

Letter to the Editor

Immunization with modified vaccinia virus Ankara prevents eczema vaccinatum in a murine model of atopic dermatitis

To the Editor:

Eczema vaccinatum (EV) is disseminated vaccinia infection in patients with atopic dermatitis (AD).¹ EV is generally self-limited and confined to the skin, but it can progress to systemic disease, which can be fatal.¹ The reason why patients with AD are at risk for EV is not known. The observation that patients with a history of AD but no active skin lesions are susceptible to EV suggests an element of immune dysfunction in addition to alterations in skin integrity.¹ We have recently reported that BALB/c mice inoculated with vaccinia virus (VV) at sites of T_H2-biased allergic skin inflammation elicited by repeated epicutaneous ovalbumin (OVA) sensitization exhibit larger primary lesions, more satellite lesions, and higher viral loads in the skin and internal organs than mice inoculated in saline-exposed skin or unsensitized skin. These results suggest that allergic skin inflammation predisposes to disseminated vaccinia infection.²

Modified vaccinia virus Ankara (MVA) strain is being considered as a candidate smallpox vaccine for patients with AD and patients with immunodeficiency because of its low virulence and poor replication in both human cells and immunosuppressed macaque monkeys.³ We investigated the efficacy of immunization with MVA in protecting against EV in mice inoculated with VV at sites of allergic inflammation in an attempt to test the hypothesis that MVA could be useful as a vaccine to protect patients with AD both from smallpox and EV caused by exposure to persons recently immunized with VV.

BALB/c mice were epicutaneously sensitized with OVA or saline as a control over 7 weeks by using three 1-week sensitization cycles separated by 2-week rest intervals, as previously described.² Three weeks into the epicutaneous sensitization period, mice in each group were immunized with 10⁷ plaque-forming units (pfu)/mouse of MVA (American Type Culture Collection, VR-1508) administered by means of intramuscular injection or were left unimmunized. Four weeks later (ie, immediately at the end of the third cycle of sensitization), mice were inoculated with VV Western Reserve strain (American Type Culture Collection, VR-1454) by means of skin scarification at the site of epicutaneous sensitization by using 10⁷ pfu/mouse (see Fig E1 in this article's Online Repository at www.jacionline.org). Seven days after VV inoculation, both saline-sensitized control animals and OVA-sensitized mice that did not receive MVA showed a modest but significant weight loss, whereas saline-sensitized or OVA-sensitized mice that received the MVA vaccine did not exhibit weight loss (see Fig E2 in this article's Online Repository at www.jacionline.org). In mice not immunized with MVA, the size of the primary lesion was significantly larger, and the number of satellite lesions was significantly higher in OVA-sensitized mice compared with that seen in saline-sensitized control animals (Fig 1, A-C), as previously reported.² Immunization with MVA virtually abolished primary and satellite skin lesions in both OVA-sensitized mice and saline-sensitized control animals (Fig 1, A-C). Quantitative PCR analysis of VV genomes demonstrated that unimmunized OVA-sensitized mice exhibited significantly higher viral loads in the skin and internal

organs than unimmunized saline-sensitized control animals (Fig 1, D), as previously reported.² Immunization with MVA caused a significant and drastic decrease in VV loads in the skin and internal organs in both OVA- and saline-sensitized mice (Fig 1, D). MVA-specific quantitative PCR did not detect MVA genomes in the skin, draining lymph nodes, lungs, kidneys, ovaries, and spleens (data not shown) of MVA-immunized mice, which is consistent with the observation that MVA is not detectable in these tissues 48 hours after systemic MVA administration.⁴

