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CLINICAL RESPONSES TO UNDILUTED AND DILUTED SMALLPOX VACCINE

SHARON E. FREY, M.D., ROBERT B. COUCH, M.D., CAROL O. TACKET, M.D., JOHN J. TREANOR, M.D., MARK WOLFF, PH.D.,
FRANCES K. NEWMAN, M.S., ROBERT L. ATMAR, M.D., ROBERT EDELMAN, M.D., CARRIE M. NOLAN, R.N., M.S.,
AND ROBERT B. BELSHE, M.D., FOR THE NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
SMALLPOX VACCINE STUDY GROUP

ABSTRACT

Background To evaluate the potential to increase the supply of smallpox vaccine (vaccinia virus), we compared the response to vaccination with $10^{8.1}$, $10^{7.2}$, and $10^{7.0}$ plaque-forming units (pfu) of vaccinia virus per milliliter.

Methods In this randomized, single-blind, prospective study, 680 adults who had not been previously immunized were inoculated intradermally with undiluted vaccine (mean titer, $10^{8.1}$ pfu per milliliter), a 1:5 dilution, or a 1:10 dilution of vaccinia virus with use of a bifurcated needle, and the site was covered with a semipermeable dressing. Subjects were monitored for vesicle formation (an indicator of the success of vaccination) and adverse events for 56 days after immunization.

Results Success rates did not differ significantly among the groups and ranged from 97.1 to 99.1 percent after the first vaccination. Both the undiluted and diluted vaccines were reactogenic. In addition to the formation of pustules, common adverse events included the formation of satellite lesions, regional lymphadenopathy, fever, headache, nausea, muscle aches, fatigue, and chills consistent with the presence of an acute viral illness. Generalized and localized rashes, including two cases of erythema multiforme, were also observed.

Conclusions When given by a bifurcated needle, vaccinia virus vaccine can be diluted to a titer as low as $10^{7.0}$ pfu per milliliter (approximately 10,000 pfu per dose) and induce local viral replication and vesicle formation in more than 97 percent of persons. (N Engl J Med 2002;346:1265-74.)

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DEVELOPING public health strategies to counter bioterrorism threats from smallpox is a high national priority.¹ In the event of an emergency, there is an insufficient supply of smallpox vaccine (vaccinia virus) available to vaccinate all U.S. residents with the recommended quantity of virus. Thus, it is important to determine whether current stocks could be diluted, administered with a traditional bifurcated needle, and still result in a high incidence of viral replication in the skin with vesicle formation.

On the basis of the results of a pilot dose-ranging study reported elsewhere in this issue of the *Journal*,² we sought to determine with greater precision (within 5 percent) the rate of success of inoculation with dilutions expected to have a moderate-to-high success rate: undiluted vaccine (geometric mean titer, $10^{8.1}$ plaque-forming units [pfu] per milliliter), a dilution of 1:5 (geometric mean titer, $10^{7.2}$ pfu per milliliter), and a dilution of 1:10 (geometric mean titer, $10^{7.0}$ pfu per milliliter). Given the reactogenicity consequent to viral replication in previously unexposed persons, we also sought to determine the frequency and range of adverse events associated with the administration of the vaccine with a bifurcated needle at these dilutions.

From the Department of Medicine, National Institute of Allergy and Infectious Diseases Vaccine and Treatment Evaluation Unit, Saint Louis University School of Medicine, St. Louis (S.E.F., F.K.N., R.B.B.); the Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston (R.B.C., R.L.A.); the Center for Vaccine Development, University of Maryland School of Medicine, Baltimore (C.O.T., R.E.); the University of Rochester School of Medicine and Dentistry, Rochester, N.Y. (J.J.T., C.M.N.); and the Emmes Corporation, Rockville, Md. (M.W.). Address reprint requests to Dr. Belshe at the Division of Infectious Diseases and Immunology, Saint Louis University Health Sciences Center, 3635 Vista Ave. (FDT-8N), St. Louis, MO 63110.

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METHODS

Vaccine and Diluent

The smallpox vaccine (Dryvax, Wyeth Laboratories, Marietta, Pa.) used in this study was produced in 1982 or earlier (lot no. 4008284) and was provided by the Centers for Disease Control and Prevention in Atlanta. The vaccine lot and diluent differed from the preparations used in the pilot study, and the vaccine contained a larger quantity of virus ($10^{8.1}$ vs. $10^{7.8}$ pfu per milliliter).² Lyophilized vaccine was reconstituted with a new formulation of diluent (lot no. 1468-1A, Chesapeake Biological Laboratories, Baltimore) consisting of 50 percent glycerin and 0.21 percent phenol in sterile water. Unlike the original vaccine diluent, this formulation did not contain brilliant green dye.

The vaccine was prepared at each site by adding 0.25 ml, 1.25 ml, or 2.5 ml of diluent directly to the vials, which corresponded to undiluted vaccine, a 1:5 dilution, and a 1:10 dilution, respectively. Vaccine was used for up to seven days after reconstitution and stored at 2 to 8°C between uses in accordance with the results of analyses of the stability of vaccinia virus. An aliquot from each dilution of vaccine used at each site was collected at the time of preparation and frozen for subsequent evaluations of the viral titer. Titers of every vaccine preparation were determined by plaque assay in the central laboratory (Saint Louis University, St. Louis).³ The quantity of virus on three representative bifurcated needles was also measured by plaque assay.

Study Design and Subjects

The study was a randomized, single-blind trial conducted at the National Institute of Allergy and Infectious Diseases Vaccine and Treatment Evaluation units in St. Louis, Baltimore, and Rochester, N.Y., and at the Respiratory Pathogens Research Unit in Houston. The study was approved by the institutional review board of each participating facility. Subjects were enrolled from November 2 to November 28, 2001, after providing written informed consent.

