Clinical and Immunological Response to Attenuated Tissue-Cultured Smallpox Vaccine LC16m8

Tomoya Saito, MD, PhD
Tatsuya Fujii, MD
Yasuhiro Kanatani, MD, PhD
Masayuki Saijo, MD, PhD
Shigeru Morikawa, DVM, PhD
Hiroyuki Yokote, MS
Tsutomu Takeuchi, MD, PhD
Noriyuki Kuwabara, MD, PhD

The threat of smallpox bioterrorism has prompted reconsideration of the need for smallpox vaccination.1-3 Serious adverse events associated with first-generation vaccines such as the New York City Board of Health (Dryvax; Wyeth, Madison, New Jersey), Lister, and Ikeda strains4-8 have raised obstacles to vaccination campaigns in the United States.7-9 Second-generation vaccines such as ACAM2000 (Acambis, Cambridge, Massachusetts) that use a first-generation seed virus but are usually accompanied by a high frequency of adverse events.10 Developing a vaccine that is safer than first-generation vaccines yet highly immunogenic is crucial to constructing a prevention plan in the event of bioterrorist attack.

LC16m8 is a live, attenuated, tissue-cultured, third-generation smallpox vaccine comprising attenuated vaccinia virus strains as well as subunit vaccines made from viral proteins or DNA.11 It is a desirable candidate for routine vaccination because of its low reactogenicity and reasonable safety profile.12 Vaccination can be conveniently accomplished with a single intraepidermal scarification alone, which usually results in a visible major skin reaction (“take”) similar to those resulting from first- and second-generation vaccines.13

LC16m8 was derived from the Lister strain used for the Intensified Smallpox Eradication Programme of the World Health Organization8 by temperature sensitivity and pock size.
through plaque cloning in rabbit kidney cells. Clones were selected as vaccine candidates based on their high immunogenicity and safety properties in animal studies. After a clinical trial enrolling approximately 10,000 children, Japanese health authorities administered LC16m8 vaccine to more than 100,000 infants between 1973 and the beginning of 1976, without serious adverse events. Evaluation of the proportion of takes and presence of neutralizing antibody titer in vaccinia-naive children given LC16m8 vaccine showed a level of immunogenicity comparable with that achieved with the original Lister vaccine. The LC16m8 vaccine has not been tested against smallpox (i.e., variola virus) in the field, but challenge studies using pathogenic orthopoxviruses in animals have been successful. We examined the clinical and immunological responses to LC16m8 vaccine in vaccinia-naive and previously vaccinated adults to assess immunogenicity and safety. We measured clinical take, neutralizing antibody response, and frequency of adverse events as part of an adult vaccination program in the Japanese Self-Defense Forces.

**METHODS**

**Vaccine**

We vaccinated participants with licensed LC16m8 vaccine (lot No. Chiba 02; Kaketsuken, Kumamoto, Japan) containing a suspension of greater than \( 1 \times 10^8 \) pock-forming units (pfu)/mL of the LC16m8 strain.

**Study Design and Participants**

The Self-Defense Forces Central Hospital planned and organized the smallpox vaccination program for select personnel in the Japan Self-Defense Forces. Candidates were participants in the International Peacekeeping Operation activities of the United Nations Disengagement Observer Force. Study participants were enrolled in a vaccination program adhering to a guideline, completing 6 rounds of vaccination from 2002 to 2005. Approximately 350 to 700 healthy adults aged 18 to 55 years were recruited in each round. Individuals providing written informed consent were screened for contraindications using a questionnaire as well as an interview with a physician before vaccination.

Inclusion criteria for vaccination were (1) normal renal and hepatic function as indicated by urine dipstick testing and measurement of serum levels of aspartate aminotransferase, alanine aminotransferase, and alkaline trans- ferase; (2) negative test results for hepatitis B surface antigen, hepatitis C antibodies, and human T-cell lymphotropic virus; (3) no history of human immunodeficiency virus infection; and (4) normal hematology parameters (white blood cell count, red blood cell count, platelet count).

