

Effects of a Reduced Dose Schedule and Intramuscular Administration of Anthrax Vaccine Adsorbed on Immunogenicity and Safety at 7 Months

A Randomized Trial

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Context In 1999, the US Congress directed the Centers for Disease Control and Prevention to conduct a pivotal safety and efficacy study of anthrax vaccine adsorbed (AVA).

Objective To determine the effects on serological responses and injection site adverse events (AEs) resulting from changing the route of administration of AVA from subcutaneous (SQ) to intramuscular (IM) and omitting the week 2 dose from the licensed schedule.

Design, Setting, and Participants Assessment of the first 1005 enrollees in a multisite, randomized, double-blind, noninferiority, phase 4 human clinical trial (ongoing from May 2002).

Intervention Healthy adults received AVA by the SQ (reference group) or IM route at 0, 2, and 4 weeks and 6 months (4-SQ or 4-IM; n=165-170 per group) or at a reduced 3-dose schedule (3-IM; n=501). A control group (n=169) received saline injections at the same time intervals.

Main Outcome Measures Noninferiority at week 8 and month 7 of anti-protective antigen IgG geometric mean concentration (GMC), geometric mean titer (GMT), and proportion of responders with a 4-fold rise in titer (%4×R). Reactogenicity outcomes were proportions of injection site and systemic AEs.

Results At week 8, the 4-IM group (GMC, 90.8 µg/mL; GMT, 1114.8; %4×R, 97.7) was noninferior to the 4-SQ group (GMC, 105.1 µg/mL; GMT, 1315.4; %4×R, 98.8) for all 3 primary end points. The 3-IM group was noninferior for only the %4×R (GMC, 52.2 µg/mL; GMT, 650.6; %4×R, 94.4). At month 7, all groups were noninferior to the licensed regimen for all end points. Solicited injection site AEs assessed during examinations occurred at lower proportions in the 4-IM group compared with 4-SQ. The odds ratio for ordinal end point pain reported immediately after injection was reduced by 50% for the 4-IM vs 4-SQ groups ($P < .001$). Route of administration did not significantly influence the occurrence of systemic AEs.

Conclusions The 4-IM and 3-IM regimens of AVA provided noninferior immunological priming by month 7 when compared with the 4-SQ licensed regimen. Intramuscular administration significantly reduced the occurrence of injection site AEs.

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SIMPLER AND BETTER TOLERATED regimens for vaccination with anthrax vaccine adsorbed (AVA) are needed. Anthrax vaccine adsorbed (BioThrax, Emergent Bio-Solutions Inc, Lansing, Michigan) is currently the only licensed anthrax vac-

cine in the United States and the only licensed aluminum-adjuvant vaccine administered subcutaneously (SQ).¹⁻³

The principal immunogen of AVA is the anthrax toxin component protective antigen (PA).⁴ The licensed AVA vac-

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nation regimen consists of 0.5-mL SQ injections at 0, 2, and 4 weeks and 6, 12, and 18 months with annual boosters thereafter. Data supporting the licensed regimen are limited and originate from animal studies and a single field evaluation of a human vaccination.^{5,6}

The paucity of clinical data compounded safety concerns raised about AVA following initiation of mandatory anthrax vaccination for personnel of the Department of Defense in 1998, and a higher reported frequency of adverse events (AEs) was experienced by female vaccine recipients compared with male recipients.⁷⁻¹² Subsequently, Pittman et al² reported a pilot clinical trial in which a reduced AVA vaccination schedule and a change to the intramuscular (IM) route elicited similar antibody responses with fewer injection site AEs than the licensed regimen. These data provided impetus for the US Congress to establish the Centers for Disease Control and Prevention (CDC) Anthrax Vaccine Research Program to study AVA in 1999. A fundamental component of the program is a randomized, double-blind, placebo-controlled, phase 4 clinical trial to assess safety and serological noninferiority of alternate schedules and routes of administration of AVA. We present analyses of clinical and serological data collected from the first 1005 subjects up to month 7 of their 43-month participation.

METHODS

Participants, Recruitment, and Time Frame

The study was sponsored by CDC under an investigational new drug application and was approved by the human investigations committees at participating clinical sites and at the CDC. Study centers included the Walter Reed Army Institute of Research, Silver Spring, Maryland; Baylor College of Medicine, Houston, Texas; Emory University School of Medicine, Atlanta, Georgia; Mayo Clinic, Rochester, Minnesota, and University of Alabama at Birmingham. Oversight

was provided by a data and safety monitoring board, a panel of experts outside of the CDC.

Participants provided consent and then were screened to determine eligibility. If eligible, they were randomized into a study group. Subjects were eligible if they were between the ages of 18 and 61 years; were healthy; had 2 intact upper arms; indicated willingness to comply with study procedures; and, if female, were not pregnant (urine pregnancy tests were done before each dose) and did not plan to become pregnant for the duration of the study.

Persons were ineligible if they had a history of anthrax infection or immunization against anthrax; had a known allergy to latex or vaccine preservatives; were receiving experimental products, live or inactivated vaccine, immunosuppressive therapy, or immunoglobulin therapy within protocol-defined windows; had an active malignancy, cardiovascular disease, hepatic or renal insufficiency, current diabetes, or severe asthma; had known infection with human immunodeficiency virus, hepatitis B, hepatitis C, or other conditions known to produce immune suppression; used high doses of inhaled steroids; or had a neuropathy, unstable mental illness, or seizure disorder. Participants were followed up for 60 days beyond the last scheduled injection.

Evaluation of the effects of race or ethnicity was included at the recommendation of the Institute of Medicine.¹³ Participants self-selected their race/ethnicity categories from a list provided at enrollment. Multirace selections were reformatted to coincide with the US Census single-race categories that existed before the year 2000 census.¹⁴

Study enrollment was staggered across participating sites. It began on May 15, 2002, and the last participant was enrolled on February 25, 2003. Administration of the first 4 injections and follow-up blood draws was outside of protocol-specified windows for some participants so that the final samples for the analysis cohort were drawn May 13,

2004. The statistical analysis plan established with the Food and Drug Administration (FDA) prior to the study initiation permitted 2 evaluations of the data, 1 at month 7 and 1 at study end. To meet specific timelines for submission of the data from the first analyses to the FDA and the manufacturer, the unimputed and imputed data were locked in August and November 2004, respectively. The sample size of 1005 was sufficient to test the primary end points using the noninferiority hypotheses. Data analyses based on the participants in the current article were submitted as a study report to the FDA in 2005. Following FDA review and comment, the data were submitted to the manufacturer in support of a biologic license application. On completion of these steps, the data could be made available for publication. The entire cohort will be evaluated at the end of the 43-month study, anticipated to be in the second quarter of 2009.

