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Immunity from Smallpox Vaccine Persists for Decades:

A Longitudinal Study

Dennis D. Taub, PhD[a], **William B. Ershler, MD**[a], **Mark Janowski, MD**[a], **Andrew Artz, MD**[a], **Michael L. Key**[a], **Julie McKelvey**[a], **Denis Muller, MS**[a], **Bernard Moss, MD, PhD**[b], **Luigi Ferrucci, MD, PhD**[a], **Patricia L. Duffey**[a], and **Dan L. Longo, MD**[a]

[a]Laboratory of Immunology and the Clinical Research Branch, National Institute on Aging—Intramural Research Program, National Institutes of Health, Baltimore, Md

[b]Laboratory of Viral Diseases, National Institute of Allergy and Infections Disease—Intramural Research Program, National Institutes of Health, Bethesda, Md.

Abstract

PURPOSE—The threat of smallpox resulting from bioterrorist action has prompted a reassessment of the level of immunity in current populations.

METHODS—We have examined the magnitude and duration of antiviral antibody immunity conferred by smallpox vaccination in 246 participants of the Baltimore Longitudinal Study of Aging. Of this population, 209 subjects were vaccinated one or more times 13 to 88 years before this evaluation, and stored serum samples were available at various intervals after vaccination. An additional 8 subjects who had documented childhood smallpox infection and 29 subjects with no history of infection or vaccination were included. We quantified the total vaccinia IgG and neutralizing antibody titers in each of these subgroups of participants over time.

RESULTS—Vaccinated participants maintained antivaccinia IgG and neutralizing antibody titers above 3 natural logs essentially indefinitely. The absolute titer of antivaccinia antibody was only slightly higher after multiple vaccinations. In 97% of the participants, no decrease in vaccinia-specific antibody titers was noted with age over a follow-up period of up to 88 years. Moreover, Baltimore Longitudinal Study of Aging participants who survived active smallpox infections in their youth retained antivaccinia antibody titers that were similar to the levels detected in vaccinated subjects.

CONCLUSION—These data suggest that multiple or recent vaccinations are not essential to maintain vaccinia-specific antibody responses in human subjects. Scarce vaccine supplies should be applied first to individuals who have not previously been vaccinated.

Keywords

Aging; BLSA; Biodefense; Smallpox; Vaccination; Vaccinia

The vaccinia virus vaccine has been used to prevent smallpox disease and control its spread since the late 18th century. Routine vaccination with vaccinia was discontinued over 30 years ago in many countries.[1-8] Despite the worldwide eradication of smallpox in 1977, the potential re-emergence of this disease from bioterrorist action has prompted international health care

Requests for reprints should be addressed to Dan L. Longo, MD, Intramural Research Program, National on Aging, National Institutes of Health, 5600 Nathan Shock Drive, Baltimore, MD 21224-6825. E-mail address: longod@grc.nia.nih.gov

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authorities to reassess the level of immunity in current populations and to evaluate the risks and benefits of revaccination programs.

Given that the majority of Americans under the age of 35 years have never been vaccinated against smallpox and the great majority of those over 35 have not received booster vaccinations since the early 1970s, immunity to smallpox is considered to be low to nonexistent in today's population. In the past, primary vaccination of individuals with vaccinia was believed to confer dependable protection for at least 5 years, with increasing protection achieved with subsequent revaccinations.[1,2] However, a major question posed today is whether those individuals vaccinated 40 or more years ago would be protected in the event of smallpox exposure. This may be a critical question because the availability of smallpox vaccines is limited and currently inadequate for a mass inoculation program.

To address this, we evaluated the stability and potency of vaccinia-specific immunity after smallpox vaccination in 209 individual participants in the National Institute on Aging's Baltimore Longitudinal Study of Aging. Longitudinal studies have an advantage over cohort studies in allowing multiple measures in an individual over time.