IFN- γ inhibits VV dissemination.⁵ In contrast, T_H2 and T_H17 cytokines promote VV replication *in vivo* and *in vitro*.^{2,6} Systemic VV-driven cytokine secretion was examined by stimulating splenocytes from VV-inoculated mice with the VV-infected B-cell line A20 as antigen-presenting cells. Splenocytes from unimmunized OVA-sensitized mice secreted more IL-4, IL-13, IFN- γ , and IL-17 in response to VV than splenocytes from saline-sensitized control animals (see Fig E3, A, in this article's Online Repository at www.jacionline.org), as previously reported.² Splenocytes from MVA-immunized mice secreted significantly more IL-4, IL-13, and IFN- γ but significantly less IL-17 in response to VV stimulation compared with that seen in their unimmunized counterparts (see Fig E3, A). Vigorous IFN- γ production in MVA-immunized mice is consistent with the observation that administration of MVA induces IFN- γ .⁷ The well-documented suppression of IL-17 production by T_H1 and T_H2 cytokines⁸ might have accounted for the inhibition of IL-17 production in MVA-immunized mice. Serum levels of IFN- γ were comparable in VV-inoculated OVA-sensitized mice and saline-sensitized control animals, whereas serum IL-17 levels were significantly higher in VV-inoculated unimmunized mice sensitized with OVA compared with those seen in saline-sensitized control animals. MVA immunization resulted in significantly higher serum levels of IFN- γ but significantly lower serum levels of IL-17 in both OVA-sensitized mice and saline-sensitized control animals (see Fig E3, B). VV-specific serum IgG₁ and IgG_{2a} levels were higher in mice immunized with MVA, which is consistent with the increased production of T_H2 and T_H1 cytokines by splenocytes from these mice (see Fig E3, C).

VV inoculation of unimmunized mice in OVA-sensitized skin induced massive neutrophil infiltration compared with VV inoculation of unimmunized mice in saline-sensitized skin, which resulted in a mononuclear infiltrate (Fig 2, A), as previously reported.² Immunization with MVA caused a drastic decrease in cellular infiltration in both OVA-sensitized and saline-sensitized skin inoculated with VV (Fig 2, A). The histology of these skin sites was comparable with that of uninfected skin, with an absence of detectable neutrophils (data not shown). After VV inoculation, mRNA expression levels of *IL4*, *IL13*, and *IL17* were higher in OVA-sensitized skin compared with those seen in saline-sensitized skin (Fig 2, B). These levels were all significantly downregulated in the skin of VV-inoculated mice previously immunized with MVA (Fig 2, B). In contrast, MVA immunization caused a significant and comparable increase in the levels of *IFNG* mRNA in VV-inoculated OVA-sensitized and saline-sensitized mice compared with those seen in their unimmunized counterparts (Fig 2, B). There were no significant differences in the levels of cathelicidin-related antimicrobial peptide (*CRAMP*) mRNA among the groups (data not shown).

