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

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Summary

Immunization with modified vaccinia virus Ankara (MVA) protects mice with allergic skin inflammation from developing eczema vaccinatum (EV), suggesting that immunization with MVA would be effective in preventing EV in patients with atopic dermatitis.

Keywords

Eczema vaccinatum; modified vaccinia virus Ankara; Th1; Th2; Th17 cytokines

To the Editor:

Eczema vaccinatum (EV) is disseminated vaccinia infection in individuals 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 Th2-biased allergic skin inflammation elicited by repeated epicutaneous (EC) ovalbumin (OVA) sensitization exhibit larger primary lesions, more satellite lesions, and higher viral loads in 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 AD patients and patients with immunodeficiency because of its low virulence and poor replication in human cells and in 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 secondary to exposure to individuals recently immunized with vaccinia virus. BALB/c mice were EC sensitized with OVA, or saline as control, over 7 weeks using three one-week sensitization cycles separated by two-week rest intervals, as previously described ². Three

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weeks into the EC sensitization period, mice in each group were immunized with 10^7 plaque forming units (pfu)/mouse MVA (ATCC, VR-1508) given by intramuscular injection, or were left unimmunized. Four weeks later, i.e. immediately at the end of the third cycle of sensitization, mice were inoculated with VV Western Reserve strain (ATCC, VR-1454) by skin scarification at the site of EC sensitization using 10^7 pfu/mouse (Figure S1). Seven days after VV inoculation, both saline-sensitized controls and OVA-sensitized mice which 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 (Figure S2). In mice not immunized with MVA, the size of primary lesions was significantly larger and the number of satellite lesions was significantly higher in OVA-sensitized mice compared to saline-sensitized controls (Figure 1A–C) as previously reported ². Immunization with MVA virtually abolished primary and satellite skin lesions in both OVA-sensitized mice and saline-sensitized controls (Figure 1A–C). Quantitative PCR (qPCR) analysis of VV genomes demonstrated that unimmunized OVA-sensitized mice exhibited significantly higher viral loads in skin and internal organs than unimmunized saline-sensitized controls (Figure 1D), 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 (Figure 1D). MVA-specific qPCR failed to detect MVA genomes in skin, draining lymph nodes, lung, kidney, ovary, and spleen (data not shown) of MVA immunized mice, 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, Th2 and Th17 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 controls (Figure S3A), 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 to their unimmunized counterparts (Figure S3A). 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 Th1 and Th2 cytokines ⁸ may 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 controls, whereas serum IL-17 levels were significantly higher in VV inoculated unimmunized mice sensitized with OVA compared to saline sensitized controls. 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 controls (Figure S3B). VV-specific serum IgG1 and IgG2a levels were higher in mice immunized with MVA, consistent with increased production of Th2 and Th1 cytokines by splenocytes from these mice (Figure S3C).

VV inoculation of unimmunized mice in OVA-sensitized skin induced massive neutrophil infiltration compared to VV inoculation of unimmunized mice in saline-sensitized skin which resulted in a mononuclear infiltrate (Figure 2A) as previously reported ². Immunization with MVA caused a drastic decrease in cellular infiltration in VV inoculated OVA-sensitized and saline-sensitized skin (Figure 2A). The histology of these skin sites was comparable to that of uninfected skin, with absence of detectable neutrophils (data not shown). Following VV inoculation, mRNA expression levels of IL-4, IL-13, and IL-17 were higher in OVA-sensitized skin compared to saline-sensitized skin (Figure 2B). These levels were all significantly downregulated in the skin of VV inoculated mice previously immunized with MVA (Figure 2B). In contrast, MVA immunization caused a significant and comparable increase in the levels of IFN- γ mRNA in VV inoculated OVA-sensitized

and saline-sensitized mice compared to those in their unimmunized counterparts (Figure 2B). There was no significant differences in the levels of cathelicidin-related antimicrobial peptide (CRAMP) mRNA among the groups (data not shown).

We propose that cutaneous inoculation of VV in MVA immunized mice selectively amplifies local IFN- γ production by Th1 memory T cells previously educated by MVA because of the action of viral products, such as DNA which engages TLR9, on antigen presenting cells in the skin. Increased local IFN- γ production would inhibit local Th2 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.

Our data demonstrates that MVA immunization is highly effective in protecting mice with allergic skin inflammation from the development of EV and suggests 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Immunization with MVA inhibits development of 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- and OVA-sensitized skin (**A**), area of primary lesions (**B**) and number of satellite lesions (**C**). Dashed circles indicate primary lesions. Arrows indicate satellite lesions. Lesion sizes were analyzed using NIH Image software Image J (National Institutes of Health, Bethesda, MD). **D.** Viral load in skin, ovary and kidney. Columns and error bars represent mean and SEM (n=5 per group). One-way ANOVA was used to determine statistical differences between groups. *p<0.05, **p<0.01, *** p<0.001. n.d.= not detectable. n.s. = not significant.

Figure 2. Cytokine and histology of VV inoculated skin of mice immunized with MVA

A. Representative H&E-stained sections of VV inoculation sites (100 $\times$ magnification. Inset 400 $\times$). **B.** Cytokine mRNA expression as fold induction relative to VV inoculated unimmunized saline skin. Cytokine expression in the skin was assessed by qPCR². One-way ANOVA was used to determine statistical differences between groups. Columns and error bars represent mean and SEM (n=5 per group). **p<0.01, *** p<0.001. n.s. = not significant.