



Patterns of antibody response in humans to the anthrax vaccine adsorbed (AVA) primary (six-dose) series[☆]

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Abstract

The antibody profile during and after the six-dose primary vaccination series with anthrax vaccine adsorbed (AVA, BiothraxTM) was characterized in 86 human volunteers. Ninety-three percent of recipients developed IgG antibodies to *Bacillus anthracis* protective antigen (PA) after two doses, and 100% were seropositive after dose #3. Geometric mean concentrations (GMC) of IgG to PA measured before and after each dose were significantly lower after injection #3 (peak GMC = 146.65 µg/mL, trough GMC = 15.16 µg/mL) than after injections #4 (peak GMC = 430.46 µg/mL, trough GMC = 94.57 µg/mL), #5 (peak GMC = 415.05 µg/mL, trough GMC = 81.94 µg/mL), or #6 (peak GMC = 401.16 µg/mL, trough GMC = 96.19 µg/mL) ($p \leq 0.0001$ for each); but not between injections #4 and #5, #5 and #6, or #4 and #6 ($p \geq 0.7923$ for each). Decay rates for IgG to PA were significantly faster after injection #3 (half life [$T_{1/2}$] = 39.21 days) than after injections #4 ($T_{1/2}$ = 72.03 days), #5 ($T_{1/2}$ = 70.14 days), and #6 ($T_{1/2}$ = 74.59 days) ($p \leq 0.0282$ for each). Toxin neutralizing assay (TNA) antibody patterns generally paralleled those for IgG to PA. The 6-month dose in the AVA primary series appears to be critical in sustaining IgG to PA concentrations in a substantial proportion of recipients.

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Keywords: Anthrax vaccine; Antibody; Antibody kinetics

At the present time, anthrax vaccine adsorbed (AVA, BiothraxTM), is the only product licensed by the U.S. Food and Drug Administration (FDA) for protecting humans against anthrax. Despite affirmation of its safety and immunogenicity through thorough review of available data by the Institute of Medicine, questions remain regarding aspects of

the vaccine's performance [1]. In particular, data regarding the antibody profile during and following administration of the primary (six-dose) vaccination series are sparse.

AVA was developed in the 1950s by investigators at the U.S. Army Medical Unit (currently the U.S. Army Medical Research Institute of Infectious Diseases [USAMRIID]) at Fort Detrick, MD [2–6]. From the time that investigational lots of the vaccine were produced for study in clinical trials, AVA and its progenitors have been used to immunize personnel at Fort Detrick and elsewhere against possible exposure to anthrax in the course of developing weapons (prior to 1969), or countermeasures against this biological weapon threat.

Vaccinations of laboratory workers and others occupationally exposed to high-hazard pathogens are conducted at USAMRIID through the institute's Special Immunization

[☆] The opinions expressed are those of the authors and should not be construed to represent those of the Department of the Army, the Department of Defense, Camber Corporation, or GoldbeltRaven LLC.

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Program (SIP). While most vaccines administered through the SIP are classified as “investigational,” several licensed products, including AVA, are provided in this venue as well. The USAMRIID occupational health program includes periodic serologic monitoring of individuals receiving vaccines through the SIP to ensure that “protective” antibody levels against pathogens of interest are maintained. Aliquots of these sera are archived for future analyses. In a previous publication, we described the antibody response to AVA after the first three doses [7]. The present study extends these findings by describing the kinetics of IgG antibodies to *Bacillus anthracis* protective antigen (PA) in AVA vaccinees receiving the entire six-dose primary series using sera obtained as part of the occupational health program and stored in the USAMRIID archive.

1. Materials and methods

1.1. Study design

A minimal-risk protocol to locate and test stored serum samples from SIP volunteer participants who received AVA during 1990–1994 was reviewed and administratively approved by the institutional review board at USAMRIID and the Human Subjects Research Review Board at the U.S. Army Surgeon General's office.