Interventions

Two lots of multidose AVA were provided by the MilVax Agency, Department of Defense, for this phase of the study: lot FAV063 was used until it expired, at which time lot FAV074 was used. Under the staggered enrollment of the study, more than 90% of participants received lot FAV063 for all vaccinations up to and including month 6. The specific concentrations of PA in the vaccine lots were not determined. Placebo injections were saline (0.9% sodium chloride, Abbott Laboratories, North Chicago, Illinois).

Subjects were randomized into 1 of 6 groups (FIGURE 1). Group 1 (4-SQ) received the first 4 doses of vaccine SQ according to the licensed schedule (0, 2, and 4 weeks and 6 months); group 2 (4-IM) received vaccine via IM route at the same intervals. Groups 3, 4, and 5 received AVA via IM route at 0 and 4 weeks and 6 months and placebo at week 2; these groups were combined for these analyses (3-IM) because their schedules do not differ until after the 6-month dose. Subgroups 6a and 6b received placebo via IM route and SQ, re-

spectively, at 0, 2, and 4 weeks and 6 months and were combined for the serological analyses. Vaccine or placebo was administered as a 0.5-mL dose.

Objectives and Hypotheses

The objectives of this study were to determine the effects on serological re-

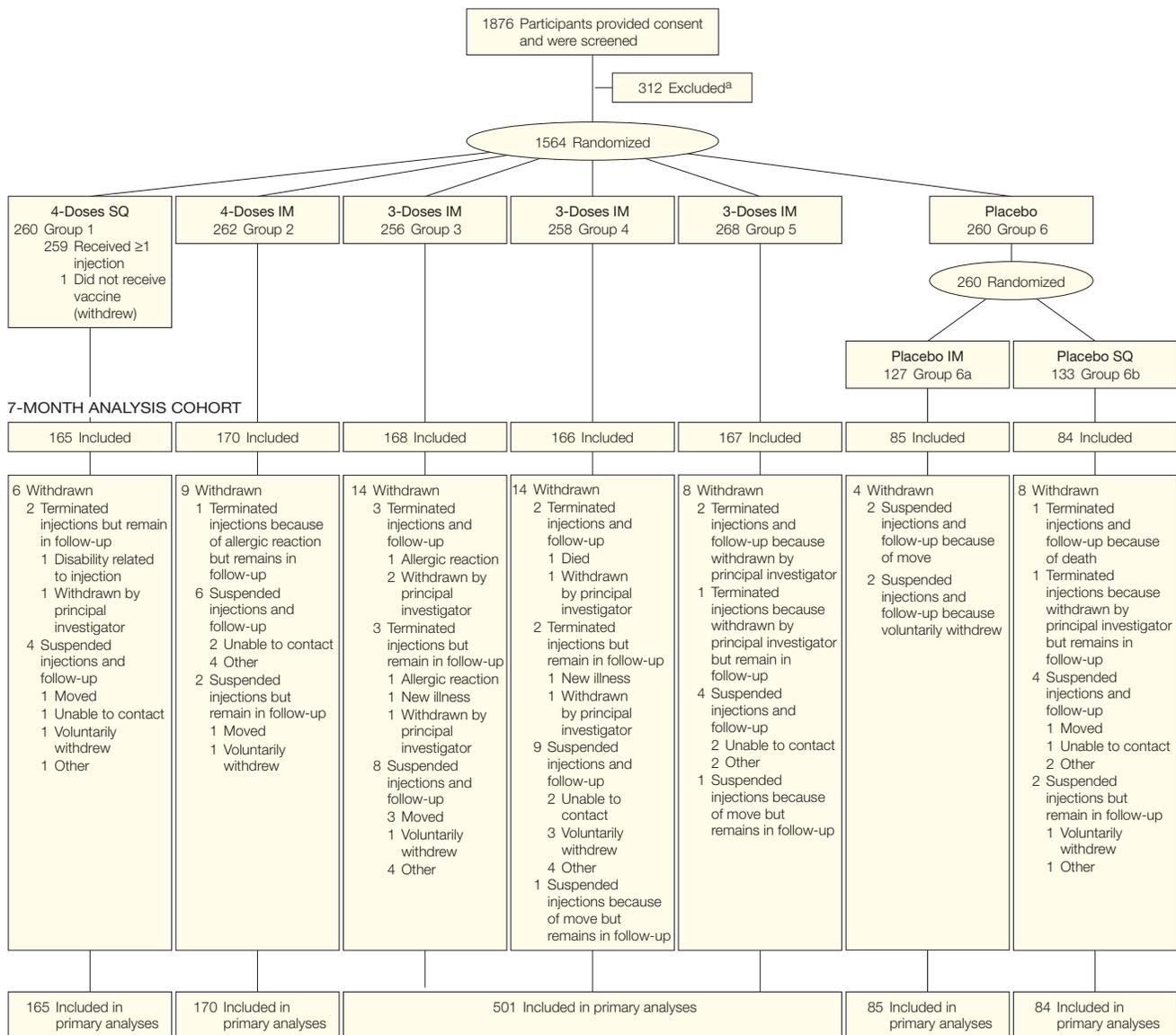
sponses and injection site AEs resulting from changing the route of administration of AVA from SQ to IM and omitting the week 2 injection from the first 4 injections of the licensed regimen of AVA. Primary serological endpoints for noninferiority were anti-PA IgG antibody geometric mean concen-

tration (GMC), geometric mean titer (GMT), and proportion of responders with a 4-fold rise in titer (%4×R).

The study hypotheses for the month 7 analyses were as follows:

1. AVA administered by the IM route at weeks 0, 2, and 4 and month 6 (4-IM) elicits antibody responses at week

Figure 1. Participant Flow for the Centers for Disease Control and Prevention Anthrax Vaccine Research Program Human Clinical Trial



"Terminated injections" means that no more injections will be received by that participant. "Suspended injections" means that the participant is anticipated, or has agreed, to resume injections later in the study. A participant listed as "unable to contact" in the suspended group may be expected to resume injections. IM indicates intramuscular; SQ, subcutaneously.

^a Indicates that reasons for exclusion included abnormal electrocardiogram results, allergy to aluminum, autoimmune disorder, chronic condition or disease, compromised injection site, current or planned pregnancy, genetic disorder, history of anthrax vaccine adsorbed injections, history of or current cancer, mental illness, military commitment, neurologic condition, ongoing immune suppression therapy, planned surgery, poor venous access, security risk, and substance abuse.

8 and month 7 for which the GMC, GMT, and $\%4\times R$ are noninferior to those achieved by the currently licensed schedule (4-SQ).

2. AVA administered by the IM route at weeks 0 and 4 and month 6 (3-IM) with placebo at week 2 elicits antibody responses at week 8 and month 7 for which the GMC, GMT, and $\%4\times R$ are noninferior to those achieved by the currently licensed schedule (4-SQ).

