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The efficacy of an anthrax vaccine licensed for human use, MDPH-PA, was tested in guinea pigs intramuscularly challenged with 10, 100 or 1,000 LD_{50}s of spores from two virulent strains of Bacillus anthracis, Vollum 1B and Ames. As demonstrated in other investigations, immunization with MDPH-PA provided better protection against challenge from the Vollum 1B strain than from the Ames strain, although vaccine efficacy against the Ames strain was better than previously reported. Enzyme-linked immunosorbent assay of serum antibody

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Efficacy of a standard human anthrax vaccine against *Bacillus anthracis* spore challenge in guinea pigs

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Keywords: anthrax, *Bacillus anthracis*, vaccine efficacy

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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SYNOPSIS

The efficacy of an anthrax vaccine licensed for human use, MDPH-PA, was tested in guinea pigs intramuscularly challenged with 10, 100 or 1,000 LD_{50} of spores from two virulent strains of Bacillus anthracis, Vollum 1B and Ames. As demonstrated in other investigations, immunization with MDPH-PA provided better protection against challenge from the Vollum 1B strain than from the Ames strain, although vaccine efficacy against the Ames strain was better than previously reported. Enzyme-linked immunosorbent assay of serum antibody titers to B. anthracis protective antigen showed no significant correlation between survival and antibody titer.

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The anthrax vaccine licensed for human use in the United States, MDPH-PA, is prepared by the Michigan Department of Public Health. This vaccine contains aluminum hydroxide-adsorbed supernatant material, consisting primarily of anthrax protective antigen (PA), from fermentor cultures of a toxinogenic, nonencapsulated strain of Bacillus anthracis, V770-NP1-R[1]. The vaccine is intended for use in individuals who are at risk of acquiring anthrax due to occupational contact with animals and animal products, such as hides, wool, meat and bones. Two problems associated with the vaccine include a high incidence of local reactions and the requirement for numerous boosters[2,3]. A third problem that has been reported is the apparent inability of the vaccine to fully protect guinea pigs from challenge by certain highly virulent strains of B. anthracis such as Ames and New Hampshire[4,5]. Turnbull et al.[4] reported that three biweekly immunizations with MDPH-PA protected only 17% of guinea pigs intramuscularly (i.m.) challenged with 500 to 1,000 spores of such B. anthracis strains. In contrast, the immunization regimen completely protected guinea pigs from a similar challenge of B. anthracis Vollum spores[4]. Little and Knudson[5] reported that three biweekly doses of MDPH-PA failed to protect guinea pigs from an i.m. challenge of 1,000 spores of B. anthracis strains such as Ames and New Hampshire, yet strongly protected the animals from challenge by other strains such as Vollum and Vollum 1B. However, the above studies were performed using equal numbers rather than equally lethal doses of challenge spores.

Furthermore, in more recent investigations, MDPH-PA has provided some protection to guinea pigs challenged by the highly virulent Ames strain of B. anthracis[6]. The study presented here was thus undertaken to clarify whether there are, in fact, measurable differences between so-called "vaccine-resistant[5]" B. anthracis strains, such as Ames, and "vaccine-sensitive" strains, such as Vollum 1B, in their ability to overcome immunization with MDPH-PA in the guinea pig.

Female, Hartley guinea pigs, 350 to 400 g, in groups of 12, were given either one i.m. dose (at 2 weeks) or two doses (at 0 weeks and 2 weeks) of 0.5 ml of MDPH-PA (lot FAV006). Two days before challenge each animal was bled by cardiac puncture, and its serum tested for antibody to PA by enzyme-linked immunosorbent assay (ELISA) as described previously[5]. At 8 weeks the animals were challenged i.m. with 10, 100 or 1,000 LD_{50} of spores from either the Vollum 1B or the Ames strain of B. anthracis. The i.m. LD_{50} of the spore preparations from the Ames and Vollum 1B strains in female Hartley guinea pigs were predetermined to be 175 and 306 spores, respectively[7]. Deaths of the guinea pigs were recorded for 3 weeks after challenge.