Specimens for the current study were identified by review of SIP vaccination records for receipt of AVA doses #1–6. Samples were retrieved from -70°C storage that met pre-established criteria: (a) Vaccinations must have occurred within defined time intervals after receipt of the initial AVA injection (day 0 [dose #1], day 14 [range 11–21], day 28 [range 25–35], day 182 [range 154–216], day 364 [range 336–413], day 546 [range 518–609]). (b) Pre-injection sera were defined as those taken on the day of each vaccine dose. If not available, the closest previous specimen available was used for analysis. The maximum interval between pre-vaccination serum and vaccination date for the baseline injection (dose #1) was 274 days (39 weeks). Postvaccination sera taken after doses #1 (day 14) and #2 (day 28) served as prevaccination sera for injections #2 and #3. For vaccine doses #4–6, if a serum specimen was not available for the day of vaccination, the closest specimen within 30 days before the vaccination was selected. (c) Sera obtained on the days of receipt for doses #2 and #3 served as postvaccination sera for doses #1 and #2, respectively. If unavailable, the closest specimen before each of these vaccine doses was chosen. After doses #3–6, specimens were sought at 28 days postvaccination. If unavailable, specimens taken within a range of 14–42 days postvaccination were used for analysis, with priority given for that specimen closest in time to the optimal 28 days postvaccination time-point. One additional sample was collected 2 years (range = 699–761 days) after dose #1. This corresponded to 17–30 weeks after dose #6.

1.2. Laboratory studies

IgG to PA antibodies were measured by using an indirect ELISA. In brief, twofold serial dilutions of serum were made in pre-defined regions of 96-well plates coated with recombinant *B. anthracis* protective antigen (rPA) (Science Applications International Corp, Frederick, MD). Twofold dilutions of an anti-AVA standard human reference serum (AVR414, CDC, Atlanta, GA) [8] were made in different wells of each coated plate. Positive serum controls with known high, medium, and low concentrations of IgG to rPA and a negative serum control were also included on each plate. Plates were incubated at 37°C for 60 min, and washed three times. A peroxidase-conjugated goat anti-human IgG antibody (Kierkegaard and Perry, Gaithersburg, MD) was then added to detect bound antigen colorimetrically. Color development was stopped after a 30-min incubation by adding Peroxidase Stop Solution (Kierkegaard and Perry, Gaithersburg, MD). Optical density (OD) values were read within 30 min by using a Bio-Tek ELx808 plate with 405 and 490 nm filters, and KC4 software (Bio-Tek Instruments, Inc., Winooski, VT). OD values were converted to immunoglobulin concentration ($\mu\text{g/mL}$) by using a standard curve calibration factor [9]. Titers were calculated as the reciprocal of the highest dilution of the test serum yielding a mean OD value $\geq$ the cut-off value for the assay.

B. anthracis lethal toxin neutralization activity was measured by using a colorimetric toxin neutralization assay (TNA) [10,11]. In brief, confluent monolayers of J774A.1 (mouse macrophage) cells were grown in 96-well plates and used after overnight incubation. Twofold dilutions of test and anti-AVA reference standard sera (initial dilution determined from antibody levels measured by ELISA) made in 96-well titration plates were combined with rPA (Science Applications International Corp, Frederick, MD) and lethal factor (LF) (List Biologicals, Campbell, CA). After a 1-h incubation at 37°C , the serum/lethal toxin mixtures were then added to the cell monolayers and incubated at 37°C for 4 h. Cell viability after exposure to the serum-toxin mixtures was determined by adding thiazolyl blue (MTT) (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma, St. Louis, MO) in 50% dimethyl formamide (Sigma) to each plate. After incubation at 37°C overnight, OD values were read at 570 nm by using a Bio-Tek ELx808 reader and KC4 software (Bio-Tek Instruments, Inc.).

A four-parameter sigmoid regression curve was used to determine serum TNA antibody titers (the dilution of serum resulting in 50% neutralization of anthrax lethal toxin, or ED_{50}). Concentration of immunoglobulin ($\mu\text{g/mL}$) was determined by using a standard curve calibration factor.