3. During the first 7 months of the study, the IM administration will be associated with a change in injection site reactivity when compared with SQ administration.

Outcomes

The criterion for the GMC and GMT outcomes was comparison of the ratio of GMC and GMT in the 4-SQ reference group (licensed route and dosage schedule) with the 4-IM or 3-IM test group. If the upper 97.5% confidence bound for this ratio was less than 1.5, then the test group was judged to be noninferior to the reference group. For the $\%4\times R$ outcome, the criterion was to consider the difference between the proportion of responders in the 4-SQ reference group and the 4-IM or 3-IM test group. If the upper 97.5% confidence bound for this difference was less than 0.10, then the test group was judged to be noninferior to the reference group.

Reactogenicity end points were injection site and systemic AEs. Adverse events were analyzed as dichotomous end points (yes/no) using logistic regression; pain on injection was analyzed as an ordinal end point. Analyses focused on differences in the occurrence of AEs between the 4-IM vs 4-SQ groups and women vs men.

Serological Evaluation

Serum samples drawn at weeks 0, 4, and 8 and months 6 and 7 were assayed using a quantitative enzyme-linked immunosorbent assay (ELISA) for human anti-PA IgG, as described previously.¹⁵⁻¹⁷ The samples drawn on week 8 and month 7 were used for

analyses of the effects of vaccination at week 4 and month 6, respectively. Dilutional titers were calculated as continuous variables using a novel mathematical approach and reported as the reciprocal of dilution.¹⁸ The ELISA reactivity threshold was 2.5 $\mu\text{g/mL}$ for concentrations of anti-PA IgG and 50 for titers.¹⁵ All samples were verified by 2 independent operators.

Safety Evaluation

An AE was defined as any untoward medical occurrence, regardless of causal relationship to vaccination. Solicited and unsolicited AE data were collected during scheduled examinations, self-reported using AE diaries, and spontaneously reported at any time during the study and through follow-up by telephone of subjects who did not return for scheduled visits. Solicited injection site and systemic AEs were predefined based on data from previous AVA studies.²

Examinations were done immediately before injection and 15 to 60 minutes and 1 to 3 days after injection. Additional examinations were performed 28 days after injections 3 and 4. Subjects recorded AEs in diaries for 14 days after each of the first 2 doses and for 28 days after all subsequent doses. Adverse events were scored by participants as mild (no interference with routine activities, or temperature $<102.3^{\circ}\text{F}$), moderate (interfered with routine activities, or temperature between 102.3°F and 104°F), or severe (incapacitating, or temperature $>104^{\circ}\text{F}$). Immediately following each injection, subjects rated any pain they experienced using a graded scale of 0 (none) to 10 (extreme pain). The following AEs were classified as serious (SAE), consistent with US regulations: death, life-threatening event, initial inpatient hospitalization or prolongation of hospitalization, significant or persistent disability or incapacity, congenital anomaly or birth defect, and a medical event that required medical or surgical intervention to prevent one of the other outcomes.

Sample Size Calculations for Serological End Points

We applied a 1-sided noninferiority hypothesis in this study. Sample size calculations were based on formulas derived from Schuirmann¹⁹ and Phillips²⁰ for GMC and GMT analyses and Farrington and Manning²¹ for $\%4\times R$. For comparison of GMCs, the null hypothesis was that the 3-IM and 4-IM study groups (alternative schedule test groups) will have a significantly lower GMC than the 4-SQ reference group (licensed route and schedule). The alternative hypothesis was that alternative schedules will be noninferior to the licensed route and schedule.

The noninferiority margins of 0.10 for the difference in 4-fold responders and 1.5 for the ratio of geometric means were derived from guidances from the FDA and ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) and the literature precedent.²²⁻²⁴ Sample size calculations were made assuming a common \log_{10} standard deviation equaling 0.45 \log_{10} units for each study group. This standard deviation was determined from data collected in the pilot study of Pittman et al.² Standard deviations were calculated for each time point in the Pittman study for the 0-2-4 SQ, 0-4 IM, and 0-4 SQ study groups ($n=80$). The 75th percentile of standard deviations was 0.45.

A 95% 1-sided hypothesis test was applied with 80% power; this was adjusted for 2 analyses. These calculations yielded a group size of 126 participants. The sample size was inflated 100% to 252 and rounded to 260 to account for loss to follow-up and temporary protocol noncompliance. For the 1005 participant analyses, variance calculations on \log_{10} -transformed anti-PA IgG concentrations and titers were done by the independent statistician from the data and safety monitoring board to confirm that this provided adequate sample size to test the noninferiority hypotheses.

For comparison of proportions of $\%4\times R$, the null hypothesis was that alternative schedule test groups will have

a significantly lower proportion of responders than the 4-SQ reference group. The alternative hypothesis was that alternative schedules will be noninferior to the licensed route and schedule. If the null hypothesis was rejected, then with stated power and level of significance, study group 4-IM or 3-IM is noninferior to 4-SQ in terms of proportion of $\%4\times R$.

For comparison of geometric means, we applied a 97.5% 1-sided, noninferiority hypothesis. The criterion was to consider the ratio of the GMC and GMT in the 4-SQ reference group to the 4-IM or 3-IM test groups to be noninferior if the upper 97.5% confidence bound for this difference was less than 1.5. For comparison of proportions of $\%4\times R$, we applied a 97.5% 1-sided, noninferiority hypothesis. The criterion was to consider the relative risk of the $\%4\times R$ in the 4-SQ reference group to the 4-IM or 3-IM test groups to be noninferior if the upper 97.5% confidence bound for this relative risk was less than 1.12. If the comparison of proportions of $\%4\times R$ were re-expressed as the difference between the 2 proportions, the relative risk of 1.12 would correspond to an approximate 0.10 difference in proportions of 4-fold responders between the licensed and study groups. The method of maximum likelihood was used to compute the variance of the difference in relative risk under the null hypothesis.²¹

Sample Size Calculations for Reactogenicity

The reactogenicity sample size calculations were based on a dichotomous measurement of any AE, regardless of causality, after a given injection. A minimum sample size of 126 per group was required to provide sufficient power to conclude that any difference in the proportion of study participants with an AE between the 4-SQ reference group and the 4-IM test group of 0.2 or greater was significant.

Random Allocation Sequence

Study participants were randomly assigned into 1 of 6 study groups

(Figure 1). To achieve equal representation of men and women in the allocation of study groups, participant randomization was stratified by sex with a separate randomization to be used for men and women at each site. Blocks of 12 participants were randomized to primary study arms within the site/sex strata. A sufficient number of randomized blocks were generated to satisfy the expected participant counts and male/female proportions at each site. The subjects in group 6 were again randomly assigned to SQ or IM. Sex-specific lists of participant identifiers were provided to the contract research organization (CRO), who made the study group assignments.