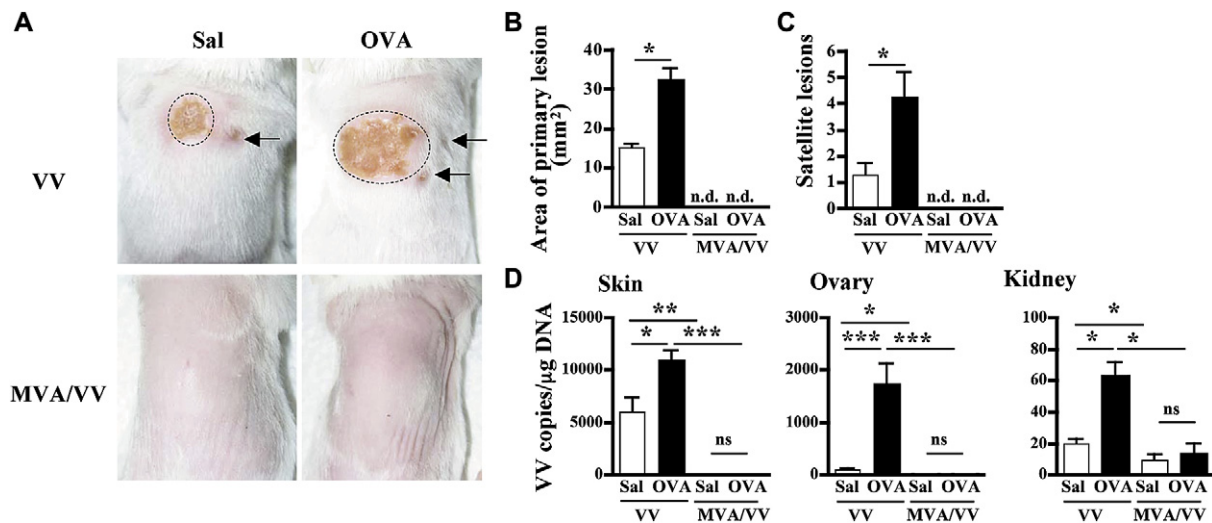


FIG 1. Immunization with MVA inhibits the development of the primary lesion, satellite lesions, and VV replication in mice inoculated with VV at the site of allergic skin inflammation. **A–C**, Primary and satellite lesions in BALB/c mice inoculated with VV in saline (Sal)- and OVA-sensitized skin (Fig 1, A), area of primary lesions (Fig 1, B), and number of satellite lesions (Fig 1, C). Dashed circles indicate primary lesions. Arrows indicate satellite lesions. Lesion sizes were analyzed with the National Institutes of Health's Image software Image J (National Institutes of Health, Bethesda, Md). **D**, Viral load in the skin, ovary, and kidney. Columns and error bars represent means and SEMs (n = 5 per group). One-way ANOVA was used to determine statistical differences between groups. **P* < .05, ***P* < .01, and ****P* < .001. n.d., Not detectable; ns, not significant.

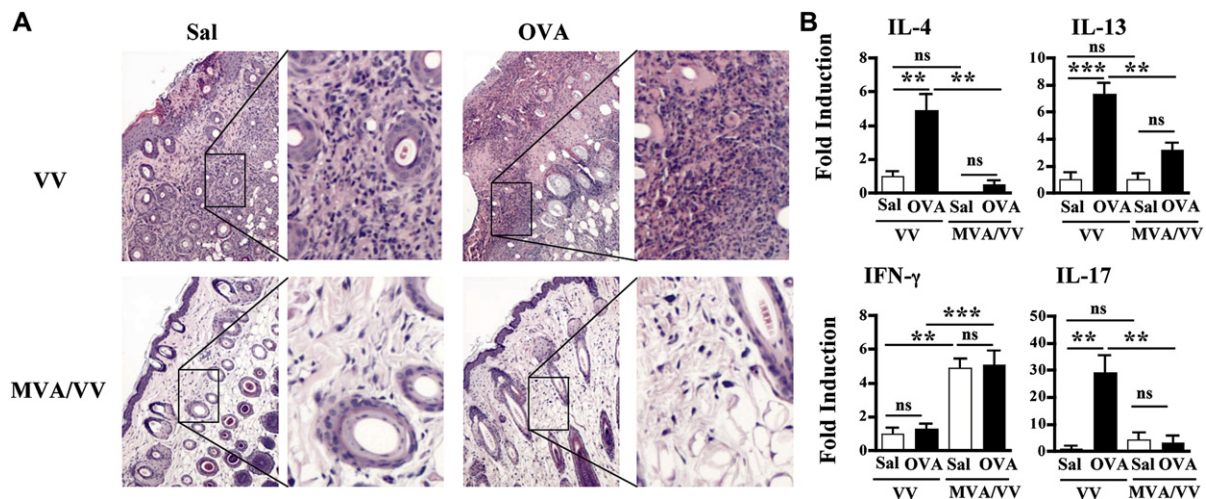


FIG 2. Cytokine expression and histology of VV-inoculated skin of mice immunized with MVA. **A**, Representative hematoxylin and eosin-stained sections of VV inoculation sites (×100 magnification; inset, ×400 magnification). **B**, Cytokine mRNA expression as fold induction relative to VV-inoculated, unimmunized, saline (Sal)-sensitized skin. Cytokine expression in the skin was assessed by means of quantitative PCR.² One-way ANOVA was used to determine statistical differences between groups. Columns and error bars represent means and SEMs (n = 5 per group). ***P* < .01 and ****P* < .001. ns, Not significant.

We propose that cutaneous inoculation of VV in MVA-immunized mice selectively amplifies local IFN- γ production by T_H1 memory T cells previously educated by MVA because of the action of viral products, such as DNA, which engages Toll-like receptor 9 on antigen-presenting cells in the skin. Increased local IFN- γ production would inhibit local T_H2 and IL-17 cytokine expression, creating a milieu unfavorable for VV replication in the skin and subsequent development of primary and satellite

lesions. Viral particles that escape from the skin of MVA-immunized mice would also be contained by a systemic milieu of high IFN- γ and low IL-17 levels.

