Immunologic Responses to Vaccinia Vaccines Administered by Different Parenteral Routes

David J. McClain,* Shannon Harrison,* Curtis L. Yeager,* John Cruz, Francis A. Ennis, Paul Gibbs, Michael S. Wright,* Peter L. Summers, James D. Arthur, and Jess A. Graham*

To develop a less reactogenic but equally immunogenic vaccine, this study of 91 human volunteers compared the safety and immunogenic potency of a new, cell culture–derived vaccinia virus vaccine administered intradermally and intramuscularly with the licensed vaccinia vaccine administered by scarification. Cutaneous pox lesions developed in a higher proportion of scarification vaccinees. Scarification and intradermal vaccine recipients who developed cutaneous pox lesions had more local reactions but also achieved significantly higher cell-mediated and neutralizing antibody responses than those who did not develop pox lesions. Although less reactogenic, intradermal or intramuscular administration of vaccinia vaccine without the concomitant development of a cutaneous pox lesion induced lower immune responses.

Vaccinia virus is a member of the Orthopox genus of the poxvirus family and has little virulence for immunocompetent humans. Apart from its critical role in the eradication of endemic smallpox, vaccinia virus has several biologic properties that make it an excellent candidate for introducing foreign genes that prompted the investigation of recombinant vaccinia vaccines [1–3]. However, cellular immunity and antibody responses to vaccinia virus administered by different routes have not been compared in humans. These issues bear importance on how such vaccines will be used in humans and perhaps why immune responses to recombinant vaccinia vaccines have required multiple injections [4–6].

The only vaccinia vaccine currently licensed in the United States was prepared from calf lymph and is stored as a freeze-dried product. Supplies of this licensed vaccine cannot be replaced, as the production method for calf lymph vaccine is archaic and there is no adequate facility in which supplies can be regenerated in compliance with Good Manufacturing Practices. Certain US military units may require smallpox vaccination during future deployments. Apart from military needs, the Centers for Disease Control and Prevention (CDC) has recommended vaccination for persons working in the laboratory with vaccinia or recombinant vaccinia viruses [7]. Therefore, it is important that modern production techniques be applied to the manufacture of a vaccinia virus vaccine to make it safe and effective as a replacement for the current calf lymph smallpox vaccine. Given the attendant risks associated with the traditional method of cutaneous inoculation of vaccinia, we sought to evaluate the clinical and immune responses of alternative routes of inoculation by using a new, cell culture–derived vaccinia vaccine.

The vaccinia vaccines used in the smallpox eradication effort were prepared on a large scale by inoculating the shaved abdomens of calves, sheep, or water buffalo with seed stocks of vaccinia virus, harvesting the infected exudative lymph from the inoculation sites, and bottling the product with phenol and brilliant green as bacteriostatic agents [8, 9]. Partly because of the nature of this production, in which bacterial contamination was expected, the vaccines were administered percutaneously with a bifurcated needle, a process that became known as scarification because of the permanent scar that resulted. Formation of a cutaneous pox lesion that healed with a scar formed an important method for verifying vaccination status during the era of endemic smallpox. This method proved effective and successful when applied by the World Health Organization to a campaign to globally eradicate smallpox [10].

As a consequence of percutaneous inoculation, infectious vaccine virus was present in the local lesion after scarification. Subsequently, there were cases of inadvertent autoinoculation and inoculation of susceptible vaccinee contacts. Additional complications included severe local spread of vaccine virus in persons with chronic skin diseases [11]. Consequently, if it is
possible to do so without loss of immunogenicity of the vaccine, it is desirable to administer vaccinia by a route that does not result in cutaneous lesions containing transmissible virus. Because of these safety concerns, human trials with recombinant vaccinia vaccines have used the injectable route.

We tested a new investigational vaccinia vaccine (BB-IND 4984) produced in cell culture. It was developed for parenteral injection to preclude the potential complication of inadvertent inoculation of virus attendant to scariﬁcation. During preclinical studies, this cell-cultured vaccinia vaccine candidate proved comparable to the Bureau of Biologics (New York Board of Health) reference strain with respect to pock formation on chorioallantoic membranes of embryonated chicken eggs, formation of lesions after adult rabbits were inoculated intradermally (id), and intracerebral and intraperitoneal virulence in adult and suckling outbred mice (unpublished data). A subsequent phase 1 dose-escalation trial evaluated this vaccine in humans given subcutaneous inoculations. However, cutaneous vesicular (pox) lesions developed in an increasing number of volunteers as the subcutaneous dose was increased. Neutralizing antibody and lymphocyte proliferation assays indicated a higher and earlier immune response in vaccinees with cutaneous lesions than in vaccinees who did not develop pox lesions (McClain DJ, unpublished observations). Since the majority of pox lesions occurred in volunteers receiving the highest dose, confounding of pox lesion formation with dose could not be excluded in the statistical analysis. Therefore, we undertook a larger study to examine whether formation of a cutaneous pox lesion was critical for optimal immune responses compared with injection of the cell-cultured vaccinia vaccine.

