Vaccination Success Rate and Reaction Profile With Diluted and Undiluted Smallpox Vaccine: A Randomized Controlled Trial

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In 2002, smallpox vaccination resumed in the United States, using stockpiled vaccine prepared in the early 1970s.1 Historically during the years of routine vaccination, 2 types of vaccinia preparations were used in the United States: a lyophilized (powdered) form and a frozen preparation, both derived from the New York Board of Health vaccinia strain. Approximately 15 million doses of the lyophilized product (Dryvax, Wyeth-Ayerst, Marietta, Pa) had been maintained within the US national stockpile in the event that widespread vaccination of the population was needed. Recent studies have demonstrated that the lyophilized vaccine dilute 10-fold still retained high vaccination take rates. Although this expanded the vaccine stockpile,2 it still was short of the stated Department of Health and Human Services goal of having a vaccine dose for every US citizen. Until newer vaccines are available, the need exists for additional vaccine doses for the United States and other countries.

Context Additional smallpox vaccine doses are needed to augment current US national stockpile. Aventis Pasteur smallpox vaccine (APSV), initially manufactured in the 1950s from the New York Board of Health vaccinia strain in a frozen preparation, appears as effective as lyophilized vaccine but the effectiveness of diluted doses of APSV is unclear.

Objective To compare the vaccination success rate and the reaction profile of various APSV dilutions.

Design, Setting, and Participants A double-blind, randomized controlled trial of 340 healthy vaccinia-naive adults aged 18 to 32 years from 3 academic medical centers who were vaccinated with 1 of 3 strengths of APSV dilutions (undiluted, 1:5, and 1:10) between October 9, 2002, and February 24, 2003. Volunteers were followed up every 3 to 5 days until the vaccination site healed for bandage changes, vaccine response assessment, and adverse event evaluation, followed by 1- and 2-month clinic evaluations and 6-month telephone interview.

Main Outcome Measures Successful vaccination, defined by presence of a vesicle or pustule at the inoculation site 6 to 11 days postvaccination, and local and systemic reactions to vaccination.

Results A total of 340 volunteers were vaccinated (vaccine dose: undiluted, n=113; 1:5 dilution, n=114; and 1:10 dilution, n=113). Following vaccination, 99.4% (95% confidence interval [CI], 97.9%-99.9%) of all volunteers had successful vaccinations. Success rates did not differ between the dilution groups (undiluted, 100.0%; 95% CI, 96.8%-100.0%; 1:5 dilution, 98.2%; 95% CI, 93.8%-99.8%; 1:10 dilution, 100.0% 95% CI, 96.8%-100.0%; P=.33). Overall, 99.7% of volunteers reported at least 1 local symptom at the vaccination site, and 61.8% had axillary lymphadenopathy, 15.0% developed satellite lesions, and 7.6% developed a rash away from the vaccination site. Fever developed in 21.5%. No differences were noted in local or systemic reactions between the 3 dilution groups (P>.05 for each comparison). A total of 25% of volunteers missed scheduled duties due to vaccine-related symptoms.

Conclusions Even at diluted doses, APSV is an effective smallpox vaccine, allowing for expansion of the current stockpile. However, reactogenicity was not reduced with dilution of the vaccine and, as with other smallpox vaccines, may impair daily activities.

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In fall 2001, Aventis Pasteur (Swiftwater, Pa) reported that multiple lots of Aventis Pasteur smallpox vaccine (APSV) had been in frozen storage for decades. An earlier head-to-head study comparing the lyophilized vaccine and APSV noted that the frozen vaccine retained potency and had similar vaccination success as the lyophilized vaccine (K.M.E., unpublished data, 2004). We conducted this study to further assess the vaccination success, safety, and reactogenicity of APSV at various dilutions in vaccinia-naïve individuals in a large multicenter clinical trial.

