

Modified Vaccinia Ankara Virus Vaccination Provides Long-Term Protection against Nasal Rabbitpox Virus Challenge

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Modified vaccinia Ankara virus (MVA) is a smallpox vaccine candidate. This study was performed to determine if MVA vaccination provides long-term protection against rabbitpox virus (RPXV) challenge, an animal model of smallpox. Two doses of MVA provided 100% protection against a lethal intranasal RPXV challenge administered 9 months after vaccination.

Modified vaccinia Ankara virus (MVA) is a safe smallpox vaccine candidate (1–3) that is being investigated in clinical trials as a viral vector for vaccination against infectious diseases (4–6) and cancers (7, 8). MVA is derived from chorioallantois vaccinia virus Ankara (CVA) that was passaged through chicken embryo fibroblasts >570 times to reduce the side effects associated with vaccinia virus immunization (9–11). Because smallpox has been eradicated, the efficacy of any smallpox vaccine, including MVA, cannot be proven directly. Instead, under the guidelines of the “animal rule,” which often requires two animal species (12, 13), vaccine efficacy may be shown indirectly by the demonstration of protection against poxviruses other than smallpox with the use of appropriate animal models relevant to the human disease. Animal models utilized to evaluate the protective efficacy of smallpox vaccines include monkeypox virus (MPXV) challenge of primates (14, 15), ectromelia virus (and other poxviruses) challenge of mice (16, 17), and rabbitpox virus (RPXV) challenge of rabbits (18, 19). Despite the numerous studies demonstrating the immunogenicity and protective efficacy of smallpox vaccines in mice, mice do not provide a physiologically relevant model for humans when utilizing maternal or intranasal immunization. Due to the similarities between rabbits and humans in regard to maternal-fetal immunoglobulin transfer (20, 21) and the volume of the upper respiratory tract (22), rabbits represent an ideal second animal model, after nonhuman primates, in which to address the animal rule for next-generation smallpox vaccines.

MVA has demonstrated efficacy against ectromelia virus (17) and RPXV (18) challenge shortly after vaccination and has demonstrated long-term protection against MPXV challenge in primates (23, 24). While MVA is known to induce protective immunity against RPXV challenge of rabbits 4 weeks after vaccination, no studies have determined the long-term protective efficacy of MVA against RPXV. Thus, the objective of our study was to determine if two doses of MVA can provide protection against lethal RPXV challenge 9 months after vaccination. We used a well-characterized intranasal RPXV challenge model that provides consistent clinical symptoms and viremia indicative of disease progression (25).

Young (3- to 4-month-old) female New Zealand White (NZW) rabbits ($n = 4$) were vaccinated intramuscularly with 1×10^8 PFU MVA in a volume of 200 μ l administered into the quadriceps femoris on days 0 and 28. Five rabbits served as naive controls. Rabbits were bled via the auricular artery on days –7, 42, 245, 289 (prechallenge) and 311 (14 days postchallenge) to monitor antigen-specific antibody responses. All procedures

were approved by the Duke University Institutional Animal Care and Use Committee.

Antibody titers to three vaccinia virus membrane proteins (B5, A33, and L1) were determined shortly after vaccination (day 42) and at day 289 by enzyme-linked immunosorbent assay (ELISA) in a 384-well format as previously described (26–28). By day 42, MVA immunization induced significantly elevated serum geometric mean IgG titers (\log_2 endpoint titers) to B5R, A33R, and L1R of 6.7×10^7 , 1.6×10^7 , and 1.7×10^5 , respectively (data not shown). By day 289, titers to B5R, A33R, and L1R remained significantly elevated compared to preimmunization titers and decreased by <10-fold compared to day 42 titers (data not shown). This decrease in antipoxvirus protein-specific serum IgG titers between days 42 and 289 was significant only for anti-B5R IgG, as determined by repeated-measures one-way analysis of variance (ANOVA) with Tukey’s multiple-comparison test. Thus, intramuscular vaccination with MVA on days 0 and 28 induced high, sustained anti-poxvirus protein-specific antibody titers for at least 289 days after vaccination.

