Effect of Vaccination with Modified Vaccinia Ankara (ACAM3000) on Subsequent Challenge with Dryvax

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(See the article by Wilck et al, on pages 1361–1370.)

Background. Despite the success of smallpox vaccination, the immunological correlates of protection are not fully understood. To investigate this question, we examined the effect of immunization with modified vaccinia Ankara (MVA) on subsequent challenge with replication-competent vaccinia virus (Dryvax).

Methods. Dryvax challenge by scarification was conducted in 36 healthy subjects who had received MVA (n = 29) or placebo (n = 7) in a previous study of doses and routes of immunization. Subjects were followed up for clinical take, viral shedding, and immune responses.

Results. MVA administration attenuated clinical takes in 21 (72%) of 29 subjects, compared with 0 of 7 placebo recipients (P < .001). Attenuation was most significant in MVA groups that received median tissue culture infectious doses (TCID50) intradermally (P < .001) and TCID50 intramuscularly (P = .001). Both duration and peak titer of viral shedding were reduced in MVA recipients. Peak neutralizing antibody responses to vaccinia virus or MVA previously induced by MVA immunization were associated with attenuated takes (P < .02) and reduced duration (P = .001) and titer (P = .005) of viral shedding.

Conclusions. MVA immunization results in clinical and virologic protection against Dryvax challenge. Protection is associated with prior induction of neutralizing antibodies to MVA or vaccinia virus. MVA administered intradermally has protective and immunologic responses similar to those of a 10-fold-higher dose given subcutaneously.

Trial registration. ClinicalTrials.gov identifier: NCT00133575.
MVA immunization study [2]. Subjects who elected to participate were followed up for the effects of Dryvax challenge, including clinical manifestations (“takes”), vaccinia virus shedding, and humoral and cellular immune responses.

METHODS

Dryvax vaccinia virus. The vaccinia virus used for challenge was Dryvax (lot 4020075; Wyeth Laboratories), provided by the Centers for Disease Control and Prevention. Dryvax was a lyophilized preparation derived from calf lymph and reconstituted with a diluent containing 50% glycerine and 0.25% phenol in sterile water (Chesapeake Biological Laboratory). The reconstituted vaccine contains 1 × 10⁸ plaque-forming units (PFUs) of vaccinia virus per milliliter.

Study design and subjects. The subjects were healthy volunteers; entry and exclusion criteria have been described elsewhere [2]. The study was approved by the institutional review board, and written informed consent was obtained from all subjects. Double-blind allocation to MVA or placebo was maintained [2]. The Dryvax challenge was to have been administered 6 months after MVA immunization; however, in discussion with the National Institutes of Health and the Food and Drug Administration, the investigational use of Dryvax was delayed because of concern for possible cardiac adverse events [4–7]. Therefore, Dryvax challenges were delayed in some subjects, and the interval between MVA vaccination and Dryvax challenge was extended to 15 months. In 2007, the Food and Drug Administration prohibited the investigational use of Dryvax in clinical studies, so the group that had previously received the highest dose of MVA intramuscularly (1 × 10⁸ median tissue culture infective doses [TCID₅₀]) was not challenged with Dryvax.

Administration of vaccinia virus. Vaccinia virus was administered by the standard scarification technique (pressing a bifurcated needle 15 times to a 5-mm area of skin over the deltoid [8]), after which a dressing was applied. Subjects were evaluated, and the vaccination site was photographed on days 4, 7, 10, 14, and 28 after vaccinia virus challenge. Subjects who had lesions later than day 14 after Dryvax challenge returned for additional visits every 3–5 days until the site healed.

Assessment of Dryvax takes. After Dryvax challenge, the vaccination site reaction was categorized into the following take categories, which were derived from standard definitions [9, 10]: category 0, no take (no skin reaction at the vaccination site); category 1, modified take without vesicle (a papule with or without surrounding erythema by the third day and without vesicle or pustule formation before resolution); category 2, modified take with vesicle (a papule by the third day that became vesicular by the fifth to seventh day and that dried shortly thereafter); and category 3, primary or full take (a papule develops and becomes vesicular, umbilicated, and pustular by the seventh to 10th day, and the lesion becomes crust and dries by the third week). Categories 1 and 2 were considered to be attenuated takes, and category 3 was considered to be a full take (no attenuation). Take category was determined by 3 physicians blinded to the volunteers’ vaccination status with respect to either MVA or placebo.