1.3. Statistical analysis

Descriptive statistics were calculated for total number of shots received per subject, number of subjects receiving specific shots, demographic variables, weeks to seroconversion,

and rate of seroconversion by sample time period. All ELISA and TNA variables were $\log_{10}$ transformed for analysis. After transformation, variables met assumptions of normality and homogeneity of variance. Results of IgG to PA concentrations and TNA antibody titers that were deemed below the limits of detection (BLD) were set to a value of 1 for purposes of analysis. BLD results of TNA concentrations were set to a value of 0.1. These values were chosen based on the observed lowest quantified values for each assay. For each vaccination and time period, the median number of weeks post-injection was calculated, along with the minimum and maximum number of weeks before the subsequent injection and after the previous injection. Geometric means, standard errors, and 95% confidence intervals were calculated for each sample time period for IgG to PA concentrations and for TNA values. *t*-Tests were used to examine differences in time to seroconversion between males and females. ANOVA was used to examine the effect of race and age on time to seroconversion. Covariates such as age, gender, and race were examined for significant effect on IgG to PA concentration. Repeated measures ANOVA was performed to assess change in IgG to PA concentration over the study period as well as changes before and after the administration of each shot. Tukey–Kramer post hoc tests were used to perform pair-wise comparisons between injections at each time point. Repeated measures ANOVA with step-down Sidak adjustment was used to perform pair-wise comparisons of change in mean concentration values between injections over time. Decay rates for IgG to PA concentrations were determined from the negative slope of the regression line of the $\log_{10}$ response variable versus time in days after injections #3–6. Rates of decay (*K*) were expressed as $\log_{10}$ $\mu\text{g/mL}$ antibody per day. Antibody $T_{1/2}$ (half life) was calculated as $\log_{10}(2)/K$. Due to a large proportion of missing observations (19%), ANOVA was not performed on TNA values. The Pearson product-moment method was used to calculate correlations of IgG to PA concentrations with TNA antibody concentrations and titers. All statistical anal-

yses were performed using SAS Version 9.1 (SAS Institute, Cary, NC, 2003).

2. Results

Pre- and post-inoculation serum samples from 86 individuals receiving the AVA primary series were identified for analysis. Most of the study subjects were male (70.9%) and Caucasian (83.7%; 11.6% were African–American). Median age of study subjects was 33 years (range 19–61).

All subjects in the study sample had received at least two AVA doses. While the mean number of injections received was 4.8, half (43) received all 6 primary series inoculations, 7 received 5, 16 received 4, 17 received 3, and 3 received 2 injections. Analyses of data from only subjects receiving all six AVA doses yielded results similar to those presented for all available serum samples. Temporal distribution of serum samples retrieved for antibody testing is shown in Table 1.

2.1. IgG to PA

A total of 671 sera were analyzed for IgG to PA. All subjects seroconverted after receiving AVA, as defined by fourfold or greater increase over baseline in dilutional IgG to PA titer. Seroconversion occurred after the first injection in 20.9% of individuals. After the second injection, a total of 93.0% had seroconverted, and by the third injection, 100% had achieved ≥ 4 -fold rises in titer over baseline levels (Table 2). Mean time after receipt of the initial AVA injection to seroconversion was 27.7 days (range = 14–63 days). No significant differences in mean time to seroconversion by gender (27.5 days for males, 28.4 days for females, $p = 0.7153$), among race groups ($p = 0.7557$), or by age ($p = 0.2224$) were observed. The distribution of IgG to PA concentrations observed during and after receipt of each of the six AVA injections is shown in Table 3.