To quantify the amount of antibody induced by MVA vaccination that would recognize RPXV, serum anti-RPXV IgG titers were determined by ELISA with plates coated with a 1:200 dilution of 5.6×10^6 PFU/ml UV-inactivated RPXV (29). RPXV (ATCC VR-1591) was grown in CV-1 cells, and the infectious titer was determined by a standard plaque assay. Vaccination with MVA induced significantly elevated anti-RPXV IgG antibodies at day 289 (geometric mean titer, 1:262,144) compared to that in control rabbits (Fig. 1). Since smallpox-neutralizing antibodies correlate with protection from smallpox in humans (30), we performed a plaque reduction neutralization assay to determine if the antibodies induced by MVA were capable of neutralizing RPXV as described previously (31), except that dilutions of heat-inactivated serum mixed with sonicated RPXV were performed overnight at

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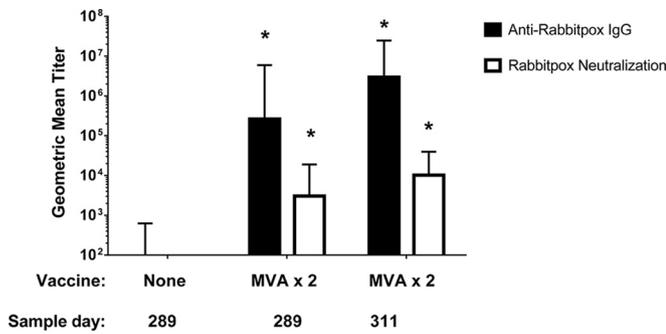


FIG 1 Intramuscular immunization with MVA induces serum IgG antibodies that bind to RPXV and antibodies that neutralize RPXV. NZW rabbits ($n = 4$) were immunized intramuscularly with 1×10^8 PFU of MVA on days 0 and 28. Naive rabbits of a similar age and housing duration were used as controls. Day 289 (prechallenge) serum for naive and MVA immunized rabbits and day 311 (postchallenge day 14) serum from MVA immunized rabbits were tested by ELISA for the presence of IgG antibodies specific for RPXV. Serum was also tested for its ability to neutralize the infectivity of RPXV. *, statistically significantly different ($P < 0.05$) from naive rabbits as determined by ANOVA with Tukey's multiple comparisons. No significant difference between prechallenge and postchallenge titers as determined by a paired parametric t test. MVA treated, $n = 4$; naive, $n = 5$.

37°C with 5% CO₂ in supplemented minimum essential medium containing 10% fetal bovine serum. Subsequently, the infectious titers of the virus suspensions were measured by plaque assay on monolayers of CV-1 cells. MVA vaccination resulted in significantly elevated RPXV neutralizing antibody titers (plaque reduction neutralization titer 50% geometric mean, 1:3,044) at day 289 compared to the results in control rabbits (Fig. 1).

To determine if MVA vaccination provides long-term protection against a lethal RPXV challenge in rabbits, we performed an intranasal RPXV challenge on day 297, 9 months after the second MVA vaccination. Rabbits were sedated with 1 mg/kg acepromazine, anesthetized with isoflurane, and then positioned in dorsal recumbency. RPXV at 1×10^5 PFU diluted in USP-grade phosphate-buffered saline for a total volume of 170 μ l was administered intranasally to a single nostril. Rabbits were checked twice daily for clinical signs, changes in body weight, and changes in temperature for 14 days postchallenge. Temperature was determined by a BMDS IPTT-300 temperature transponder implanted intrascapularly. Beginning on the third day postchallenge, the treatment-naive rabbit group, comprising three rabbits tested in parallel with the MVA-vaccinated rabbits, and two rabbits tested in a separate pilot study (to confirm challenge virus phenotype and dose) experienced significant reductions in body weight compared to MVA-vaccinated rabbits (Fig. 2). Comparison of postchallenge absolute body weight to the prechallenge weight showed that MVA-vaccinated rabbits did not exhibit significant changes while naive rabbits exhibited significant weight loss. Additionally, naive rabbits exhibited increased body temperature starting 2 days postchallenge compared to the temperature of MVA-vaccinated rabbits (Fig. 2). Comparison of postchallenge body temperature to the prechallenge temperature showed that MVA-vaccinated rabbits did not exhibit any significant change in temperature while naive rabbits exhibited significantly increased body temperature. Coinciding with changes in temperature and body weight, naive rabbits developed clinical signs of ocular and nasal discharge. On the 4th day postchallenge, two of the naive rabbits were eutha-