Exclusion criteria were (1) pregnancy; (2) underlying immunosuppression or treatment with immunosuppressive drugs; (3) current eczema or other skin problems; (4) household contact with a person having any of the above; (5) receipt of another live attenuated-virus vaccine within 30 days; and (6) occupational exposure to pregnant women and newborn infants. Individuals with atopic dermatitis were vaccinated if skin lesions were dry and stable.

Participants were inoculated with approximately 4 \( \mu \)L of vaccine suspension \((1 \times 10^8 \) pfu/mL) using a disposable bifurcated needle (JMS Co Ltd, Hiroshima, Japan). Vaccinia-naive individuals (primary vaccinees) received 5 strokes, whereas previously vaccinated individuals (revaccinees) received 10 strokes. We defined vaccination history by visual examination of the scar before vaccination by the physician. Distinction between previous smallpox vaccination and bacille Calmette-Guérin can be made in Japan, because subcutaneous multiple puncture has been used for bacille Calmette-Guérin inoculation and results in a distinctively different scar.

For comparison, vaccinees were divided into 4 age groups (A-D) that most reasonably captured birth cohorts with different histories of previous vaccination (Table 1). In Japan, universal smallpox vaccination programs included 3 doses (birth, age 6 years, and age 12 years). Because of the cessation of routine vaccination in early 1976, participants born after this (group A) were considered primary vaccinees. Based on the routine vaccination schedule before 1976, the birth cohort born from 1970 through 1975 (group B) was vaccinated once with the Lister strain. Birth cohorts from 1964 through 1969 (group C) were vaccinated with the Ikeda strain for the first dose and the Lister strain for the second. Participants born before 1964 (group D) were vaccinated with the Ikeda strain for the first and second doses and with either the Ikeda or Lister strain for the third.

We monitored participants for 30 days postvaccination. Successful vaccination was determined by the appearance of a major skin reaction (vaccine take), defined as a “pustular lesion or an area of definite induration or congestion surrounding a central lesion, which could be a scab or an ulcer,” to 10 to 14 days postvaccination. To precisely assess the size of skin reaction yet avoid excessive reporting burden in a single vaccination round, the diameter of the local skin reaction on day 14 was measured among third-round vaccinees.

**Table 1. Age Group and Previous Vaccination History**

<table>
<thead>
<tr>
<th>Group</th>
<th>Birth Year</th>
<th>First Dose</th>
<th>Second Dose</th>
<th>Third Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1976-1984</td>
<td>Not vaccinated</td>
<td>Not vaccinated</td>
<td>Not vaccinated</td>
</tr>
<tr>
<td>B</td>
<td>1970-1975</td>
<td>Lister</td>
<td>Not vaccinated</td>
<td>Not vaccinated</td>
</tr>
<tr>
<td>C</td>
<td>1964-1969</td>
<td>Ikeda</td>
<td>Lister</td>
<td>Not vaccinated</td>
</tr>
<tr>
<td>D</td>
<td>1953-1963</td>
<td>Ikeda</td>
<td>Ikeda</td>
<td>Ikeda or Lister</td>
</tr>
</tbody>
</table>
Serum samples were collected before and 30 days after vaccination from individuals vaccinated in the second round and were stored at −80°C until testing for neutralizing antibody titers and levels of troponin T.

The protocol was approved by the institutional review board of the Self-Defense Forces Central Hospital (No. 16-004; August 30, 2004).

Analysis of Serum Antibody Response
Neutralizing antibody levels to LC16m8 were assessed by plaque-reduction neutralization testing of serum specimens collected before and 30 days after vaccination. Effective seroconversion or booster response was defined as a 4-fold increase in plaque-reduction neutralization titer at day 30 after vaccination, compared with the titer before vaccination.

