

## Impaired immune response to vaccinia virus inoculated at the site of cutaneous allergic inflammation

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**Background:** Patients with atopic dermatitis (AD) exposed to the vaccinia virus (VV) smallpox vaccine have an increased risk of developing eczema vaccinatum.

**Objective:** To investigate the effects of local allergic skin inflammation on vaccinia immunity.

**Methods:** BALB/c mice were epicutaneously sensitized with ovalbumin (OVA) to induce allergic skin inflammation or with saline control, then inoculated with an attenuated VV strain by skin scarification or intraperitoneally. After 8 days, serum IgG anti-VV and cytokine secretion by splenocytes were measured.

**Results:** Mice inoculated with VV at sites of epicutaneous sensitization with OVA, but not control mice inoculated at saline exposed sites, developed satellite pox lesions and had impaired secretion of  $T_H1$  cytokines in response to VV, decreased VV specific serum IgG<sub>2a</sub>, increased VV specific serum IgG<sub>1</sub>, and impaired upregulation of IFN- $\alpha$ , but not the cathelicidin-related antimicrobial peptide, at the infection site. The VV immune response of OVA-sensitized mice inoculated with VV at distant skin sites or intraperitoneally was normal. **Conclusion:** Local immune dysregulation at sites of allergic skin inflammation underlies the impaired  $T_H1$  immune response to VV introduced at these sites and the increased susceptibility to develop satellite pox lesions, a characteristic of eczema vaccinatum in patients with AD.

**Clinical implications:** In a mouse model of AD, inoculation of VV at inflamed skin sites is associated with increased numbers of satellite pox lesions and an abnormal immune response to the virus. This may contribute to the susceptibility of patients with AD to virus dissemination after smallpox vaccination. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

**Key words:** Eczema vaccinatum, allergy, vaccinia virus, smallpox vaccination, viral response,  $T_H1/T_H2$  cells

Smallpox, which is caused by infection with variola virus, has a fatality rate as high as 30%.<sup>1</sup> Prophylactic smallpox vaccination with vaccinia virus (VV) is the only known protection. Smallpox was eradicated in 1979 by mass immunization with VV. The only known stocks of variola virus are stored near Novosibirsk, Russia, and at the Centers for Disease Control and Prevention in Atlanta. Concern has recently increased about the potential misuse of variola virus as a bioterrorism weapon and has led to consideration of resuming mass immunization against smallpox. Although effective, VV immunization is associated with significant complications.<sup>2</sup> One of these complications is eczema vaccinatum (EV), which is caused by localized and/or systemic viral dissemination that can be lethal. Patients with AD are predisposed to develop EV, which occurs in roughly 10 to 40 per million vaccinees with an approximately equal number of secondary cases in contacts of vaccinees.<sup>2,3</sup> Although EV is usually confined to the skin and self-limited, it can progress to systemic disease. Mortality may be as high as 6% in cases involving vaccinees, and as high as 4% in secondary cases.<sup>4,5</sup> Although smallpox vaccination is contraindicated in patients with atopic dermatitis (AD), they may develop EV after contact with a recently immunized individual, as has been documented lately in a child whose father had received the vaccine.<sup>6</sup> The immunologic basis of the enhanced susceptibility of patients with AD to EV is not well understood.

Atopic dermatitis is a genetically determined pruritic chronic relapsing inflammatory skin disease that currently affects as many as 20% of children in the United States.<sup>7</sup> In acute lesions, CD4<sup>+</sup> cells are predominantly of the  $T_H2$

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Supported by federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract number HHSN266200400030C (Atopic Dermatitis Vaccinia Network).

Disclosure of potential conflict of interest: J. Scott has consulting arrangements with Alicare Medical Management, owns Pharmaceutical holder trust stock, and is on the speakers' bureau for Verus Pharmaceuticals. The rest of the authors have declared that they have no conflict of interest.

Received for publication April 9, 2007; revised July 30, 2007; accepted for publication August 1, 2007.