Methods

Vaccines. The licensed vaccinia (or smallpox) vaccine (Wyeth-Ayerst Laboratories, Philadelphia) is a lyophilized vaccinia virus derived from the New York Board of Health strain [7] and is the only remaining licensed vaccinia vaccine in the United States. The cell-cultured vaccinia vaccine was manufactured according to Good Manufacturing Practices after three successive passages in MRC-5 cells. This vaccine lot was derived from the master seed of the smallpox vaccine previously licensed by Connaught Laboratories (Philadelphia). The Connaught vaccine was one of several licensed vaccinia products used in the United States until the end of smallpox vaccination in the early 1970s.

The cell culture-derived vaccinia vaccine was administered in a dose of 5.1 log_{10} pfu, either as 0.1 mL id or 0.5 mL intramuscularly (im). The licensed vaccinia vaccine was administered by scarification (as directed in the package insert) by dipping a sterile bifurcated needle into the vaccine and then pricking the skin inoculation site three times. The licensed vaccinia vaccine has a virus titer of \( \sim 10^8 \) pfu/mL, and the administration of 1 drop via scarification with a bifurcated needle is estimated to deliver 2.5 \( \mu \)L. Therefore, \( \sim 10^5 \) pfu is delivered by this method. All vaccinations were administered into the deltoid region of the arm.

Subjects. The vaccines were administered in an open-label study to healthy vaccinia-naive volunteers. Volunteers were screened by medical history and physical examination before being enrolled, with specific attention to the presence of a vaccinia vaccination scar or any contraindication to vaccination. Laboratory parameters before enrollment included HIV serologic test, serum chemistries, a complete blood cell count, and a pregnancy test. Subjects were accepted if they were in good health, had no vaccinia scar or history of vaccination, and had no significant abnormalities that indicated an increased risk for vaccinia immunization (i.e., exfoliative skin disease or disorders of cellular immunity). The criteria that excluded a person from participating in the protocol were the same as those recommended by the CDC [7]. Volunteers who lacked a vaccination scar but were subsequently proved to be vaccinia-immune by their baseline serologic test (50% plaque-reduction neutralization titer [PRNT] of \( \geq 1:20 \)) were excluded from statistical analyses.

Vaccinations and study design. Volunteers were randomized into 3 groups, with one-third receiving licensed vaccinia vaccine by scarification, the cell culture–derived vaccinia vaccine id, or the cell-cultured vaccinia vaccine id. In addition, one-half of volunteers vaccinated id were randomly selected to have the inoculation site wiped with alcohol immediately after injection. This procedure was to determine if immune recognition afforded by processing of antigen id could be achieved without the formation of a cutaneous pox lesion. All volunteers vaccinated id underwent alcohol wiping at the injection site after inoculation to minimize the chance of accidental dermal inoculation with the vaccine. All inoculation sites were initially covered with a semipermeable dressing until any pox lesion had scabbed or until day 10 after inoculation (if no pox lesion developed). During the month following vaccination, subjects were seen as outpatients twice a week during the first 2 weeks after vaccination, and then weekly for the next 2 weeks. These outpatient examinations consisted of clinical examinations and laboratory tests to assess adverse reactions, potential complications, and immunogenicity.

Clinical assessments. Volunteers underwent semiweekly assessment for local signs or symptoms around the inoculation site and for potential systemic symptoms related to vaccination. Any local reaction at the inoculation site was measured to determine the diameter of any local erythema and induration and scored for the presence or absence of warmth, tenderness, lymphadenopathy, subcutaneous nodule, or a vesicle (pox) lesion. Systemic reactions were assessed by questioning and examining the volunteers for the presence or absence of warmth, tenderness, lymphadenopathy, arthralgia, loss of appetite, nausea, vomiting, diarrhea, pruritus, or rash. These symptoms were quantitatively scored based upon severity (0 = no symptom; 1 = mild, symptom can be ignored; 2 = moderate, symptom affects activity but is relieved by analgesics; and 3 = severe, symptom cannot be relieved by analgesics). Data were entered into a database for subsequent statistical analysis. The total local or systemic symptom score for a given vaccinee was defined as the sum of all scores for either local or systemic symptoms.