Assessment of viral shedding. Viral cultures of the inoculation sites were performed to assess viral shedding at each visit (see the Appendix, which appears only in the online version of the Journal).

Immunogenicity assays. Humoral antibody assays (neutralization assay, comet-reduction assay, and enzyme-linked immunosorbent assay [ELISA]) and T cell interferon γ (IFN-γ) enzyme-linked immunospot (ELISPOT) assays were conducted as described elsewhere [2].

Statistical analysis. The Fisher exact test was used to test for association between categorical values. The Wilcoxon rank-sum test or the Kruskal-Wallis test was used to assess group differences in continuous measures. All tests were 2-sided. To investigate the effect of pre-Dryvax immune responses on post-Dryvax viral shedding, a simple group comparison as well as a linear regression analysis was performed. The maximum values of pre-Dryvax MVA and vaccinia virus antibody titers, the maximum values of titer and duration of virus shed, and ELISPOT baseline-adjusted counts for each subject were used. In the linear model, dose and route of MVA vaccination were adjusted. Maximum pre-Dryvax immune response measurements and maximum post-Dryvax viral titers were log₁₀ transformed before modeling. The interaction between dose and route was also examined in each of these models. Locally weighted scatterplot smoothing [11, 12] was used to describe the effect of the immune response to MVA vaccination on swab titer and viral shedding over time after Dryvax challenge.

RESULTS

Characteristics of subjects. Thirty-six subjects were challenged with Dryvax 6–15 months after receiving 2 doses of MVA vaccine; 29 of these subjects had received MVA vaccination as part of our previous study [2], and 7 had received placebo and were thus still vaccinia virus–naïve before Dryvax challenge. For the 29 subjects who had previously received MVA vaccination, the median time of challenge (scarification) with Dryvax was 190 days (range, 154–385 days) after the first immunization. Of the 36 subjects, 18 (50%) were male, 30 (83%) were white, and 4 (11%) were Latino; the median age was 27 years (range, 20–34 years).

Clinical responses to Dryvax challenge. All 36 subjects who were challenged with Dryvax experienced local reactions at the site of inoculation. Prior MVA vaccination was significantly associated with attenuation of takes in 21 (72%) of 29 MVA recipients. None of the placebo recipients (0/7) demonstrated attenuation of takes (P = .001). Of note, 2 of 2 MVA recipients

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Table 1. Cutaneous Responses to Dryvax Challenge in Modified Vaccinia Ankara (MVA)–Vaccinated Groups

| Group | MVA regimen | No. of subjects (n = 36) | No. with Attenuated response | Unattenuated response | P
|-------|-------------|--------------------------|-----------------------------|----------------------|---
| A     | 1 × 10^6 ID | 2                        | 0                           | 2                    | NA |
| B     | 1 × 10^7 IM  | 5                        | 5                           | 0                    | .001 |
| C     | 1 × 10^7 SC  | 7                        | 4                           | 3                    | .07 |
| D     | 1 × 10^8 SC  | 6                        | 4                           | 2                    | .02 |
| E     | 1 × 10^7 ID  | 9                        | 8                           | 1                    | .001 |
| Placebo |             | ...                      | 7                           | 0                    | 7 |

**NOTE.** ID, intradermal; IM, intramuscular; NA, not applicable; SC, subcutaneous.

* Median tissue culture infectious doses given on days 0 and 28; Dryvax challenge by scarification occurred at a median of 194 days after the first vaccination, including both MVA and placebo recipients (range, 154–438 days after the first vaccination).

**Figure 1.**

A, Magnitude and duration of viral shedding in relation to Dryvax take category (as defined in the main text [9, 10]) in subjects who had received modified vaccinia Ankara (MVA) vaccination. Swabs of Dryvax-induced lesions were obtained at the indicated time points after challenge, and viral shedding was determined by tissue culture plaque-forming unit (PFU) assay. Data are median values for groups; error bars indicate interquartile ranges. The dashed line represents the limit of detection (10 PFU/mL). All subjects who received placebo had full takes and are plotted separately.