Blinding

Unblinded clinical site staff included only those involved in vaccine or placebo preparation and administration of injections. The CRO provided participant identifiers with vaccine group assignments to unblinded individuals at the sites who then used these lists to sequentially assign participant identification numbers and study group assignments. Site investigators, persons involved in monitoring AEs, and other site staff remained blinded to group assignments. Laboratory personnel received blood specimens that could be linked back to the subject from whom they originated to prevent errors in sample identification; however, the laboratory personnel did not have access to study group assignments or visit numbers. The statisticians were blinded and did not have access to the data until the analyses, at which time the data were received with masked participant identifiers from the CRO. This masking was done to prevent unblinding of data collected after the month 7 analyses cutoff.

Statistical Methods

The statistical analysis plan was filed with the FDA before the study statisticians received any study data. Time points for serological noninferiority analyses were week 8 and month 7; the responses to injections, up to week 4

and month 6, respectively. Primary serological end points were GMC, GMT, and $\%4\times R$. Antibody concentrations and titers that were below the assay reactivity thresholds were reported as 1.25 $\mu\text{g/mL}$ and 1/25 respectively.²⁵ Noninferiority criteria are described earlier in the "Methods" section. Analysis-of-variance models were constructed to analyze log-transformed antibody data. Models allowed for the longitudinal nature of the data and included adjustments for study site, age group, sex, race, and significant interactions. Analyses of proportion of 4-fold and threshold responses were stratified by the time points. Alternate regimens were considered noninferior to the licensed regimen as described earlier in the "Methods" section. Rates of antibody decay were calculated by taking the difference of the GMCs at 2 time points and dividing this difference by the time interval, in weeks, between the 2 time points. The study was not powered to investigate differences between vaccine lots.

For analyses of injection site and systemic AEs, models were fitted using generalized estimating equations for longitudinal data, and hypothesis testing was performed using a 2-sided significance level of $\alpha = .05$. Factors considered in all models were study site, study group, sex, race, time, and interactions of treatment group by sex and race. Time was a continuous variable defined as the number of days between dose 1 and doses 2, 3, and 4. Study site, study group, sex, and race remained in the models regardless of significance. Other nonsignificant factors and interactions were removed in a stepwise fashion. Age and other possible risk factors for reactogenicity will be evaluated at the end of the study. All AEs were assessed and included in the analyses; however, this study was not designed to evaluate possible associations between AVA and rare SAEs.

Multiple imputation of missing data was performed to correct for potential nonresponse bias in the interpretation of serological and reactogenicity data.²⁶⁻²⁹ Intention-to-treat analyses

were done on the imputed data to preserve randomization of the treatment groups. All analyses were conducted using SAS software (SAS Institute Inc, Cary, North Carolina).

RESULTS

Participant Flow, Recruitment, and Demographics

Study enrollment was from May 15, 2002, to February 25, 2003. A total of 1876 participants provided consent and were screened (TABLE 1). Of these, 312 were excluded from enrollment, and the remaining 1564 participants were randomized into 7 study groups (Figure 1). For the month 7 analyses of the first 1005 participants, the mean study group size was 168 (range, 165-170). The combined 3-IM group size was 501. The proportions of men (n=505) and women (n=500) were similar. The mean age was 38.4 years. The proportion of participants (percentage of total) in each age group was similar for age categories of younger than 30 years (n=301, 30.0%), 30-39 years (n=241, 24.0%), and 40-49 years (n=281, 28.0%) but was lower for 50-61 years (n=182, 18%). Neither the distribution of age categories ($\chi^2 P=.35$) nor the proportion of males/females ($\chi^2 P=.98$) were statistically significantly different across treatment groups. Overall race distribution was 76% white

(n=768), 19% black (n=187), and 5% other (n=50). Ninety-five percent were non-Hispanic and 5% were Hispanic. The distributions of these 3 race categories were not significantly different across treatment groups ($\chi^2 P=.85$).

Serological Evaluation

Anti-PA IgG antibody responses were determined at weeks 0, 4, and 8 and at months 6 and 7. There was a strong positive correlation between anti-PA IgG antibody concentration and antibody titers ($r=0.991$; $P<.001$). Of the potential 1005 serological data points at each respective time point, 0%, 2.1%, 5.2%, 4.6%, and 8.6% of data points were missing and replaced with multiply imputed values. The responses at week 8 and month 7 were used for noninferiority analyses of vaccinations at week 4 and month 6, respectively (TABLE 2 and TABLE 3, FIGURE 2).

Week 8 antibody responses were significantly higher in women than men within the 4-IM and 3-IM vaccination groups but not in the 4-SQ group ($P=.12$). At month 7, there were no significant differences between the sexes in any group (TABLE 4). Antibody levels were significantly higher in white compared with black individuals at week 8 ($P\leq.05$) but not at month 7 (data not shown).

In general, at week 8 and month 7, there was a decrease in antibody response with increase in age. At week 8 across all age groups (<30 years, 30-39 years, 40-49 years, and 50-61 years), the 3-IM GMC was significantly lower than the GMC for both 4-SQ and 4-IM. Antibody levels for vaccinated groups declined at similar rates between weeks 8 to 26 (month 6) (range, -2.7 to -5.4 $\mu\text{g/mL/week}$). At month 7, there were no significant differences in anti-PA GMC among the 3 study groups for those aged 49 years or younger ($P\leq.05$). However, in study participants younger than 50 years, the 4-IM study group GMC was significantly different from the 3-IM group GMC ($P=.03$), although the ability to detect small differences as significant may be a consequence of the 3-fold larger number of subjects in 3-IM.

Reactogenicity

Solicited injection site AEs assessed during examinations occurred at lower proportions in the 4-IM group compared with 4-SQ. These proportions are provided in TABLE 5. There was a significant odds ratio (OR) reduction of occurrence for warmth, tenderness, itching, erythema, induration, edema, and nodules (Table 5). Mantel-Haenszel analyses comparing ordinal AE duration data (0, 1-3, and >3 days)

Table 1. Baseline Characteristics for 1005 Participants in the CDC Anthrax Vaccine Research Program Human Clinical Trial^a

Group ^b	Participants, No.										
	Overall	Sex, F/M ^c	Age, y ^d				Race ^e			Ethnicity ^f	
			<30	30-39	40-49	50-61	White	Black	Other	Hispanic	Non-Hispanic
1	165	81/84	58	30	50	27	129	28	8	157	8
2	170	87/83	42	44	52	32	126	32	12	163	7
3	168	85/83	52	55	29	32	131	31	6	161	7
4	166	82/84	54	38	41	33	125	32	9	156	10
5	167	82/85	43	39	58	27	127	33	7	158	9
6a	85	42/43	28	17	25	15	65	15	5	81	4
6b	84	41/43	24	18	26	16	65	16	3	83	1

Abbreviation: CDC, Centers for Disease Control and Prevention.