Clinical laboratories. Blood samples were obtained by weekly phlebotomy of volunteers, beginning before vaccination until \( \sim 1 \) month after vaccination. A complete blood cell count with a five-part differential cell count was done using a cell counter (CellDyn 3000; Abbott Laboratories, Abbott Park, IL). Serum samples were analyzed by an Ektachem 700XR (Eastman Kodak, Rochester, NY) for a panel of chemistry measurements (sodium, potassium, ...
chloride, bicarbonate, urea nitrogen, creatinine, glucose, calcium, phosphorus, lactate dehydrogenase, aspartate transaminase, alanine transaminase, γ-glutamyl transferase, alkaline phosphatase, total bilirubin, and creatine phosphokinase). Test value means were calculated for each group on a given day of measurement. The data from each hematologic and serum chemistry test were analyzed using Statistical Analysis System procedure GLM (version 6.10; SAS, Cary, NC) repeated measures analysis of variance (ANOVA). The overall differences between groups over the course of the study period were compared by using the univariate tests of hypothesis for within-subjects effects.

Serologic assays. Serum specimens from days 0, 10, 14, 20, and 27 were frozen for subsequent ELISA and PRNT assay. An ELISA was performed as previously described [12] to assay for antibodies reactive with cell lysate antigens from vaccinia virus–infected cells, with the modification of human sera as the test specimen and goat anti–human IgG as the detector antibody (Kirkegaard & Perry, Gaithersburg, MD). Given a lower limit of detection of the assay at a 1:100 serum dilution, negative titers (i.e., optical density comparable to background) were reported as equal to 1:50.

PRNT, an in vitro test of serum’s ability to neutralize the Wyeth strain of vaccinia virus, was determined using a modification of the method of Earley et al. [13]. Briefly, each coded serum sample was incubated at 56°C for 30 min, then diluted 1:10 in Eagle MEM (EMEM) containing 10% heat-inactivated fetal bovine serum (FBS). A suspension of vaccinia virus, calculated to yield a dose of ~40–100 pfu/0.1 mL, was prepared in Hanks’ balanced salt solution (HBSS) with 40 mM HEPES. Two-fold dilutions of serum samples were then mixed 1:1 with ~40–100 pfu of vaccinia virus suspension and incubated at 37°C for 1 h. After incubation, test samples and controls were inoculated onto monolayers of Vero cells in 12-well cell culture plates. After adsorption for 1 h at 37°C, each monolayer was overlaid with 1 mL of 0.5% agarose (FMC Bioproducts, Rockland, ME) containing HEPES-buffered Eagle basal medium with Earle's salts, and 5% heat-inactivated FBS. After incubation for 2 days at 37°C in a humidified atmosphere of 5% (vol/vol) carbon dioxide (CO2), each monolayer was stained with 1 mL of the agarose overlay containing 0.167 mg/mL neutral red dye. The plates were returned to the incubator for 24–36 h and the plaques were counted. The number of plaques for each test sample was entered into a computer program to determine the PRNT using probit analysis. The end-point titer was the highest serum dilution demonstrating >50% reduction in the number of plaques for the average dose.

Lymphocyte proliferation assays. Proliferative responses of peripheral blood mononuclear cells (PBMC) to live vaccinia virus and heat-inactivated antigen were tested as previously described [14, 15]. Results were expressed as a stimulation index, derived by dividing counts in wells containing antigen by counts in wells without vaccinia antigen or virus.