B, Correlation between prior vaccination with MVA and reduced titer and duration of viral shedding after Dryvax challenge. Data are individual swab titers for individuals grouped as MVA or placebo recipients. A locally weighted scatterplot smoothing fit line is plotted for each group. The limit of detection is 10 PFU/mL.

in the 1 × 10^7 intradermal group experienced full takes, indicative of no attenuation. Each of the other groups experienced a significantly higher rate of attenuation compared with the placebo group, except for the 1 × 10^7 subcutaneous group (Table 1). The most statistically significant differences in rates of attenuation occurred in the 1 × 10^7 intradermal group (P = .001) and the 1 × 10^8 intramuscular group (P = .001), compared with the placebo group. Systemic reactions to Dryvax challenge occurred in 14 (48%) of 29 MVA recipients and in 6 (86%) of 7 placebo recipients, were either mild or moderate in severity, and did not differ in characteristics among any of the MVA and placebo groups.

**Viral shedding from Dryvax lesions.** Viral shedding from Dryvax lesions was highly correlated with take categorization (Figure 1A). Prior immunization with MVA was significantly correlated with a lower peak titer of viral shedding (median, 2.9 × 10^4 PFU/mL for MVA recipients vs 4.8 × 10^3 PFU/mL for placebo recipients; P < .004) and with a shorter duration of viral shedding (median [range], 14 [2–21] days for MVA recipients vs 21 [20–29] days for placebo recipients; P < .001) (Figure 1B). MVA recipients with category 3 takes had peak viral titers that were similar to those of placebo recipients but had significantly shorter durations of shedding (median, 17 vs 21 days; P = .003). Furthermore, MVA recipients with category 1 and 2 attenuated takes had lower peak titers of viral shedding (median, 3.5 × 10^2 and 3.6 × 10^2 PFU/mL; P = .001 and P = .006 for the comparison with placebo, respectively) and shorter duration of viral shedding (median, 7 and 14 days; P = .001 and P < .001 for the comparison with placebo, respectively).

**Neutralizing antibody responses after Dryvax challenge.** The development of neutralizing antibody responses to vaccinia virus Western Reserve (VV:WR) in placebo recipients was consistent with a primary immune response (Figure 2). In these subjects, serum neutralizing antibody titers were first detected on day 14 after challenge and continued to rise through day 28. In contrast, all MVA recipients had detectable anti-vaccinia virus neutralizing antibody titers by day 7 after challenge. The responses in the 1 × 10^6 subcutaneous, 1 × 10^7 intradermal, 1 × 10^7 intramuscular, and 1 × 10^8 subcutaneous groups peaked...
Figure 2. Neutralizing antibody responses after Dryvax challenge. Serum samples were obtained on days 0, 4, 7, 14, and 28 after Dryvax challenge. Serial dilutions were tested for neutralizing activity against VV:Luc. Data are 50% inhibitory dose (ID_{50}) titers for each dose and route-of-administration group, with the number of individuals in each group indicated; error bars indicate interquartile ranges. The dashed line represents the limit of detection (serum ID_{50} titer, 10). ID, intradermal; IM, intramuscular; SC, subcutaneous.

on day 14 and remained stable on day 28. The magnitude of the anti–vaccinia virus neutralizing antibody responses in the 2 subjects in the 1 × 10^6 intradermal group was lower than that in the other MVA vaccine groups on day 14 but was equivalent on day 28. On days 7 and 14, neutralizing antibody titers were significantly higher in MVA recipients (50% inhibitory dose [ID_{50}] titers, 42 and 803, respectively) than in placebo recipients (ID_{50} titers, <10 and 58, respectively) (P = .002 and P < .001 for day 7 and 14, respectively). Serum samples were also tested for neutralizing activity against MVA virus, and the kinetics and magnitude of responses were similar to those observed against VV:WR (data not shown).

