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Serum IgG antibody response to the protective antigen (PA) of *Bacillus anthracis* induced by Anthrax Vaccine Adsorbed (AVA) among U.S. military personnel

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Abstract

The seroconversion rates and geometric mean concentrations (GMC) of IgG anti-PA for stored sera from U.S. military personnel immunized 3, 4, and 6 times with the U.S. licensed anthrax vaccine adsorbed were studied. Anti-PA IgG concentrations were measured by ELISA. All 246 vaccinees had low but detectable pre-immunization anti-PA IgG (GMC 1.83μg/mL). Three doses elicited a GMC of 60 μg/mL and a seroconversion rate of 85.3%, four doses elicited a GMC of 157 μg/mL and 67.9% and the sixth of 277 μg/mL and 45.5% respectively. The forth dose elicited 100% seroconversion compared to the pre-immunization level. These results should facilitate comparison between different immunization schedules and new vaccines.

Keywords

Anthrax; vaccine; IgG; bacillus anthracis; military

1. Introduction

Worldwide, the threat of bioterrorism with biological weapons against military and civilian populations is a major concern. *Bacillus anthracis*, the causative agent of anthrax, is one of the many infectious agents identified as a potential bioterrorist weapon as demonstrated by recent events in the United States [1]. Inhalation anthrax is difficult to diagnose and must be rapidly treated in order to avoid its fatality rate of almost 100%. The signs of cutaneous anthrax may not be typical and if not treated has a 25% fatality rate [2]. Based upon limited clinical data

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[3] the Department of Defense (DoD) announced plans to vaccinate U.S. military personnel with Biothrax™ (anthrax vaccine adsorbed [AVA]) through the Anthrax Vaccine Immunization Program (AVIP) [4]. This announcement was reaffirmed in September 30, 1999 by Executive Order #13139 [5] mandating that all personnel in military operations, where they could be exposed to biological weapons or endemic disease, be provided with safe and effective vaccines and treatments. From 1998 to 2006, 1.2 million US military personnel have received AVA [6].

The signs and symptoms of anthrax are mediated by an extracellular toxin that conforms to the AB model of bacterial toxins. The B, or binding component, is called Protective Antigen (PA). The two enzymatically active A components are Lethal Factor (LF) and Edema Factor (EF). PA alone is nontoxic and a critical (protective) level of serum antibodies to this protein confers immunity to inhalational and cutaneous anthrax in laboratory and wild animals. Some protection to mice and rats was shown by antibodies, mostly mAbs, to LF & EF but the contribution of antibodies to these two proteins to immunity to B. anthracis is an open question [7]. Limited evidence of protection by anti EF against challenge with anthrax toxin or with spores of non-capsulated *B. anthracis* strain Sterne was published recently [8].

AVA is produced from the culture supernatant of a mutant strain of *B. anthracis*, V63340 77/-NP1-R that expresses mostly PA and does not induce antibodies to the LF or the EF [9]. Patients convalescent from anthrax have antibodies to all three components of anthrax toxin [10]. The current schedule for administering AVA is by subcutaneous dose of 0.5 mL at 0, 2, 4 weeks and at 6, 12 and 18 months for the primary series with annual boosters for as long as the individual remains at risk for infection [11]. It has been suggested that increasing the time interval to two months between the first three immunizations, such as is recommended for diphtheria and tetanus toxoids will induce comparable levels of antibodies as the current schedule [12-14].

The objective of this study was to characterize the seroconversion rates (> 4-fold rise) and GMC of anti-PA IgG for stored samples from U.S. military personnel immunized 3, 4, and 6 times with AVA according to the licensed vaccine schedule

2. Materials and methods

2.1. Data sources

The Department of Defense Serum Repository (DoDSR) collects and stores serum samples from U.S. military personnel. The DoDSR has more than 39 million sera linked to the Defense Medical Surveillance System, a central system of medical surveillance data. The majority of these samples are the remainder of sera drawn for mandated HIV-1 testing. Stored sera are labeled with a unique identifier and are included in DMSS along with testing dates, personnel information, medical events, deployment data, inpatient and outpatient records, and immunization data [15].

2.2. Study subjects

All active duty or former active military personnel with both AVA vaccination information and appropriate serum samples available in the DoDSR were selected as study subjects, as of October 2002. Military service members who received at least three, four, or six doses of AVA, compliant with the immunization schedule [11], and who were seronegative for HIV infection in sera pre and post dose, were eligible for this study. These sample sets are the result of a convergence of the individuals having the AVA immunization and being tested for HIV-1 infection within the time period defined by the protocol's methods. These samples are not necessarily representative of the individuals immunized under the AVIP or the military

population as a whole, but rather represent an occurrence of immunization and HIV testing. Primary vaccination consists of three subcutaneous injections at zero, two, and four weeks, and three doses at six, twelve, and eighteen months. Pre-immunization sera were the closest samples drawn prior to the first vaccination. Pre-dose samples considered for the 4th and 6th dose were drawn up to 56 days prior to that vaccination with AVA. Post-dose samples for the 3rd, 4th, and 6th doses were drawn 14 to 42 days after vaccination. These samples were chosen to represent to primary and long term antibody responses. Samples following the 1st and 2nd doses were not included for laboratory analysis as antibody levels following these doses were shown previously to be low and unlikely suitable for statistical evaluation.