Healthy adults 18 to 32 years of age were eligible if they had no vaccination scar, no history of vaccinia virus vaccination, and no antibodies against human immunodeficiency virus. In the wake of the September 11, 2001, terrorist attacks, public announcement of the study resulted in a vigorous public response among young adult volunteers. Many were students at the participating trial sites. Exclusion criteria included the contraindications against vaccination noted in the package insert (pregnancy, immunosuppression, and eczema), a history of vaccination with any type of live attenuated virus within 60 days before the study, the receipt of blood products or immune globulin within 6 months before the study, and household contact, sexual contact, or occupational exposure to pregnant women, immunosuppressed persons, persons with eczema, or infants less than 12 months of age.

A total of 680 subjects were randomly assigned to receive undiluted vaccine, a 1:5 dilution of vaccine, or a 1:10 dilution of vaccine. The subjects were inoculated with a bifurcated needle that held a drop of vaccine and that was pressed 15 times into the skin of the upper arm. The vaccination sites were covered with folded gauze and a semipermeable adhesive membrane to avoid autoinoculation or exposure of personal contacts to vaccinia virus (Fig. 1).² Dressings were changed and the vaccination sites were assessed every three to five days until the lesions dried and an eschar formed. Subjects were evaluated for adverse events for 56 days after immunization.

The primary end point was the rate of success of vaccination. Success was defined by the presence of a primary vesicle at the inoculation site seven to nine days after scarification, as described previously.⁴ To increase the rate of success of vaccination, subjects who had no evidence of vesicle formation by days 7 through 9 were revaccinated between days 7 and 17 with the same dilution of vaccinia virus.



Figure 1. Semipermeable Adhesive Dressing.

This dressing was used on all subjects; gauze covered the lesion. The bandages, which protect the subjects and their contacts from further inoculation with vaccinia virus, were changed every three to five days.

Neutralizing Antibody and Vaccine-Stability Assays

Serum samples were collected before vaccination (day 0) from all subjects. Serum neutralizing antibody titers were determined in prevaccination samples from all 15 subjects in whom the initial vaccination failed, as well as in 28 subjects in whom vaccination was successful. The end point was a 60 percent reduction in the number of plaque-forming units, as described previously.³ In addition, plaque assay was used to determine the stability of the vaccine reconstituted with the new diluent after storage at 2 to 8°C.⁵

Statistical Analysis

The goal of the study was to establish precise estimates of the rates of response to a 1:5 dilution and a 1:10 dilution and secondarily to test the hypothesis of the noninferiority of these dilutions as compared with undiluted vaccine. Noninferiority was defined by an alpha level of 0.05 and a difference in success rates (the rate for undiluted vaccine minus the rate for diluted vaccine) of no more than 5 percent. The sample size was based on the results of the pilot study.² We estimated that 684 subjects would need to be enrolled — 107 in the group given undiluted vaccine, 241 in the group given a 1:5 dilution, and 336 in the group given a 1:10 dilution. We used exact methods to calculate the 95 percent confidence intervals for the success rates in each group and for the differences in these rates among the groups.⁶ We used a one-sided t-test to calculate the confidence intervals for the difference in rates, in keeping with the nature of the study as an evaluation of noninferiority.

The groups were compared with the use of analysis of variance or the Kruskal–Wallis test⁷ for continuous outcomes. Categorical outcomes were compared with the use of exact or asymptotic contingency-table tests and logistic regression. Unless otherwise noted, longitudinal outcomes, such as the reactogenicity of the vaccine, reflect the worst possible (most severe) outcome, rather than the best possible outcome. The statistical significance of these results was confirmed with the use of generalized estimating equations. According to the study protocol, the vaccination had to be evaluated at least once on day 7, 8, or 9 and again on day 13, 14,

or 15 and could be evaluated at other follow-up visits at the discretion of the evaluators. In addition, one clinical center evaluated all vaccination sites on day 10, 11, or 12. To allow for the possibility that a small number of subjects had been exposed to vaccinia virus before the study, we analyzed the data according to the intention-to-treat principle.

No subjects were lost to follow-up. One subject who had no response to the initial vaccination was not revaccinated because of logistic constraints.

RESULTS

Characteristics of the Subjects

A total of 680 subjects were enrolled: 106 received undiluted vaccine, 234 received a 1:5 dilution, and 340 received a 1:10 dilution. Five hundred eighty-four subjects were white (85.9 percent), 39 were black (5.7 percent), 26 were Asian (3.8 percent), 16 were Hispanic (2.4 percent), 3 were Native American (0.4 percent), 1 was a Pacific Islander (0.1 percent), and the racial or ethnic background of 11 subjects (1.6 percent) was unknown. There were 346 women (50.9 percent), and the mean (\pm SD) age of the subjects was 24.8 ± 3.7 years. There were no significant differences ($P=0.51$ by Pearson's exact chi-square test⁶) in these characteristics among the groups.

Titers and Stability of Vaccine

The geometric mean titers of vaccinia virus were $10^{8.1}$ pfu per milliliter in the case of undiluted vaccine (range, $10^{7.8}$ to $10^{8.4}$), $10^{7.2}$ pfu per milliliter in the case of the 1:5 dilution (range, $10^{6.9}$ to $10^{7.5}$), and $10^{6.8}$ pfu per milliliter in the case of the 1:10 dilution (range, $10^{6.0}$ to $10^{7.2}$). At 1 site two of the three vaccine preparations of the 1:10 dilution had lower titers ($10^{6.0}$ and $10^{6.2}$ pfu per milliliter, as compared with a mean titer of $10^{7.0}$ pfu per milliliter at the 12 other sites). The reason for the lower titers among 2 of 14 preparations of the 1:10 dilution of vaccine is unknown, but vaccination was successful in all the subjects who received these preparations; handling and shipping of the vaccine to the central laboratory may have led to the decrease in titer in these 2 instances. Therefore, throughout this article we use $10^{7.0}$ pfu per milliliter as the mean titer for the 1:10 dilution.