Our data demonstrate that MVA immunization is highly effective in protecting mice with allergic skin inflammation from the development of EV and suggest that MVA could be useful as a safe smallpox vaccine in patients with AD, particularly those with a history of eczema herpeticum who

demonstrate abnormalities in their production of and response to IFN- γ .⁹

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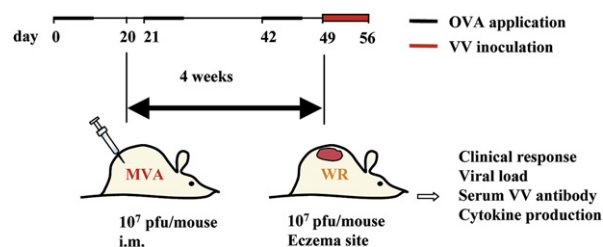


FIG E1. Experimental design of MVA immunization using a murine model of EV. Mice were epicutaneously sensitized with OVA or saline for three 1-week cycles with 2-week rest intervals (total of 7 weeks) followed by inoculation of 10^7 pfu/mouse by means of skin scarification. Mice were killed after 7 days of VV inoculation and examined for clinical responses, viral load, serum levels of VV antibodies, and cytokine production, as previously described.² MVA was administered by means of intramuscular (*i.m.*) injection in the outer thigh 4 weeks before VV inoculation. *WR*, Western Reserve.

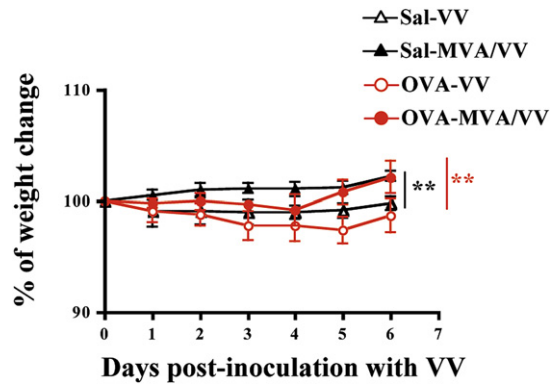


FIG E2. Immunization with MVA protects saline (*Sal*)-sensitized and OVA-sensitized mice from weight loss caused by VV inoculation at the sites of allergic skin inflammation. The percentage of weight change relative to day 0 of VV inoculation is shown. *Error bars* represent means and SEMs ($n = 5$ per group). The 2-tailed Student *t* test was used to determine statistical differences between each set of 2 groups. $**P < .01$.

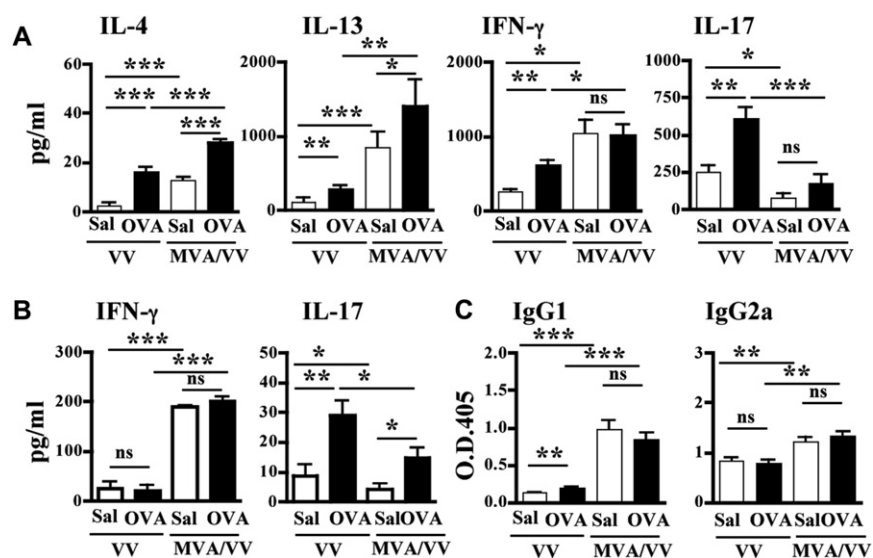


FIG E3. VV-specific systemic immune responses in mice immunized with MVA. **A**, VV-specific cytokine production by splenocytes after stimulation with VV-infected A20 cells. **B**, Serum levels of IFN- γ and IL-17. **C**, Serum levels of VV-specific IgG₁ and IgG_{2a} antibodies. VV-specific IgG levels were analyzed as described previously.² Columns and error bars represent means and SEMs ($n = 5$ per group). One-way ANOVA was used to determine statistical differences between groups. * $P < .05$, ** $P < .01$, and *** $P < .001$. *ns*, Not significant; *Sal*, saline.