Efficacy of a standard human anthrax vaccine against *Bacillus anthracis* aerosol spore challenge in rhesus monkeys

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In conducting research using animals, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals, of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, revised 1985)

The views, opinions and/or findings contained in this publication are those of the authors and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

Summary

The efficacy of a licensed human anthrax vaccine was tested in rhesus monkeys challenged by an aerosol of virulent *Bacillus anthracis* spores. Adult rhesus monkeys were injected intramuscularly at 0 and 2 weeks with 0.5 ml of vaccine or phosphate-buffered saline. At 8 weeks, 38 weeks or 100 weeks, the animals were challenged by *B. anthracis* aerosolized spores. All immunized animals survived challenge at either 8 weeks or 38 weeks, and seven of eight animals survived challenge at 100 weeks. All control animals died 3 to 5 days after challenge. Serum from immunized animals possessed demonstrable antibodies to protective antigen by ELISA.

Introduction

*Bacillus anthracis* is the causative agent of anthrax, a disease primarily of herbivores, but one which humans can acquire through contact with infected animals or animal products. The anthrax vaccine licensed for human use in the United States, MDPH (manufactured by the Michigan Department of Public Health, Lansing, Michigan, U.S.A.), consists of aluminum hydroxide-adsorbed supernatant material, principally protective antigen (PA), from fermentor cultures of a toxigenic, nonencapsulated strain of *B. anthracis* V770-NP1-R. Several recent studies described an aerosol inhaled dose of 5.5 X 10² CFU/ml, then heat-shocked at 60°C for 45 min. Eight-mI aliquots of the spores were used for aerosol challenge with a three-jet Collison nebulizer as previously described. The concentration of spores in the aerosol (sampled in water in an all-glass impinger) and the aerosol inhaled dose (expressed as LDa) were also determined as previously described. An aerosol inhaled dose of 5.5 X 10² spores of the *B. anthracis* Ames strain was previously determined to be 1 LDa in rhesus monkeys (B. Ivins, unpublished observations).

Results

All 10 of the immunized monkeys challenged at 8 weeks survived a small-particle aerosolized dose of spores (255 to 760 LDa), whereas all five PBS controls died 3 to 5 days after challenge (Table 1). Similarly, at 38 weeks, all three monkeys survived an aerosol spore challenge of 161 to 247 LDa. At 100 weeks, the final group of eight immunized and two control monkeys were aerosol challenged with 739 to 535 LDa of spores. Seven of eight immunized monkeys survived. Of the all surviving immunized animals, only one had a demonstrable, transient bacteremia, which lasted from days 2 to 6 after challenge. The bacteremia never exceeded 200 CFU per ml on the days assayed. The two control monkeys died 4 days after challenge. Terminal bacteremias in control monkeys that died during the study ranged from 4.7 X 10⁹ to 5.5 X 10⁹ CFU per ml.

Weekly pre- and postchallenge bleeds were drawn on all animals, and the sera were assayed for antibodies to PA by enzyme-linked immunosorbent assay (ELISA) either by an indirect method using baculovirus-produced PA, in which mouse monoclonal antibody to PA was first bound to the ELISA plates, or by a direct method in which *B. anthracis*-produced PA was bound to the ELISA plates (C. Rossi, personal communication). Blood was cultured quantitatively for 10 days after challenge as described previously.

Spore challenge

The virulent Ames strain of *B. anthracis* was obtained from the U.S. Department of Agriculture, Ames, Iowa. It was grown in Leighton-Doi medium, and spores were harvested and washed in sterile, distilled water as previously described. The spores were purified by centrifugation through 58% Renografin-76, washed again, then resuspended in 1% phenol and stored at 4°C.

For aerosol challenge, spores were suspended to a concentration of approximately 1.5 X 10⁷ CFU/ml, then heat-shocked at 60°C for 45 min. Eight-mI aliquots of the spores were used for aerosol challenge with a three-jet Collison nebulizer as previously described. The concentration of spores in the aerosol (sampled in water in an all-glass impinger) and the aerosol inhaled dose (expressed as LDa) were also determined as previously described. An aerosol inhaled dose of 5.5 X 10² spores of the *B. anthracis* Ames strain was previously determined to be 1 LDa in rhesus monkeys (B. Ivins, unpublished observations).