To understand the amount of virus potentially delivered, we measured the number of plaque-forming units of virus on three representative bifurcated needles. The needles with undiluted vaccine had a mean of $10^{5.0}$ pfu (range, $10^{4.8}$ to $10^{5.2}$), the needles with the 1:5 dilution of vaccine had a mean of $10^{4.3}$ pfu (range, $10^{4.1}$ to $10^{4.5}$), and the needles with the 1:10 dilution of vaccine had a mean of $10^{3.9}$ pfu (range, $10^{3.8}$ to $10^{4.1}$).

Assessments of the stability of the vaccinia virus demonstrated that when reconstituted in the new diluent, it remained viable, with consistent titers (within $10^{0.3}$ pfu per milliliter), for seven days at 2 to 8°C. The titers of the undiluted vaccine were $10^{7.8}$, $10^{7.9}$, and $10^{7.4}$ pfu per milliliter on day 0, on day 7, and at four

months, respectively. The titers of the 1:5 dilution were $10^{7.5}$, $10^{7.2}$, and $10^{6.6}$ pfu per milliliter on day 0, on day 7, and at four months, respectively. The titers of the 1:10 dilution were $10^{6.9}$, $10^{6.9}$, and $10^{6.5}$ pfu per milliliter on day 0, on day 7, and at four months, respectively.

Success Rate

The initial vaccination was successful in 665 of the 680 subjects (97.8 percent). There was no significant difference in the rate of vesicle formation over the range of titers tested — $10^{8.1}$, $10^{7.2}$, and $10^{7.0}$ pfu per milliliter (Table 1). When the results of the initial vaccination were combined with those of revaccination, the upper limit of the one-sided 95 percent confidence interval for the difference in values was 0.5 percentage point for the 1:5 dilution and 1.7 percentage points for the 1:10 dilution; thus, the noninferiority of the diluted vaccines as compared with the undiluted vaccine was established. This criterion was also achieved when the analysis included only the results of the initial vaccination (the upper limit of the one-sided confidence interval for the difference in values was 1.8 percentage points for the 1:5 dilution and 4.6 percentage points for the 1:10 dilution). The criterion was essentially met with the use of a more conservative two-sided confidence interval (data not shown), except that the upper limit of the 95 percent confidence interval for the difference in values was 5.1 percentage points for the comparison of a single vaccination of a 1:10 dilution with a single vaccination of undiluted vaccine.

At three of the clinical centers, the first vaccination with each preparation was successful for every subject. All 665 subjects with a response to the first vaccination had a primary response, except 1 subject with an accelerated response⁴ that rapidly resolved after the first vaccination. On further questioning, this subject reported a history of international travel and vaccinia virus vaccination at the age of 16 months. At one center, 15 of 173 subjects (8.7 percent) had no response to the first vaccination. Six of these subjects (40.0 percent) had neutralizing antibodies in serum samples obtained before vaccination, in contrast to 0 of 28 randomly selected subjects in whom vaccination was successful ($P<0.001$ by Fisher's exact test). Fourteen of the 15 subjects were revaccinated with the same titer between 7 and 17 days after the first vaccination. The second vaccination was successful in 7 of the 14 subjects (0 of 3 given undiluted vaccine, 2 of 2 given a 1:5 dilution of vaccine, and 5 of 9 given a 1:10 dilution of vaccine).

Reactogenicity

Local signs and symptoms of vaccinia virus replication among all 665 subjects in whom the initial

vaccination was successful are summarized in Table 2. The diameter of the pustule was maximal on day 13 or 14 (mean, 12.4 mm) (Fig. 2A, 2B, and 2K). Erythema and induration were maximal on day 10, 11, or 12 (mean, 51.4 and 48.1 mm, respectively), then decreased rapidly (Fig. 2A, 2B, 2I, and 2J). However, some subjects had very large areas of redness and swelling. Ten percent had diameters of redness exceeding 10 cm on day 10, 11, or 12, and 50 percent had diameters of redness exceeding 4 cm. Local satellite lesions near the site of inoculation were observed in 5.8 percent of subjects on day 13 or 14 (Fig. 2B and 2C) and were more common among subjects who had received diluted vaccine. Regional lymphadenopathy was common, occurring in 30.5 percent of subjects on day 7, 8, or 9.

The frequency and severity of systemic signs or symptoms of vaccinia virus replication and pain at the vaccination site among all 665 subjects in whom the initial vaccination was successful are summarized in

Table 3. Fever (a temperature of at least 37.7°C [100°F]) occurred in 59 of 665 subjects (8.9 percent) on day 7, 8, or 9. However, a temperature of 38.3°C (101°F) or higher was uncommon, occurring in only 20 of 665 subjects (3.0 percent) on day 7, 8, or 9. Beyond day 14, fever was recorded in only two subjects (0.3 percent). Headaches were common at all times. Severe headache was present in 14 subjects at some time during the first six days and on day 7, 8, or 9 (2.1 percent) and in 17 subjects (2.6 percent) on day 10, 11, or 12. Muscle aches and chills were also common; 137 subjects (20.6 percent) reported moderate or severe muscle aches on day 7, 8, or 9, and 43 subjects (6.5 percent) reported moderate or severe chills during this period. Moderate or severe nausea (incidence, 3.9 percent) and moderate or severe fatigue (incidence, 19.7 percent) were common on day 7, 8, or 9 after vaccination.

