

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

B. E. IVINS*‡, P. F. FELLOWS*, M. L. M. PITT†, J. E. ESTEP†, S. L. WELKOS*, P. L. WORSHAM* and A. M. FRIEDLANDER*

Bacteriology Division and Applied Research Division†, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD, 21702-5011, USA*

‡To whom correspondence should be addressed

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).

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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 toxinogenic, nonencapsulated strain of *B. anthracis*, V770-NP1-R². Several recent studies demonstrated the partial efficacy of MDPH in guinea pigs challenged parenterally with *B. anthracis* spores of the virulent Ames strain^{7-10,13}, and, in a field evaluation in humans, a vaccine similar to MDPH showed protection against anthrax². Unfortunately, no study has been reported on the efficacy of the vaccine in nonhuman primates against an aerosol spore challenge. Thus, the research reported here was conducted to determine the short-term, mid-term, and long-term efficacy of MDPH against inhalation anthrax in rhesus monkeys.

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.

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 PA⁵, 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 previously⁴.

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 described⁶. 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 1.5×10^9 CFU/ml, then heat-shocked at 60°C for 45 min. Eight-ml aliquots of the spores were used for aerosol challenge with a three-jet Collison nebulizer as previously described^{3,4,11}. The concentration of spores in the aerosol (sampled in water in an all-glass impinger) and the aerosol inhaled dose (expressed as LD₅₀) were also determined as previously described^{3,4,11}. An aerosol inhaled dose of 5.5×10^4 spores of the *B. anthracis* Ames strain was previously determined to be 1 LD₅₀ in rhesus monkeys (B. Ivins, unpublished observations).

Results

All 10 of the immunized monkeys challenged at 8 weeks survived a small-particle aerosol inhaled dose of spores (255 to 760 LD₅₀), 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 LD₅₀. At 100 weeks, the final group of eight immunized and two control monkeys were aerosol challenged with 239 to 535 LD₅₀ of spores. Seven of eight immunized monkeys survived. Of all the 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 4.7×10^6 to 5.5×10^8 CFU per ml.

Mean anti-PA ELISA titers before and after challenge are presented in Table 2. Immunized animals exhibited a substantial increase in titer after the 2-week booster and also after challenge at 8 weeks. By 99 weeks, titers dropped to a barely detectable level, but 2 weeks after challenge at 100 weeks, they rose sharply to a geometric mean of 28,265.

Blood was drawn for clinical evaluation every other day after challenge for 10 days from the three monkeys challenged at 38 weeks. The white blood cell counts increased, whereas red blood cell counts, hematocrit, and hemoglobin decreased. Other parameters such as fibrin degradation products, fibrinogen, activated partial thromboplastin time, prothrombin time, and platelets were not affected.

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 1. Protection of rhesus monkeys by MDPH from aerosol challenge by *B. anthracis* Ames spores

Time after first Immunization ^a	LD ₅₀	Survived/total (%)	Time to death in days (range)
8 weeks			
MDPH	255-760 ^b	10/10 ^c (100)	—
PBS	189-435 ^d	0/5 (0)	3-5
38 weeks			
MDPH	161-247 ^e	3/3 ^e (100)	—
100 weeks			
MDPH	239-535 ^f	7/8 ^g (88)	4
PBS	511-535 ^h	0/2 (0)	4

^aMonkeys were immunized intramuscularly at 0 and 2 wk with 0.5 ml of MDPH human anthrax vaccine. ^bMean LD₅₀ = 437. ^cAll surviving monkeys had negative bacterial cultures through 10 days after challenge. ^dMean LD₅₀ = 303. ^eMean LD₅₀ = 203. ^fMean LD₅₀ = 330. ^gOne of the seven surviving monkeys had a positive bacterial culture (days 2-6) after challenge. ^hMean LD₅₀ = 523.

Table 2. Anti-PA ELISA titers of immunized monkeys

Time after first immunization	Geometric mean titers
0 weeks ^a	ND ^b
2 weeks ^a (before second immunization)	14
8 weeks ^a (before 8-wk challenge)	919
10 weeks ^a (2 weeks after challenge)	7,879
99 weeks ^a (1 week before 100-wk challenge ^c)	200
102 weeks ^d (2 weeks after 100-wk challenge ^c)	28,265

^a ELISA performed by using indirect method.

^b ND = Not detectable.

^c Not previously challenged at 8 weeks or 38 weeks.

^d ELISA performed by using direct method. Titers obtained by the direct method gave values which were approximately 1.71-fold greater than those obtained by the indirect method.

PA is a major component of MDPH, and previous efficacy studies^{6,10} 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 as an adjuvant aluminum hydroxide (Alhydrogel), which is a good stimulator of humoral immunity, but not cell-mediated immunity¹. 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^{7,10,13}.

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.

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