METHODS
Study Design
This double-blind, randomized controlled trial was conducted at 3 sites: Vanderbilt University School of Medicine, Nashville, Tenn; Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio; and University of Iowa, Iowa City. Volunteers were enrolled from October 9, 2002, to February 24, 2003. Approval for the trial was granted by the institutional review boards of each participating organization. Written informed consent was obtained from all volunteers. Healthy adults aged 18 to 32 years with no prior history of smallpox vaccination and the absence of a vaccination scar were eligible for enrollment. In addition, volunteers were excluded if they had a history of any of the following conditions: autoimmune disease, human immunodeficiency virus infection, solid organ or bone marrow transplantation, malignancy, eczema, prior vaccination with any vaccinia-vectored or other pox-vectored experimental vaccine, or allergies to the vaccine components. Volunteers with a history of or current illegal injection drug use, current exfoliative skin disorders, a history of immunosuppressive conditions noted above, ongoing pregnancy, or children younger than 1 year were also prohibited from study participation. All volunteers underwent a comprehensive screening history, physical examination, and laboratory evaluation, including serologic testing for hepatitis B surface antigen and antibodies to human immunodeficiency virus and hepatitis C virus. Those volunteers with abnormal screening test results were excluded. All women were required to have a negative urine pregnancy test result within 48 hours of vaccination.

Vaccine Specifics
The APSV used in this study (lot 2243) was initially derived from the New York Board of Health vaccinia strain and manufactured between 1956 and 1957. Approximately 85 million doses had been reserved for the Department of Defense and maintained at −20°C since manufacture. Potency determinations of the lot revealed chorionic allantoic membrane titer of 107.6 plaque forming units per milliliter, maintained following 4 freeze-thaw cycles (data on file at Aventis Pasteur).

Vaccination Methods
Eligible volunteers were randomized using an in-house, custom-built program with fixed blocks of size 6 to receive 1 of 3 strengths of APSV dilutions (undiluted, 1:5, and 1:10). Each vial of frozen APSV was thawed to room temperature immediately before first use. Vaccine was diluted with sterile water diluent containing 50% glycerin and 0.25% phenol (Chesapeake Biological Laboratories, Baltimore, Md) and was administered to the deltoid area via scarification by 15 punctures with a bifurcated needle. The site was covered with 2 occlusive bandages, as described previously. Study staff and volunteers were blinded to vaccine-dose assignment.

Follow-up Assessments
Demographic data and information on race were recorded for each volunteer. Race was determined by volunteer self-declaration and was collected to assess for potential racial differences in vaccine success and reactogenicity. Volunteers were observed every 3 to 5 days for scheduled bandage changes, vaccine response assessment, and adverse event evaluation. Vaccination success was measured by the development of a vaccination site take, defined as the presence of a vesicle or pustule at the inoculation site 6 to 11 days postvaccination (FIGURE 1A). At each follow-up visit, study staff inspected and measured the vaccination lesion, the surrounding erythema and induration, and any regional lymphadenopathy. Volunteers were questioned at each follow-up visit for the presence of any vaccine-related adverse events and instructed to note via symptom diary daily oral temperatures and the presence and severity of various local (site pain and pruritus, axillary pain, and swelling) and systemic (malaise, fatigue, chills, myalgias, nausea, headache, joint pain, anorexia) symptoms for at least 2 weeks after vaccination until resolution of all symptoms. Fever was defined as oral temperature of at least 37.8°C. Daily absence from work or school due to adverse events after vaccination was recorded. As this study occurred before the identification of cardiac adverse events in the civilian and military populations, specific screening for signs and symptoms of cardiac disease were not actively solicited at each follow-up visit.

Volunteers were trained on bandage removal and application in the event an unscheduled bandage change was needed. Sterile gloves and bandages were provided if the bandage required changing. Careful hand washing was stressed. There were no restrictions on work activities following vaccination, per Centers for Disease Control and Prevention recommendations. An assessment of vaccinia shedding from the inoculation site, outside of the site dressing, and the vaccinee’s hands was conducted in volunteers at the Vanderbilt University School of Medicine site and has been published elsewhere. Follow-up visits occurred until the vacci-
nation site was considered healed by study staff. Additional 1-month and 2-month clinic visits and a 6-month telephone interview were conducted to evaluate for any delayed adverse events. In addition, volunteers were encouraged at the 6-month telephone interview to report to the study staff any concerning symptoms should they occur in the future; if such symptoms were considered clinically significant, volunteers would then be evaluated in the clinic. An independent data and safety monitor at each site promptly reviewed all adverse events to ensure volunteer safety.