Rashes occurred at sites other than the vaccination site in 37 subjects (5.6 percent) on day 7, 8, or

TABLE 1. RATE OF SUCCESS OF INITIAL AND REPEATED VACCINATION WITH VACCINIA VIRUS.*

VACCINE AND TITERT	NO. OF SUBJECTS	INITIAL VACCINATION SUCCESSFUL‡		INITIAL OR SUBSEQUENT VACCINATION SUCCESSFUL	
		NO. OF SUBJECTS	PERCENT (95% CI)§	NO. OF SUBJECTS	PERCENT (95% CI)¶
Undiluted, 10 ^{8.1} pfu/ml	106	103	97.2 (92.0–99.4)	103	97.2 (92.0–99.4)
1:5 Dilution, 10 ^{7.2} pfu/ml	234	232	99.1 (97.0–99.9)	234	100.0 (98.4–100.0)
1:10 Dilution, 10 ^{7.0} pfu/ml	340	330	97.1 (94.7–98.6)	335	98.8 (97.0–99.7)

*Success was defined by vesicle formation seven to nine days after intradermal inoculation with a bifurcated needle. Subjects who had no initial response were revaccinated between days 7 and 17. CI denotes confidence interval.

†The titer in each group is the mean titer of all vials of vaccine prepared for use in the study except for the 1:10 dilutions, for which 2 of 14 preparations were excluded (as explained in the Results section). The mean quantities of vaccinia virus on representative bifurcated needles were 10^{5.0} pfu in the case of undiluted vaccine, 10^{4.3} pfu in the case of the 1:5 dilution, and 10^{3.9} pfu in the case of the 1:10 dilution.

‡The mean age of the 15 subjects with no response to vaccination was significantly older than that of the subjects with a response (27.1 vs. 24.6 years, P=0.03). Six of the 15 had neutralizing antibody against vaccinia virus before the initial vaccination. On further questioning, three seropositive subjects reported a history of international travel before 1985 or military service.

§The value in the group given undiluted vaccine minus the value in the group given the 1:5 dilution was –2.0 percentage points (upper limit of the one-sided 95 percent confidence interval, 1.8 percentage points). Similarly, the value in the group given undiluted vaccine minus the value in the group given the 1:10 dilution was 0.1 percentage point (upper limit of the one-sided 95 percent confidence interval, 4.6 percentage points). The value in the group given undiluted vaccine minus the value in the two groups given diluted vaccine combined was –0.7 percentage point (upper limit of the one-sided 95 percent confidence interval, 2.3 percentage points). When the two groups given diluted vaccine were combined, the difference in values between this group and the group given undiluted vaccine was –0.7 percentage point (upper limit of the one-sided 95 percent confidence interval, 2.3 percentage points).

¶The value in the group given undiluted vaccine minus the value in the group given the 1:5 dilution was –2.8 percentage points (upper limit of the one-sided 95 percent confidence interval, 0.5 percentage point). Similarly, the value in the group given undiluted vaccine minus the value in the group given the 1:10 dilution was –1.7 percentage points (upper limit of the one-sided 95 percent confidence interval, 1.7 percentage points). The value in the group given undiluted vaccine minus the value in the two groups given diluted vaccine combined was –2.1 percentage points (upper limit of the one-sided 95 percent confidence interval, 0.6 percentage point). When the two groups given diluted vaccine were combined, the difference in values between this group and the group given undiluted vaccine was –2.1 percentage points (upper limit of the one-sided 95 percent confidence interval, 0.6 percentage point).

||One of the 340 subjects could not be revaccinated because of logistic constraints.

TABLE 2. LOCAL SIGNS AND SYMPTOMS OF VACCINIA VIRUS REPLICATION AMONG THE 665 SUBJECTS WITH VESICLE FORMATION AFTER THE FIRST VACCINATION.

VARIABLE	DAYS AFTER VACCINATION		
	7-9	10-12*	13-14
Pustule size — mm			
Mean	9.6	11.9	12.4
Range	0-23	4-19	0-28
Diameter of erythema — mm			
Mean	17.6	51.4	17.0
Range	0-100	7-165	0-120
Diameter of induration — mm			
Mean	12.0	48.1	11.8
Range	0-150	7-125	0-120
Local satellite lesions — no. of subjects/total no. (%)†	16/659 (2.4)	5/76 (6.6)	38/656 (5.8)
Regional lymphadenopathy — no. of subjects/total no. (%)†‡			
None	455/655 (69.5)	37/80 (46.2)	530/653 (81.2)
Mild	173/655 (26.4)	39/80 (48.8)	119/653 (18.2)
Moderate	27/655 (4.1)	4/80 (5.0)	4/653 (0.6)
Severe	0/655	0/80	0/653

*The results are reported for all subjects at one center; therefore, the number of subjects is smaller than 665.

†Data were missing for some subjects.

‡Mild lymphadenopathy was easily tolerated; moderate lymphadenopathy was bothersome but did not preclude the performance of routine activities. Severe lymphadenopathy precluded the performance of routine activities.

9 and in 67 subjects (10.1 percent) on day 10, 11, or 12. In all, 95 subjects (14.3 percent) had a rash (Fig. 2D, 2E, 2F, and 2G) at a site other than the vaccination site at some point during follow-up; 18 were described as moderate and 5 as severe. Pustular or vesicular rashes were the most common rash and were most commonly observed on the chest and back. The rashes resolved spontaneously in all subjects. At least two subjects had rashes that were consistent with a diagnosis of erythema multiforme (Fig. 2H). Some subjects had small papules (1 to 2 mm in diameter) on the trunk (Fig. 2D). These papules were not considered to represent disseminated vaccinia, and the lesions resolved in a few days without treatment.