^a Mean study group size was 168 (range, 165-170).

^b Groups 3 through 5 (n = 501) and groups 6a and 6b (n = 169) were combined for analyses.

^c Proportions of men (n = 505) to women (n = 500) were not statistically significantly different across treatment groups ($\chi^2, P = .98$).

^d Mean age was 38.4 years. Proportions of participants (% of total) in each age group were similar for age categories of younger than 30 years (n = 301, 30.0%), 30-39 years (n = 241, 24.0%), and 40-49 years (n = 281, 28.0%) but were lower for the category 50-61 years (n = 182, 18%). The distributions of age were not statistically significantly different across treatment groups ($\chi^2, P = .35$).

^e Overall race distribution was 76% white (n = 768), 19% black (n = 187), and 5% other (n = 50). The distributions of these 3 race categories were not significantly different across treatment groups ($\chi^2, P = .85$).

^f Overall ethnicity was 95% non-Hispanic and 5% Hispanic.

demonstrated that injection site AEs in the 4-IM group were of shorter duration when compared with 4-SQ (data not provided). Logistic model analyses of severity demonstrated that individuals in the 4-IM group experienced fewer moderate and severe injection site AEs (10.2% vs 7.0%; $P=.04$). Analyses incorporating additional data collected from subjects' diaries and dur-

ing telephone follow-up showed similar results for injection site AE comparisons between 4-IM and 4-SQ, but the overall AE frequencies were higher. An exception was frequency of injection site bruising, which was not significantly different between 4-IM and 4-SQ when considering the in-clinic data alone (4.6% vs 4.3%; $P=.86$) but was found to occur less frequently in

the 4-IM group when the diary, telephone follow-up, and in-clinic data were combined (18.2% vs 8.9%; $P<.001$). The OR for ordinal end point pain reported immediately after injection was reduced by 50% for the 4-IM vs 4-SQ groups ($P<.001$).

Across all treatment groups, women were almost twice as likely as men to experience any injection site AE

Table 2. Vaccination Regimens and Serum Anti-Protective Antigen IgG Responses for the Week 4 and Week 8 Time Points

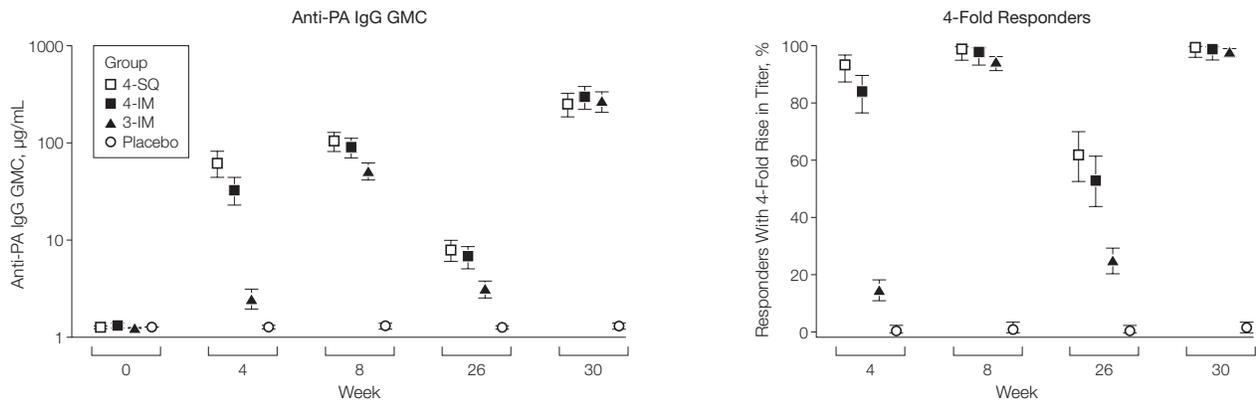
Group (n = 1005)	Full Study Vaccination Schedule	Dose Schedule to Month 7	Analysis Designation	Serum Anti-PA IgG Responses (95% CI) ^a					
				Week 4			Week 8		
				GMC, µg/mL	GMT	4-Fold Rise in Titer Response, %	GMC, µg/mL	GMT	4-Fold Rise in Titer Response, %
1 (n = 165)	SQ 0, 2, 4 wk; 6, 12, 18, 30, 42 mo	0, 2, 4 wk; 6 mo	4-SQ	61.8 (45.4-84.1)	753.9 (565.1-1005.7)	93.3 (87.6-97.0)	105.1 (83.8-131.8)	1315.4 (1047.8-1651.4)	98.8 (95.2-99.9)
2 (n = 170)	IM 0, 2, 4 wk; 6, 12, 18, 30, 42 mo	0, 2, 4 wk; 6 mo	4-IM	32.6 (23.5-45.4)	408.7 (302.9-551.5)	84.1 (76.8-89.9)	90.8 (71.9-114.6) ^c	1114.8 (881.9-1409.3) ^c	97.7 (93.5-99.5) ^c
3 (n = 168)	IM 0, 4 wk; 6, 12, 18, 30, 42 mo	0, 4 wk; 6 mo	3-IM ^b	2.5 (2.0-3.2)	38.9 (31.8-47.6)	14.6 (11.2-18.5)	52.2 (42.6-63.9)	650.6 (533.3-793.7)	94.4 (91.6-96.5) ^c
4 (n = 166)	IM 0, 4 wk; 6, 18, 42 mo								
5 (n = 167)	IM 0, 4 wk; 6, 42 mo								
6a (n = 85)	SQ 0, 2, 4 wk; 6, 12, 18, 30, 42 mo	0, 2, 4 wk; 6 mo	Placebo ^b	1.30 (1.25-1.36)	25.3 (24.8-25.9)	0 (0.0-2.6)	1.33 (1.24-1.43)	26.0 (24.5-27.6)	0.6 (0.01-3.7)
6b (n = 84)	IM 0, 2, 4 wk; 6, 12, 18, 30, 42 mo								

Abbreviations: CI, confidence interval; GMC, geometric mean concentration; GMT, geometric mean titer; IM, intramuscular route; PA, protective antigen; SQ, subcutaneously.
^aGeometric means and CIs were adjusted for study site, age group, sex, race, and significant interactions. Geometric means for the control group and titer fold response proportions and CIs were stratified by time period.
^bGroups 3 through 5 and groups 6a and 6b were combined for these analyses.
^cNoninferiority was achieved when the upper bound of the 95% CI for the ratio of the geometric means of the 4-SQ group to that of the test groups (groups 3-5) was less than 1.5 and if the analogous upper bound for the differences in proportions of 4-fold response was less than 0.10.