**Serum Antibody Response**

Specimens to examine the postvaccination serologic responses were collected from each volunteer at baseline before vaccination and at 28 and 56 days postvaccination. Due to the high volume of samples collected from this trial and from several other concurrently conducted smallpox vaccine studies, serum antibody data for all volunteers in this trial are not yet available. Serum neutralizing antibody data, however, were assessed on a subset of volunteers from the Vanderbilt University School of Medicine site who were invited to participate in a site-specific substudy, investigating various detailed aspects of the immune response, including cell-mediated immunity and cytokine responses postvaccination. Serum antibody responses were measured by plaque reduction neutralization assay as described previously. Serial 4-fold dilutions of serum collected at baseline and 1 month following vaccination were incubated at 37°C for 15 hours with vaccinia virus diluted to contain 50 to 70 plaque forming units of virus. Triplicate virus-serum mixtures at each dilution were then plated onto confluent BSC-40 (African green monkey kidney) cell monolayer cultures, incubated at room temperature for 1 hour, and overlaid with media containing 2% fetal bovine serum. Following a 48-hour incubation, the monolayer was fixed with 10% formalin and stained with crystal violet. Results were expressed as reciprocal 60% neutralization titers using a standard linear regression curve. Seroconversion was defined as a 5-fold or more increase in titers from baseline. Negative and both low-titered and high-titered serum positive controls were run with each assay to determine assay acceptance. The study investigators decided that providing the humoral immunity data from these volunteers at this time, although they represent a subset of all study participants, would provide useful information on reactogenicity and vaccine success.

**Statistical Analysis**

Based on an estimated 90% vaccine take frequency in each dilution group, a take frequency 95% confidence interval (CI) half-width of 5%, and a potential 5% loss to follow-up, the study sample size was calculated at 148 volunteers per study group (N=444). On February 24, 2003, the US Food and Drug Administration temporarily suspended all vaccinia trials due to adverse events noted in the ongoing civilian and military vaccination campaigns; therefore, the study was terminated. At that time, 340 volunteers had been vaccinated and evaluated for clinical take status. At the time of suspension, only 2 volunteers had not developed a take and no volunteers were lost to follow-up, providing a minimum possible take rate in any of the dilution groups of 98%, surpassing the confidence interval half-width criterion of 5%. Thus, with the higher-than-expected take rates and lower-than-expected percentage of volunteers lost to follow-up, the sample size of 340 provided power similar to the original calculations. Because the objectives of the trial had been met, the study investigators and the US National Institute of Allergy and Infectious Diseases project leaders chose to terminate study enrollment.

Vaccination success rates and safety measurements were compared between dilution groups. The uncorrected $\chi^2$ test was used to compare reactogenicity rates. Symptom severity and extent of induration and erythema were compared using the analysis of variance. Take rates were evaluated using Fisher exact test and exact methods were used to determine 95% CIs. Comparisons of the mean neutralizing antibody titers at 1 month after vaccination between the dilution groups were performed using the Kruskal-Wallis test and, when a significant difference was detected by this test, Mann-Whitney U test was performed between the individual dilution groups. SAS statistical software version 8.2 (SAS Institute Inc, Cary, NC) was used for all
analyses; \( P<.05 \) was considered statistically significant.

**RESULTS**

**Baseline Characteristics**

A total of 340 volunteers were vaccinated before study closure (n=148 at Vanderbilt University School of Medicine, n=150 at University of Iowa, and n=42 at Cincinnati Children’s Hospital Medical Center) (Figure 2). Mean (SD) age of the cohort was 24 (3) years, 42.2% were men, and the cohort was 94.4% white. Significant differences in volunteer age, sex, and racial characteristics were not detected between the 3 dilution groups (data not shown). The specific dilution of vaccine received was evenly distributed among volunteers (undiluted, n=113; 1:5 dilution, n=114; and 1:10 dilution, n=113). Volunteers had a mean (range) of 7.6 (6-11) follow-up visits with a mean (range) follow-up time of 55 (44-102) days. All volunteers completed follow-up.

**Vaccination Success**

Following vaccination, overall 99.4% (95% CI, 97.9%-99.9%) of volunteers developed a clinical take (Table 1). Success rates did not differ between the different dilution groups \( (P=.33) \). Mean vaccination lesion size was 16 mm and median (range) peak size was 15 (5-30) mm; lesion size peaked at 11 (3-15) days postvaccination. Ranges of lesion sizes are listed in Table 2. Measurements did not differ across all dilution groups \( (P=.30 \text{ and } P=.47, \text{ respectively}) \). The 2 volunteers without a clinical take each developed a detectable papule at the vaccination site that did not progress to a vesicle or pustule.