METHODS

Study Population

Subjects included in this study are participants in the National Institute on Aging Baltimore Longitudinal Study of Aging (BLSA), a longitudinal study of normative aging established in 1958 which continues to gather extensive information on health and physiologic function on a cohort of individuals examined at 1-5 year intervals, depending on age.[9] All of the BLSA participants had fasting serum samples banked at each visit. Included in this study was a subset of the total BLSA cohort: all those with a known history of either smallpox infection (n = 8) or documented smallpox vaccinations (n = 209). Also included were 29 nonvaccinated BLSA participants. Of the 209 BLSA participants previously vaccinated, serum samples were available that had been stored for 13 to 88 years (median, 61 years) after the first vaccination. The median number of samples per subject was 4 (range 2-13). All vaccination histories were verified through medical and military records. Usually, information on the number of vaccinations was documented by more than one source. This substudy of the BLSA was approved by the Institutional Review Board, and all participants signed informed consent.

Antivaccinia Antibody Determination

Antivaccinia antibody was determined in all serum specimens by both enzyme-linked immunosorbant assay (ELISA) and virus neutralization assay.

ELISA

Vaccinia Virus (1×10^7 plaque-forming units [pfu]/mL [Lister strain; Advanced Biotechnologies Inc., Columbia, MD]) was adhered to the wells of a 96-well flat-bottomed plate to which 2-fold serial dilutions of sera were added. Using species-specifire antihuman conjugate (Sigma, St. Louis, Mo), the colorimetric response was assessed on an ELISA plate reader at wavelenths 340 and 492 nm. Antibody titers were determined by logarithmic transformation of the linear portion of the curve, with 0.025-0.030 optical density units used as the endpoint to convert the final values. The coefficient of variation of repeated samples ranged from 2.3% to 5.4%. Specificity and background controls were performed with each assay and plate run. A vaccinia immunoglobulin (VIG) reference standard, generously donated by Dorothy Scott (CBER/FDA), was used as a positive control. This standard demonstrated reproducible IgG titers of 1:20,480-40,960 by ELISA, comparable to the VIG vial

concentration of 50 μg/mL. The mean antibody titer of serum samples from nonvaccinated individuals was <1:4.

Neutralization Assay

Vaccinia neutralization assays were performed using a modification of a published protocol.[10] Serial 2-fold dilutions of sera, starting with a 1:8 dilution, were incubated with 5×10^3 pfu of green fluorescent protein-expressing vaccinia virus-infected BS-C-1 cells at a final concentration 0.5 pfu per cell in 96-well plates. Fluorescence intensity in infected cells was compared with wells containing cells and virus, but no serum (positive control) and cells and serum containing no virus (background control). The neutralizing titer-50 (NT_{50}) was defined as the reciprocal of the serum dilution required for 50% reduction in green fluorescent protein expression apparent on the vaccinia-infected BS-C-1 cells. Logarithmic transformation of the data was used to calcualte the titer. Negative serum controls were run with each assay. The sample-to-sample variations of these titers typically deviated by only ±1 titer dilution in samples under repeated runs. In addition, VIG (50 mg/mL) was used as an internal reference standard with an established ID50 at 20-30 μg/mL. This standard demonstrated NT_{50} titers of 1:1024-2048, which calculated to neutralizing concentrations of VIG between 24 and 49 μg/mL of VIG.

Statistical Analysis

Statistical analyses were perfomed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC). Vaccinia-specific IgG and NT_{50} titers were converted to natural logarithms to normalize their distributions. Regression and repeated-measures linear mixed-effects analyses were used to determine significant changes over time in these variables after vaccination, the differences in antibody titers in subjects with 0, 1, 2, 3, 4, or 5 or more vaccinations, and the relationship between the log of the vaccinia-specific IgG and NT_{50} titers. A P value $\leq .05$ was considered statistically significant.

RESULTS

Characteristics of Subjects

Subjects were selected from the total BLSA population and included all subjects with a documentable history of one or more smallpox vaccinations (n = 209) or a known history of small pox infection (n = 8). An additional 29 subjects were included who had no history of smallpox vaccination or infection (Table). The latter group was generally younger, having been born after smallpox vaccination was no longer recommended practice.