We determined the plaque-reduction neutralizing titer to LC16m8 (PRNLC16m8 titer) as follows. Heat-inactivated serum samples (20 minutes at 60°C) were serially diluted 4-fold, mixed with an equal volume of vaccinia LC16m8 virus that had been freshly sonicated, and then diluted to contain approximately 30 to 50 pfu/mL of virus. Serum-virus mixtures were incubated overnight (approximately 15-18 hours) at 35°C with 5% CO2, inoculated onto duplicate RK13 cell monolayers, and incubated 2 additional hours under identical conditions. After adsorption, RK-13 monolayers were overlaid with minimum essential medium supplemented with 5% fetal bovine serum and 0.8% agarose and then returned to the 35°C incubator for 3 days. At the end of the incubation period, monolayers were stained with neutral red, further incubated overnight at 35°C with 5% CO2, and the plaques counted.

We defined the end point titer as the reciprocal of the highest dilution of serum with a mean plaque count of 50% or less plaque reduction. The 50% neutralization titer for each sample was calculated using Minitab Probit statistical analysis (Minitab Inc, State College, Pennsylvania) and converted to the dose required to produce infection in 50% of participants based on the concentration of undiluted product. Titers less than 1:4 were expressed as 2 and those less than 1:8 as 4 in the LC16m8 assay.

Frequency of Adverse Events
Vaccinates were interviewed for general health at the examination for take 10 to 14 days postvaccination. Vaccinates underwent electrocardiography (ECG) 30 days postvaccination. Those reporting symptoms suggestive of severe adverse events within 30 days postvaccination and those who exhibited a serious abnormality on ECG were hospitalized, monitored, and their severe adverse events reported. The incidence of minor adverse events was reviewed retrospectively using postvaccination records from individuals vaccinated during the third and fourth rounds. Serum samples obtained before and 30 days after vaccination were assayed for levels of troponin T, using electrochemiluminescence immunoassay (BML Inc, Tokyo, Japan), to screen for asymptomatic myocarditis.

Statistical Analysis
We measured (1) vaccine take, (2) PRNLC16m8 titer, (3) seroconversion or effective boosting, (4) incidence of a specific adverse event, and (5) post-vaccination day of adverse events. Outcomes 1, 3, and 4 were measured as dichotomous variables, whereas outcomes 2 and 5 were modeled as continuous variables. Outcomes 1, 2, and 3 were compared by age group (ordinal variable) and vaccination history (dichotomous). Dichotomous outcome variables and explanatory variables were compared using the χ² test. Neutralizing antibody titers were compared following logarithmic transformation, because the distribution of titers tended to skew toward the right.

We compared the PRNLC16m8 (or PRNDryvax) titers from different age groups using an independent-sample t test following the F test. We used the Mann-Whitney test for comparison between 2 groups because the postvaccination day of an adverse event is a discrete variable. P = .05 was considered significant; all statistical tests were 2-tailed. All analyses were performed using STATA version 9.2 (StataCorp, College Station, Texas).

Sample Size for Adverse Events
A total of 3221 vaccinates were monitored for severe adverse events; 1239 individuals vaccinated in the third and fourth rounds were more closely monitored for minor adverse events. To ensure the absence of a severe adverse event, believed to occur with a frequency of 2 per 100 000 individuals,24 the study would require monitoring of 124 600 vaccinated individuals for a 95% level of significance. Our study yielded a significance level of 12.6% and an upper confidence limit of 2.5 cases. If the expected frequency were much greater (eg, 1 in 5000), the level of significance to ensure the absence of a severe adverse event in the present study would be increased to 72.4%.

The expected frequency of a common minor adverse event (eg, fever) is 4% to 6%,2 and provided that vaccination with LC16m8 can reduce this frequency by 40% to 60% (ie, reduction of relative risk), the detection of a minor event with statistical power of 80% to 90% suggests that the minimum number of samples would be between 184 and 1021 individuals. This was met by our cohort of patients vaccinated in the third and fourth rounds (n = 1239).

We assessed the representativeness of third- and fourth-round vaccinates compared with those in the other rounds by comparison of demographics (age, sex, vaccination history) and outcome (ie, take).