**Reactogenicity**

Local induration and erythema of the vaccination site occurred in all volunteers, including the 2 who did not develop a clinical take, and reached their peaks on postvaccination day 10 (erythema: range, 4-15 days; induration: range, 5-15 days). Women had significantly less local erythema (mean, 46 vs 58 mm; \( P=.02 \)) and induration (mean, 38 vs 44 mm; \( P=.02 \)) than men did. Axillary lymphadenopathy was detected in 61.8% of volunteers after vaccination. Differences were not detected in the maximum erythema, maximum induration, time to maximum erythema, time to maximum induration, and frequency of axillary lymphadenopathy between the 3 dilution groups \( (P>.05 \text{ for each comparison}) \).

Satellite lesions surrounding the vaccination site were noted in 51 volunteers (15.0%) and did not differ between dilution groups (Table 2 and Figure 1B). At the Vanderbilt University School of Medicine site, suspected cases of satellite lesions were cultured for vaccinia and 8 of 16 lesions tested positive. Rashes away from the vaccination site were noted in 26 volunteers (7.6%), including postvaccinia folliculitis, as described previously, in 15 volunteers. The incidence of these rashes did not differ between dilution groups. One volunteer noted migratory arthralgias, fever, and nodular skin lesions 2 months after vaccination; a complete rheumatologic evaluation and serologic panel were negative. This condition resolved with anti-inflammatory therapy. No episodes of autoinoculation were detected.

### Table 1. Success of Initial Vaccination With Various Dilutions of APSV in Vaccinia-Naive Adults

<table>
<thead>
<tr>
<th>Group</th>
<th>Total No. of Volunteers</th>
<th>No. of Volunteers</th>
<th>% (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>113</td>
<td>113</td>
<td>100.0 (96.8-100.0)</td>
</tr>
<tr>
<td>1:5 Dilution</td>
<td>114</td>
<td>112</td>
<td>98.2 (93.8-99.8)</td>
</tr>
<tr>
<td>1:10 Dilution</td>
<td>113</td>
<td>113</td>
<td>100.0 (96.8-100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>340</td>
<td>338</td>
<td>99.4 (97.9-99.9)</td>
</tr>
</tbody>
</table>

Abbreviation: APSV, Aventis Pasteur smallpox vaccine.

*Success was defined by vesicle or pustule formation at the initial inoculation site 6 to 11 days after vaccination.
Volunteer-reported symptoms during the 2 weeks after vaccination are shown in Table 3. Overall, 99.7% of volunteers reported at least 1 symptom localized to the vaccination site; 92.1% reported local site pain, 96.8% described localized pruritus, and 87.1% noted axillary pain. No significant differences in these local symptoms between the various dilution groups were noted. In the 2 weeks postvaccination, volunteers also noted a variety of systemic symptoms, including fever (21.5%; mean peak, 38.5°C) and malaise (79.1%). In general, symptoms occurred with greatest frequency between 6 to 11 days after vaccination. Due to vaccine-related symptoms, 25% of volunteers missed scheduled activities at work or school. A difference in systemic symptoms, including headache, fatigue, site pruritus, and axillary pain, was noted axillary pain. Physical examination was without evidence of a pericardial rub and lung fields were clear. The volunteer took ibuprofen as needed for all systemic symptoms, which resolved 6 days later.

A fourth volunteer noted periodic episodes of chest tightness beginning on the fifth postvaccination day that lasted for several minutes at a time. This volunteer also had numerous symptoms of systemic inflammatory response to the vaccine, such as headache, fatigue, axillary pain, and nausea. Physical examination did not reveal any signs of pericarditis or ischemic heart disease. Acetaminophen was taken as needed and these symptoms resolved after 8 days.

### Reports of Chest Pain, Pressure, or Dyspnea

Although symptoms of cardiac disease were not actively elicited in volunteers in this study, a post hoc analysis of volunteer symptom reports revealed 4 volunteers who reported chest pain or tightness and 1 additional volunteer who reported exercise-associated dyspnea and tachycardia in the 2 weeks after vaccination.

The first volunteer with chest pain noted 2 episodes of exercise-associated stabbing chest pain 6 days after vaccination that lasted for approximately 20 minutes. These episodes resolved without specific therapy and were not reported by the volunteer until the following day. Examination of the volunteer at that time revealed mild tachycardia (pulse of 100) but no evidence of a cardiac rub or gallop, an enlarged axillary lymph node, as well as a normal pulmonary, extremity, and vascular examination.