Vaccinia-Specific Antibody Titers

Among those individuals with prior vaccination (n = 209), vaccinia-specific IgG titers persisted and remained relatively stable at the various assessed time points, with titers ranging from 1:32 to 1:256 (Ln 3.46-5.54) for periods up to 88 years after an initial vaccine (Figure 1). Comparison of antiviral antibody IgG titers elicited by additional vaccinations demonstrated a small but statistically significant ($P < .05$) increase in the mean IgG titer that was produced after 2 or more vaccinations compared with subjects receiving only one vaccination. Additional vaccinations of 3, 4, or 5 inoculations resulted in a further small but significant increase in the IgG maintenance titers out to 88 years after the final vaccine administration (Figure 1).

While these IgG titers demonstrate the persistence of vaccinia-reactive antibodies, they do not indicate the antibody's ability to neutralize viral infectivity. To assess viral neutralization by antibody, we used a modified vaccinia neutralization assay. Similar to the IgG titers, the vaccinia-specific neutralizing antibody levels were quite stable after single or multiple

vaccinations for up to 88 years (Figure 2). In the majority of examined samples (124/209, 59%), the NT_{50} ranged from 1:256 to 1:512 (Ln 5.55-6.24) and remained stable. Only 3 patients (1.4%) had no measurable neutralizing antibody. Of note, and in contrast to the ELISA findings, there was no significant difference in neutralizing antibody level in those vaccinated once compared with those receiving additional vaccines (Figure 2). Control non-vaccinated subjects reproducibly yielded NT_{50} titers of <1:16 (Ln <2.77), with the majority of samples reproducibly measuring below 1:8 (Ln 2.08). We also assessed the stability of sequential measurements of neutralizing antibody among the 62 subjects who had received only a single vaccination. The median line through the values obtained on these subjects had a small negative slope (−0.0003/year) (data not shown), suggesting that even individuals who have had only a single vaccination maintain protective levels of neutralizing antibody indefinitely.

Overall, these data document the persistence of vaccinia-specific antibody levels for extended time periods after initial and subsequent vaccinations in nearly all vaccinated donors.

Vaccinia-Specific Titers in Smallpox Survivors

Smallpox infection survivors are known to have lifelong protection from reinfection.[11,12] We expected, therefore, that individuals with history of infection would have higher levels of immunity than those merely vaccinated. However, we found no differences in antivaccinia antibody titers between patients who recovered from smallpox and subjects who were vaccinated one or more times with vaccinia. Subjects with a prior history of smallpox retained vaccinia-specific IgG and neutralization titers similar to those subjects vaccinated one or more times during their lifetime (Figure 3).

Vaccinia-Specific Antibody Titers as a Biomaker of Protection

With the extinction of naturally occuring smallpox, it may be impossible to definitively establish the immunological correlates of protection against this disease in humans. Based on older data,[11] the 1:32 titer is a reasonable biomarker of protective immunity. Our results demonstrated that >97% of the samples tested exhibited neutralization titers above 1:32 at all time points tested. Less than 1% of the donors demonstrated titers initially above 1:32 that subsequently fell below this cutoff value.

Similarly, as subjects previously diagnosed with smallpox possess lifelong protection against the smallpox virus,[11-21] we also have used as a threshold the lowest neutralization titer from the 8 BLSA participants with documented medical histories of smallpox infections. This titer was found to be 1:48. Using this level of neutralizing antibody as a marker for smallpox resistance, we found that 95% of the subjects demonstrated titers above this level for all samples examined.

DISCUSSION

Our longitudinal study of the durability of immune responses to vaccinia immunization suggests that nearly all individuals who have been vaccinated maintain both specific and neutralizing antivaccinia antibodies at levels that suggest protection against smallpox. Multiple vaccinations produced slightly higher levels of antibody, but levels of antibody do not decrease significantly over time after vaccination.

The dogma on smallpox vaccinations has been that individuals with repeated exposure to smallpox (eg, travelers to endemic countries) should be revaccinated every 5 years.[10-12] However, several cross-sectional epidemiological studies have suggested that smallpox vaccination provided recipients with protection against lethal smallpox for longer periods of time and that multiple inoculations might not be necessary.[11,13-21] Our longitudinal data

support this argument; a single vaccination elicits functional antibody that remains stable over the lifetime.

