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Vaccinia immune globulin ameliorates eczema vaccinatum in a murine model of atopic dermatitis

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To the Editor

Recent concern that vaccinia virus (VV) might be used as a bioterror weapon has led to contingency plans for mass vaccination; however, a major concern is that patients with atopic dermatitis (AD) are susceptible to eczema vaccinatum (EV), a complication of smallpox vaccination (Boguniewicz and Leung; Copeman and Banatvala, 1971). This has prompted the National Institutes of Health/National Institute of Allergy and Infectious Diseases to form the Atopic Dermatitis Vaccinia Network for EV research. In affected individuals, VV spreads through skin, resulting in large primary lesions and satellite lesions, and infects internal organs. Vaccinia immune globulin (VIG) has been used to prevent and treat EV in AD patients accidentally exposed to, or inoculated with, VV (Copeman and Banatvala, 1971; Goldstein *et al.*, 1975). Studies in the literature reveal a mean EV mortality rate of 4% in AD patients treated with VIG, compared to 5% and 40% in two studies of historical controls (Hopkins and Lane, 2004). Four controlled studies and one observational study reported promising results with the use of VIG to prevent smallpox in contacts of patients with documented smallpox (Hopkins and Lane, 2004). However, there have been no controlled trials to establish the efficacy of VIG in the prevention and treatment of EV in AD patients.

We previously reported that BALB/c mice inoculated with VV at sites of Th2-biased allergic skin inflammation elicited by epicutaneous (EC) ovalbumin (OVA) sensitization exhibit features of EV, including satellite lesions and VV dissemination (Oyoshi *et al.*, 2009). We used this mouse model to examine the efficacy of VIG in attenuating EV. BALB/c mice were EC sensitized with OVA or saline over 7 weeks (49 days), then

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Conflict of Interest

The authors state no conflict of interest.

immediately inoculated with VV Western Reserve strain (ATCC, VR-1454) by skin scarification at the site of EC sensitization using 10^7 PFU/mouse, and euthanized seven days later. OVA-sensitized mice were divided into four groups: Three received a single intraperitoneal injection of 10 mg/mouse of polyclonal anti-VV immunoglobulin (ATCC, NR-2632) on day-1, +1, or +3 of VV inoculation; a fourth group received on day-1 the same dose of control immune globulin (CIG) prepared in-house from donors never vaccinated with VV (Figure S1). VV inoculation of CIG-treated mice in OVA-sensitized skin sites resulted in modest, but significant, weight loss compared to mice inoculated with VV at saline-exposed sites. All three groups of VIG-treated OVA-sensitized mice were protected against weight loss (Figure S2). Mice injected with VIG at days-1 and +1, but not +3, developed significantly smaller primary lesions and lower numbers of satellite lesions compared to CIG-treated mice (Figure 1a–c). Quantitative PCR analysis of VV genomes demonstrated that all three groups of VIG-treated mice had significantly decreased viral loads in inoculated skin and internal organs compared to CIG-treated controls (Figure 1d). The reduction of satellite lesions in day+1 treated mice is consistent with a previous study (Shearer *et al.*, 2005). The failure of day+3 VIG administration to affect skin lesions despite drastically reducing viral load suggests that delayed VIG treatment could have allowed greater early viral replication resulting in more robust cutaneous inflammation.

VV-encoded epidermal growth factor and anti-apoptotic protein F1L promote cell survival (Postigo *et al.*, 2009) and VV antigens colocalize with proliferating keratinocytes (Fisher *et al.*). VV inoculation in OVA-sensitized skin resulted in a significant increase in epidermal thickness in CIG-treated mice (Figure 2a,b). This was inhibited by VIG treatment. IFN-γ inhibits (Combadiere *et al.*, 2004), while Th2 and Th17 cytokines promote VV replication *in vivo* and *in vitro* (Howell *et al.*, 2006; Oyoshi *et al.*, 2009). OVA-sensitized skin of CIG-treated mice exhibited an intense infiltrate with neutrophils, and an increase in local mRNA expression of Th2 cytokines (IL-4 and IL-13), IL-17, with no significant change in IFN-γ (Figure 2c). Cellular infiltration at VV-inoculated sites, which was predominantly neutrophilic, was decreased more in mice treated with VIG at days-1 and +1, than day+3 (Figure 2a, b). All three groups of VIG-treated mice showed decreased levels of Th2 cytokines in VV-inoculated skin compared to CIG-treated mice, with no change in IFN-γ (Figure 2c). Mice treated with VIG at days-1 and +1, but not day+3, exhibited significantly decreased levels of IL-17 mRNA expression in VV-inoculated skin (Figure 2b). The failure of day+3 VIG-treated mice to decrease IL-17 levels correlates with the persistence of neutrophils and of inflammatory lesions in their skin, and is consistent with IL-17 being critical for neutrophil infiltration in our EV model (Oyoshi *et al.*, 2009).

Splenocytes from all three groups of VIG-treated mice secreted significantly less IL-4, IL-13, IFN-γ and IL-17 in response to VV stimulation compared to splenocytes from CIG-treated mice ($p<0.01$, Figure S3a). This is likely due to the decreased antigenic stimulation in VIG-treated mice. However, the protective VV-specific IgG2a antibody response (Xu *et al.*, 2004), was comparable in all groups, indicating that VV-treated mice generated sufficient T-helper cell activity to drive normal IgG2a production (Figure S3b). In humans, VIG prophylaxis does not interfere with the vaccination reaction (Nanning, 1962).