A second volunteer presented with anterior chest and axillary pain 10 days after vaccination. This was not associated with dyspnea, diaphoresis, or radiation and lasted a few hours. This volunteer had also noted multiple systemic symptoms compatible with a vigorous vaccine response, including headache, fatigue, site pruritus, and axillary pain. Full physical examination, including auscultation of the heart and lungs and extremity assessment, revealed only an enlarged axillary lymph node. These symptoms resolved with rest.

A third volunteer noted chest pain starting 5 days after vaccination along with other signs of systemic reactogenicity, including headache, fatigue, and axillary pain. Physical examination was without evidence of a pericardial rub and lung fields were clear. The volunteer took ibuprofen as needed for all systemic symptoms, which resolved 6 days later.

A fourth volunteer noted periodic episodes of chest tightness beginning on the fifth postvaccination day that lasted for several minutes at a time. This volunteer also had numerous symptoms of systemic inflammatory response to the vaccine, such as headache, fatigue, axillary pain, and nausea. Physical examination did not reveal any signs of pericarditis or ischemic heart disease. Acetaminophen was taken as needed and these symptoms resolved after 8 days.

### Table 2. Reactogenicity of Various Dilutions of APSV in Vaccinia-Naive Adults*

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Total (N = 340)</th>
<th>Undiluted (n = 113)</th>
<th>1:5 Dilution (n = 114)</th>
<th>1:10 Dilution (n = 113)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vaccination lesion size, mm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>16 (4)</td>
<td>15 (4)</td>
<td>16 (3)</td>
<td>15 (4)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>15 (5-30)</td>
<td>15 (8-30)</td>
<td>16 (7-26)</td>
<td>15 (5-27)</td>
</tr>
<tr>
<td>Peak days postvaccination, median (range)</td>
<td>11 (3-15)</td>
<td>11 (3-15)</td>
<td>11 (3-15)</td>
<td>11 (3-15)</td>
</tr>
<tr>
<td><strong>Diameter of surrounding erythema, median (range), mm</strong></td>
<td>39 (10-210)</td>
<td>40 (10-210)</td>
<td>36 (12-172)</td>
<td>38 (10-210)</td>
</tr>
<tr>
<td><strong>Diameter of surrounding induration, median (range), mm</strong></td>
<td>35 (7-175)</td>
<td>35 (10-175)</td>
<td>36 (10-175)</td>
<td>33 (7-118)</td>
</tr>
<tr>
<td><strong>Axillary lymphadenopathy, No. (%)</strong></td>
<td>210 (61.8)</td>
<td>69 (61.1)</td>
<td>75 (65.8)</td>
<td>66 (58.4)</td>
</tr>
<tr>
<td><strong>Satellite lesion development, No. (%)</strong></td>
<td>51 (15.0)</td>
<td>21 (18.6)</td>
<td>16 (14.0)</td>
<td>14 (12.4)</td>
</tr>
<tr>
<td><strong>Development of rash away from vaccination site, No. (%)</strong></td>
<td>26 (7.6)</td>
<td>9 (8.0)</td>
<td>7 (6.1)</td>
<td>10 (8.8)</td>
</tr>
</tbody>
</table>

*Abbreviation: APSV, Aventis Pasteur smallpox vaccine.

**There were no significant differences in these findings between dilution groups.

### Table 3. Frequency of Self-reported Local and Systemic Symptoms During the 2 Weeks Following Vaccination With Various Dilutions of APSV in Vaccinia-Naive Adults