We further assessed anti–extracellular enveloped virion (EEV) neutralizing antibody activity in serum samples from MVA and placebo recipients by comet-reduction assay 14 days after Dryvax challenge (Figure 3). Placebo recipients had detectable anti-EEV neutralization activity by day 14 after challenge (median, 49% comet reduction), but responses were higher in the 1 × 10^6 subcutaneous, 1 × 10^7 intradermal, 1 × 10^5 intramuscular, and 1 × 10^6 subcutaneous groups (P = .05, P = .04, P = .03, and P = .005, respectively). Only minimal comet-reduction activity was observed in serum from the 2 subjects in the 1 × 10^6 intradermal group at this time point. Analysis of anti-EEV neutralization on day 28 after challenge showed similar levels of activity in all MVA groups and the placebo group (data not shown).

**Binding antibody responses after Dryvax challenge.** Serum antibody binding titers to the intracellular mature virion (IMV)–associated protein antigens, L1R and A27L, and the EEV-associated protein antigens, A33R and B5R, were measured by ELISA on days 14 and 28 after challenge (Figure 4). For these analyses, end-point titers measured in all MVA recipients as a group were compared with those measured in placebo recipients. On day 14 after challenge, serum end-point titers against B5R, A33R, and L1R were higher in MVA recipients than in placebo recipients (P = .003, P = .02, and P = .009, respectively), whereas end-point titers against A27L were similar among these groups. Of note, we had previously observed only low-level antibody responses against A27L after MVA prime-boost vaccination [2]. Antibody titers against all 4 proteins were similar by day 28 after challenge in MVA and placebo recipients.

T cell responses after Dryvax challenge. T cell responses in MVA and placebo recipients after Dryvax challenge were assessed by IFN-γ ELISPOT assay (Figure 5). In the placebo group, T cell responses peaked by day 14 after Dryvax challenge and declined by nearly 50% by day 28. After Dryvax challenge, T cell responses were found to be quite variable among MVA recipients. Of note, peak T cell responses in the 1 × 10^6 subcutaneous and 1 × 10^7 intradermal groups were only slightly elevated compared with prechallenge baseline and were significantly lower than peak responses measured in the placebo group.
Effect of MV A on Challenge with Dryvax

Figure 4. Antibody responses to intracellular mature virion (IMV)–associated and extracellular enveloped virion (EEV)–associated antigens after Dryvax challenge. Serum samples were obtained on days 14 and 28 after Dryvax challenge. Serial dilutions were tested for binding antibody activity against 2 IMV-associated protein antigens (A27L and L1R) and 2 EEV-associated protein antigens (A33R and B5R) by enzyme-linked immunosorbent assay. Data are individual end-point titers; horizontal lines indicate the median titer for each group. MVA, modified vaccinia Ankara.