Pain at the vaccination site was common and was moderate or severe in 225 subjects (33.8 percent) on day 7, 8, or 9 and in 202 subjects (30.4 percent) on day 10, 11, or 12. More than one third of the subjects (36.4 percent) were sufficiently ill to miss school, work, or recreational activities or to have trouble sleeping.

The differences in local signs of vaccinia virus replication after vaccination with any of the three titers of vaccine are summarized in Table 4. There was no significant difference in the mean diameter of the pustule on day 13 or 14 among the three groups ($P=0.10$). However, subjects given undiluted vaccine had

significantly larger areas of erythema ($P<0.001$) and induration ($P=0.004$) on day 7, 8, or 9 and a significantly higher incidence of regional lymphadenopathy on day 7, 8, or 9 ($P=0.003$) but a significantly lower incidence of satellite lesions on day 13 or 14 ($P=0.003$).

Twelve subjects had serious adverse events, defined by the need for a visit to the clinic or emergency department or for hospitalization. Seven serious adverse events were classified as unrelated to vaccination. Two were classified as probably not related to vaccination: one case of bronchitis 12 days after vaccination and an episode of syncope 4 days after vaccination. In one subject, a high fever (temperature, 40.1°C [104.2°F]) on day 11 was classified as possibly related to the vaccine. Two serious adverse events were classified as definitely related to vaccination: one subject had a severe headache and nausea beginning five days after vaccination that resolved after day 14, and one subject had a large area of erythema (29 by 6.5 cm) around the vaccination site, which developed eight days after vaccination and had resolved by day 15.

DISCUSSION

Our results suggest that the current stocks of vaccinia virus can be diluted to a titer of approximately $10^{7.0}$ pfu per milliliter and still induce local viral rep-

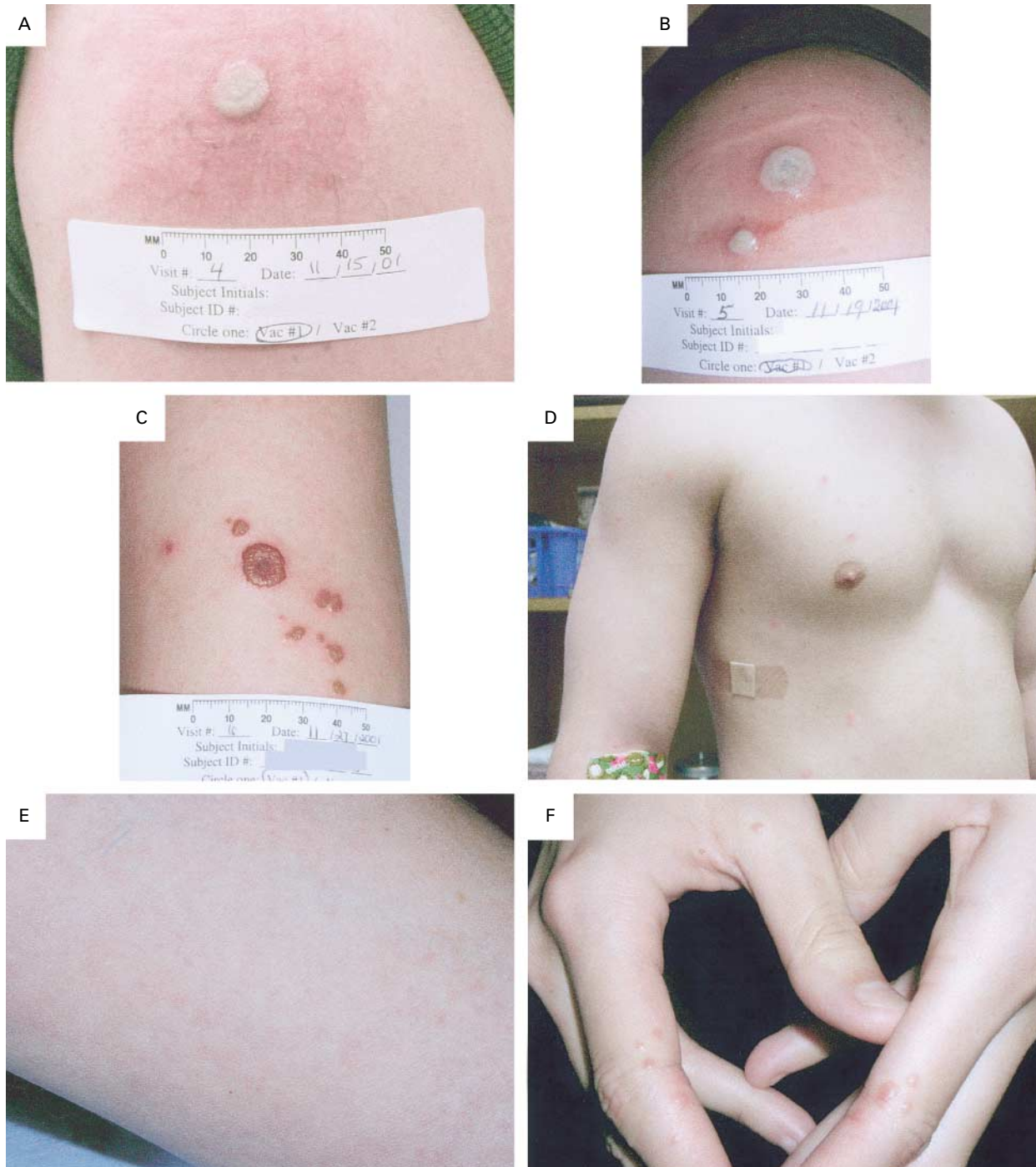
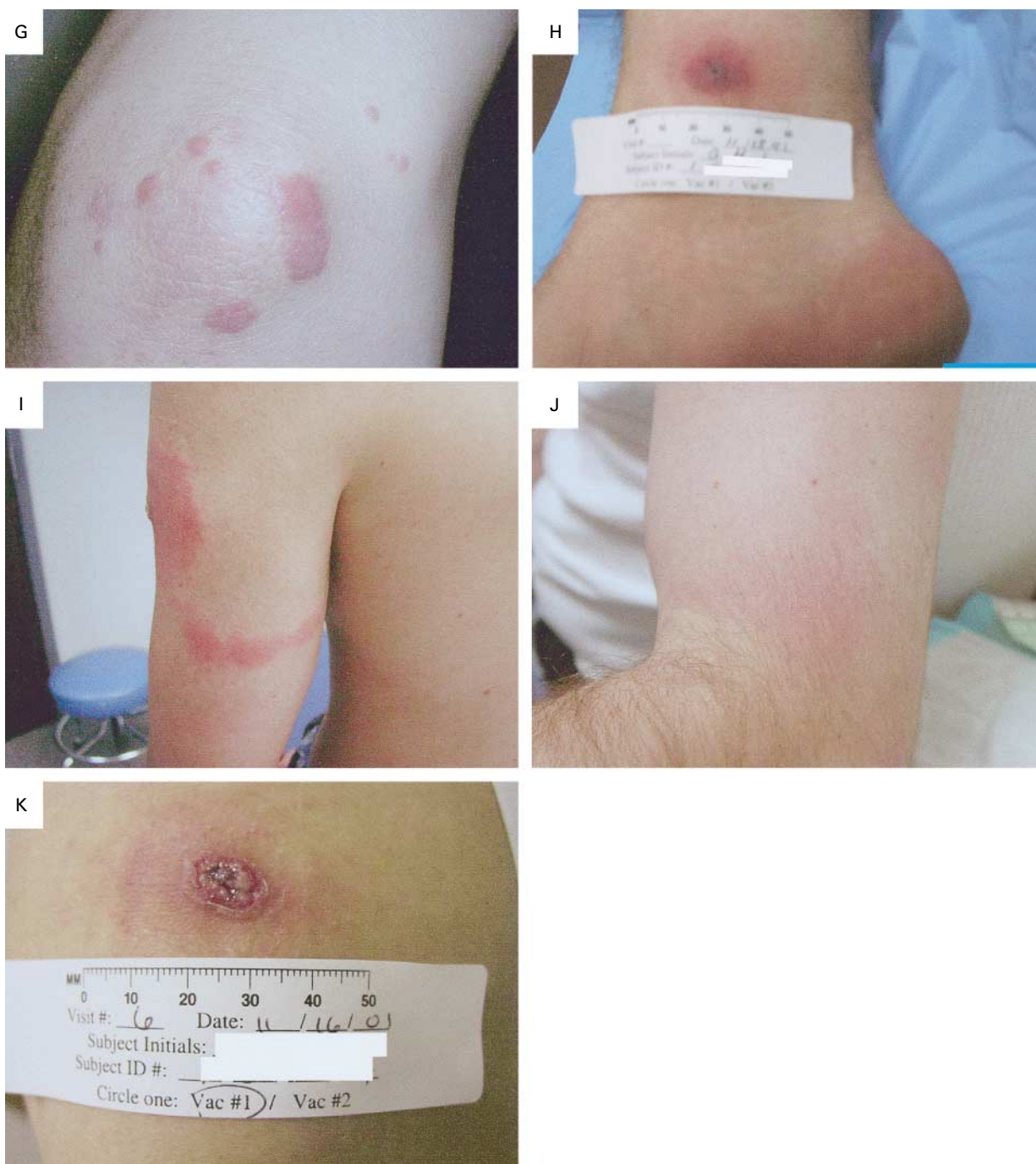