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Total (N = 340)</th>
<th>Undiluted (n = 113)</th>
<th>1:5 Dilution (n = 114)</th>
<th>1:10 Dilution (n = 113)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Any symptom</strong></td>
<td>339 (99.7)</td>
<td>113 (100)</td>
<td>113 (99.1)</td>
<td>113 (100)</td>
</tr>
<tr>
<td><strong>Any local symptom</strong></td>
<td>113 (99.7)</td>
<td>113 (100)</td>
<td>113 (99.1)</td>
<td>113 (100)</td>
</tr>
<tr>
<td><strong>Vaccination site pain</strong></td>
<td>313 (92.1)</td>
<td>104 (92.0)</td>
<td>104 (91.2)</td>
<td>105 (92.9)</td>
</tr>
<tr>
<td><strong>Vaccination site pruritus</strong></td>
<td>329 (96.8)</td>
<td>110 (97.3)</td>
<td>108 (94.7)</td>
<td>111 (98.2)</td>
</tr>
<tr>
<td><strong>Axillary pain</strong></td>
<td>296 (87.1)</td>
<td>98 (86.7)</td>
<td>96 (84.2)</td>
<td>102 (90.3)</td>
</tr>
<tr>
<td><strong>Any systemic symptom</strong></td>
<td>323 (95.0)</td>
<td>110 (97.3)</td>
<td>105 (92.1)</td>
<td>108 (95.6)</td>
</tr>
<tr>
<td><strong>Fever</strong></td>
<td>73 (21.5)</td>
<td>24 (21.2)</td>
<td>29 (25.4)</td>
<td>20 (17.7)</td>
</tr>
<tr>
<td><strong>Myalgias</strong></td>
<td>266 (78.2)</td>
<td>88 (77.9)</td>
<td>90 (78.9)</td>
<td>88 (77.9)</td>
</tr>
<tr>
<td><strong>Chills</strong></td>
<td>164 (48.2)</td>
<td>53 (46.9)</td>
<td>61 (53.5)</td>
<td>50 (44.2)</td>
</tr>
<tr>
<td><strong>Headache</strong></td>
<td>255 (75.0)</td>
<td>93 (82.3)</td>
<td>83 (72.8)</td>
<td>79 (69.9)</td>
</tr>
<tr>
<td><strong>Nausea</strong></td>
<td>128 (37.6)</td>
<td>37 (32.7)</td>
<td>45 (39.5)</td>
<td>46 (40.7)</td>
</tr>
<tr>
<td><strong>Fatigue</strong></td>
<td>269 (79.1)</td>
<td>94 (83.2)</td>
<td>84 (73.7)</td>
<td>91 (80.5)</td>
</tr>
<tr>
<td><strong>Missed activities due to any symptom</strong></td>
<td>85 (25.0)</td>
<td>23 (20.4)</td>
<td>38 (33.3)*</td>
<td>24 (21.2)</td>
</tr>
</tbody>
</table>

*Abbreviation: APSV, Aventis Pasteur smallpox vaccine.

**Comparison with other dilution groups, P = .04. All other symptoms did not differ between dilution groups.

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Finally, 8 days after vaccination, 1 volunteer noted a transient episode of dyspnea and tachycardia during exercise that lasted for several minutes and resolved with rest. Examination after symptom resolution revealed mild tachycardia (pulse of 101), axillary lymphadenopathy, and normal cardiac, pulmonary, and vascular examination results.

Because of the low index of suspicion for myopericarditis at the time, further cardiac evaluation, including electrocardiogram and echocardiogram, was not conducted. Each of the preceding volunteers was contacted at the 6-month telephone follow-up. All symptoms resolved without residual sequelae and had not recurred by 6 months postvaccination.

Serious Adverse Events

One study volunteer required hospitalization 1 week after vaccination for nausea and dehydration that resolved completely within 24 hours after intravenous hydration. A second volunteer presented with orthostatic dizziness 10 days after vaccination that rapidly cleared with oral hydration. Right facial nerve palsy developed in 1 volunteer 37 days after vaccination, which fully resolved with corticosteroid treatment. Another volunteer with a history of ulcerative colitis diagnosed 10 years before vaccination presented with a colitis flare 2 months after vaccination. Reinstitution of immunosuppressive therapy with mesalamine was associated with resolution of symptoms.

Pregnancies Detected Following Vaccination

Despite a negative urine pregnancy test on the day of vaccination, verbal confirmation of a normal menstrual period the month before vaccination, and continued counseling by study staff to avoid pregnancy, 1 volunteer had a positive pregnancy test noted 26 days after vaccination. Her estimated date of conception was 16 days before vaccination. The volunteer was counseled on the risks of fetal vaccinia and opted to continue with the pregnancy. The volunteer moved from the area before delivery and was lost to follow-up, despite numerous attempts to locate her. Two other volunteers also became pregnant at the end of the 2-month postvaccination follow-up (estimated dates of conception, postvaccination days 38 and 56, respectively). One person delivered a healthy neonate at term, while the second volunteer terminated her pregnancy during the first trimester.