Table 3. Vaccination Regimens and Serum Anti-Protective Antigen IgG Responses for the Month 6 and Month 7 Time Points

Group (n = 1005)	Full Study Vaccination Schedule	Dose Schedule to Month 7	Analysis Designation	Serum Anti-PA IgG Responses (95% CI) ^a					
				Month 6			Month 7		
				GMC, µg/mL	GMT	4-Fold Rise in Titer Response, %	GMC, µg/mL	GMT	4-Fold Rise in Titer Response, %
1 (n = 165)	SQ 0, 2, 4 wk; 6, 12, 18, 30, 42 mo	0, 2, 4 wk; 6 mo	4-SQ	7.9 (6.2-10.2)	99.6 (79.0-125.6)	61.8 (52.8-70.3)	252.2 (190.8-333.5)	2986.4 (2288.1-3897.8)	99.4 (96.2-100.0)
2 (n = 170)	IM 0, 2, 4 wk; 6, 12, 18, 30, 42 mo	0, 2, 4 wk; 6 mo	4-IM	6.8 (5.2-8.8)	86.1 (68.2-108.8)	52.9 (44.1-61.7)	298.5 (227.5-391.7) ^c	3491.9 (2699.6-4516.7) ^c	98.8 (95.3-99.9) ^c
3 (n = 168)	IM 0, 4 wk; 6, 12, 18, 30, 42 mo	0, 4 wk; 6 mo	3-IM ^b	3.2 (2.6-3.9)	43.8 (36.5-52.7)	25.0 (20.7-29.6)	270.6 (212.4-344.7) ^c	3342.7 (2642.1-4229.1) ^c	98.2 (96.4-99.3) ^c
4 (n = 166)	IM 0, 4 wk; 6, 18, 42 mo								
5 (n = 167)	IM 0, 4 wk; 6, 42 mo								
6a (n = 85)	SQ 0, 2, 4 wk; 6, 12, 18, 30, 42 mo	0, 2, 4 wk; 6 mo	Placebo ^b	1.29 (1.24-1.34)	25.4 (24.8-26.0)	0 (0.0-2.6)	1.33 (1.24-1.43)	25.8 (24.4-27.2)	0.6 (0.01-3.7)
6b (n = 84)	IM 0, 2, 4 wk; 6, 12, 18, 30, 42 mo								

Abbreviations: CI, confidence interval; GMC, geometric mean concentration; GMT, geometric mean titer; IM, intramuscular route; PA, protective antigen; SQ, subcutaneously.
^aGeometric means and CIs were adjusted for study site, age group, sex, race, and significant interactions. Geometric means for the control group and titer fold response proportions and CIs were stratified by time period.
^bGroups 3 through 5 and groups 6a and 6b were combined for these analyses.
^cNoninferiority was achieved when the upper bound of the 95% CI for the ratio of the geometric means of the 4-SQ group to that of the test groups (groups 3-5) was less than 1.5 and if the analogous upper bound for the differences in proportions of 4-fold response was less than 0.10.

Figure 2. Anti-Protective Antigen IgG Geometric Mean Concentration and Proportion of Responders With a 4-Fold Rise in Titer Response Profiles

Time points for serological noninferiority analyses were week 8 and month 7 (week 30); the responses to injections up to week 4 and month 6 (week 26), respectively. Primary serological end points were geometric mean concentration (GMC), geometric mean titer (GMT), and proportion of responders with a 4-fold rise in titer (%4×R). Because of the strong positive correlation between anti-protective antigen (PA) IgG antibody concentration and antibody titers ($r=0.991$; $P<.001$), only GMC data are presented in the figure. Analysis-of-variance models were constructed to analyze log-transformed antibody data. Models allowed for the longitudinal nature of the data and included adjustments for study site, age group, sex, race, and significant interactions. Analyses of proportion fold and threshold responses were stratified by the time points. A, Scatterplots of GMC by regimen at weeks 0, 4, 8, 26 (month 6), and 30 (month 7). B, Scatterplots of proportions of anti-PA IgG antibody titer 4-fold responders by regimen at weeks 0, 4, 8, 26 (month 6), and 30 (month 7). Error bars indicate 95% confidence intervals (CIs).

(OR=1.93, $P<.001$); however, the absolute differences between women and men for warmth, itching, erythema, induration, and nodules were largest in 4-SQ (Table 5). Women had a significant increase in the odds of experiencing pain immediately after injection compared with men (OR=1.61, $P<.001$).

Route of administration did not significantly influence the occurrence of systemic AEs (TABLE 6). These findings were consistent with analyses incorporating data from diaries and telephone follow-up.

Women had a significantly increased OR for occurrence of solicited systemic AEs vs men (Table 6). The sex × treatment group interaction term for systemic AEs was not significant in any of the models, indicating that the differences in systemic AEs between men and women were generally consistent across all study groups.

During the 7 months of participation summarized here, there were 51 SAEs reported among 47 subjects of the 1005, including 3 deaths. Using World Health Organization causality assessment criteria, the data and safety monitoring board concluded after a blinded review that none of these 51 SAEs was

Table 4. Serum Anti-Protective Antigen IgG GMC for Men and Women at Week 8 and Month 7

Group	Time Point	Antibody GMC, µg/mL (95% CI) ^a		
		Men	Women	P Value
4-SQ	Week 8	94.8 (74.9-120.0)	116.4 (91.9-147.6)	.12
	Month 7	250.1 (186.8-334.9)	254.4 (192.3-336.5)	.91
4-IM	Week 8	73.4 (57.1-94.3)	112.2 (87.8-143.5)	.003
	Month 7	289.1 (217.4-384.4)	308.2 (233.4-407.1)	.68
3-IM ^b	Week 8	44.7 (36.7-54.4)	60.9 (49.0-74.8)	.002
	Month 7	243.8 (191.9-309.8)	300.3 (236.0-382.0)	.07
Placebo ^b	Week 8	1.3 (1.3-1.3)	1.4 (1.2-1.5)	ND
	Month 7	1.3 (1.2-1.5)	1.3 (1.3-1.4)	ND

Abbreviations: CI, confidence interval; GMC, geometric mean concentration; IM, intramuscular route; ND, not done; SQ, subcutaneously.

^aGeometric means and CIs were adjusted for study site, age group, sex, race, and significant interactions. Geometric means for the control group were stratified by time period.