In situ ELISA viremia assay. Volunteers underwent periodic serum sampling within the first 2 weeks after inoculation, with an aliquot frozen at −70°C until later assay for vaccinia viremia. Serum was assayed for viremia by a modification of an in situ ELISA. Briefly, Vero cells grown in 24- or 96-well plates were inoculated with 0.1 mL or 50 μL, respectively, of a serum sample. After adsorption for 1 h at 35°C, the 24-well plates were re-fed with 1 mL (100 μL for the 96-well plates) of EMEM containing 2% heat-inactivated FBS. Cultures were incubated at 35°C in a humidified atmosphere of 5% CO2 for 4–6 days. Cultures were decanted and fixed with 1 mL (200 μL for the 96-well plates) of 10% formalin at 15 min at room temperature. Plates were incubated with 1 mL (200 μL for the 96-well plates) of HBSS containing 1% bovine serum albumin (BSA) for 30 min. The blocker buffer was removed and 0.5 mL (50 μL for the 96-well plates) of a 1:1000 dilution of vaccinia mouse hyperimmune ascitic fluid (ATCC, Rockville, MD) or normal mouse ascitic fluid was added to the appropriate wells for 1 h at 35°C. After three washes, 0.2 mL (35 μL for the 96-well plates) of a 1:2000 dilution of peroxidase-labeled anti–mouse IgG (Kirkegaard & Perry) was added to all wells for 1 h at 35°C. Plates were then washed five times and incubated 30 min at 35°C with 0.5 mL/well (80 μL/well for the 96-well plates) of ABTS (2,2'-azino-di[3-ethyl-benzthiazoline sulphonate]) substrate (Kirkegaard & Perry). Test samples and positive and negative controls were tested in duplicate. A positive control was prepared using 10-fold dilutions of vaccinia virus in negative antibody serum. Using these “spiked” serum samples, 2 wells were inoculated with each dilution. Two wells were also inoculated with normal (noninfected) human serum as a negative control. Results were read visually or spectrophotometrically at 414 nm. Titers were calculated as TCID50 values according to the method of Reed and Muench [16] or as the highest specimen dilution with an optical density of 0.2 units over that of the negative controls.

Statistical methods. The PRNT and ELISA antibody responses were analyzed by variance with repeated measures followed by multiple comparisons for the study groups using the Tukey-Kramer adjustment for multiplicity [17]. The data from each hematologic and serum chemistry test and lymphocyte transformation assays were analyzed for each study group by using SAS procedure GLM ANOVA. The overall differences between groups over the course of the study were compared by using the univariate tests of hypothesis for within-subjects effects. All statistical tests were performed at the α = .05 level unless otherwise indicated.

Results

Subjects. Ninety-one volunteers participated in this phase II study. Data from 8 volunteers were excluded from statistical analysis because of preexisting vaccinia immunity as determined by baseline serologies. Of the remaining 83 subjects, 11 were female and 72 were male. During the study, 3 volunteers developed medical problems that were judged as unrelated to vaccine administration. One volunteer developed folliculitis 1 week after inoculation; another developed lower extremity cellulitis 2 weeks after inoculation secondary to an infected leg laceration; and a third developed nausea, vomiting, and abdominal pain, which led to subsequent laparoscopic appendectomy on day 8 after inoculation, with normal appendiceal histopathology and eventual full recovery.

Clinical assessments. ANOVA for vaccine effects at each week yielded no significant differences between the study groups’ temperature profiles (temperature maximums recorded each week).

758 McClain et al. JID 1997;175 (April)
The vaccinees inoculated id who received alcohol wiping after inoculation and the vaccinees inoculated im did not significantly differ with respect to incidence of pox lesion (25% vs. 4.5%, \(P = .141\)). However, failure to wipe with alcohol increased the incidence in the group inoculated id to 62.5%, which was significantly higher than that in the group inoculated im (\(P < .001\)). This was significantly less than the incidence of pox lesions in the scarification group (96.6%), which was higher than that in all the other groups (\(P < .001\)).

The scarification group had the highest incidence of local reactions, the group inoculated im experienced the fewest, and the subgroups inoculated id were intermediate and indistinguishable from each other. Local symptom analysis indicated highly significant statistical differences between the study groups, using both ANOVA and nonparametric Wilcoxon analyses (\(P < .001\)). There was a higher total symptom score for the scarification group (mean, 169.2) and a significantly lower score for the group inoculated im (mean, 3.0). Mean total scores for vaccinees inoculated id with and without alcohol wiping were 61.7 and 90.4, respectively. Additional analysis exclusive of the group inoculated im revealed that the scarification group still differed from the subgroups inoculated id (\(P < .001\) by ANOVA or nonparametric analysis). When the two subgroups inoculated id were compared, no differences were found in local symptom scores (\(P = .215\) by ANOVA, \(P = .406\) by nonparametric analysis). When local symptom scores were compared between volunteers who did and did not develop cutaneous pox lesions, overall and weekly score differences were significant (\(P = .0001\), nonparametric Wilcoxon test).