Figure 2. Photographs of Primary Reactions and Common Adverse Events after Vaccination with Vaccinia Virus.

A different subject is shown in every panel except Panels F and G. Panel A shows a typical primary reaction with pustule formation, erythema, and edema on day 7 after vaccination. Panel B shows a primary reaction with a satellite lesion, edema, and erythema on day 11. Panel C shows a primary reaction on day 14; the multiple satellite lesions are beginning to heal. Panel D shows a generalized vesicular rash on day 11. Panel E shows a generalized flat erythematous rash on the leg of a subject on day 13. Panel F shows a localized vesicular rash on the hands and Panel G a localized papular rash on the elbow of one subject on day 18. Panel H shows a rash consistent with a diagnosis of erythema multiforme above the ankle on day 8. Panel I shows extensive erythema and induration with a linear streak posteriorly on day 9, and Panel J shows extensive erythema and induration extending from the pustule to the elbow on day 14. This type of inflammation may be confused with cellulitis and treated with antibiotics; however, these lesions resolved spontaneously in a few days in most subjects. Panel K shows a typical healing lesion on day 14. The eschar will be followed by scar formation within four to eight weeks.



lication and vesicle formation in a high proportion of persons who had never before vaccinated. The lot of vaccine we used in our pilot study had a lower titer ($10^{7.8}$ pfu per milliliter), and a 1:10 dilution yielded a titer of $10^{6.5}$ pfu per milliliter.² The higher rate of response to the 1:10 dilution in the

current study indicated that a titer of approximately $10^{7.0}$ pfu per milliliter is needed to obtain a success rate of 95 percent or more.