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doi:10.1016/j.jaci.2007.08.004

*Abbreviations used*

|        |                                            |
|--------|--------------------------------------------|
| AD:    | Atopic dermatitis                          |
| CRAMP: | Cathelicidin-related antimicrobial peptide |
| DC:    | Dendritic cell                             |
| DMEM:  | Dulbecco modified Eagle medium             |
| EV:    | Eczema vaccinatum                          |
| LN:    | Lymph node                                 |
| OVA:   | Ovalbumin                                  |
| pDC:   | Plasmacytoid dendritic cell                |
| PFU:   | Plaque-forming unit                        |
| VV:    | Vaccinia virus                             |

type and express IL-4, IL-5, and IL-13, whereas in chronic lesions, IFN- $\gamma$ -producing T<sub>H</sub>1 cells may dominate.<sup>7</sup> AD is associated with a systemic allergic response evidenced by elevated serum IgE levels, peripheral blood eosinophilia, and a propensity of T cells to secrete T<sub>H</sub>2 cytokines in response to anti-CD3 stimulation.<sup>7</sup>

Both the T<sub>H</sub>1 cytokine IFN- $\gamma$  and the IgG<sub>2a</sub> antibody response it drives have been shown to be important for viral clearance and containment in mice.<sup>8-10</sup> In contrast, the T<sub>H</sub>2 cytokines IL-4 and IL-10 inhibit viral clearance and increase susceptibility for VV dissemination.<sup>11,12</sup> We hypothesized that the susceptibility of patients with AD to EV may result from an altered T<sub>H</sub> immune response to VV. This could be caused by local immune dysregulation in the inflamed skin where the virus is introduced. Alternatively, it may result from a globally altered systemic immune response.

To address these questions, we took advantage of a murine model of allergic skin inflammation we have established that mimics many aspects of the allergic skin inflammation and the systemic T<sub>H</sub>2 response observed in patients with AD.<sup>13-15</sup> We compared the clinical and immune response of epicutaneously sensitized mice to VV inoculation at sites of allergic skin inflammation, distant skin sites, and intraperitoneally. We used an attenuated VV strain because efforts are currently aimed at examining the safety and efficacy of attenuated VV strains (eg, Modified Vaccinia Ankara) for the immunization of immunodeficient subjects and patients with AD.<sup>16</sup> Our results show that inoculation of an attenuated VV strain at sites of allergic skin inflammation, but not at distant skin sites or intraperitoneally, was associated with a impairment of the T<sub>H</sub>1 immune response to the virus and the development of satellite pox lesions, a characteristic feature of EV.

## METHODS

### Virus source and expansion

The VV Western Reserve Thymidine kinase mutant strain (VTK-79L) was obtained from American Type Culture Collection (Manassas, Va; VR-2056). CV1 cells (American Type Culture Collection; CCL-70) grown to 80% confluence in Dulbecco modified Eagle medium (DMEM) supplemented with 10% FCS for propagation of the virus were rinsed and overlaid with DMEM/2.5% FCS containing VV at a ratio of 1 plaque-forming unit (PFU) per cell. Then a 10-fold volume of DMEM/2.5% FCS was added, and infected

cells were incubated at 37°C in 5% CO<sub>2</sub> for 2 days. VV was harvested after disruption of the cells by 3 freeze-thaw cycles, and its titer was assessed by a standard plaque-forming assay using CV1 cells.<sup>17</sup>

### EC sensitization and vaccinia infection protocols

Female BALB/c mice 4 to 6 weeks old were EC sensitized as described previously.<sup>13</sup> After five 1-week exposures to the patches separated by 2-week intervals, mice were inoculated with VV at the site of EC sensitization or at a site on the upper back removed as far as possible from the sensitization patch (Fig 1, A). Ten microliters of PBS containing  $1 \times 10^7$  PFU VV was placed on the skin, followed by administration of 20 superficial scratches using a 27½-gauge needle, and the inoculation site was covered with a gauze patch and Tegaderm (Westnet Inc, Canton, Mass). Mice were weighed daily and examined for lethargy and the development of satellite lesions and were killed 8 days after infection or at days 1, 2, 4, and 8 to determine viral loads in skin. In other experiments, EC sensitized mice were inoculated with VV intraperitoneally using  $5 \times 10^6$  PFU in 200  $\mu$ L DMEM, and their immune response was examined 8 days later. All experiments were reviewed and authorized by the Children's Hospital Institutional Animal Care and Use Committee.