Table 1
Schedule of sample collection for AVA vaccinees

Sample number	N ^a	Interval after first injection		Previous injection number	Interval after previous injection		Next injection number	Interval before next injection	
		Median (weeks)	Min/max (weeks)		Median (weeks)	Min/max (weeks)		Median (weeks)	Min/max (weeks)
1 (Pre-injection #1) (baseline)	85	n/a ^b	n/a	n/a	n/a	n/a	1	1	0–39
2 (Pre-injection #2)	84	2	1–3	1	2	1–3	2	0	0–1
3 (Pre-injection #3)	83	4	3–5	2	2	1–3	3	0	0–2
4 (Post-injection #3)	80	8	6–10	3	4	2–6	4	18	16–20
5 (Pre-injection #4)	60	26	26–28	3	22	22–24	4	0	0–1
6 (Post-injection #4)	60	31	28–33	4	4	2–6	5	22	21–28
7 (Pre-injection #5)	46	53	48–55	4	26	22–28	5	0	0–4
8 (Post-injection #5)	44	56	55–59	5	4	2–5	6	23	22–28
9 (Pre-injection #6)	46	80	78–86	5	27	23–32	6	0	0–4
10 (Post-injection #6)	46	84	82–89	6	4	2–5	n/a	n/a	n/a
11 (Post-injection #6)	37	106	100–109	6	26	17–30	n/a	n/a	n/a

^a Number of samples tested at each time point.

^b Not applicable.

Table 2
Intervals from first injection to seroconversion^a among AVA vaccinees

Weeks	Vaccinations received prior to seroconversion			Cumulative frequency	
	1 (N)	2 (N)	3 (N)	N	%
2	15	0	0	15	17.4
3	3	3	0	21	24.4
4	0	51	0	72	83.7
5	0	8	0	80	93.0
6	0	0	1	81	94.2
8	0	0	3	84	97.7
9	0	0	2	86	100.0

^a Seroconversion defined as ≥ 4 -fold increase in IgG to PA dilutional titer from baseline (pre-injection #1) sample.

Table 3
Distribution of IgG to *B. anthracis* PA concentrations among AVA vaccinees

Sample	N ^a	Geometric mean	Percentiles		
			75th	90th	95th
Pre-injection #1 (baseline)	85	1.16	1.00	1.73	2.87
Pre-injection #2	84	2.56	4.76	11.38	13.29
Pre-injection #3	83	84.57	166.20	351.26	404.37
Post-injection #3	80	146.65	275.87	370.45	508.33
Pre-injection #4	60	15.16	28.72	35.59	54.35
Post-injection #4	60	430.36	778.69	1145.08	1594.40
Pre-injection #5	46	94.57	165.02	333.72	398.44
Post-injection #5	44	415.05	524.41	964.75	1055.45
Pre-injection #6	46	81.94	104.06	215.95	273.07
Post-injection #6	46	401.16	577.67	844.01	869.28
6-Months post-injection #6	37	96.19	130.00	200.00	281.00

^a Number of samples tested at each time point.

A serum concentration of IgG to PA $\geq 3 \mu\text{g/mL}$ was observed in all subjects after vaccination. This level of antibody, which represents the lower limit of quantification for undiluted serum using an ELISA analogous to that applied in this study [9], was reached by 39.5% of subjects after the first injection, by a total of 96.5% after the second injection, and by 100% after the third injection (Table 4). The mean number of days after the first vaccine dose needed to reach $3 \mu\text{g/mL}$ was 24.2 (range=13–63 days). No significant differences in mean time to achieving an IgG to PA

Table 4
Intervals from first injection to detect IgG to *B. anthracis* PA concentration $\geq 3 \mu\text{g/mL}$ ^a among AVA vaccinees

Weeks	Vaccinations received prior to seroconversion			Cumulative frequency	
	1 (N)	2 (N)	3 (N)	N	%
2	30	0	0	30	34.9
3	4	2	0	36	41.9
4	0	41	0	77	89.5
5	0	6	0	83	96.5
8	0	0	2	85	98.8
9	0	0	1	86	100.0

^a Lower limit of quantification for EIA¹⁰.