While the specific roles of humoral and cellular immunity in the long-term protection against smallpox are not completely defined, 2 prospective studies indicate that high levels of neutralizing antibodies may be associated with protective immunity against smallpox.[22,23] One study[22] demonstrated that subjects who were in contact with smallpox victims possessing vaccinia virus neutralizing titers <1:32 were more susceptible to smallpox infection (3 of 15, or 20% of contacts infected) compared with subjects with antibody titers of ≥1:32 (0 of 127 or <1% of contacts infected. In a smaller study,[23] 6 of 43 (14%) contacts with neutralizing titers <1:20 contracted smallpox. whereas 0 of 13 contats with titers of 1:20 or higher contracted the disease. Thus, these serum-neutralizing antibody levels might be useful at least as biomarkers of protective immunity, regardless of whether the protection is mediated by humoral or cellular immunity or both.

In a study by Hammarlund et al,[24] >90% of volunteers vaccinated between the ages of 25 and 75 years retained substantial humoral or T-cell immunity against vaccinia virus. Antiviral antibody responses were present 1 to 75 years after vaccination, while antivaccinia T-cell responses decreased 8 to 15 years after the last vaccination.

Another study found that virus-specific T-cell memory can persist for up to 50 years in the presumed absence of antigen.[25] We similarly observed the maintenance of high levels of circulating antivaccinia antibody at many different time points in an individuals's life span over a 13- to 88-year period. In contrast, studies by Hsieh and coworkers[26] revealed that the longevity of vaccinia-specific T-cell-specific immune memory in a cohort of 220 Taiwanese individuals (aged 18-70 years) was easily detectable in subjects previously vaccinated 23-30 years earlier but more difficult to detect in subjects vaccinated 31-40 years earlier. These findings differ somewhat from the reports where vaccinia-reactive T cells could be detected as long as 50-75 years after vaccination.[24] The reasons for such differences remain unclear.

In a more recent study,[27] 159 healthy, previously vaccinated adults between the ages of 24 and 52 years were revaccinated; of these, 88% with clinical "take" (skin reactivity to vaccine) were found to seroconvert, while subjects with no clinical take demonstrated little to no antibody response. Moreover, the level of preexisting antibodies inversely correlated with the rates of clinical take and sero-conversion with the group of vaccinees with the lowest preexisting levels of antibodies (86%) developing antibody responsese to vaccine challenge. This study also demonstrated that the time since the last vaccination was significantly associated with the rates of clinical take and antibody seroconversion. The longer the interval since the last vaccination, the greater the rate of clinical take and seroconversion. Unfortunately, in our longitudinal study, precise information on clinical take was not retained over the ex-tanded time periods. However, a small cohort of subjects did have such documentation and demonstrated similar antibody maintenance patterns as subjects without such documentation (dada not shown).

Individuals who have survived a smallpox infection are thought to maintain lifelong protection; by contrast, vaccination generally induces a response that diminishes over time. Thus, we expected that individuals with a history of infection would have higher levels of immunity than those who were vaccinated. However, we found that vaccinia-specific antibody titers were comparable in these 2 groups (Figure 3). The mechanism of the lifelong persistence of vaccinia-elicited smallpox immunity is undefined.

The morbidity associated with vaccine administration and the risk of mortality among elderly and immune-compromised populations also are critical factors that will influence a vaccination policy. Little information is available about the safety and efficacy of smallpox vaccines in elderly populations. Furthermore, the aging process is associated with immune response

abnormalities.[28-30] Development of immunization guidelines for the population will need to consider these factors.

Protecting the population against smallpox in the face of limited supplies of vaccine may be a challenge. Frey et al[31] have shown that 1:10 dilutions of the vaccine also are highly efficient at eliciting clinical takes in previously unvaccinated individuals. Our revealed that nearly all the individuals who have been vaccinated one or more times maintained antivaccinia IgG and neutralizing antibody titers above 3 natural logs indefinitely. Titers were stable for up to 88 years. Moreover, those who survived active smallpox infections in their youth retained vaccinia-specific immunity throughout their lives and their antivaccinia antibody titers were similar to the levels of vaccinated subjects. Thus, vaccinated subjects remain immune to vaccinia indefinitely and do not require booster vaccinations even if they are many decades removed from primary vaccination. These data imply that limited supplies of vaccine can be more usefully applied (perhaps in diluted form) to individuals who have never been vaccinated, primarily individuals born after 1972.