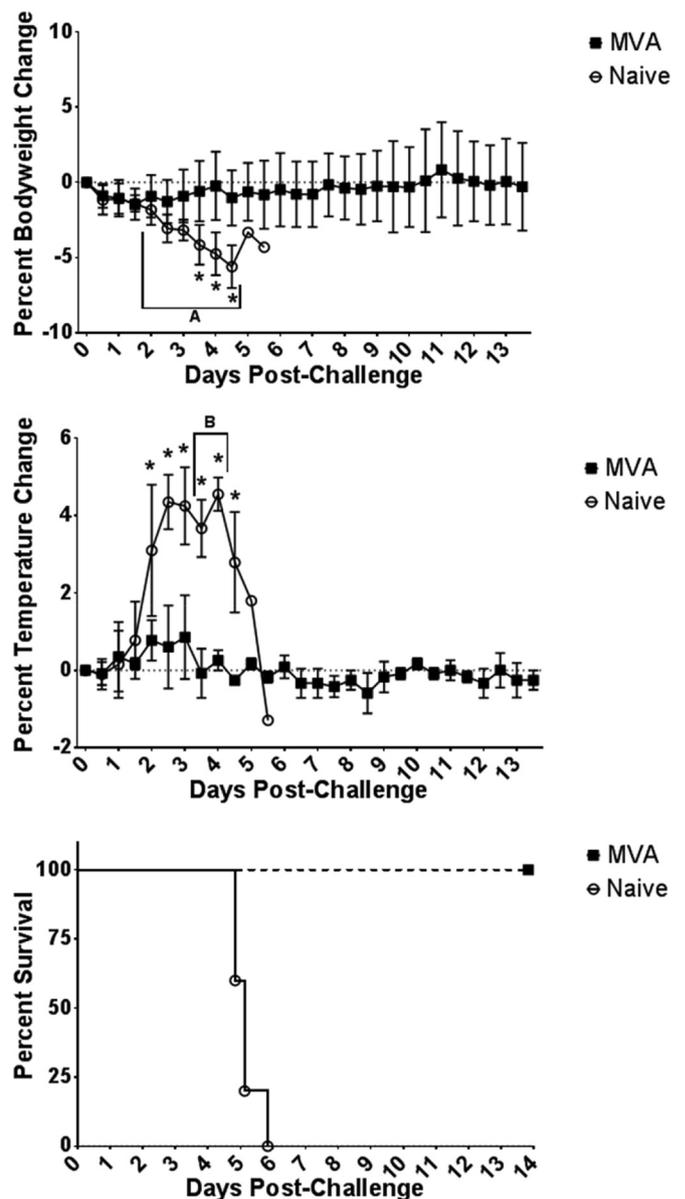


FIG 2 Intramuscular immunization with MVA provides long-term protection against RPXV. NZW rabbits ($n = 4$) were immunized intramuscularly with 1×10^8 PFU of MVA on days 0 and 28. On day 297, rabbits were intranasally inoculated with 1×10^5 PFU of rabbitpox, Utrecht strain. Rabbit body weight, temperature, and clinical signs were monitored twice each day. Rabbits reaching the humane endpoint of 5% weight loss combined with nasal or ocular discharge were euthanized. *, statistically significantly different ($P < 0.05$) from MVA-immunized rabbits as determined by repeated-measures 2-way ANOVA with Sidak's multiple-comparison test. Comparisons of weight and temperature were not made after day 4.5 due to $<100\%$ survival. In naive rabbits, there was a significant loss of body weight (A) and significant increase in body temperature (B) when comparing postchallenge to prechallenge absolute body weight or temperature as determined by repeated-measures one-way ANOVA ($P < 0.05$). Survival was statistically significantly different from that in naive rabbits, as determined by the Mantel-Cox log-rank test ($P < 0.005$). naive, $n = 5$; MVA, $n = 4$ for body weight and survival, and $n = 3$ for temperature.

nized because they met the humane endpoint criteria (5% weight loss and ocular or nasal discharge). By the sixth day, all naive rabbits were euthanized (Fig. 2). Our observed course of clinical symptoms in naive rabbits is consistent with the pathogenesis after

intranasal challenge previously reported by others (25, 32). MVA-vaccinated rabbits were monitored for a total of 14 days postchallenge. None of the MVA-vaccinated rabbits developed clinical symptoms or any temperature or body weight changes of >2.5%. Additionally, RPXV-specific serum IgG and neutralization increased after challenge (postchallenge day 14), although the increases were not significantly different from the day 289 titers (Fig. 1). Therefore, two intramuscular doses of MVA provided 100% protection against a lethal high-dose intranasally administered RPXV challenge 9 months after vaccination. Our results indicate the ability of MVA to induce durable protection against an RPXV challenge in rabbits. These results parallel those showing the ability of MVA to produce long-term protection against MPXV in nonhuman primates and support the use of rabbits as a host species suitable for evaluating next-generation smallpox vaccines developed for maternal or intranasal immunization (23, 24).

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The following reagents were obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID: recombinant from baculovirus, NR-2622; vaccinia virus (WR) A33R protein with C-terminal histidine tag, recombinant from baculovirus, NR-545; vaccinia virus (WR) B5R protein with N-terminal histidine tag, recombinant from baculovirus, NR-546; vaccinia virus (WR) L1R protein with C-terminal histidine tag, recombinant from baculovirus, NR-2625; and polyclonal anti-vaccinia virus (immunoglobulin G, human), NR-2632.

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