Materials and methods

**Animals**

Adult male and female rhesus monkeys (*Macaca mulatta*), weighing 4.4 to 16.8 kg, were immunized intramuscularly at 0 and 2 weeks with 0.5 ml, the standard human dose, of the MDPH human anthrax vaccine. Control animals were given 0.5 ml of phosphate-buffered saline (PBS). Animals were challenged by an aerosol of *B. anthracis* spores of the virulent Ames strain at either 8 weeks, 38 weeks, or 100 weeks. Survival for 3 months after challenge was noted, and moribund animals were euthanized.

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For aerosol challenge, spores were suspended to a concentration of approximately 1.5 X 10⁷ CFU/ml, then heat-shocked at 60°C for 45 min. Eight-mI aliquots of the spores were used for aerosol challenge with a three-jet Collison nebulizer as previously described. The concentration of spores in the aerosol (sampled in water in an all-glass impinger) and the aerosol inhaled dose (expressed as LDa) were also determined as previously described. An aerosol inhaled dose of 5.5 X 10² spores of the *B. anthracis* Ames strain was previously determined to be 1 LDa in rhesus monkeys (B. Ivins, unpublished observations).

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Discussion

A human anthrax vaccine must protect against all forms of anthrax, including inhalation anthrax, which, although rare, is usually fatal. The data in this study demonstrates that the MDPH vaccine is highly efficacious against inhalation anthrax in rhesus monkeys. The rhesus monkey is a useful model for inhalation anthrax in humans, although there is currently no known surrogate marker or in vitro correlate of immunity that allows direct comparison of immunity in humans to that in monkeys. Although the current vaccine regimen in humans calls for doses at 0, 2, and 4 weeks, 6 months, 12 months, 18 months, and then yearly thereafter, in this study only two doses of vaccine, at 0 and 2 weeks, were required to provide substantial protection for almost two years. Based on this study’s data, the MDPH human anthrax vaccine confers substantial protection against inhalation anthrax, and the recommended immunization regimen may be able to be reduced with respect to the number of doses.

Table 2 Anti-PA ELISA titers of immunized monkeys

<table>
<thead>
<tr>
<th>Time after first immunization</th>
<th>Geometric mean titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 weeks</td>
<td>ND</td>
</tr>
<tr>
<td>2 weeks</td>
<td>14</td>
</tr>
<tr>
<td>(before second immunization)</td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>919</td>
</tr>
<tr>
<td>(before 8-wk challenge)</td>
<td></td>
</tr>
<tr>
<td>10 weeks</td>
<td>7,879</td>
</tr>
<tr>
<td>(2 weeks after challenge)</td>
<td></td>
</tr>
<tr>
<td>99 weeks</td>
<td>200</td>
</tr>
<tr>
<td>(1 week before 100-wk challenge)</td>
<td>28,265</td>
</tr>
<tr>
<td>102 weeks</td>
<td></td>
</tr>
<tr>
<td>(2 weeks after 100-wk challenge)</td>
<td></td>
</tr>
</tbody>
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* ELISA performed by using indirect method
* ND = Not detectable
* Not previously challenged at 8 weeks or 38 weeks
* ELISA performed by using direct method

PA is a major component of MDPH, and previous efficacy studies demonstrated that PA must be present in a non-living anthrax vaccine or produced in a live vaccine. Other components such as edema factor, lethal factor, and cell-surface antigens may be present in some lots of MDPH and might affect the vaccine’s efficacy. MDPH contains an adjuvant aluminum hydroxide (Alhydrogel), which is a good stimulator of humoral immunity, but not cell-mediated immunity. The high level of efficacy of MDPH in rhesus monkeys suggests that humoral immunity is important in the specific resistance of rhesus monkeys to anthrax. In guinea pigs, however, intramuscular immunization with MDPH only partially protects against a challenge with anthrax spores.

These findings suggest the importance of various, specific immune mechanisms against inhalation anthrax may vary in different animal species, or that the ability of the licensed human anthrax vaccine to stimulate cell-mediated immunity may be greater in some species than others.

References