Statistically, the survival distribution function was estimated by using the product-limit method to describe the distribution of lifetimes of animals within each treatment group. Comparison of survival curves was tested by using two non-parametric rank tests, Log-rank and Wilcoxin. Both rank tests are reported because the Log-rank tends to give more weight to

deaths that occur later in the observation period[3]. Differences in death rates between treatment groups were examined by Fisher's exact test. The statistical tests and parameter estimates were produced by SAS®, Version 6.04 (SAS Institute, Inc., Cary, NC).

The data in Table 1 demonstrate the differences between strains Vollum 1B and Ames in their ability to kill guinea pigs immunized with MDPH-PA. The Ames strain has been described as "vaccine-refractory" or "vaccine resistant[5]", and in the present study, the Ames strain indeed proved to be more virulent in immunized guinea pigs than the Vollum 1B strain, even though equivalent LD_{50} of spores of the two strains were used for challenge. These data are consistent with the data of Turnbull et al.[4] and Little and Knudson[5] In the present study, however, the MDPH-PA vaccine was more protective against the Ames strain than previously reported[4,5].

Animals challenged with the Ames strain survived at a significantly lower rate ($P<0.05$) in both 1- and 2-dose immunization schedules at the 100 and 1000 LD_{50} levels of challenge dose, and also when the data from both the 1- and 2-dose immunization schedules and all three challenge dose levels were combined ($P = .0002$). The estimated survival distribution functions in Figure 1 show that the distribution of postchallenge survival times also differed significantly between strains (Wilcoxin, $P = 0.0003$; Log-Rank, $P = 0.0002$), with an increased number of deaths occurring between 4 and 7 days postchallenge in the Ames-challenged group. It is unknown why one or two doses of

MDPH-PA do not completely protect guinea pigs challenged with B. anthracis Ames strain spores. Other studies[6,9,10] suggest that the relevant epitopes required for inducing a protective immune response are present in the PA antigen contained in MDPH-PA, as immunizing guinea pigs with MDPH-PA combined with potent adjuvants substantially protected guinea pigs from an Ames strain spore challenge. Indeed, in these previous investigations[6,9,10] the protective efficacy of MDPH-PA was greatly augmented by adjuvants such as Freund's adjuvant, killed Corynebacterium ovis or Bordetella pertussis, or monophosphoryl lipid A. These adjuvants are known to enhance cell-mediated as well as humoral immune responses, and it is therefore probable that the diminished efficacy of MDPH-PA (not combined with other adjuvants) is due to its inability to stimulate sufficiently the full complement of immune mechanisms responsible for protection against anthrax.

Guinea pigs immunized with either one or two doses of MDPH-PA had demonstrable serum anti-PA titers (Table 2). However, as in other studies, there was no significant correlation between titer and survival[3,5,6]. Furthermore, there was no particular anti-PA titer identified, above which guinea pig survival to challenge was assured, although six of seven animals with antibody titers less than 100 died from the Ames spore challenge. The observation that anti-PA titers by themselves cannot reliably predict survival from spore challenge further suggests either that immune mechanisms in addition to antibody formation play a

significant role in specific immunity to anthrax, or that the ELISA is not a reliable measure of the host's humoral response to specific protective antigenic epitopes.

The data in this study indicate that in guinea pigs the human anthrax vaccine, MDPH-PA, is only partially protective against i.m. challenge by spores of the B. anthracis Ames strain. However, the level of protection for MDPH-PA against Ames strain spores was greater than that previously reported[4,5]. Possible reasons for these reported differences include i) genetic or physiological differences in the guinea pigs or possible underlying subclinical diseases in the animals, ii) differences in challenge strain preparations and iii) variations among individual lots of MDPH-PA. Although the lot of MDPH-PA used for vaccination in the studies of Little and Knudson[5] was not available for this study, we have found that other lots of MDPH-PA vary significantly in their efficacy in guinea pigs (B. Ivins, unpublished data). In addition, although the basis of vaccine resistance in strains such as Ames and vaccine sensitivity in strains such as Vollum 1B has not been elucidated, Welkos et al.[11] have demonstrated that virulence differences in B. anthracis strains are both plasmid- and chromosome-mediated. Finally, results presented in this study clearly emphasize the need for a human anthrax vaccine to be efficacious against all virulent B. anthracis strains, including those strains that in guinea pigs are refractory to immunization with MDPH-PA.

