, U.S. Army Medical Research and Development Command, under Contract DA-49-193-MD-2588.

The cooperation of J. M. McGuire of Eli Lilly & Co. and E. B. Seligman of the National Institutes of Health in providing tetanus toxin, toxoid, and antitoxin is gratefully acknowledged.