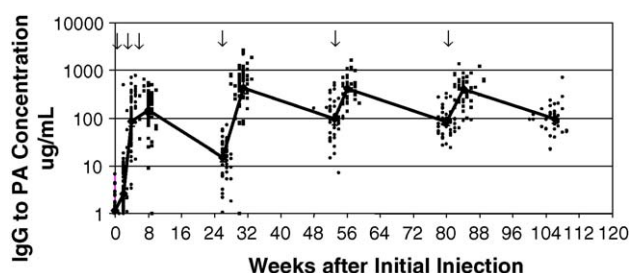


Fig. 1. IgG to PA concentrations in 650 serum samples from 86 individuals receiving AVA. Large symbols represent geometric mean concentration of IgG to PA before and after each injection in the primary vaccination series. Small symbols represent individual antibody concentrations before (circles) and after (squares) AVA injections. Arrows indicate time-points for each injection in the vaccination series. Note seven samples with IgG to PA concentration $< 3 \mu\text{g/mL}$ at time points before injection #3 and after injection #4; these values were set to $1 \mu\text{g/mL}$ for purposes of display and analysis.

concentration $\geq 3 \mu\text{g/mL}$ were observed between males and females ($p = 0.4463$), among race groups ($p = 0.3013$), or by age ($p = 0.0834$).

Significant changes in IgG to PA concentrations occurred over time from pre-injection #1 samples to those obtained at both bleed points after injection #6 ($p < 0.0001$) (Fig. 1). There were also significant changes in concentration observed before and after each individual injection ($p = 0.0014$ for injection #3; $p = 0.0006$ for injections #1, #2, and #4–6). No effects for gender ($p = 0.9431$), race ($p = 0.3765$), or age ($p = 0.6454$) were seen over time or before and after any individual injection.

Changes in IgG to PA concentrations before and after injections were compared between injections #3 and #6. There were significant differences in antibody concentrations between serial injections ($p < 0.0001$) and between pre- and post-injection sera ($p < 0.0001$). Geometric mean IgG to PA concentrations at peak levels measured 14–42 days after each vaccination were significantly lower after injection #3 than after injections #4–6 ($p \leq 0.0001$ for each), but not between injections #4 and #5 ($p = 0.9969$), #5 and #6 ($p = 0.9979$), or #4 and #6 ($p = 0.9777$) (Table 3). Likewise, IgG to PA concentrations (GMC) at trough levels measured within 30 days of the next vaccination were significantly different between injections #3 and #4 ($p < 0.0001$), between injections #3 and #5 ($p < 0.0001$), and between injection #3 and 17–30 weeks after injection #6 ($p \leq 0.0001$), but not between injections #4 and #5 ($p = 0.8182$), injections #4 and #6 ($p = 0.9997$), and injections #5 and #6 ($p = 0.7923$) (Table 3).

Slopes of IgG to PA decay curves were significantly different between injections ($p = 0.0007$). The rate of decay ($0.008 \log_{10} \mu\text{g/mL/day}$ and $T_{1/2}$ (39.21 days) after injection #3 were much faster than those after injections #4 ($0.004 \log_{10} \mu\text{g/mL/day}$ and 72.03 days, respectively), #5 ($0.004 \log_{10} \mu\text{g/mL/day}$ and 70.14 days, respectively), and #6 ($0.004 \log_{10} \mu\text{g/mL/day}$ and 74.59 days, respectively). Pair-wise comparisons of slopes showed significant differences between injections #3 and #4 ($p = 0.0282$), between injections #3 and #5 ($p = 0.0282$), and between injections