^bGroups 3 through 5 and groups 6a and 6b were combined for these analyses.

related or possibly related to the study agent. Since enrollment began, there have been 229 SAEs in the entire 1563 participant cohort involving 186 participants, with 7 deaths. Causes of death included atherosclerotic cardiovascular disease, intracranial aneurysm, motor vehicle accident, suicide, AIDS-related illness, accident, and gunshot wound. Nine SAEs (involving 7 participants) were rated as possibly related to the study agent (TABLE 7). All other events were considered unrelated or unlikely to be related to the in-

vestigational agent. A complete and unblinded analysis of SAEs will be conducted at the study conclusion when all participants complete the 43-month visit.

COMMENT

Prior studies and an extensive Institute of Medicine review have found AVA to be effective at preventing anthrax and reasonably safe.³⁰⁻³² This article provides analyses of the first 1005 participants at month 7 in a congressionally mandated phase 4 clinical trial

Table 5. Proportions of Injection Site Adverse Events Reported by Dose During In-Clinic Examinations by Sex and Route

Adverse Event	Sex	Incidence, No./Total Doses (%)		Comparison	Odds Ratio (95% CI) ^b	P Value ^b
		4-IM ^a	4-SQ ^a			
Warmth ^c	F	34/333 (10.2)	156/314 (49.7)	F vs M (4-SQ)	5.18 (3.26-8.23)	<.01
				F vs M (4-IM)	2.84 (1.33-6.03)	.007
	M	11/300 (3.7)	50/322 (15.5)	4-IM vs 4-SQ (F)	0.10 (0.06-0.17)	<.001
				4-IM vs 4-SQ (M)	0.19 (0.09-0.40)	<.001
Tenderness	F	180/340 (52.9)	220/315 (69.8)	F vs M	1.44 (1.22-1.71)	<.001
	M	128/304 (42.1)	168/321 (52.3)	4-IM vs 4-SQ	0.57 (0.43-0.77)	<.001
Itching	F	17/332 (5.1)	71/312 (22.8)	F vs M	2.62 (1.72-4.00)	<.001
	M	7/300 (2.3)	22/319 (6.9)	4-IM vs 4-SQ	0.22 (0.12-0.40)	<.001
Pain ^d	F	79/334 (23.7)	67/312 (21.5)	F vs M	1.62 (1.29-2.03)	<.001
	M	38/301 (12.6)	36/319 (11.3)	4-IM vs 4-SQ	1.14 (0.79-1.65)	.49
Arm motion limitation	F	44/335 (13.1)	44/312 (14.1)	F vs M	1.34 (1.00-1.78)	.05
	M	19/301 (6.3)	20/317 (6.3)	4-IM vs 4-SQ	0.99 (0.63-1.55)	.95
Erythema ^c	F	96/335 (28.7)	234/317 (73.8)	F vs M (4-SQ)	4.22 (2.61-6.81)	<.001
				F vs M (4-IM)	2.47 (1.53-4.01)	<.001
	M	41/301 (13.6)	135/322 (41.9)	4-IM vs 4-S (F)	0.13 (0.08-0.20)	<.001
				4-IM vs 4-SQ (M)	0.21 (0.13-0.35)	<.001
Induration	F	33/332 (9.9)	134/312 (42.9)	F vs M	2.03 (1.54-2.68)	<.001
	M	18/300 (6.0)	72/321 (22.4)	4-IM vs 4-SQ	0.16 (0.11-0.24)	<.001
Edema	F	50/334 (15.0)	86/312 (27.6)	F vs M	1.68 (1.30-2.16)	<.001
	M	21/300 (7.0)	69/320 (21.6)	4-IM vs 4-SQ	0.36 (0.25-0.51)	<.001
Nodules ^c	F	21/332 (6.3)	161/313 (51.4)	F vs M (4-SQ)	3.33 (2.09-5.29)	<.001
				F vs M (4-IM)	3.52 (1.24-10.01)	.02
	M	5/300 (1.7)	72/319 (22.6)	4-IM vs 4-SQ (F)	0.05 (0.03-0.11)	<.001
				4-IM vs 4-SQ (M)	0.05 (0.02-0.12)	<.001
Bruise	F	15/333 (4.5)	18/310 (5.8)	F vs M	2.14 (1.49-3.07)	<.001
	M	12/300 (4.0)	11/317 (3.5)	4-IM vs 4-SQ	0.95 (0.52-1.72)	.86

Abbreviations: 4-SQ, full dose regimen given SQ; 4-IM, full dose regimen given IM; CI, confidence interval; IM, intramuscular route; SQ, subcutaneously.

^aIncidence was calculated using the unimputed intention-to-treat cohort.

^bOdds ratios and *P* values were determined from multivariate modeling using the imputed intention-to-treat cohort and were adjusted for all factors in the model. If there were 5 or fewer occurrences in the placebo groups, then they were removed prior to model fit. If interactions were significant, then comparisons were conducted within groups. Significance level was set at *P* ≤ .05.

^cInjection site adverse events with a significant sex and treatment interaction.

^dPain is defined as a subjective feeling of discomfort in the area of the injection site; this is not pain on injection, which is assessed immediately following the injection using a visual scale.

Table 6. Systemic Adverse Events Reported During In-Clinic Examinations by Sex and Route

Adverse Event	Sex	Incidence, No./Total Doses (%)		Comparison	Odds Ratio (95% CI) ^b	P Value ^b
		4-IM Incidence ^a	4-SQ Incidence ^a			
Fatigue	F	40/336 (11.9)	32/310 (10.3)	F vs M	1.34 (1.01-1.78)	.04
	M	20/302 (6.6)	19/319 (6.0)	4-IM vs 4-SQ	1.09 (0.69-1.73)	.72
Muscle ache	F	32/335 (9.6)	22/310 (7.1)	F vs M	1.60 (1.19-2.16)	.002
	M	21/302 (7.0)	12/320 (3.8)	4-IM vs 4-SQ	1.58 (0.93-2.69)	.09
Headache	F	28/335 (8.4)	33/310 (10.6)	F vs M	1.96 (1.39-2.76)	<.001
	M	12/302 (4.0)	14/319 (4.4)	4-IM vs 4-SQ	0.78 (0.45-1.34)	.36
Fever	F	0/331	0/309	F vs M	NA	NA
	M	0/300	0/319	4-IM vs 4-SQ	NA	NA
Tender/painful axillary adenopathy	F	2/334 (0.6)	8/309 (2.6)	F vs M	NA	NA
	M	1/300 (0.3)	3/319 (0.9)	4-IM vs 4-SQ	NA	NA

Abbreviations: 4-IM, full dose regimen given IM; 4-SQ, full dose regimen given SQ; CI, confidence interval; IM, intramuscular route; NA, analyses that could not be conducted due to insufficient number of occurrences (≤5) in the vaccine groups; SQ, subcutaneously.

^aIncidence was calculated using the unimputed intention-to-treat cohort.