RESULTS
Vaccine Take
We enrolled 3468 persons in the program from 2002 to 2005, of whom 229 and 18 were not vaccinated owing to nonmedical or medical reasons, respectively (FIGURE 1). Those who did not receive vaccination owing to...
nonmedical reasons accounted for only 6.6% of the total number of participants. Among those who did not receive vaccination owing to medical reasons, 5 had active atopic dermatitis. Four other individuals with a history of atopic dermatitis did receive vaccination because their skin lesions were not active.

In total, 3221 individuals (92.8% of all candidates) were vaccinated, and all underwent follow-up for 10 to 14 days to determine take. Nearly half (1529/3221 [47.5%]) had never been vaccinated, almost all were men (3168/3221 [98.4%]), and all were Asian.

The overall proportion of clinical take was significantly higher in primary vaccinees (1443/1529 [94.4%; 95% confidence interval {CI}, 93.2%-95.9%]) than in revaccinees (1465/1692 [86.6%; 95% CI, 85.0%-88.2%]) (P < .001) (Table 2). The proportion of takes induced by LC16m8 vaccine in primary vaccinees appeared comparable with the proportion induced by past vaccination with Lister strains and appeared slightly smaller than that induced by Dryvax. The proportion of takes did not significantly differ among age groups in primary vaccinees (Table 2). Among revaccinees, the 20- to 29-year-old age group had a significantly lower proportion of takes than the other age groups (P < .001) (Table 2).

### Table 2. Proportion of Vaccine Take by Age and Vaccination Round in the Smallpox Vaccination Program With LC16m8

<table>
<thead>
<tr>
<th>Round</th>
<th>% Take (95% CI)</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;20</td>
<td>20-29</td>
</tr>
<tr>
<td>Primary Vaccinees</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>0/0</td>
<td>106/113</td>
</tr>
<tr>
<td>Second</td>
<td>1/1</td>
<td>105/109</td>
</tr>
<tr>
<td>Third</td>
<td>2/2</td>
<td>223/238</td>
</tr>
<tr>
<td>Fourth</td>
<td>3/3</td>
<td>204/222</td>
</tr>
<tr>
<td>Fifth</td>
<td>1/1</td>
<td>187/196</td>
</tr>
<tr>
<td>Sixth</td>
<td>0/0</td>
<td>236/244</td>
</tr>
<tr>
<td>Total</td>
<td>7/7</td>
<td>1061/1122</td>
</tr>
<tr>
<td>Revaccinees</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>0/0</td>
<td>1/1</td>
</tr>
<tr>
<td>Second</td>
<td>0/0</td>
<td>54/54</td>
</tr>
<tr>
<td>Third</td>
<td>0/0</td>
<td>11/14</td>
</tr>
<tr>
<td>Fourth</td>
<td>0/0</td>
<td>10/10</td>
</tr>
<tr>
<td>Fifth</td>
<td>0/0</td>
<td>4/5</td>
</tr>
<tr>
<td>Sixth</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Total</td>
<td>0/0</td>
<td>80/84</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NA, not available (not calculable).

aBy χ² test.
group experienced a marginally higher proportion of takes compared with older age groups.

**Size of Skin Reaction**
We examined the local skin reaction and measured the diameter of the erythematous area in 665 third-round vaccinates. Among 569 participants with take, 444 (78.0%) local skin reactions were reported. The mean diameter of the erythematous area on day 14 was 12.0 (SD, 7.1) mm (n=192) in primary vaccinees and 7.5 (SD, 5.2 mm) (n=252) in revaccinees (Figure 2), both of which were considerably smaller than the 15.9 mm reported in infants on day 14 after vaccination.14

**Level of Neutralizing Antibodies in Serum**
Antibody testing performed on 200 serum samples from a total of 366 (54.6%) available from second-round vaccinees included 45, 47, 45, and 63 samples from age groups A, B, C, and D, respectively. The age at which revaccinees were sampled, stratified by previous vaccination history, was significantly older compared with nonsampled vaccinees (P=.40 and P < .001 for previously unvaccinated and vaccinated, respectively). We therefore stratified the analyses by age group whenever we analyzed the results of serum samples. Four of 45 individuals (8.9%) in age group A did not show clinical take and were therefore omitted from the analyses of the 4 individuals without take, 1 revealed seroconversion with PRNLC16m8, although all did not seroconvert with PRNDryvax.