Systemic symptom analysis indicated no significant differences in the study groups with respect to total symptom scores either by ANOVA (\(P = .352\)) or by nonparametric Wilcoxon test (\(P = .412\)). However, higher mean systemic scores occurred 2 weeks after inoculation in volunteers who developed a cutaneous pox lesion (\(P = .038\)).

All volunteers initially had their immunization sites covered with a vapor-permeable surgical dressing until pox lesions scabbed. However, 4 volunteers with primary vesicles from their vaccinations subsequently developed secondary pox lesions adjacent to the inoculation site, underneath the dressing. We noted that the dressings were occlusive enough to accumulate perspiration underneath and around the inoculation site. Consequently, in the majority of volunteers with pox lesions, vesicular exudate accumulated under these dressings despite frequent dressing changes. This virus-containing exudate seeped under the dressing and covered areas much greater than that of the primary pox lesion, with subsequent secondary or autoinoculation. Subsequently, a nonocclusive and smaller dressing (i.e., dry gauze) was applied over the inoculation sites of the remaining volunteers. Dressings were changed if there was a scheduled clinical check, the bandage became wet, or there were any visible signs of dried exudate on the exterior surface of the bandage. After the institution of the dry gauze dressings, no further incident of secondary pox lesions or large areas of contact dermatitis from dressing adhesive were observed.

Clinical laboratories. Laboratory findings were based on the analysis of the weekly mean for each subject for weeks 1, 0, 1, 2, 3, and 4. Due to some missing values when volunteers omitted scheduled phlebotomies, no repeated measures ANOVA could be done. Hence, an ANOVA was done at each week. Only sporadic differences were detected for any post-treatment week (noted in table 1.)

Serologic and virologic assays. PRNTs were significantly greater for the scarification group than for the groups inoculated im or id beginning at day 13 after inoculation (\(P < .001\)) using a repeated measures ANOVA. Moreover, volunteers from either scarification or groups inoculated id who developed cutaneous pox lesions had significantly higher neutralization titers than those without pox lesions in comparisons adjusted for prevaccination baseline. There was no significant difference between volunteers inoculated by scarification or id who had developed a cutaneous vesicle. However, there was a statistically insignificant trend toward higher titers in the scarification (Wyeth vaccine) recipients. These PRNT responses are illustrated in figure 1.

As illustrated in figure 2, vaccinia ELISA antibody responses were significantly higher for the scarification group at day 27 after vaccination than for either of the groups inoculated id or im (\(P < .001\)). In contrast to PRNT responses, significantly higher ELISA titers were found in scarification vaccinees than in those inoculated id who formed a cutaneous pox lesion (\(P < .001\)). There was greater standard error in this assay than in the PRNT assay. No viremia was detected in any volunteer.

Lymphocyte proliferation assays. Lymphocyte proliferation assays examined stimulation indices before and after vaccination to both live vaccinia virus and heat-inactivated antigen. For the heat-inactivated antigen, responses to scarification were lower than to live vaccinia virus, although the response to scarification was still statistically significantly higher for the scarification group than for the groups inoculated id or im (\(P < .001\)).

**Table 1.** Statistically significant differences in laboratory values of groups of subjects inoculated against vaccinia virus intradermally (id) or intramuscularly (im) or by scarification (scar).

<table>
<thead>
<tr>
<th>Test</th>
<th>Study week</th>
<th>With wipe</th>
<th>Without wipe</th>
<th>im</th>
<th>Scar</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg%)</td>
<td>1</td>
<td>1.2</td>
<td>1.08</td>
<td>1.12</td>
<td>1.18</td>
<td>.0175</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>3</td>
<td>4.2</td>
<td>4.49</td>
<td>4.25</td>
<td>4.49</td>
<td>.0043</td>
</tr>
<tr>
<td>Phosphorus (mEq/L)</td>
<td>4</td>
<td>4.05</td>
<td>4.09</td>
<td>4.57</td>
<td>4.45</td>
<td>.0274</td>
</tr>
<tr>
<td>% monocytes</td>
<td>2</td>
<td>7.23</td>
<td>8.53</td>
<td>7.6</td>
<td>9.34</td>
<td>.0032</td>
</tr>
<tr>
<td>% monocytes</td>
<td>1</td>
<td>6.16</td>
<td>8.1</td>
<td>8.24</td>
<td>7.54</td>
<td>.0392</td>
</tr>
</tbody>
</table>