^bOdds ratios and *P* values were determined from multivariable modeling using the imputed intention-to-treat cohort and were adjusted for all factors in the model. If there were 5 or fewer occurrences in the placebo groups, then they were removed prior to model fit. If interactions were significant, then comparisons were conducted within groups. Significance level was set at *P* ≤ .05.

designed to evaluate alternate regimens for AVA. These data demonstrate that it is possible to eliminate the dose administered at week 2 and to change the route of administration to IM, which will reduce injection site AEs without impacting the anamnestic anti-PA IgG response at month 7. The data from the remaining 559 participants, including analyses of omitting additional vaccine doses at 12, 18, and 30 months, will be included in the analyses at the end of the study in 2009.

A critical part of this activity has been the development and standardization of clinical and laboratory protocols, production of qualified source and reference standard reagents, establishment of validated serological assays, and development of new mathematical models for the quantification of anti-PA antibody responses. The broad application of these technologies and procedures has facilitated direct, standardized comparison of serological data from this and other anthrax vaccine clinical trials and from clinical *Bacillus anthracis* infection.³³⁻³⁶

The serological data indicate that at week 8 in response to the first 2 injec-

tions of the 3-IM schedule, AVA elicited a significant rise in anti-PA IgG GMC compared with controls. The response was almost 50% less than that observed for 3 AVA injections at that time point in the full schedule (4-IM) and licensed regimen (4-SQ). In contrast, Pittman et al² reported that differences in antibody responses between analogous regimens in a smaller pilot study of route change and schedule reduction were not significant at their peak levels (week 6). The divergence between Pittman et al and the present study is possibly attributable to several factors. For example, sample size differences (n=22-28 in Pittman et al compared with n=165-170) and higher assay precision will result in smaller variances and shorter confidence intervals for these data, thus increasing power to detect statistical differences, and vaccine lot variation may result in changed antibody response profiles. In addition, differences in study population may have an important impact, for example, an exclusively military cohort in Pittman et al vs a more diverse cohort. Testing these hypotheses would

require further investigation. Nonetheless, the levels of anti-PA IgG elicited by the first 2 AVA injections of the 3-IM regimen match or exceed those reported to protect 90% of animals (9/10) vaccinated with 2 doses of diluted AVA and exposed at week 10 to high doses (>200 median lethal dose [LD₅₀] equivalents) of lethal *B anthracis* aerosol challenge.³⁷ Similar anti-PA IgG levels in rabbits vaccinated with 1 dose of a recombinant PA vaccine followed by a challenge at week 4 protected more than 90% (28/30).³⁸ Recognizing the potential differences in susceptibility between humans and other animals—humans are reported to have relatively low susceptibility—it is nonetheless highly probable that the antibody levels elicited by 2 doses of AVA (weeks 0, 4) would confer protection against anthrax in humans.³⁴ This interpretation is supported by both the magnitude and noninferiority of the anamnestic responses at month 7, which clearly demonstrate that a 3-IM schedule provides immunological priming equivalent to the 4-SQ and 4-IM schedules. In addition, the CDC Anthrax Vac-

Table 7. Serious Adverse Events Rated as Possibly Associated With the Study Agent Since Study Initiation

Participant	Description of SAE	Status	Outcome	Medical Monitor Causality Assessment ^a
1	Tear of supraspinatus tendon	Study injections discontinued; continuing follow-up	Symptoms resolved after surgery and physical therapy	Possible
2	Generalized allergic reaction	Study injections discontinued; continuing follow-up	Resolved	Possible
3	Bilateral pseudotumor cerebri with bilateral disc edema	Study injections discontinued; continuing follow-up	Treated and improving	Possible
4	New onset of generalized seizure; hospitalized for generalized clonic seizure; MRI confirmed hydrocephalus consistent with aqueductal stenosis; hospitalized endoscopic third ventriculostomy secondary to aqueductal stenosis	Study injections initially suspended and then later discontinued; continuing follow-up	Discharged in stable condition	Possible
5	Bilateral ductal carcinoma of the breast	Completed study	Undergoing further testing (at time of report)	Possible
6	Onset of new bilateral arthralgia of the metacarpophalangeal joints; ANA positive	Completed study	Continuing treatment	Possible
7	Invasive ductal carcinoma of the breast	Study injections discontinued; continuing follow-up	Positive outcome reported by participant following mastectomy and chemotherapy treatment	Possible

Abbreviations: ANA, anti-nuclear antibody; MRI, magnetic resonance imaging; SAE, serious adverse event.
^aBlinded review using World Health Organization causality assessment criteria.

cine Research Program correlates of immune protection studies in rhesus macaques have demonstrated an 80% to 100% efficacy of the 3-IM regimen against high-dose 102-772 LD₅₀ equivalents (5.6×10^6 – 4.2×10^7 spores) of virulent *B anthracis* aerosol challenge at 52 months after vaccination (8/10 and 9/9 surviving animals that received a full human dose or a 1/5 diluted dose, respectively) (C. P. Quinn, PhD; N. Marano, DVM; R. E. Hunt, DVM; R. Barnewall, DVM; G. S. Sivko, DVM, PhD; unpublished data, January 2006).

Previous AVA studies reported that both injection site and systemic AEs occurred more frequently in women compared with men.^{1-3,39,40} In the present study, the proportions of women and men in the 4-IM group with an injection site AE during an in-clinic exam were 69% and 53%, respectively, similar to those from safety evaluations of other aluminum-containing vaccines given IM and comparable with the AVA study by Pittman et al.^{2,30-32,41-43} The data presented here demonstrate that changing from SQ to IM reduces the frequency of most injection site AEs in men and women and results in substantially diminished absolute differences in AEs between men and women. Additional analyses are under way to explore the reasons for these sex differences. These include evaluating the influence of sex hormone levels, HLA type, and pre-injection anti-PA IgG levels on the frequency, severity, and duration of AEs.

Additional immunological data will be available when the full study is unblinded in 2009. These data will include an assessment of the impact on the magnitude and duration of the anamnestic anti-PA IgG responses at month 42 when omitting booster doses at months 12, 18, and 30 and the in vitro anthrax lethal toxin neutralization efficacy of the antibody responses for the entire study. These data, together with parallel AVA efficacy studies in rhesus macaques, may help elucidate a surrogate marker of protection against anthrax in humans.

CONCLUSION

Our data demonstrate that a 3-IM regimen (omission of the week 2 dose) elicits serum antibody responses at month 7 that are noninferior when compared with regimens containing 4 doses of AVA (SQ or IM). Intramuscular administration was associated with a significant reduction in injection site AEs. Changing the injection route from SQ to IM may increase vaccine acceptability. Reducing the number of doses in the AVA regimen would have the added benefit of increasing the number of doses available for prophylactic use.

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