### Microscopic analysis of skin

Skin sections of 4  $\mu$ m were stained with hematoxylin and eosin. CD4 staining of frozen skin sections was performed as previously described.<sup>13</sup> Positive cells were counted blinded in 10 to 15 high-power fields.

### Quantitative PCR analysis of VV genome copies

Tissue samples were immediately frozen and stored at -80°C. Quantification of VV genomes was performed as previously described.<sup>18</sup>

### In vitro cytokine synthesis of splenocytes

One million splenocytes were incubated with  $1 \times 10^6$  A-20 (H2<sup>d</sup>) B cells derived from a mouse B-cell lymphoma line that had been infected with VV for 24 hours or left uninfected, then irradiated. As positive control, cells were incubated in plates coated with 0.2  $\mu$ g/mL anti-CD3 $\epsilon$  chain (clone 145-2C11) mAb (BD Bioscience Pharmingen, San Diego, Calif). Supernatants were collected after 24 hours (for IFN- $\gamma$  and IL-2 detection) and 72 hours (for IL-4, IL-5, and IL-13 detection), and cytokine concentration was determined by ELISA (BD Bioscience Pharmingen).

### Vaccinia specific IgG<sub>2a</sub> and IgG<sub>1</sub> antibody ELISA

Flat-bottom 96-well microtiter plates (Immulon 2HB, Thermo Scientific, Milford, Mass) were coated overnight with  $1 \times 10^6$  PFU/well of purified inactivated VV (Advanced Biotechnologies, Columbia, Md). After blocking with 1% BSA/PBS for 2 hours, sera were added, diluted 1:10 for IgG<sub>2a</sub> and 1:1000 for IgG<sub>1</sub> in duplicate, and incubated overnight at 4°C. Biotin-conjugated goat antibodies against mouse IgG<sub>2a</sub> and IgG<sub>1</sub> (BD Bioscience Pharmingen) were used for detection at 2  $\mu$ g/mL. Washing steps between incubations were performed with 0.05% Tween/PBS. ODs were measured at 405 nm.

### Quantitative PCR for cytokines, cathelicidin-related antimicrobial peptide, IFN- $\alpha$ , and IFN- $\gamma$

RNA was prepared from skin homogenates, and quantitative PCR was performed as previously described<sup>19</sup> by using primers and an ABI Prism 7300 sequence detector from Applied Biosystems,