The Army Medical Surveillance Activity part of the US Army Center for Health Promotion and Preventive Medicine generated a dataset, based upon the following search criteria: the subject number, date of birth, gender, service component, dates of immunization and bleeding, and serum availability. In this retrospective study, doses and serum collection took place from January 1996 to October 2002. This study did not involve notifying the study participants.

2.3. Assay procedures

All serum samples were assayed for anti-PA IgG by ELISA as described [16] with the following modifications: PA antibodies were detected using mAb HP6043 anti-human IgG followed by alkaline phosphatase-labeled rat anti-mouse IgG [17].

2.4. Statistical analysis

Seroconversion was defined as a >4-fold rise of serum anti-PA IgG post each immunization. Geometric mean concentrations (GMC) and 95% CI were estimated for each dose group. Seroconversion rates were compared by Fisher's exact test. GMC were compared using the Mann-Whitney U test or the Signed-rank test. Statistical analyses were performed with a StatXact version 6.1 (Cytel Software Corporation, Cambridge, MA).

3. Results

A total of 246 US military personnel were selected. Of these, 88% (216) were male, 48% (118) were 18-24 years old and 52% 128 were 25-50 year old (mean age). Based upon our search criteria, there were 129 samples in the 3 dose group, 84 in the 4 dose group and 33 in the 6 dose group (Table 1).

Table 2 shows low GMC pre-immunization levels (1.83 μg/mL, CI 1.5-2.3 μg/mL) among vaccinees. Overall, there was a 151-fold rise in GMC, from 1.83 μg/mL in the pre-first dose to 276.95 μg/mL in the post-sixth dose ($p < 0.001$). Significant rises ($p < 0.001$) of GMC were observed among men (203-fold, 1.75 *vs.* 355.14 μg/mL), women (41-fold, 2.71 *vs.* 109.95 μg/mL), the 18-24 year-olds (221-fold, 1.94 *vs.* 429.73 μg/mL), and the 25-50 year-olds (115-fold, 1.75 *vs.* 200.36 μg/mL).

After the 3rd dose, there were significant increases in the overall GMC from 1.83 to 59.92 μg/mL (33-fold, $p < 0.001$). Significant increases were also observed for men from 1.75 to 59.99 μg/mL (34-fold, $p < 0.001$), for women from 2.71 to 59.33 μg/mL (22-fold, $p = 0.003$), and for the 18-24 year-olds from 1.94 to 83.91 μg/mL (43-fold, $p < 0.001$) and for those 25-50 years-olds from 1.75 to 45.52 μg/mL (26-fold, $p < 0.001$). The seroconversion rate was 85.3% (CI 77.9-90.9%). The men had a slightly higher but not statistically significant seroconversion rate than the women (86.2% *vs.* 76.9%, $p = 0.406$); however, the seroconversion rate was significantly higher for the 18-24 year-olds than for the 25-50 years old (93.1% *vs.* 78.9%, $p = 0.043$).

After the 4th dose, there was a significant 6.4-fold overall increase in the GMC from 24.61 to 157.44 μg/mL ($p < 0.001$). Significant increases ($p < 0.01$) in the GMC were found among men (7-fold, from 23.46 to 157.00 μg/mL) and the two age groups (18-24 years old: 5-fold, from 30.14 to 139.60 μg/mL; and 25-50 years old: 9-fold, from 19.26 to 182.10 μg/mL). The seroconversion rate 3rd to 4th dose, 67.9% (CI 56.8-77.6%), was lower than that of 'pre' to 3rd dose. There were no significant differences in the seroconversion rates between men, women, or the age groups.

After the 6th dose, there was an overall significant 3-fold increase in the GMC from 92.39 to 276.95 μg/mL ($p < 0.001$). Among men, the GMC increased from 105.56 to 355.14 μg/mL (3-fold, $p < 0.001$). Among women the increase, from 56.31 to 109.95 μg/mL, was not significant (2-fold, $p = 0.091$). There was a lesser overall seroconversion rate 4th to 6th dose of 45.5% (CI 28.1-63.6%) with 50.0% in the males and 28.6% in the females. A lower seroconversion rate was observed for the 25-50 year-olds (36.8%) than for the 18-25 years-olds (57.1%). The pre-immunization GMC was 1.83 μg/mL and the post-sixth GMC was 276.95 μg/mL for a 151.4-fold increase; 100% of the vaccinees had a ≥ 4-fold rise.