CLINICAL SIGNIFICANCE

- Vaccinia elicits antiviral antibody levels and virus neutralizing activity that remain elevated for the life of the patient.
- Multiple vaccinations achieve only marginally higher levels of antibody and virus neutralizing activity than single vaccination.
- Levels of antibodies and virus neutralizing activity are comparable in vaccinated individuals and those who developed smallpox and recovered.
- Vaccinia should be used first on individuals who have never been vaccinated before.

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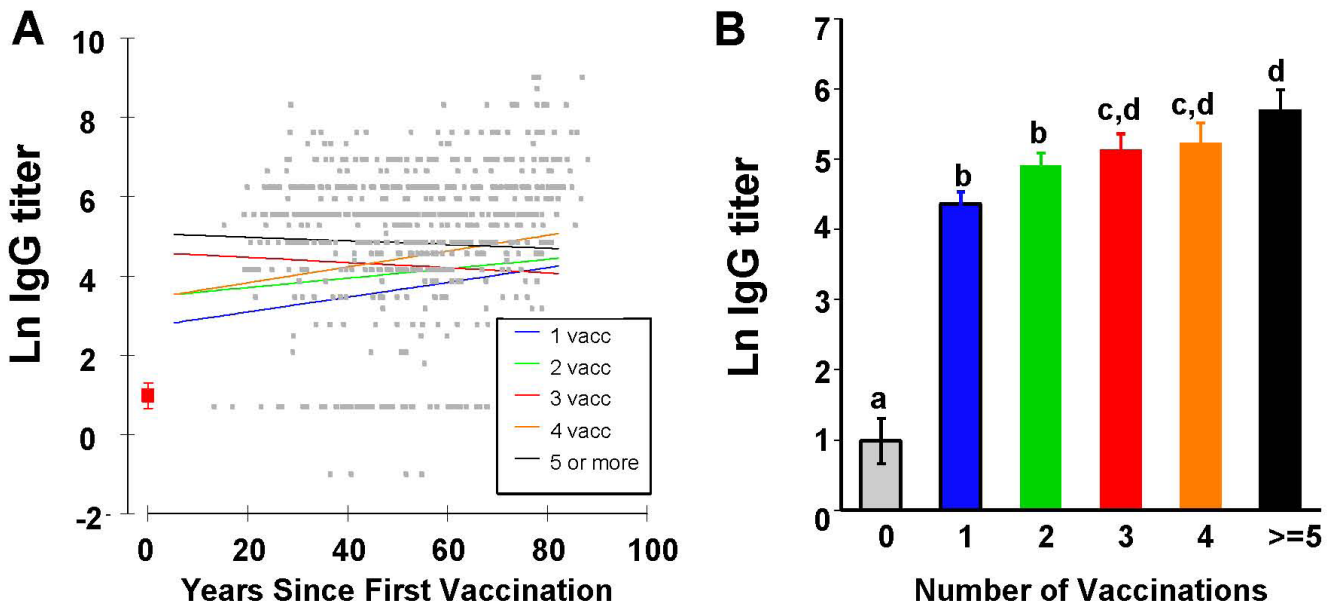

Figure 1.

Antivaccinia IgG titers in participants vaccinated one or more times over an 88-year period. (A) Effect of time on vaccinia-specific IgG antibody responses for each group of singly or multiply vaccinated individuals using the banked serum of Baltimore Longitudinal Study of Aging subjects. Serum banked over a 45-year time period on 209 Baltimore Longitudinal Study of Aging study participants was examined using a vaccinia-specific IgG ELISA to determine the antibody titers in subjects vaccinated one or more times over individual follow-up times between 13 and 88 years. Several time points were examined to assess how titers actually changed over time. All serum assay results are represented by individual dots. The lines indicate the repeated measures linear-effects model projection by number of vaccines. The bar (± SEM) at time zero represents the mean titer of the 29 non-vaccinated subjects (B) Vaccinia-specific IgG titers were compared with the total number of vaccinations received. Serum samples obtained from unvaccinated volunteers (n = 29) demonstrated titers <1:4 (Ln 1.39). Bars show the average±SE of antibody by each group. Bars with different superscripts (a, b, c, d) are significantly different from each other, $P < .05$.