Humoral Immune Response

Of the 148 volunteers enrolled at Vanderbilt University School of Medicine site, informed consent and successful serum collection at each time was obtained from 109 volunteers for the immune response substudy measuring serum neutralizing responses (undiluted, 34; 1:5 dilution, 36; 1:10 dilution, 39), all of whom developed evidence of a clinical take (Figure 3). There were no significant differences between mean age, race, ethnicity, or sex among those who consented for the substudy and those who did not consent. Before vaccination, all volunteers had baseline reciprocal antibody titers of less than 40; 1 month after vaccination all, except 1 volunteer who received 1:10 dilution of vaccine, developed a neutralizing response. At 1 month after vaccination, volunteers receiving the 1:5 dilution of vaccine had significantly higher neutralizing titers than volunteers administered the 1:10 dilution (P = .007). However, significant differences in neutralizing antibody responses between the undiluted and 1:5 or 1:10 dilution groups were not detected.

COMMENT

Although manufactured nearly 50 years ago, APSV is associated with high vaccination success rates, even at a 1:10 dilution. Therefore, the existing supply of approximately 85 million doses of APSV can be expanded, leaving an ample stockpile of smallpox vaccine to protect the entire US population in the event widespread vaccination is imminently needed. With adequate supplies of vaccine for the population of the United States, the potential exists for sharing additional supplies with other countries as well. Reactogenicity to the vaccine was also similar between the different dilutions of vaccine.

The ability to dilute vaccinia vaccines without a resultant decline in success rates has also been reported with other vaccine preparations. Investigators recently discovered that various dilutions of lyophilized vaccine had similar vaccination success rates as undiluted doses, with more than 97% of vaccinees, who received a 1:10 vaccine dilution, successfully developing a clinical take after initial vaccination. In contrast with the current study, a previous study with lyophilized vaccine reported that local reactogenicity was significantly reduced with 1:5 and 1:10 dilutions. Those individuals vaccinated with undiluted lyophilized vaccine had significantly larger areas of surrounding erythema and induration and higher incidence of regional lymphadenopathy when compared with those receiving 1:5 and 1:10 vaccine dilutions. Interestingly, those volunteers vaccinated with the diluted lyophilized vaccine doses had a higher inci-
The clinical success rate of APSV in our study was mirrored by the humoral immune response to vaccination observed in a subsample of the study. All volunteers except 1 exhibited a 5-fold or more increase in plaque reduction neutralization titers 1 month after vaccination, regardless of dilution of vaccine received. These results are similar to immune responses reported after dilutions of lyophilized vaccine. However, in contrast with our study in which antibody titers were significantly lower in those volunteers who received the 1:10 dilution compared with those vaccinated with the 1:5 dilution, Belshe et al noted significantly higher antibody titers between volunteers receiving a 1:10 dilution of vaccine and volunteers receiving undiluted vaccine. The clinical significance of the difference in titers between the 1:10 and the 1:5 dilution groups is unclear as all volunteers in both groups developed a clinical take.

Clinical symptoms were common after APSV vaccination, as with other vaccinia preparations, with all but 1 volunteer exhibiting local symptoms. Systemic symptoms after vaccination were also quite common, as a majority of volunteers reported malaise, headache, and fatigue. Seventy-three volunteers (21.5%) developed fevers. This degree of reactogenicity, observed with other vaccinia preparations, is not unexpected, as all smallpox vaccines are live viral vaccines. Vaccine inoculation introduces live vaccinia virus intradermally and leads to a localized infection. Many of the postvaccination symptoms result from the vigorous inflammatory and immune response after vaccination. However, reactogenicity to APSV appears greater than what was observed in a recent trial of vaccinia-naive volunteers vaccinated with the lyophilized vaccine. Mean lesion size was larger in those volunteers vaccinated with APSV (16 mm) than those vaccinated with lyophilized vaccine (12.4 mm). More volunteers in this study also developed fever (21.5% vs 8.9%) and had detectable lymphadenopathy and satellite lesions than volunteers vaccinated with the lyophilized vaccine. Nonetheless, although both vaccines are derived from similar vaccinia stock, such comparisons must be performed with caution, as our study did not directly compare APSV with other vaccinia vaccines.

