Humoral Immunity to F1 and V Correlates with Protection Against Pneumonic Plague in Mice

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Abstract

Background

Several strains of plague (P. pestis) are the etiological agent of bubonic and pneumonic plague. Bubonic vaccinia approaches comprising recombinant toxins of the F1 (F) and V (V) proteins have demonstrated protection against pneumonic plague in mice. Here, we perform a dose-ranging efficacy study in mice using an F1/V-based vaccine in attempts to identify immune correlates of protection following inhalation challenge with F. pestis CO92.

Methods

On Days 0 and 21, Balb/c mice (n=19) in groups 1-6 were vaccinated with 10, 2.5, 6.25, 12.5, 25, and 50 µg vaccine plus Alumgluec, respectively. Blood was obtained on Days 7, 14, 21, 28, 36, and 49 for analysis for F1- and V-specific antibodies by ELISA. On Day 43, all surviving mice were challenged with a mean lethal concentration of 1.0×10⁸ CFU/mL. F. pestis CO92 via naso- or conjunctival exposure and monitored for 14 days post-exposure. The immunological responses were measured by ELISA and western blotting.

Results

Vaccine doses of 10 and 2.5 µg induced robust antibody titers to both F1 and V, F1-specific antibodies were detected as early as Day 7 post-vaccination. Concomitantly, V-specific antibodies were not detected until Day 21. No survivors were observed following the challenge dose on Day 43 (50 µg CO92).

Conclusions

F1 and V antibodies induced by an F1/V-based vaccine correlated with protection against pneumonic plague.

Introduction

Despite considerable research efforts, no vaccine is currently available to protect humans against pneumonic plague. A formalin-killed (F1/V)-based vaccine was produced in large quantities by the U.S. Army, however, due to its reactivity and failure to protect against pneumonic plague it is no longer used.

Methods

Experimental Design

Six groups of mice were vaccinated with decreasing concentrations of 10 µg F1/V antigen. In Group 1 to 0 µg/mL, in Group 3 the F1/V-based vaccine was shown in Table 1. Mice were vaccinated on Days 0 and 21. Five mice from each vaccinated group and 2 mice from the control group were euthanized on Days 7, 14, 21, 28, 36, and 49. Titers were measured and the mice were challenged on Day 49 and euthanized on Day 55.

Table 1. Description of test article administration to each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Test/Control Article Administered</th>
<th>Test/Control Article Dose Level</th>
<th>No. of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1/V-based vaccine 10 µg</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>F1/V-based vaccine 2.5 µg</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>F1/V-based vaccine 0.625 µg</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>F1/V-based vaccine 0.125 µg</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>F1/V-based vaccine 0.0625 µg</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Vehicle + Adjuvant</td>
<td>18</td>
<td>10</td>
</tr>
</tbody>
</table>

* Group 6 received the same concentration of adjuvant (0.1%) as Group 1 (the highest concentration of adjuvant delivered).

Bacterial Production

F. pestis CO92 working stocks were thawed and inoculated on tryptic blood agar base plates. Plates were incubated at 28 ± 1°C for approximately 6 to 72 hours and bacterial growth was harvested in 1% proteose. The concentration of the bacterial suspension was estimated based on an optical density (OD562) reading with the actual concentration of the challenge material determined by retrospective plate counts.

Inhalation Exposure System and Procedure

Mice were exposed to a mean aerosol concentration of 1.0×10⁶ CFU/mL of F. pestis CO92 for 30 minutes in a rake nose-only inhalation exposure system on Day 43 and monitored for up to 14 days post-exposure. The immunological responses were measured by ELISA and western blotting.

Immunological Analysis

Figure 3. The survival curves of all vaccinated mice at 35 days post-exposure are shown below. The mice were vaccinated on Day 0 and the second boost immunization on Day 21. The horizontal bar indicates immune response. The curve indicates the median for each group.

Microbiological Analysis

Figure 4. Survival of mice vaccinated with F1/V-based vaccine and challenged with F. pestis CO92.

Figure 5. Average concentration of F. pestis CO92 in the blood and spleen collected at euthanasia. The number of samples that were included in the analysis are shown above the bar.