We compared PRNLC16m8 titer as well as the percentage of participants who seroconverted or experienced an effective booster response among the primary vaccinees and revaccinees as well as within revaccinees in different age groups (Table 3). Before vaccination, the geometric mean PRNLC16m8 titer in revaccinees (age groups B-D) was significantly higher than that in primary vaccinees (group A) (P < .001); the titer among participants in group D was higher than that in any other age group (B vs D, P = .003; C vs D, P = .04). After vaccination, the geometric mean PRNLC16m8 titer was not significantly different between primary vaccinees and revaccinees (P = .40). Among revaccinees, the postvaccination PRNLC16m8 titer in group B was marginally higher than that in group D (P = .05).

The percentage of primary vaccinees who seroconverted (37/41 [90.2%]) was significantly higher than that of revaccinees (93/155 [60.0%]) (P < .001). Among revaccinees, the percentage who seroconverted was higher in group B than in group D (P = .002). The geometric mean plaque-reduction neutralization titer was significantly higher among those who did not seroconvert (36.6 [95% CI, 28.0-47.8]) than among those who seroconverted (10.7 [95% CI, 8.8-13.1]) (P < .001).

**Adverse Events**
Throughout the vaccination program, no severe adverse event (eg, autoin-
occlusion/contact inoculation, eczemata vaccinatum, progressive vaccinia, generalized vaccinia, encephalitis, and symptomatic myopericarditis) was reported, and there was no need to use vaccinia immune globulin. In the 30 days after vaccination, 4 participants experienced illness thought to be consistent with an adverse event; 2 were possibly severe. One was a 26-year-old male primary vaccinee who experienced rash onset on the third day postvaccination; the rash spread from his extremities to his trunk. The patient was hospitalized 20 days after vaccination. A skin biopsy from the rash was consistent with allergic dermatitis, which did not disprove a causal relationship with vaccination. The second participant was a 29-year-old male primary vaccinee who developed a rash on his trunk 10 days postvaccination and was diagnosed with erythema multiforme. The other 2 participants were suspected of having vaccine-induced minor adverse events; one reported pain from a swollen axillary lymph node, and the other reported groin pain resulting from a bacterial infection.

No abnormal ECG findings or symptomatic heart disease were reported during the vaccination program. Three hundred forty-seven second-round vaccinees from the total 366 (94.8%) underwent assay of serum troponin T levels to assess asymptomatic myopericarditis. The distributions by age and sex in this sample were not significantly different from the whole samples ($P = .22$ and $P = .75$, respectively). Troponin T levels were below the limit of detection (0.01 ng/mL) before and after vaccination in all participants.

Among those vaccinated in the third and fourth rounds, the prevalence of minor adverse events, along with the date of onset for each event, were retrospectively reviewed using postvaccination records. In total, 491 and 575 previously unvaccinated and vaccinated individuals out of the totals of 647 (75.9%) and 592 (97.1%), respectively, were successfully monitored, resulting in a total sample of 1066 (86.0%). Participants in the third and fourth rounds had a sex distribution similar to those in the other rounds ($P = .91$) but were older ($P < .001$). No significant difference was revealed for the proportions of take (stratified by previous vaccination history) between those in the third and fourth rounds and other vaccinees ($P = .09$ for primary vaccinees, $P = .11$ for revaccinees). One hundred forty-eight minor incidents were reported, 96 (65%) of which were swelling of the axillary lymph nodes (Table 4). The frequency of swelling of the axillary lymph nodes was significantly smaller in revaccinees (20/575 [3.5%]) than in primary vaccinees (76/491 [15.5%]) ($P < .001$). The mean postvaccination day of onset of adverse events (swollen lymph nodes and fever) was significantly earlier in revaccinees than in primary vaccinees ($P < .001$) (Table 5).