**NOTE.** Data were analyzed for each study group by using SAS Procedure GLM analysis of variance. Overall differences between groups over study period were compared by using univariate tests of hypothesis for within-subjects effects (\(a = .05\)). Monocytes are expressed as % of total white blood cells in complete blood cell count. With wipe = inoculation id with local alcohol wiping after inoculation; without wipe = inoculation id without alcohol wiping after inoculation.
vaccines is not a new idea. Between 1930 and 1975, at least 8 strains of vaccinia virus were developed for parenteral administration in order to decrease virulence [18]. All were attenuated when compared with standard lymph strains, and some were possibly overattenuated, producing low neutralizing antibody levels after primary and booster inoculations [19]. With the eradication of smallpox following closely upon initial development of most of these vaccines, comparable safety and efficacy data were not obtained on a scale comparable to that for lymph vaccines. In addition, data about cell-mediated responses were not documented for these injectable products.

In this study, vaccination reactions were readily apparent from clinical observation alone. Extensive laboratory monitoring of vaccinees identified no specific safety concerns or issues about the routes of vaccine administration. There was no evidence of viremia in any volunteer. Serum chemistry or hematology value differences between study groups were sporadic and relatively small. Given the number of data points and comparisons in this study, these were likely to be type I errors. As these statistical differences do not represent medically important differences in a contiguous time frame, we do not attribute them to causal events related to respective treatments.

The study indicated that, although not severe, local reactions corresponded to formation of a cutaneous pox lesion. As expected, pox lesion incidence was significantly higher in the scarring group than in the other groups, as were local symptoms. In addition, higher systemic scores occurred 2 weeks after inoculation in volunteers who developed a cutaneous pox

Discussion

This human study compared the safety and immunogenicity of administration id and im of a cell culture–derived vaccinia vaccine with those of the licensed vaccine administered by the traditional route of scarification. The use of injectable vaccinia vaccines is not a new idea. Between 1930 and 1975, at least 8 strains of vaccinia virus were developed for parenteral administration in order to decrease virulence [18]. All were attenuated when compared with standard lymph strains, and some were possibly overattenuated, producing low neutralizing antibody levels after primary and booster inoculations [19]. With the eradication of smallpox following closely upon initial development of most of these vaccines, comparable safety and efficacy data were not obtained on a scale comparable to that for lymph vaccines. In addition, data about cell-mediated responses were not documented for these injectable products.

In this study, vaccination reactions were readily apparent from clinical observation alone. Extensive laboratory monitoring of vaccinees identified no specific safety concerns or issues about the routes of vaccine administration. There was no evidence of viremia in any volunteer. Serum chemistry or hematology value differences between study groups were sporadic and relatively small. Given the number of data points and comparisons in this study, these were likely to be type I errors. As these statistical differences do not represent medically important differences in a contiguous time frame, we do not attribute them to causal events related to respective treatments.

The study indicated that, although not severe, local reactions corresponded to formation of a cutaneous pox lesion. As expected, pox lesion incidence was significantly higher in the scarring group than in the other groups, as were local symptoms. In addition, higher systemic scores occurred 2 weeks after inoculation in volunteers who developed a cutaneous pox

Discussion

This human study compared the safety and immunogenicity of administration id and im of a cell culture–derived vaccinia vaccine with those of the licensed vaccine administered by the traditional route of scarification. The use of injectable vaccinia vaccines is not a new idea. Between 1930 and 1975, at least 8 strains of vaccinia virus were developed for parenteral administration in order to decrease virulence [18]. All were attenuated when compared with standard lymph strains, and some were possibly overattenuated, producing low neutralizing antibody levels after primary and booster inoculations [19]. With the eradication of smallpox following closely upon initial development of most of these vaccines, comparable safety and efficacy data were not obtained on a scale comparable to that for lymph vaccines. In addition, data about cell-mediated responses were not documented for these injectable products.

In this study, vaccination reactions were readily apparent from clinical observation alone. Extensive laboratory monitoring of vaccinees identified no specific safety concerns or issues about the routes of vaccine administration. There was no evidence of viremia in any volunteer. Serum chemistry or hematology value differences between study groups were sporadic and relatively small. Given the number of data points and comparisons in this study, these were likely to be type I errors. As these statistical differences do not represent medically important differences in a contiguous time frame, we do not attribute them to causal events related to respective treatments.