In our study, reactogenicity to the vaccine was not reduced with decreasing strengths of vaccine. This may be explained by the fact that once inoculation succeeds in creating a nidus of vaccinia infection and subsequent host immune response, the resultant local and systemic reactions occur regardless of the initial quantity of virus inoculated. The impact of the postvaccination symptoms are not trivial, as 25% of vaccinees missed regularly scheduled activities due to vaccine-related symptoms. Fortunately, these symptoms were generally short-lived, with most volunteers returning to full function within 1 to 2 days. Although symptoms of cardiac disease were not actively sought from volunteers at follow-up, 5 volunteers reported cardiac symptoms 5 to 14 days postvaccination that resolved without sequelae. Retrospective analysis of these symptoms and physical examination conducted at the time showed no evidence of moderate or severe myopericarditis or cardiac ischemia. The finding that local reactogenicity was significantly lower in women is also unexpected. Although improved immune responses have been observed in women following some vaccinations, studies of other vaccines, such as the adsorbed anthrax vaccine, have noted increased reactogenicity in women compared with men. Therefore, potential sex differences in vaccine response require further investigation.

Limitations

Our investigation has a few limitations. As the study occurred before the increased awareness of postsmallpox vaccination cardiac complications, we did not prospectively elucidate the occurrence of such adverse events following vaccination, and all descriptions of symptoms potentially cardiac were analyzed retrospectively. Nonetheless, volunteers in our study were observed in person every 3 to 5 days for the first month after vaccination. At each visit, volunteers were actively assessed for systemic reactions to the vaccine and were reminded at each study visit to report any visits to other clinicians. Thus, it is unlikely that volunteers with clinically moderate to severe myopericarditis or cardiac ischemia would have been missed during the postvaccination assessments. Another limitation of our study is that, due to the early study closure, the smaller sample size may have limited our ability to detect subtle differences in reactivity between the dilution groups. However, given the excellent compliance of the study participants, comprehensive evaluation of the study population was achieved.

Conclusion

The future of smallpox vaccination in the United States and other countries is unclear. From experiences in the 1960s and 1970s, smallpox vaccination is an effective tool to prevent smallpox in persons before as well as after exposure. Through its use and the concerted efforts of many individuals, wild smallpox virus was eradicated from the globe. More recently, the US military vaccination campaigns in 2003 successfully vaccinated more than half a million persons, but the unique adverse events and expected inflammatory reactions to the vaccine resulted in concern about the use of smallpox vaccine in its current form. The civilian campaign, likely impacted by these concerns, concluded in late 2003 after having vaccinated more than 39,000 persons, a total well below initial targets. Newer vaccine candidates, such as those derived from cell-culture grown vaccinia virus, more attenuated vaccinia virus strains, and component vaccines, are under development.

The results of our study show that a frozen preparation of APSV has a high
VACCINATION SUCCESS RATE WITH SMALLPOX VACCINE

vaccination success rate and is an available option for smallpox vaccination of vaccinia-naive persons, even at 10-fold diluted doses. This allows for amplification of the current smallpox vaccine stockpile, if needed.

Author Contributions: Dr Edwards had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Stapleton, Brady, Crowe, Edwards. Acquisition of data: Talbot, Stapleton, Brady, Winokur, Bernstein, Yoder, Rock, Crowe, Edwards. Analysis and interpretation of data: Talbot, Stapleton, Brady, Germainson, Rock, Crowe, Edwards. Drafting of the manuscript: Talbot, Rock, Crowe, Edwards. Critical revision of the manuscript for important intellectual content: Talbot, Stapleton, Brady, Winokur, Bernstein, Germainson, Yoder, Rock, Crowe, Edwards. Statistical analysis: Germainson, Rock. Obtained funding: Stapleton, Brady, Bernstein, Crowe, Edwards. Administrative, technical, or material support: Stapleton, Bernstein, Yoder, Crowe. Study supervision: Talbot, Stapleton, Brady, Bernstein, Crowe, Edwards.

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REFERENCES

CORRECTIONS

Incorrect Wording: In the Original Contribution entitled “Vaccination Success Rate and Reaction Profile With Diluted and Undiluted Smallpox Vaccine: A Randomized Controlled Trial” published in the September 8, 2004, issue of THE JOURNAL (2004;292:1205-1212), there was incorrect wording in a sentence. On page 1206, under Vaccine Specifics, the third sentence should read “...pock forming units per milliliter...” instead of “...plaque forming units per milliliter...”

Financial Disclosure Omitted: In the Preliminary Communication entitled “Repetitive Bilateral Arm Training and Motor Cortex Activation in Chronic Stroke” published in the October 20, 2004, issue of JAMA (2004;292:1853-1861), a financial disclosure was omitted. Drs McCombe-Waller and Whitall are named as inventors on a patent application for the bilateral arm trainer. The patent will be held by the University of Maryland but not by the authors.