Our results are in agreement with a recent study that used neutralizing human monoclonal antibodies in VV-infected NC/Nga mice (Tomimori *et al.*, 2011). Several mechanisms may account for the efficacy of VIG in limiting viral spread. Direct neutralization of the virus is likely. The IgG Fc region in VV-VIG antigen-antibody complexes and anti-idiotypic-anti-VIG complexes might activate $Fc\gamma R^+$ effector cells (e.g. macrophages and NK cells) to limit VV replication (Ballow, 2011). Further studies are needed to understand the precise mechanisms of action of VIG in attenuating EV.

The dose of VIG we used is equivalent to a dose of 500 mg/kg in humans, which is close to recommended dose for treatment of EV (400 mg/kg). Thus, our data suggest that VIG may be effective in preventing and treating EV. We recently showed that attenuated VV strain Modified Vaccinia virus Ankara (MVA) protects mice inoculated with VV in OVA-sensitized skin from VV infection (Oyoshi *et al.*). A dual strategy of MVA immunization and VIG administration should be evaluated in preventing EV in patients with AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AD	atopic dermatitis
CIG	control immune globulin
EC	epicutaneous
EV	eczema vaccinatum
OVA	ovalbumin
VIG	vaccinia immune globulin
VV	vaccinia virus

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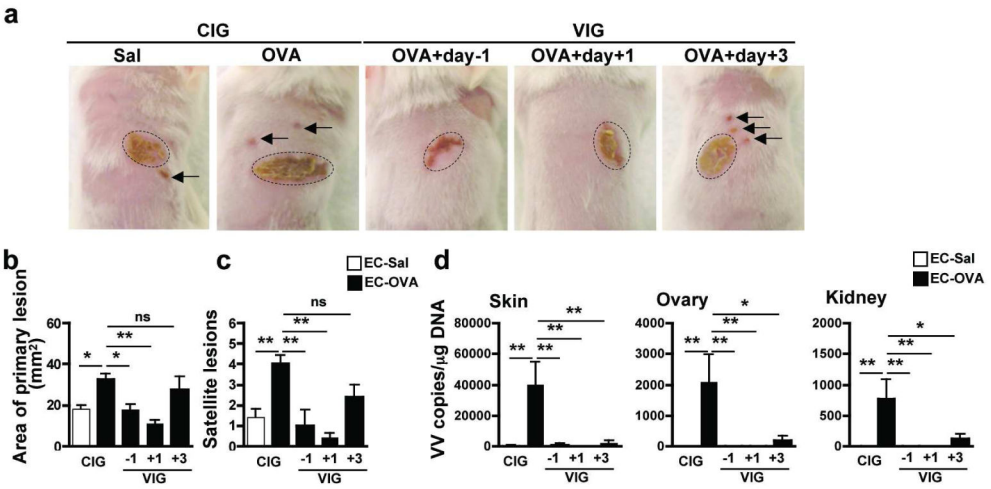

Figure 1. Treatment with VIG decreases the size of primary lesions, the number of satellite lesions, and VV dissemination caused by VV inoculation at the sites of allergic skin inflammation

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)** 7 days after VV inoculation. Mice were treated with control immunoglobulin (CIG) or vaccinia immunoglobulin (VIG) on day −1, +1, or +3 of VV inoculation. Polyclonal Anti-Vaccinia Virus (immune globulin G, Human), NR-2632 was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID. Dashed circles indicate primary lesions. Arrows indicate satellite lesions. Lesion sizes were analyzed using NIH Image software Image J. **d.** Viral load in skin and internal organs. Viral genomes were quantified by real-time PCR as described previously (Oyoshi *et al.*, 2009). 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. ns = not significant.

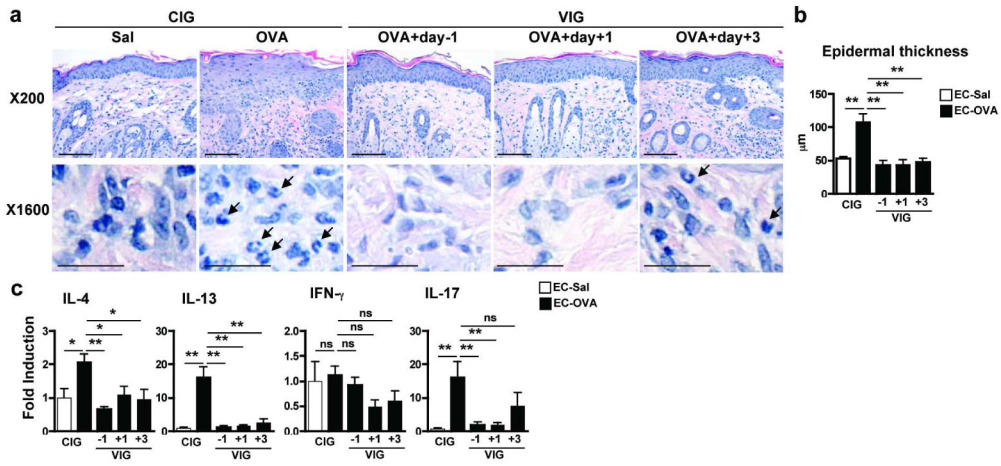

Figure 2. Cytokine and histology of VV-inoculated skin of mice treated with VIG

a. Representative H&E-stained sections of VV inoculation sites. Scale bars indicate 100 μm (X200 magnification) or 25 μm (X1600 magnification). Arrows indicate neutrophils. **b.** Epidermal thickness. **c.** Cytokine mRNA expression as fold induction relative to VV-inoculated saline-exposed skin. Cytokine expression in the skin was assessed by quantitative real-time PCR (Oyoshi *et al.*, 2009). One-way ANOVA was used to determine statistical differences between groups. Columns and error bars represent mean and SEM (n=5 per group). *p<0.05, **p<0.01. ns = not significant.