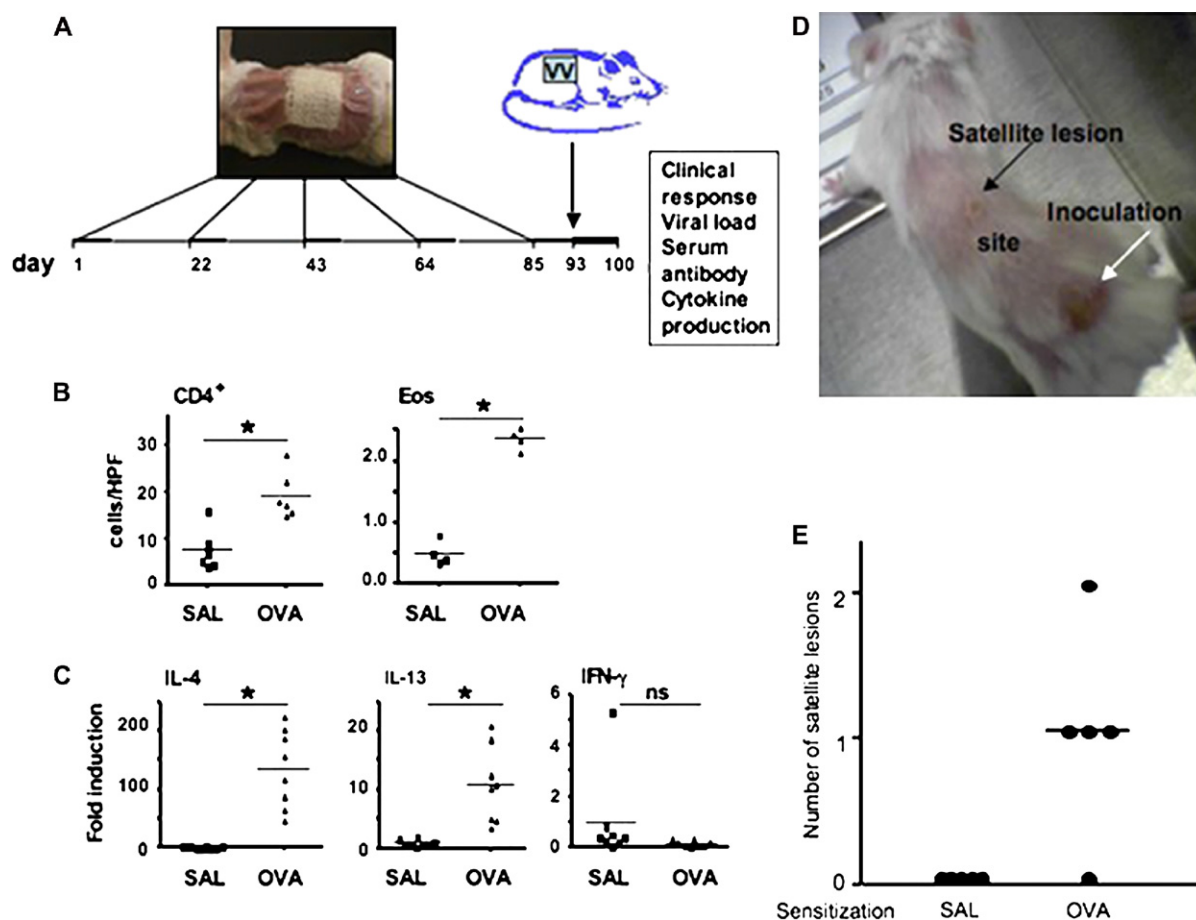


**FIG 1.** A, EC sensitization and VV infection protocol. **B and C,** Skin infiltration with CD4<sup>+</sup> T cells and eosinophils and expression of T<sub>H</sub>2 and T<sub>H</sub>1 cytokines at the time of VV inoculation. **D,** Representative satellite skin lesion. **E,** Satellite lesions in mice inoculated with VV at OVA versus saline-sensitized sites. \**P* < .05. HPF, High-power field; *Eos*, eosinophils; SAL, saline; ns, not significant.

Foster City, Calif. Relative expression levels were calculated by the relative standard curve method as outlined in the manufacturer's technical bulletin (Applied Biosystems). Quantities of all targets in test samples were normalized to the corresponding glyceraldehyde 3-phosphate dehydrogenase (GAPDH) levels in the skin and expressed as Target Gene normalized to GAPDH.

### Statistical analyses

Differences in values between experimental groups were examined for significance with GraphPad Prism software using the 2-tailed Student *t* test.

## RESULTS

### Clinical manifestations of BALB/c mice infected with vaccinia by skin scarification at the site of EC sensitization

Fig 1, *B and C*, shows that skin sites EC sensitized with ovalbumin (OVA) for five 1-week cycles exhibit allergic skin inflammation similar to that previously documented in skin sites EC sensitized with OVA for three 1-week cycles.<sup>13,14</sup> This allergic inflammation is characterized by infiltration with eosinophils and CD4<sup>+</sup> cells (Fig 1,

*B*) and increased expression of the T<sub>H</sub>2 cytokines IL-4 and IL-13, with no increase in IFN-γ expression (Fig 1, *C*).