### Table 4. Prevalence of Adverse Events by Previous Vaccination History With LC16m8 Vaccine

<table>
<thead>
<tr>
<th>Adverse Events (%)</th>
<th>Primary Vaccinees (n = 491)</th>
<th>Revaccinees (n = 575)</th>
<th>Total (N = 1066)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swelling of axillary lymph node</td>
<td>76 (15.5)</td>
<td>20 (3.5)</td>
<td>96 (9.0)</td>
</tr>
<tr>
<td>Low-grade fever (&gt;37.5°C)</td>
<td>13 (2.6)</td>
<td>8 (1.4)</td>
<td>21 (2.0)</td>
</tr>
<tr>
<td>Skin itching/urticaria</td>
<td>4 (0.8)</td>
<td>3 (0.5)</td>
<td>7 (0.7)</td>
</tr>
<tr>
<td>Influenza-like symptom</td>
<td>5 (1.0)</td>
<td>1 (0.2)</td>
<td>6 (0.6)</td>
</tr>
<tr>
<td>Headache</td>
<td>5 (1.0)</td>
<td>0</td>
<td>5 (0.5)</td>
</tr>
<tr>
<td>Myalgia of neck, breast, upper arm</td>
<td>3 (0.6)</td>
<td>1 (0.2)</td>
<td>4 (0.4)</td>
</tr>
<tr>
<td>Swelling of cervical lymph node</td>
<td>2 (0.4)</td>
<td>1 (0.2)</td>
<td>3 (0.3)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>Acute sensorineural deafness</td>
<td>1 (0.2)</td>
<td>0</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>0</td>
<td>1 (0.2)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Swelling around orbital area</td>
<td>0</td>
<td>1 (0.2)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>0</td>
<td>1 (0.2)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Total</td>
<td>110 (22.4)</td>
<td>38 (6.6)</td>
<td>148 (13.9)</td>
</tr>
</tbody>
</table>

### Table 5. Timing and Frequency of Low-Grade Fever (>37.5°C) and Swelling of Axillary Lymph Nodes

<table>
<thead>
<tr>
<th>Event</th>
<th>Total</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>Unknown</th>
<th>Mean</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-grade fever (&gt;37.5°C)</td>
<td>Primary vaccinees</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>5.8</td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Revaccinees</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swelling of axillary lymph node</td>
<td>Primary vaccinees</td>
<td>76</td>
<td>2</td>
<td></td>
<td>6</td>
<td>6</td>
<td>12</td>
<td>12</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>22</td>
<td>5.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Revaccinees</td>
<td>20</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>3.2</td>
<td></td>
</tr>
</tbody>
</table>

### COMMENT

We evaluated the immunogenicity of LC16m8 vaccine, measuring clinical take and neutralizing antibody titer in a large-scale adult vaccination program. The immunogenicity of LC16m8 in vaccinia-naive adults was similar to that observed with the older Lister strains, and there was an adequate booster response in previously vaccinated individuals. Although the total sample size in our study limited our ability to conclusively confirm the absence of severe adverse events, no patient appeared to experience a severe
adverse event. This finding is consistent with the concept that LC16m8 vaccine causes minimal local manifestations and systemic adverse effects.\textsuperscript{14}

**Immunogenicity of LC16m8 Vaccine**

We used vaccine take and the development of neutralizing antibody titer as benchmarks of immunogenicity. The overall proportion of takes with LC16m8 appeared comparable with that seen with Lister strains in the past but, compared with Dryvax, was lower among revaccines, which may be partly attributable to the lower proliferation property in peripheral skin (see eSupplement at http://www.jama.com). The proportion of takes among primary vaccinees did not significantly differ with age, although the numbers in all age categories other than 20 to 29 years were small, limiting statistical power. Appropriate training in vaccination technique may help achieve a higher proportion of takes, because we observed a higher proportion of takes in later vaccination rounds (Table 2). This was presumably a result of improved practice with a bifurcated needle. The improvements in take could also be observed by increasing the number of strokes given per vaccination.\textsuperscript{25}

Another component of the take evaluation was assessment of the diameter of erythema among third-round vaccinees. We found that the diameter was greater among primary vaccinees than among revaccines. Considering that prevaccination plaque-reduction neutralization titer was negatively associated with seroconversion, modified reactions such as smaller skin lesions and a lower proportion of takes among revaccines could have been caused by residual immunity that prevented viral replication. Although third-round vaccinees, among whom the diameter of the local skin reaction was assessed, were older than other vaccinees, the issue of representativeness of third-round vaccinees did not influence the finding of modified reaction among revaccines.