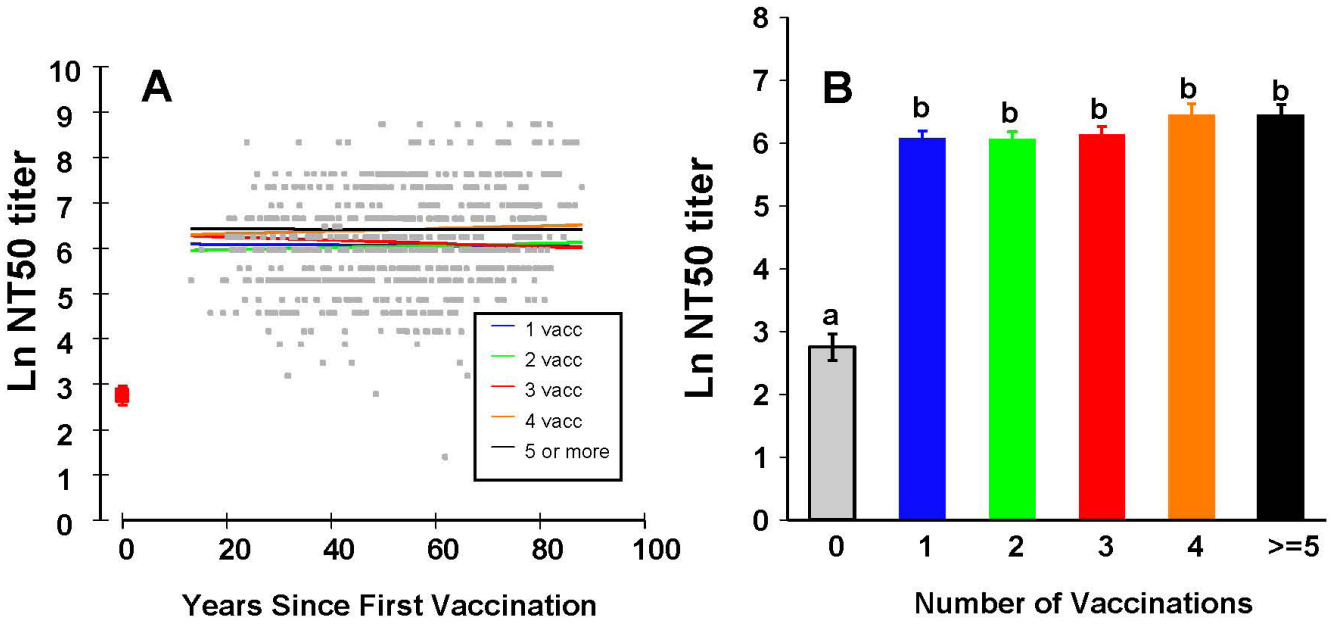

Figure 2.

Neutralizing antivaccinia titers in participants vaccinated one or more times over an 88-year period. (A) Effect of time on neutralizing antivaccinia titers for each group of singly or multiply vaccinated individuals using the banked serum of Baltimore Longitudinal Study of Aging subjects. Serum banked over a 45-year time period on Baltimore Longitudinal Study of Aging study participants was examined using a vaccinia-specific neutralization assay to determine the antibody titers in subjects vaccinated one or more times between over individual follow-up times between 13 and 88 years. For the majority of participants, several time points were examined to assess how titers may actually change over time. All serum assay results are represented by individual dots. The lines indicate the repeated measures linear-effects model projection by number of vaccines. The bar (± SEM) at time zero represents the mean titer of the 29 non-vaccinated subjects. (B) Neutralizing antivaccinia titers were compared with the total number of vaccinations received. The serum samples obtained from unvaccinated volunteers (n = 29) demonstrated titers <1:16 (Ln 2.77), with the majority of samples reproducibly measuring below 1:8 (Ln 2.08). Bars show the average ± SE of antibody production by each group. Bars with different superscripts (a, b) are significantly different; $P < .05$