After VV inoculation, mice in both OVA and saline-sensitized groups maintained baseline weights (data not shown). However, only OVA-sensitized mice developed lethargy and exhibited satellite skin lesions (Fig 1, *D and E*). VV genomes were detected at the inoculation site on days 1, 2, 4, and 8, with no significant differences between OVA and saline-sensitized groups (data not shown). We were unable to detect VV genomes in satellite skin lesions, suggesting that the virus was cleared from these sites by day 8. Viral loads in lung and kidney at day 8 were not significantly different between the 2 groups (data not shown).

### Cytokine expression by splenocytes of vaccinia infected BALB/c mice

There is evidence that IFN-γ promotes whereas T<sub>H</sub>2 cytokines impair the clearance of VV.<sup>11,12,20,21</sup> Splenocytes from mice inoculated with VV at saline-exposed sites mounted a robust IFN-γ and IL-2 response to VV stimulation. In contrast, splenocytes from mice inoculated with VV at OVA-sensitized sites had significantly diminished production of IFN-γ and IL-2 in response to VV (Fig

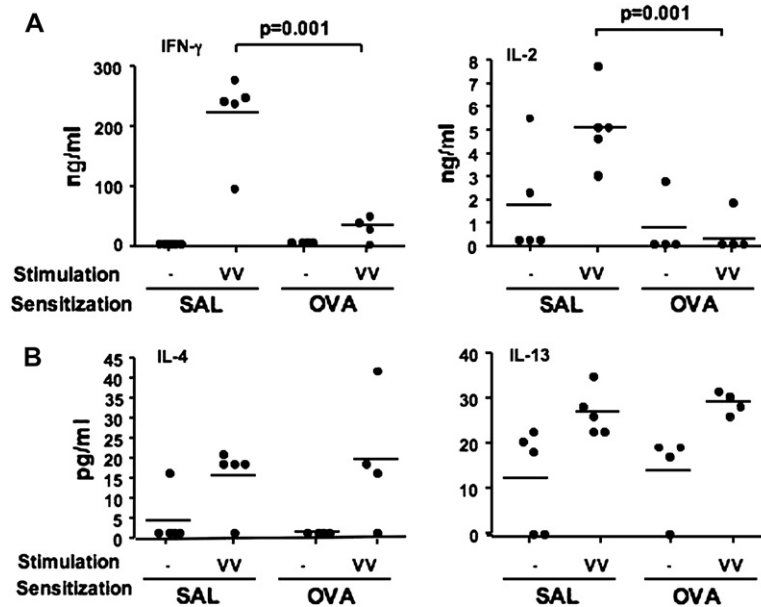


FIG 2.  $T_H1$  and  $T_H2$  cytokine production by splenocytes of mice inoculated with VV at EC sensitization sites. SAL, Saline.

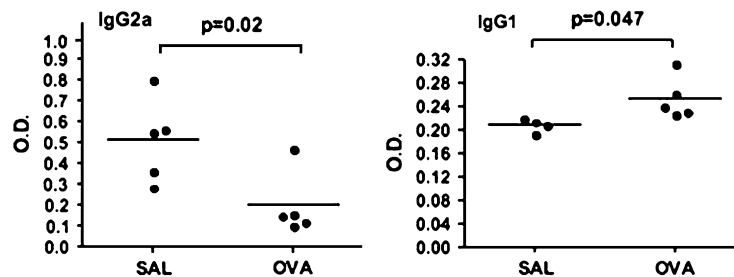


FIG 3. Serum IgG<sub>2a</sub> and IgG<sub>1</sub> anti-VV antibody levels in mice inoculated with VV at EC sensitization sites. SAL, Saline.

2, A) but comparable production of IL-4 and IL-13 (Fig 2, B). Production of IFN- $\gamma$  and IL-2 in response to anti-CD3 was comparable between OVA and saline-sensitized mice infected with VV (data not shown), ruling out a generalized impairment of  $T_H1$  cytokine production in OVA-sensitized mice.