We evaluated the neutralizing antibody response as another measure of immunogenicity.\textsuperscript{16-28} Before vaccination, plaque-reduction neutralization titers were higher in revaccines than in primary vaccinees, suggesting that neutralizing antibody persists in individuals vaccinated more than 30 years ago, as has recently been demonstrated.\textsuperscript{29} Such a finding may inform the duration of vaccine-induced immunity,\textsuperscript{30} which would help determine the interval to revaccinate individuals responding first. Residual neutralizing antibody titers were also higher among individuals in the older age group, despite the time elapsed since their last vaccination. This may be because they were previously revaccinated more frequently than younger individuals, vaccinated with different vaccinia strains having different immunogenicities, or both.

After vaccination, a high percentage of seroconversion (90.2\%) in primary vaccinees with clinical take was noted. Among revaccinees, higher post-vaccination plaque-reduction neutralization titer was observed among those who did not show seroconversion than among those who seroconverted, which is consistent with the literature.\textsuperscript{31} Rather than the time elapsed since the last vaccination, our plaque-reduction neutralization titer suggests that previous vaccination with different vaccinia strains probably determined the plaque-reduction neutralization titer and seroconversion with LC16m8.

One possible limitation of this study involves the representativeness of serum samples taken from second-round vaccinees. The participants in each round were similar in terms of sex, ethnicity (Asian), and occupation (military), but the mean age of the sampled population was significantly younger in the second round than in other rounds ($P = .02$) (Table 2). Considering that previous vaccination history appeared to be a more important factor in determining seroconversion than age and that our analysis was stratified by previous vaccination history, the potential influence of previous vaccination was accounted for in our analyses of serum samples.

Plaque-reduction neutralization titer should be evaluated with caution in previously vaccinated individuals, because the titer and response can vary depending on the strain of virus used in the test. LC16m8 vaccination significantly increased the booster response of the neutralizing antibody more for LC16m8 than for Dryvax in age group B (individuals vaccinated once with the Lister strain). This may be explained by the high booster response to Lister-like epitopes of LC16m8, a response derived from the similarity of the injected LC16m8 to the immune memory resulting from the Lister vaccine. Future studies should investigate differences in the epitopes of antibodies raised by booster shots within individuals with different previous vaccinia strains. This would promote an understanding of cross-protection among orthopox virus species. Testing for plaque-reduction neutralization using variola virus would facilitate more valid and direct evaluation of immunogenicity by vaccinia immunization but is not possible in most laboratories.

**Safety of LC16m8 Vaccine**

We assessed serious and minor adverse reactions through participant report of symptoms suggestive of adverse events. We observed no severe adverse events. This is consistent with past studies in children (with sample size > 100 000),\textsuperscript{14} suggesting that LC16m8 would be safe in a large-scale vaccination program. The attenuated characteristics of LC16m8 and findings from preclinical studies support a higher safety profile compared with other first- and second-generation vaccines. Nevertheless, the small sample size ($N = 3221$) in our study bears consideration. The frequency of a severe adverse event related to vaccination is believed extremely rare (<1-40 per million vaccinations),\textsuperscript{45} such that a sample size of 124 600 individuals would be needed to ensure absence of a severe event with a 95\% level of significance.
Minor adverse events were reviewed from clinical records of participants in the third and fourth rounds. In general, frequencies of minor adverse events were smaller than those obtained with conventional first-generation vaccines. Rather than total sample size, representativeness was better reflected by correctly interpreting minor adverse events; i.e., the mean age of the sampled vaccinees appeared to be significantly higher than that of nonsampled vaccinees. We did not observe a significant difference in the outcome (i.e., take) between sampled and nonsampled vaccinees, and we stratified analyses of minor adverse events by previous vaccination history (which mirrors ages; see Table 1). We therefore believe that our findings of minor adverse events are valid.