The study indicated that, although not severe, local reactions corresponded to formation of a cutaneous pox lesion. As expected, pox lesion incidence was significantly higher in the scarring group than in the other groups, as were local symptoms. In addition, higher systemic scores occurred 2 weeks after inoculation in volunteers who developed a cutaneous pox

Discussion

This human study compared the safety and immunogenicity of administration id and im of a cell culture–derived vaccinia vaccine with those of the licensed vaccine administered by the traditional route of scarification. The use of injectable vaccinia vaccines is not a new idea. Between 1930 and 1975, at least 8 strains of vaccinia virus were developed for parenteral administration in order to decrease virulence [18]. All were attenuated when compared with standard lymph strains, and some were possibly overattenuated, producing low neutralizing antibody levels after primary and booster inoculations [19]. With the eradication of smallpox following closely upon initial development of most of these vaccines, comparable safety and efficacy data were not obtained on a scale comparable to that for lymph vaccines. In addition, data about cell-mediated responses were not documented for these injectable products.

In this study, vaccination reactions were readily apparent from clinical observation alone. Extensive laboratory monitoring of vaccinees identified no specific safety concerns or issues about the routes of vaccine administration. There was no evidence of viremia in any volunteer. Serum chemistry or hematology value differences between study groups were sporadic and relatively small. Given the number of data points and comparisons in this study, these were likely to be type I errors. As these statistical differences do not represent medically important differences in a contiguous time frame, we do not attribute them to causal events related to respective treatments.

The study indicated that, although not severe, local reactions corresponded to formation of a cutaneous pox lesion. As expected, pox lesion incidence was significantly higher in the scarring group than in the other groups, as were local symptoms. In addition, higher systemic scores occurred 2 weeks after inoculation in volunteers who developed a cutaneous pox
lesion. Either vaccine dissimilarities or the route of vaccination could account for the symptomatic differences between scarification vaccinees and vaccinees inoculated id who developed a pox lesion: Impurities and animal proteins peculiar to calf lymph exudate could explain in part the reactivity of the licensed vaccinia vaccine, or scarification could permit greater viral replication than would intradermal inoculation. Given comparable results in preclinical tests and the fact that both vaccines studied derived from New York Board of Health strains, it is unlikely that the cell culture–derived vaccinia virus has enough sequence variation or intrinsic biologic disparities from the calf lymph–derived Wyeth vaccine to account for such differences. The vaccinees inoculated id who received alcohol wiping after inoculation and those inoculated im did not significantly differ with respect to incidence of pox lesions, but the latter group had significantly fewer local symptoms than did those inoculated id, who in turn had fewer than the scarification vaccinees.

This study strongly indicated that, although less reactogenic, vaccinia vaccine administered im at a dose of 10^5 pfu fails to induce an immune response comparable to that elicited by standard scarification. Although a higher dose of vaccinia virus might have been attempted im, the WHO Smallpox Eradication Campaign [10] and other clinical trials [18–21] had supported the safety of a parenteral dose of 10^3 pfu, as used in this trial. In a phase I dose-escalation human trial, the cell culture–derived vaccinia vaccine was used safely subcutaneously up to a dose of 10^7.8 pfu, but with an attendant increased risk of a cutaneous pox lesion. Although the crossing of an additional tissue plane with injection im may have lessened this occurrence, we were concerned that injection im of a similar high dose of vaccinia virus might risk viremia.

The study also demonstrated that inoculation id will not reliably prevent the formation of a cutaneous pox lesion. Phase I data had raised similar concerns regarding the subcutaneous route of inoculation. In a study by Connor et al. [21], typical Jennerian vesicles were seen in 4%–9% of children vaccinated subcutaneously with 10^3, 10^4, or 10^5 pfu. Furthermore, it is apparent that the presence of such a lesion, although undesirable for safety reasons, is necessary for the most robust immune responses. By cleansing the inoculation site after inoculation, we sought to determine if immune recognition afforded by intradermal processing of antigen could be achieved without the safety risks attendant to a cutaneous pox lesion. This concept stemmed from a previous observation that the cutaneous vesicle was prevented after inoculation id with vaccinia if the needle wound was immediately cleansed with alcohol [22]. These results indicate that replication of the virus in the skin, not merely exposure to antigen-presenting cells (i.e., Langerhans cells) in that location, is essential to generate optimal immune responses to vaccinia antigens.