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Table 1 Protection of guinea pigs from *B. anthracis* Vollum 1B and Ames spore challenge by MDPH-PA[a]

	Percent survival after challenge with			Combined
	10 LD_{50}	100 LD_{50}	1,000 LD_{50}	totals
One immunization				
Vollum 1B challenge	88	83	83	84
Ames challenge	58	40[b]	58	53[b]
Two immunizations				
Vollum 1B challenge	92	92	83	89
Ames challenge	90	58	42[b]	63[b]
PBS controls				
Vollum 1B challenge	17	17	0	11
Ames challenge	36	0	0	11

[a]Guinea pigs were immunized at 0 and 2 weeks (two doses) or 2 weeks (one dose), then challenged i.m. with anthrax spores at 8 weeks.

[b]Survival value significantly less ($P<0.05$) than that seen in the corresponding group of Vollum 1B strain-challenged guinea pigs.

Table 2 Serological response of guinea pigs immunized with MDPH-PA and challenged with either B. anthracis Ames or Vollum 1[illegible] spores[illegible]

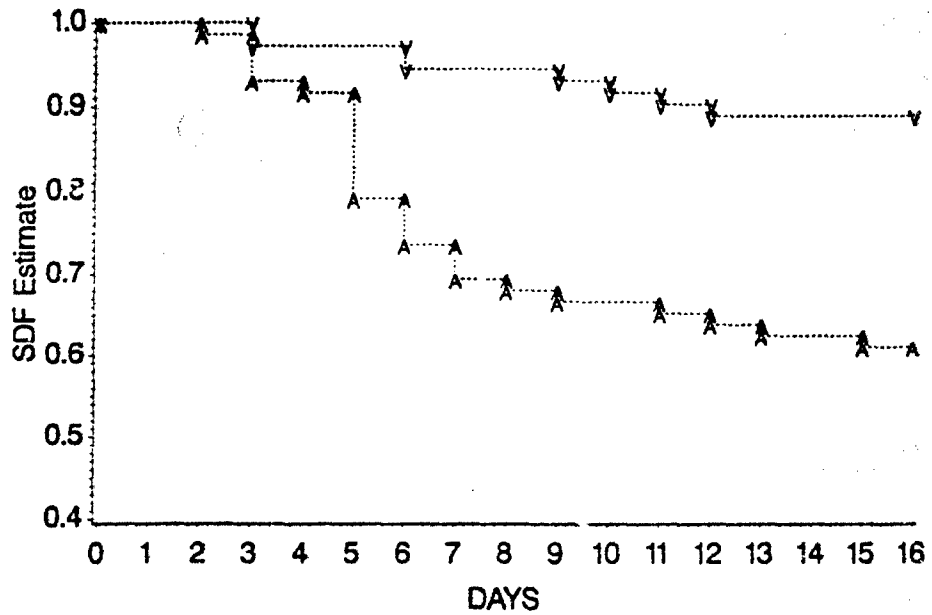
	One immunization[b]	Two immunizations[c]	Com[illegible] totals
Survivors	2,307	5,937	[illegible]776
Non-survivors	263	4,436	964
Total	1,337	5,532	2,636

[a]Reciprocal geometric mean anti-PA ELISA titers of sera from guinea pigs bled 2 days before challenge. Although anti-PA titers appeared somewhat higher in survivors, there was no statistically significant correlation between survival and antibody titer.

[b]Titers in both survivors and non-survivors ranged between 3 and 10,000.

[c]Titers in both survivors and non-survivors ranged between 1,000 and 10,000.

Figure 1 Survival distribution function (SDF) estimates of guinea pigs immunized with MDPH-PA and challenged with either B. anthracis Ames or Vollum 1B spores.



A = Ames
V = Vollum 1B