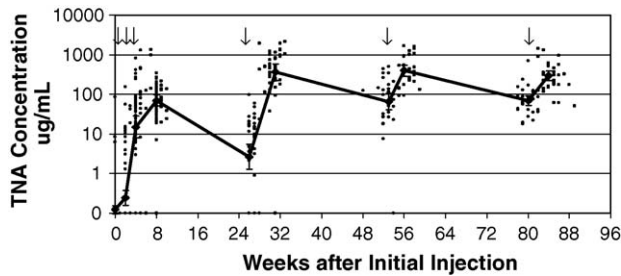


Fig. 2. Toxin neutralization assay (TNA) antibody concentrations in 532 serum samples from 86 individuals receiving AVA. Large symbols represent geometric mean TNA concentration before and after each injection in the primary vaccination series. Small symbols represent individual antibody concentrations before (circles) and after (squares) AVA injections. Arrows indicate time-points for each injection in the vaccination series. Note 18 samples with TNA antibody concentration below the limit of detection at time points between pre-injection #3 and pre-injection #5; these values were set to 0.1 $\mu\text{g/mL}$ for purposes of display and analysis.

#3 and #6 ($p=0.0060$), but not between injections #4 and #5 ($p=0.8925$), #5 and #6 ($p=0.6492$), and #4 and #6 ($p=0.8925$).

2.2. TNA antibodies

For 515 (77%) of the 671 serum samples available for testing, volumes were adequate for measuring TNA antibodies. Samples collected 17–30 weeks after injection #6 were unavailable for TNA testing. Among samples collected through approximately 1 month after injection #6, there was a relatively large number (19%) with volumes insufficient for TNA testing. Therefore, inferential statistics for this antibody could not be performed. The TNA antibody response after AVA vaccination generally paralleled that of IgG to PA for the time points measured (Fig. 2). Using samples for which both IgG to PA and TNA antibody concentrations could be measured, Pearson correlation coefficients were calculated over all sample times and for each sample time. While the overall correlation between IgG to PA and TNA concentrations was

high ($r=0.891$, $p<0.0001$), correlations for specific sample times showed more variability ($r=0.031$ – 0.852 , $p=0.7902$ to <0.0001) (Table 5).

3. Discussion

Central to the pathogenesis of anthrax is a toxin-mediated immune dysfunction that allows the bacteria to multiply rapidly and disseminate within the infected host [12]. Two principal *B. anthracis* toxin components, lethal factor (LF, a Zn^{2+} -protease) and edema factor (EF, a calmodulin- and Ca^{2+} -dependent adenylate cyclase) are enzymes transported into cells by a third polypeptide, PA; combinations of PA with LF and EF yield *B. anthracis* lethal toxin (LT) and *B. anthracis* edema toxin (ET), respectively, that function as bacterial virulence factors in producing many of the symptoms of anthrax [13–18]. The pivotal role played by PA in toxin cell entry forms the basis for the current licensed anthrax vaccine, AVA (BiothraxTM), a sterile culture filtrate of avirulent, non-encapsulated, *B. anthracis* (stimulated to produce relatively large amounts of PA) mixed with formalin and benzethonium chloride, and combined with aluminum hydroxide [19]. Vaccination with this relatively crude preparation stimulates antibodies that bind to PA. Binding of PA impedes toxin entry into cells, and is important in neutralizing *B. anthracis* LT in vivo and in vitro [20]. We and others have shown that there is strong correlation between IgG to PA and toxin-neutralizing activity after human infection with virulent anthrax, and vaccination with AVA [21,22].

Since initial licensure by the U.S. Food and Drug Administration in 1970, AVA has been used to immunize veterinarians, laboratory workers, textile workers, and more recently, hundreds of thousands of military personnel, against the threat of exposure to natural and/or weaponized anthrax. No cases of anthrax disease have been observed among individuals receiving the 6-month dose of AVA [23,24]. In the Brachman paper, 21 cases of cutaneous anthrax were observed (15 among placebo recipients, 3 in the observational group, and 3 who received anthrax vaccine). Of the three cases of cutaneous anthrax among partially vaccinated subjects, none had received the 6-month dose of anthrax vaccine [23]. One case occurred 5 months after third dose, but before the first of three 6-month boosters. Another case occurred 13 months after the initial three doses, but this individual failed to receive the 6-month dose. The third case occurred just prior to the third vaccine inoculation. The authors concluded “The occurrence of the single vaccinated case 5 months after the initial series may indicate that the immunity resulting from the initial three inoculations had fallen significantly by that time . . . It appears that a booster response occurs after the first booster inoculation, raising immunity to protective levels which are stable for at least 6 months. The single case in the incompletely immunized individual, 13 months after the initial series, further supports the importance of the first booster inoculations in securing adequate protective levels.” [23].