Vaccinees who developed cutaneous pox lesions had significantly higher ELISA and neutralizing antibody responses. The poor responses to inoculation id (in those without pox lesions) and im confirm and expand on earlier observations concerning injectable vaccinations in children [19]. The investigators of that study concluded that “...until more is known about the importance of neutralizing antibody in the immunity to smallpox, it would seem unwise to administer vaccine by the subcutaneous route...” [21]. This conclusion was based on the relatively poor rates of seroconversion (67%), as measured by vaccinia-neutralizing antibodies, in children vaccinated subcutaneously compared with their percutaneously vaccinated counterparts (87%), and the unimpressive neutralizing antibody responses upon successful percutaneous revaccination of children whose primary vaccination was subcutaneous.

In addition to antibody responses, cell-mediated responses were also superior in volunteers who developed pox lesions (whether inoculated id or by scarification). There was no significant difference in lymphocyte stimulation responses between those who formed pox lesions from the licensed vaccinia vaccine by scarification and those with pox lesions from the cell culture–derived vaccinia vaccine. Inferior cell-mediated responses of vaccination without a pox lesion, as observed in this study, may explain why Connor et al. [21] reported that children who had initially received an injectable (subcutaneous) vaccine exhibited >30% incidence of “primary-type” responses to percutaneous revaccination. This study also confirms the observation by Cherry et al. [20] of a correlation between neutralizing antibody and lymphocyte stimulation responsiveness to vaccinia after vaccination.
An unexpected event in this phase II study was the high incidence of secondary pox lesions adjacent to the inoculation site in the first set of volunteers (4/31). The use of semipermeable dressings appeared to have caused this complication, as no further instances occurred after instituting the use of dry gauze dressings. Although vapor-permeable, the semipermeable dressings caused accumulation of perspiration around the inoculation site despite frequent dressing changes, permitting virus-containing exudate to secondarily inoculate areas proximate to the primary pox lesion. The large adhesive area of this dressing caused various degrees of contact dermatitis in several volunteers, but this did not trigger autoinoculation, since the secondary lesions were under the dressing area and not at the point of adhesive contact. Ironically, the use of these semipermeable dressings was originally mandated as a protective measure against contact and environmental spread of the vaccine virus. Apart from the four instances of autoinoculation precipitated by semipermeable dressings, there were no serious or unexpected complications from the study; all inoculation site lesions healed completely.

If it is necessary to achieve immunity to vaccinia or related Orthopoxviridae such as variola or monkeypox, these results warrant vaccination by scarification in a population without contraindications, despite the attendant risks. Formation of a cutaneous pox lesion (i.e., a vaccine “take”), although less desirable from a safety standpoint, engenders higher PRNT titers and cell-mediated responses and has been historically validated as indicating protective immunity [10]. This observation holds true when comparing administration id or im of the cell culture–derived vaccinia vaccine when such does not result in the development of a cutaneous pox lesion. These data appear to reflect the epithelial tropism of Orthopoxviridae, which may explain many of the disappointing immune responses in humans to vaccinia–vectored gene inserts [23, 24]. In addition, it remains dubious whether the greater risk of high-dose (e.g., 10^7 pfu) inoculation im would be warranted in an effort to achieve higher PRNT responses without a cutaneous pox lesion, despite evidence from both preclinical and clinical studies that the cell culture–derived vaccinia vaccine is biologically comparable to other New York Board of Health vaccines. Although multiple injections of vaccinia via a parenteral route might improve immune responses closer to those seen with scarification, repeated injections would increase costs and logistic burdens and thereby negate the typical advantages of a live vaccine, yet with greater safety concerns.

These results have important implications for the use of recombinant poxvirus vaccines as vectors for immunogens and may explain why immune responses to some of these vaccines have required multiple injections [4–6]. Although deliberate inoculation by scarification may provide improved immune responses for vaccinia virus–vectored immunogens, this is with a greater risk of local reactions and possible secondary inoculation. This rationale would not apply to highly attenuated poxvirus vectors that are incapable of productive replication in human-derived cell lines [25, 26].

When administration id of the cell-cultured vaccinia resulted in a cutaneous pox lesion, immune responses were not significantly different from those seen with scarification with the licensed vaccinia vaccine. Although there is a statistically insignificant trend towards higher neutralization titers with the latter, future studies will examine the cell-cultured vaccine’s immunogenicity by this same scarification route.

Acknowledgment

We gratefully acknowledge the skillful assistance of Tamara Lewis in the completion of ELISAs.

References