Effect of prior MV A-elicited immune responses on subsequent takes and viral shedding after Dryvax challenge. We examined the relationship between immune responses elicited by MV A immunization before Dryvax challenge and the attenuated takes and decreased viral shedding that were subsequently observed after Dryvax challenge. The immune responses we examined included neutralizing antibody responses to MV A and vaccinia virus, ELISA antibody responses to MV A and vaccinia virus, protein-specific antibody responses to IMV and EEV antigens, and anti–vaccinia virus T cell responses as measured by IFN-γ ELISPOT assay. The only predictor of an attenuated take among the immune responses before Dryvax challenge was the peak anti-MV A neutralizing antibody responses after MV A immunization: median ID₅₀ titers were 353, 82, and 139 for take categories 1, 2, and 3, respectively (P < .001). We further investigated this association by categorizing peak prechallenge anti-VV:WR neutralizing antibody ID₅₀ titers as ≤20, 21–100, or >100. As shown in Figure 6, the maximum titer of virus shed was inversely correlated to increasing antibody category: ≤20, 21–100, and >100 PFU/mL for each increasing antibody category, respectively (P < .001). The magnitude of the peak challenge anti-VV:WR neutralizing antibody category was also inversely correlated to duration of viral shedding: median durations were 21 (range, 8–29), 14 (range, 4–21), and 7.5 (range, 2–18) days for each, respectively (P < .001). A similar statistically significant association was seen between decreased duration and titer of viral shedding and the maximum vaccine-elicited neutralizing antibody responses to MV A. For peak anti-MV A neutralizing antibody categories of ≤20, 21–100, and >100 ID₅₀, virus was shed for a median of 21 (range, 18–29), 14 (range, 8–19), and 10.5 (range, 2–21) days, respectively (P < .001) and was shed at median titers of, respectively (P < .001). Thus, elicitation of a neutralizing antibody response to MV A or vaccinia virus before Dryvax challenge is associated with a decrease in duration of vaccinia shedding by 50%–67% and a reduction in maximum titer of virus shed by 10–100-fold. When the above-described analyses were adjusted for dose group (for both comparisons, P < .001). A more rapid cellular immune response was observed in the 1 × 10⁶ intradermal, 1 × 10⁷ subcutaneous, and 1 × 10⁶ intramuscular MVA groups compared with placebo recipients, with responses being measurable by day 7 after challenge. Responses in the 1 × 10⁷ subcutaneous and 1 × 10⁶ intramuscular groups remained stable through day 28, whereas ELISPOT responses for the 2 individuals in the 1 × 10⁶ intradermal group peaked on day 14 after challenge. Taken together, these data suggest that anamnestic T cell responses in MVA recipients after Dryvax challenge are variable and are possibly influenced by the dose and route of MVA used in the vaccination regimen, in which the lower-dose groups demonstrate a more robust recall response.
Figure 5. Cellular immune responses elicited by Dryvax challenge. Peripheral blood mononuclear cells (PBMCs) were isolated on days 0, 4, 7, 14, and 28 after Dryvax administration by scarification and tested by an interferon γ enzyme-linked immunospot assay against autologous vaccinia virus Western Reserve–infected target cells isolated on day 4 after the first modified vaccinia Ankara or placebo inoculation. Data are the median number of spot-forming cells (SFCs) per effector PBMCs and interquartile ranges for each dose and route-of-administration group after subtraction of responses to medium alone. The number of SFCs at baseline are subtracted from each data point.

DISCUSSION

Vaccination with ACAM3000 MVA conferred protection against challenge with Dryvax administered by scarification, as reflected by attenuation of Dryvax takes and decreased titer and duration of viral shedding. Attenuation of takes was observed with MVA vaccination by each of the 3 routes (intradermal, subcutaneous, and intramuscular) and was most significantly associated with the highest dose administered by each route. Of particular note, the protective effects were obtained with intradermal administration of MVA at a dose of \(1 \times 10^8\) TCID₅₀, which was 10-fold lower than the highest dose given subcutaneously (\(1 \times 10^9\) TCID₅₀).

Studies of other MVA candidate vaccines in humans have also reported an attenuation of takes and decreased viral shedding after Dryvax challenge. Frey et al [13] administered MVA-BN (Bavarian Nordic) to 90 vaccinia virus–naïve volunteers either subcutaneously at doses ranging from \(2 \times 10^7\) to \(1 \times 10^8\) TCID₅₀ or intramuscularly at \(1 \times 10^8\) TCID₅₀ and then challenged them with Dryvax by scarification 112 days later. MVA vaccination was associated with decreased lesion size and decreased titers of virus shed after Dryvax challenge, but no dose or route relationship was observed. Parrino et al [10] studied 76 vaccinia virus–naïve and 68 vaccinia virus–immune volunteers vaccinated with TBC-MVA (Therion Biologics) intramuscularly in multiple-dose regimens. A somewhat lower dose of TBC-MVA than that originally intended was administered (\(1 \times 10^6\) PFUs), and subjects were challenged with Dryvax by scarification 3 months after MVA vaccination. MVA vaccination reduced the severity of lesion formation and the systemic reactogenicity induced by Dryvax in both the vaccinia virus–naïve and vaccinia virus–immune subjects, and 2 or 3 doses of MVA reduced the duration of viral shedding. Protective effects were seen in both the vaccinia virus–naïve and vaccinia virus–immune subjects but were more profound in the former [10].