Only eight subjects (1.2 percent) did not have a response after two vaccinations. Five of these eight were among the subjects who had preexisting neu-

TABLE 3. FREQUENCY AND SEVERITY OF SYSTEMIC SIGNS AND SYMPTOMS OF VACCINIA VIRUS REPLICATION AND PAIN AMONG ALL THE SUBJECTS WITH VESICLE FORMATION AFTER THE FIRST VACCINATION.*

VARIABLE	DAYS 0-6 (N=665)	DAYS 7-9 (N=665)	DAYS 10-12 (N=665)	DAYS 13-14 (N=665)	DAYS 15 AND BEYOND (N=205)†
	number of subjects (percent)				
Oral temperature‡					
≥37.7°C (100°F)	15 (2.3)	59 (8.9)	35 (5.3)	4 (0.6)	2 (1.0)
≥38.3°C (101°F)	6 (0.9)	20 (3.0)	19 (2.9)	2 (0.3)	0
≥38.8°C (102°F)	2 (0.3)	5 (0.8)	2 (0.3)	0	0
Headache					
None	371 (55.8)	395 (59.4)	412 (62.0)	559 (84.1)	173 (84.4)
Mild	194 (29.2)	178 (26.8)	161 (24.2)	76 (11.4)	21 (10.2)
Moderate	86 (12.9)	78 (11.7)	75 (11.3)	25 (3.8)	8 (3.9)
Severe	14 (2.1)	14 (2.1)	17 (2.6)	5 (0.8)	0
Muscle aches					
None	400 (60.2)	330 (49.6)	381 (57.3)	603 (90.7)	191 (93.2)
Mild	204 (30.7)	198 (29.8)	176 (26.5)	52 (7.8)	10 (4.9)
Moderate	55 (8.3)	120 (18.0)	94 (14.1)	7 (1.1)	1 (0.5)
Severe	6 (0.9)	17 (2.6)	14 (2.1)	3 (0.5)	0
Chills					
None	575 (86.5)	547 (82.3)	562 (84.5)	653 (98.2)	199 (97.1)
Mild	68 (10.2)	75 (11.3)	59 (8.9)	8 (1.2)	2 (1.0)
Moderate	16 (2.4)	31 (4.7)	34 (5.1)	2 (0.3)	0
Severe	6 (0.9)	12 (1.8)	10 (1.5)	2 (0.3)	0
Nausea					
None	560 (84.2)	572 (86.0)	586 (88.1)	640 (96.2)	195 (95.1)
Mild	77 (11.6)	67 (10.1)	52 (7.8)	17 (2.6)	4 (2.0)
Moderate	20 (3.0)	19 (2.9)	21 (3.2)	6 (0.9)	2 (1.0)
Severe	8 (1.2)	7 (1.1)	6 (0.9)	2 (0.3)	1 (0.5)
Fatigue					
None	314 (47.2)	348 (52.3)	380 (57.1)	549 (82.6)	166 (81.0)
Mild	246 (37.0)	186 (28.0)	184 (27.7)	88 (13.2)	32 (15.6)
Moderate	89 (13.4)	114 (17.1)	84 (12.6)	21 (3.2)	6 (2.9)
Severe	16 (2.4)	17 (2.6)	17 (2.6)	7 (1.1)	0
Rash at sites other than vaccination site					
None	643 (96.7)	628 (94.4)	598 (89.9)	631 (94.9)	182 (88.8)
Mild	17 (2.6)	31 (4.7)	51 (7.7)	31 (4.7)	16 (7.8)
Moderate	5 (0.8)	5 (0.8)	12 (1.8)	3 (0.5)	1 (0.5)
Severe	0	1 (0.2)	4 (0.6)	0	2 (1.0)
Pain at vaccination site					
None	301 (45.3)	156 (23.5)	155 (23.3)	488 (73.4)	157 (76.6)
Mild	311 (46.8)	284 (42.7)	307 (46.2)	163 (24.5)	44 (21.5)
Moderate	51 (7.7)	212 (31.9)	181 (27.2)	14 (2.1)	2 (1.0)
Severe	2 (0.3)	13 (2.0)	22 (3.3)	0	0

*There were no significant differences among the groups, with the exception that the group given undiluted vaccine had a higher incidence of muscle aches on days 0 through 6 ($P=0.01$ by the Kruskal-Wallis test) and a higher incidence of local pain on days 10 through 12 ($P=0.0037$ by the Kruskal-Wallis test) than did the other two groups. Mild symptoms were easily tolerated; moderate symptoms were bothersome but did not preclude the performance of routine activities; severe symptoms precluded the performance of routine activities.

†Data were missing for some of the 205 subjects who reported data after those obtained from the 14-day diary card.

‡The temperatures are nested.

tralizing antibodies, suggesting that they had been vaccinated as infants. The use of a 1:10 dilution of vaccine in persons who have never been vaccinated, followed by a second vaccination in those with no response after seven days, could potentially protect nearly 10 times as many persons as would be protected by the administration of undiluted vaccine.

The rate of adverse reactions to vaccinia vaccine is

dependent on age and immune status.^{4,5,8-16} Although there were no life-threatening adverse events, such as encephalitis or progressive vaccinia, in our study, there was a substantial degree of reactogenicity. The high frequency of local pain and erythema at the inoculation site, regional lymphadenopathy, and fever reflect active replication of vaccinia virus, with resultant acute viral illness. For the most part, the symp-

TABLE 4. DIFFERENCES IN LOCAL SIGNS OF VACCINIA VIRUS REPLICATION AFTER VACCINATION IN 665 SUBJECTS WITH VESICLE FORMATION AFTER THE FIRST VACCINATION.