Table 5

Correlations between IgG to *B. anthracis* PA and TNA concentrations in serum samples from AVA vaccinees

Serum sample	N ^a	IgG to PA vs. TNA concentration	
		<i>r</i>	<i>p</i>
Overall	515	0.891	<0.0001
Pre-injection #1 (baseline)	75	0.031	0.7902
Pre-injection #2	78	0.361	0.0012
Pre-injection #3	72	0.750	<0.0001
Post-injection #3	72	0.572	<0.0001
Pre-injection #4	45	0.421	0.0039
Post-injection #4	43	0.149	0.3414
Pre-injection #5	37	0.852	<0.0001
Post-injection #5	28	0.747	<0.0001
Pre-injection #6	32	0.710	<0.0001
Post-injection #6	33	0.427	0.0132

^a Number of samples tested using both assays at each time point.

The present study serves to quantify the antibody response to *B. anthracis* in healthy adults following receipt of each of the six doses in the AVA primary vaccination series. The population included in this analysis reflects the demographics of current vaccinees in the USAMRIID SIP [P. Pittman, pers. commun.], but is somewhat older than the active duty military, the largest user of AVA [25]. Our analysis confirms that AVA is an effective immunogen; more than 1/3 of vaccinees developed detectable IgG to PA after a single inoculation, >95% after the second injection, while 100% of our study sample was seropositive after three doses. Significant increases in antibody concentration occurred after each injection, with peak responses achieved after the fourth (6-month) dose. Neutralizing activity against LT generally paralleled the IgG to PA response. Importantly, neither age, race, nor gender influenced response to vaccination.

A threshold concentration of IgG to PA correlating with protection against anthrax has not been established for humans. Examination of data from studies in rabbits vaccinated with AVA [26] and rPA [27] suggests that >75% of animals with IgG to PA levels between ~40 µg/mL (after receipt of rPA) and ~120 µg/mL (after receipt of AVA) were protected against anthrax spore challenges ranging from 84 to 467 LD₅₀ delivered by aerosol. In our study, a GMC of IgG to PA exceeding 120 µg/mL was achieved after three injections, but only after receiving the 6-month dose (fourth injection) did the GMC remain above 80 µg/mL between doses (Table 3). Moreover, while GMCs at both peak and trough levels were similar between the fourth and fifth, fourth and sixth, and fifth and sixth injections, there were significant differences at both peak and trough levels between injections #3 and #4, injections #3 and #5, and injections #3 and #6 in the vaccination series. These data, together with our finding that antibody decay rates were significantly slower after the 6-month dose compared with previous doses, indicate that receipt of the 6-month dose in the primary series is critical for anchoring a sustained IgG to PA response using the present AVA licensed schedule. Previously, we demonstrated that increasing the interval between the first and second doses in individuals receiving AVA enhances the IgG to PA response [7]. A subsequent study indicated that neither the proportion of seroconverters nor the overall antibody levels (GMC) were compromised by eliminating the second (2 weeks) dose altogether [10]. The Centers for Disease Control and Prevention is currently conducting a pivotal study to confirm these findings (P. Pittman, pers. commun.). Future attempts to reduce the number of AVA injections should consider the importance of the 6-month dose in promoting a durable antibody response.

The next generation of anthrax vaccines based upon rPA is currently under active investigation in human clinical trials [28,29]. Our findings should prove useful as background against which to compare vaccine responses as attempts continue to optimize immunization against this highly pathogenic microorganism.

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