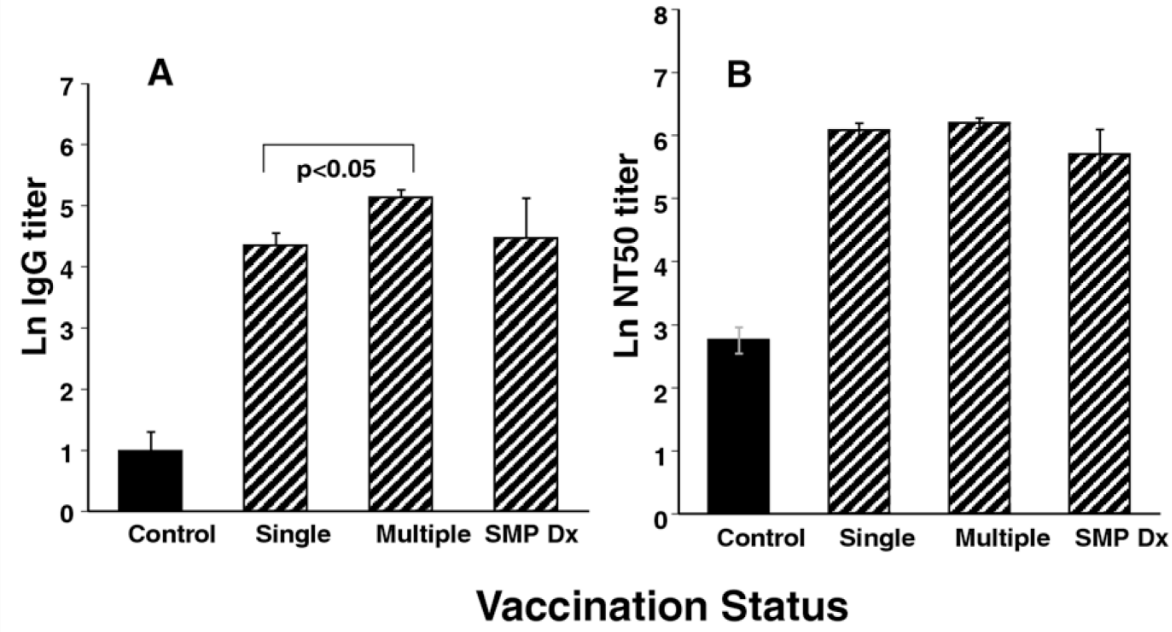

Figure 3.

Comparison of the natural log of vaccinia-specific IgG and neutralization titers among participants with single vaccination, multiple vaccinations, and previous smallpox diagnosis. Bars show the average ± SE of antibody production by each group. Vaccinia-specific IgG (A) and neutralization (B) titers in participants with previous smallpox diagnosis were not significantly different from titers in participants with single or multiple vaccinations. However, similar to Figure 1, the vaccinia-specific IgG responses (A) were significantly ($P > .05$) different between singly versus multiply vaccinated Baltimore Longitudinal Study of Aging subjects.

Table

Cohorts of Vaccinated and Control Subjects Examined in the Current Study*

Number of Vaccinations	Controls	Years Since Last Vaccination†			Totals
		≤40 years	41-50 years	51-60+ years	
0	29	—	—	—	29
1	—	12	21	29	62
2	—	37	12	10	59
3 or more		79	6	3	88
Smallpox‡	—	—	—	—	8
Total§	29	128	39	42	246

*Participants were selected based on their medical histories, docu-mented vaccination status, and the intervals since last vaccination.

†Many of these subjects (209) have a number of serum samples banked at 1-5 year intervals over extended time periods. Samples were selected so that vaccinia-specific IgG and neutralization antibody responses could be examined before and at 5-10 year intervals after their last vaccination. Control non-vaccinated subjects (29) also were recruited for these studies.

‡Eight participants in the BLSA study were diagnosed with smallpox in their youth. Smaples from these subjects also were examined to assess the presence of vaccinia-reactive antibody.

§In all, over 800 samples were screened and titered for vaccinia-specific IgG and neutralizing antibody levels.