MVA has also afforded protection against high-dose challenge with monkeypox virus, administered either intravenously or intratracheally, to nonhuman primates. In a study by Earl et al [14], 2 intramuscular inoculations of MVA at \(1 \times 10^6\) PFUs significantly reduced viral load and poxvirus lesions after challenge. MVA-vaccinated animals remained clinically well compared with nonvaccinated animals, which became extremely ill. Anamnestic binding and neutralizing antibody responses were observed after challenge, although no statistically significant correlation could be established because of the small number of animals. Postchallenge T cell responses were not measured in the study by Earl et al because of biocontainment issues.
another study, monkeys vaccinated with 2 inoculations of an MVA-based candidate human immunodeficiency virus vaccine (1 × 10^6 PFUs given intramuscularly) similarly demonstrated protection against viral load, the number of poxvirus lesions, and death after monkeypox virus challenge 3 years later [15]. The magnitude of the postchallenge anti–monkeypox virus neutralizing antibody response significantly correlated with reduced peak viral load, whereas no statistically significant correlations were observed for postchallenge T cell responses.

In the present study, rapid anamnestic neutralizing antibody responses to both MVA and VV:WR were seen in all previously MVA-immunized groups after challenge with Dryvax, and the time course was consistent with a recall response. In contrast, placebo recipients who were challenged with Dryvax had a pattern more consistent with a primary response. Importantly, we demonstrated a rapid antibody response in recipients of MVA to key protein antigens associated with protection against the IMV and EEV forms of infectious vaccinia virus [16–20] and also demonstrated effective in vitro neutralization against these viruses. The titers of neutralizing antibodies after Dryvax challenge were ultimately similar by day 28 at all doses and routes of MVA vaccination studied. However, the group that received 1 × 10^4 TCID50 intradermally had anamnestic antibody responses that were similar in magnitude and kinetics to those in the group that received a 10-fold-higher dose of MVA subcutaneously.

The present study of the effect of MVA immunization on Dryvax challenge showed a strong correlation between the presence of neutralizing antibodies to MVA or VV:WR before Dryvax challenge and attenuation of takes and decreased viral shedding. The relationship between neutralizing antibody titers and reduction of viral shedding was maintained even when dose and route were adjusted in linear models. Consistent with these observations, other studies have found antibody responses to be important for protection against orthopoxvirus infection and disease [21–23], and high titers of neutralizing antibodies against vaccinia virus have been correlated with protection against smallpox [24–26]. Neutralizing antibody protected nonhuman primates against lethal intravenous challenge with monkeypox, and the protection afforded by immunization with vaccinia virus was abrogated by B cell depletion [27].

Other clinical and experimental animal studies have implicated T cell as well as B cell immunity in protection against orthopoxvirus infection. Individuals with either genetic T cell or B cell deficiencies have an increased frequency and severity of complications with smallpox vaccination, although patients with T cell abnormalities appear to be more at risk than those with agammaglobulinemia [28, 29]. Adoptive transfer of lymphocytes from vaccinia virus–immunized donors was associated with a resolution of vaccinia necrosis that was unresponsive to vaccinia immune globulin [29], and protection against vaccinia virus infection has been achieved via adoptive transfer with virus-specific T cells [30, 31]. In mice, protection against orthopoxvirus infection has been associated with both B and T cell responses [32, 33]. In the present study, T cell responses measured by IFN-γ ELISPOT assay using autologous VV:WR-infected target cells were seen after Dryvax challenge and showed varied patterns with respect to dose and route of MVA administration. Interestingly, the lowest T cell responses measured after Dryvax challenge were in groups receiving high-dose MVA vaccination (1 × 10^6 subcutaneously and 1 × 10^4 intradermally), which also had the highest titer of neutralizing antibody responses before challenge [2]. It is possible that preexisting neutralizing antibodies or the rapid expansion of such responses subsequently blunted cellular immune responses that require replication of virus for optimal stimulation. Although the present study did not show a correlation between T cell responses and protection, additional studies entailing more detailed analyses of T cell responses in terms of epitope specificity and phenotypic and functional characteristics will be required to adequately assess the relationship of such responses to protection against Dryvax challenge.

In summary, MVA immunization by intradermal, subcutaneous, and intramuscular routes resulted in attenuation of takes and decreased viral shedding after Dryvax challenge. The protective effects of intradermal administration were seen at a 10-fold-lower dose of MVA than that administered subcutaneously and suggest that intradermal administration may thus offer a dose-sparing effect in vaccination regimens. Among the immune responses elicited by MVA vaccination, the presence of neutralizing antibodies against MVA or vaccinia virus was highly associated with decreased viral shedding after challenge.

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