LOCAL SIGN*	UNDILUTED VACCINE (N=103)	1:5 DILUTION (N=232)	1:10 DILUTION (N=330)	P VALUE†
Mean pustule size on days 13–14 — mm	11.7	12.5	12.6	0.10
Mean diameter of erythema on days 7–9 — mm	24.1	17.0	16.1	<0.001
Mean diameter of induration on days 7–9 — mm	16.4	11.8	10.6	0.004
Local satellite lesions on days 13–14 — no. of subjects (%)	0	10 (4.4)	28 (8.6)	0.003
Regional lymphadenopathy on days 7–9 — no. of subjects (%)‡				
None	56 (54.9)	171 (74.7)	228 (70.4)	0.003
Mild	39 (38.2)	47 (20.5)	87 (26.8)	0.003
Moderate	7 (6.9)	11 (4.8)	9 (2.8)	0.003

*Data were missing for some subjects.

†P values were calculated with the use of the Kruskal–Wallis test.

‡Mild lymphadenopathy was easily tolerated; moderate lymphadenopathy was bothersome but did not preclude the performance of routine activities. None of the subjects had severe lymphadenopathy.

toms were mild to moderate. Although these adverse events were not serious, in more than one third of subjects they were the cause of missed work, school, sleep, or recreational activities. Autoinoculation of the eyes or the genitalia may occur in vaccinated children, and vaccinated persons may also inadvertently inoculate unvaccinated persons; we avoided these potentially serious events by applying semipermeable-membrane bandages to the inoculation sites.

There were significant differences in the incidence of local reactions between the group given undiluted vaccine and the groups given diluted vaccine. Undiluted vaccine resulted in larger areas of inflammation and a higher incidence of regional lymphadenopathy, whereas diluted vaccine resulted in a higher incidence of satellite lesions. These two observations may be related; increased inflammation may limit viral replication and therefore reduce the formation of local satellite lesions in persons who receive a high-titer vaccine. These clinical observations are also consistent with the observation in the pilot study that, although all subjects in whom vaccination was successful had cellular immune responses, the responses were more vigorous in the group that received undiluted vaccine. Whether or not the increased local inflammation associated with undiluted vaccine is due to a more rapid rate of replication soon after vaccination or to the inoculation of a minor subpopulation of more virulent vaccinia is not known. The morphology of vaccinia

virus plaques in tissue culture is highly variable, suggesting that a mixed population of virus is present in the vaccine.

Whether or not persons vaccinated more than 30 years previously can be successfully revaccinated with diluted vaccine (e.g., doses as low as $10^{7.0}$ pfu per milliliter) remains to be determined. Our observation that 6 of 14 subjects with no response after the initial vaccination had preexisting neutralizing antibodies suggests that the success rates in this population may be lower. Studies are being designed to determine whether protection is associated with preexisting antibody levels, cellular immunity, or both and whether a significant boost in immunity can occur in the absence of a primary response to vaccination.

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APPENDIX

In addition to the authors, the National Institute of Allergy and Infectious Diseases Smallpox Vaccine Study Group includes Stephen P. Heyse, M.D., M.P.H., Holli Hamilton, M.D., M.P.H., Pamela McInnes, D.D.S., Walla Dempsey, Ph.D., Lydia Falk, Ph.D., and Wendy Fanaroff-Ravick, R.N., M.S.N., all of the National Institute of Allergy and Infectious Diseases, Bethesda, Md.

REFERENCES

1. Henderson DA. The looming threat of bioterrorism. *Science* 1999;283:1279-82.
2. Frey SE, Newman FK, Cruz J, et al. Dose-related effects of smallpox vaccine. *N Engl J Med* 2002;346:1275-80.
3. Katz JB. The effect of the virus-serum incubation period upon vaccinia virus serum neutralization titers. *J Biol Stand* 1987;15:389-92.
4. Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001. *MMWR Morb Mortal Wkly Rep* 2001;50(RR-10):1-25.
5. Graham BS, Belshe RB, Clements ML, et al. Vaccination of vaccinia-naïve adults with human immunodeficiency virus type 1 gp160 recombinant vaccinia virus in a blinded, controlled, randomized clinical trial. *J Infect Dis* 1992;166:244-52.
6. StatXact-3 for Windows: statistical software for exact nonparametric inference. Cambridge, Mass.: CYTEL Software, 1995.
7. Holander M, Wolfe D. Nonparametric statistical methods. New York: John Wiley, 1973:115-6.
8. Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968: results of ten statewide surveys. *J Infect Dis* 1970;122:303-9.
9. Fulginiti VA, Kempe CH, Hathaway WE, et al. Progressive vaccinia in immunologically-deficient individuals. *Birth Defects Orig Artic Ser* 1968;5:129.
10. O'Connell CJ, Karzon DT, Barron AL, Plaut ME, Ali VM. Progressive vaccinia with normal antibodies: a case possibly due to deficient cellular immunity. *Ann Intern Med* 1964;60:282-9.
11. Redfield RR, Wright DC, James WD, Jones TS, Brown C, Burke DS. Disseminated vaccinia in a military recruit with human immunodeficiency virus (HIV) disease. *N Engl J Med* 1987;316:673-6.
12. Freed ER, Duma RJ, Escobar MR. Vaccinia necrosum and its relationship to impaired immunologic responsiveness. *Am J Med* 1972;52:411-20.
13. Feery BJ. Adverse reactions after smallpox vaccination. *Med J Aust* 1977;2:180-3.
14. Connor JD, McIntosh K, Cherry JD, et al. Clinical and serologic study of four smallpox vaccines comparing variations of dose and route of administration: primary subcutaneous vaccination. *J Infect Dis* 1977;135:167-75.
15. Cherry JD, McIntosh K, Connor JD, et al. Clinical and serologic study of four smallpox vaccines comparing variations of dose and route of administration: primary percutaneous vaccination. *J Infect Dis* 1977;135:145-54.
16. Sinclair MC, Lane JM, Donald WJ, Esco LC. Complications of smallpox vaccination in Alabama, 1968. *South Med J* 1972;65:41-4.

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