4. Discussion

Our study sought to evaluate serum antibody response to AVA outside of a clinical trial setting. Clinical trials do not necessarily reflect under military conditions. We found that military personnel had responded to AVA with high PA antibody levels after 3, 4, and 6 doses. The antibody levels increased with increasing number of doses without an observed plateau. With the increase in GM antibody levels the additional seroconversion rate decreased. Although none of the study subjects had anthrax or a history of receiving an anthrax vaccine all had low but detectable levels of anti-PA IgG prior to vaccination with AVA, confirming previous reports [18]. These antibodies were likely induced by cross-reactive antigens of non-anthrax bacilli or non-pathogenic *Clostridia* [19-21].

Each of the 3rd, 4th, and 6th doses elicited a rise in the GMC of anti-PA IgG from 59.9 μg/mL after the 3rd dose to 157.4 μg/mL, after the fourth and 277.0 μg/mL, after the 6th dose. The 18-24 year-olds responded consistently with higher antibody levels than the 25-50 year-olds. Males responded with higher GMC levels than the females, after the 6th dose only. The number of females in the study was too small for establishing gender differences.

The seroconversion rates decreased in parallel to the increases in antibody levels, indicating that this schedule was approaching a maximal response as has been previously observed in hyperimmunized animals and in people [22]. The protective level of anti-PA against natural infection is unknown. Considering the unpredictability of infective doses that might occur in a bioterrorist attack, the "protective" level of vaccine-induced anti-PA should be the highest possible, have a long duration, and be induced as readily as possible.

Three factors should be considered when evaluating these and other immunogenicity data of anthrax vaccines. First, to be effective, antibiotic therapy has to be administered within 24 hours of an inhalational challenge, as should vaccination. Second, immunization of the military and other at-risk individuals should be designed to maintain the highest level of serum anti-PA IgG possible [23], while reducing the burden of the five doses within the first year. Third, the level required for protection from an inhalational anthrax exposure is unidentified. Studies to quantify this level could be only derived from studies of humans and for obvious reasons such studies cannot be conducted.

Based upon current assumptions of protection using the AVA vaccine, we suggest that levels of IgG anti-PA comparable to those achieved with the current 6 dose schedule could be achieved by administration of AVA at 0, 2, 4 months for the primary series as is recommended

for protein toxoid vaccines in infants with boosters at every year thereafter. Data from Pittman et al. showed higher antibody levels achieved with longer intervals between immunizations, and that antibody levels and the proportion of seroconverters were not compromised by longer intervals support such a schedule [24]. Pittman et al. also showed that with the currently US used schedule anti PA levels peaked after the 4th dose and the GM levels stayed above 80ug/mL (the estimated protective level derived from experiments in rabbits) in all pre-vaccination assays [25]. Further doses brought back the levels to those achieved after the 4th dose. The need for the 4th, 6 months, dose using the 0, 2, 4 months schedule is not known. There seems to be a need for annual vaccinations.

Lastly, there are no clinical or animal data that identify the age-related infectiousness of *B. anthracis* or the vaccine-induced serum antibody responses of infants or of young children. This lack of information is a serious deficiency of our preparedness program.

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Table 1

Characteristics of the study participants.

Feature	All participants		Three-doses		Four-doses		Six-doses	
	No.	%	no.	%	no.	%	no.	%
All participants	246	100	129	100	84	100	33	100
Gender								
Male	216	87.8	116	89.9	74	88.1	26	78.8
Female	30	12.2	13	10.1	10	11.9	7	21.2
Age group (yrs)								
18-24	118	48	58	45	46	54.8	14	42.4
25-50	128	52	71	55	38	45.2	19	57.6

Table 2

Geometric mean concentrations (GMC) by injecting group.

	Three Doses Group				Four Doses Group				Six Doses Group			
	Pre-immunization		Post-immunization		Pre-immunization		Post-immunization		Pre-immunization		Post-immunization	
Feature	**GMC**	**95% CI**	**GMC**	**95% CI**	**GMC**	**95% CI**	**GMC**	**95% CI**	**GMC**	**95% CI**	**GMC**	**95% CI**
All participants	1.83	(1.46-2.30)	59.92	(46.45-77.30)	24.61	(19.85-30.51)	157.44	(120.03-206.49)	92.39	(70.42-121.20)	276.95	(185.65-413.05)
Gender												
Male	1.75	(1.37-2.24)	59.99	(46.13-78.02)	23.46	(18-65-29.52)	157.00	(118.01-208.93)	105.56	(82.07-135.74)	355.14	(259.72-485.51)
Female	2.71	(1.53-4.82)	59.33	(20.00-176.04)	35.00	(17.73-69.14)	160.58	(57.20-450.92)	56.31	(20.92-151.53)	109.95	(22.06-547.90)
Age group (yrs)												
18-24	1.94	(1.35-2.80)	83.91	(62.16-113.29)	30.14	(22.63-40.13)	139.6	(93.20-209.07)	104.18	(80.35-135.11)	429.73	(251.36-734.51)
25-50	1.75	(1.30-2.34)	45.52	(30.90-67.05)	19.26	(13.97-26.55)	182.1	(126.74-261.64)	84.55	(53.85-132.74)	200.36	(113.82-352.61)

Note: 95% CI, confidence interval at 95 percent.