One of the major concerns with adult smallpox vaccination has been the myopericarditis observed in the United States program. Inflammatory cardiomyopathy was recognized in adult recipients of Dryvax and the ACAM2000 vaccine in the United States, but we observed no abnormal ECG tracings or symptomatic heart diseases in the present study. As discussed previously, the sample size was limited to sufficiently verify the absence of myopericarditis. In addition, asymptomatic myopericarditis could not be excluded, because the sensitivity of ECG and measurement of troponin T levels 30 days postvaccination is limited.

A more appropriate time to perform ECG and quantify troponin T levels to detect myopericarditis is 7 to 14 days after vaccination. We recognize this issue after starting the vaccination program but could not revise the protocol. Future studies performing ECG and measuring troponin T levels 7 to 14 days postvaccination could confirm our findings and may strengthen the evidence of the safety of LC16m8. Other explanations for the difference between our study and investigations with other vaccines are the younger age of vaccinees compared with those in a previous study as well as the possible influence of the ethnic and genetic backgrounds.

Vaccination of individuals diagnosed with atopic dermatitis is another concern for implementation of widespread vaccination. In our program, individuals with atopic dermatitis were vaccinated if their lesions were stable, and we observed no features suggestive of eczema vaccinatum. A preclinical study using a murine atopic dermatitis model supports the safety of LC16m8 vaccine for immunocompromised individuals as well as for those with atopic dermatitis. The low proliferation rate of LC16m8 in the peripheral skin may make it safer than first- and second-generation vaccines. Given the absence of severe adverse events, further evaluation of the performance of LC16m8 in individuals with atopic dermatitis is warranted.

CONCLUSION

We demonstrated the immunogenicity of LC16m8 vaccine in vaccinia-naive adults by a single vaccination. LC16m8 vaccine also induces a good booster response in previously vaccinated individuals. Our study also offers supportive evidence for the safety of LC16m8 vaccine in adults; LC16m8 vaccine appears to be a viable alternative to first-, second-, and other third-generation vaccines in a smallpox preparedness program.

Author Contributions: Dr Kanatani 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: Kanatani, Yokote, Kuwabara.

Acquisition of data: Fujii, Saijo, Morikawa.

Analysis and interpretation of data: Saito, Takeuchi.

Drafting of the manuscript: Saito, Fujii, Kanatani, Takeuchi.

Critical revision of the manuscript for important intellectual content: Saito, Kanatani, Saijo, Morikawa, Yokote, Kuwabara.

Statistical analysis: Saito.

Obtained funding: Kanatani.

Administrative, technical, or material support: Yokote, Kuwabara.

Study supervision: Kanatani.

All authors commented on early versions of the manuscript and approved the final version.

Financial Disclosures: None reported.

Funding/Support: This study was supported by research funding from the Japan Ministry of Health, Labour, and Welfare within fiscal year 2004-2006.

Role of the Sponsor: The study sponsor had no role in the design and conduct of the study; the collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript.


Additional Contributions: We thank Takuya Matsumura, MD, PhD (Department of Internal Medicine, Self-Defense Forces Hanshin Hospital, Hyogo, Japan), Fumihiro Tomo Nagai, MD, PhD (Department of Internal Medicine, Self-Defense Forces Central Hospital, Tokyo, Japan), for collecting clinical data and Isao Arita, MD (Agency for Cooperation in International Health [emeritus], Kumamoto, Japan), and J. Michael Lane, MD, MPH (Emory University School of Medicine [emeritus], Atlanta, Georgia), for useful comments and critical reading of an early draft of the manuscript. None of these individuals received any extra compensation for their contributions.

REFERENCES


There are no new truths, but only truths that have not been recognized by those who have perceived them without noticing. A truth is something that everyone can be shown to know and to have known, as people say. all along.
—Mary McCarthy (1912-1989)