#### Decreased vaccinia specific IgG<sub>2a</sub> antibody response in mice inoculated with VV in OVA-sensitized skin sites

Immunization with live VV induces protective IgG antibody.<sup>9</sup> The vaccinia-specific serum IgG<sub>2a</sub> antibody level was significantly decreased in mice inoculated at OVA-sensitized sites relative to mice inoculated at saline-exposed sites ( $P = .02$ ), whereas the vaccinia-specific serum IgG<sub>1</sub> antibody level was modestly increased with borderline significance ( $P = .047$ ; Fig 3). Because IFN- $\gamma$  is important for IgG<sub>2a</sub> responses and inhibits IgG<sub>1</sub> responses,<sup>22</sup> these findings support the observation that the IFN- $\gamma$  response to VV is impaired in mice inoculated with VV in OVA-sensitized skin.

Vaccinia virus inoculation at a skin site distant from the site of EC sensitization with OVA or intraperitoneally elicits a virtually normal immune response to VV in EC sensitized mice. The impaired  $T_H1$  response of mice to VV infection at the site of EC sensitization with OVA may be a result of a global predisposition of these mice to mount a  $T_H2$  response or/and to a local effect of allergic skin inflammation on the response to VV introduced in EC sensitized skin. To distinguish between these possibilities, we examined the response to VV inoculated at a distant skin site. OVA-sensitized mice inoculated at a distant skin site exhibited no lethargy and did not develop satellite skin lesions, and their splenocytes exhibited only a modest, albeit significant, 2-fold decrease in IFN- $\gamma$  production in response to VV (Fig 4, A). Secretion of the  $T_H2$  cytokines IL-4 and IL-13 as well as IgG<sub>2a</sub> and IgG<sub>1</sub> responses were comparable in the 2 groups (Fig 4, B, and data not shown).

To confirm that EC sensitization has little effect on the response to VV introduced at sites other than the sensitization site, we examined the immune response to VV

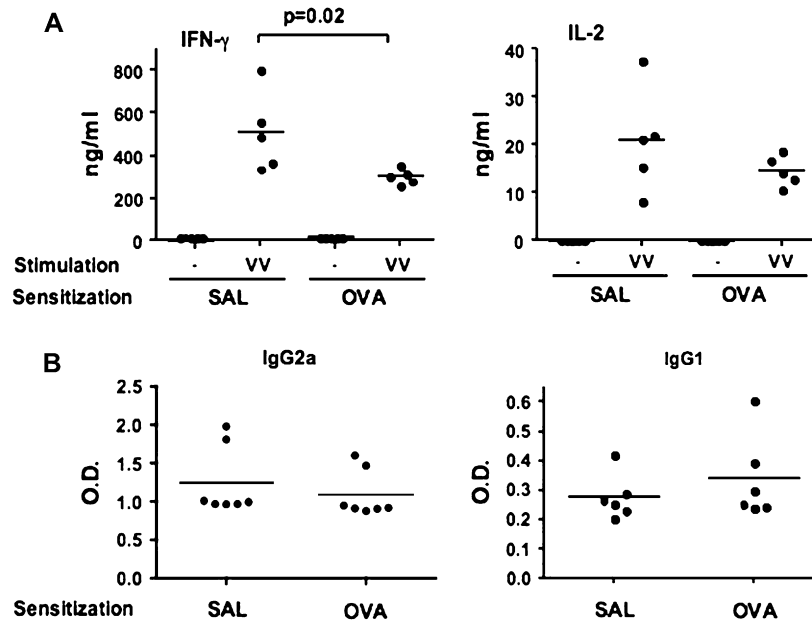


**FIG 4.** T<sub>H</sub>1 cytokine production (A) and serum anti-VV Ab levels (B) in EC sensitized mice inoculated with VV at a distant skin site. SAL, Saline.

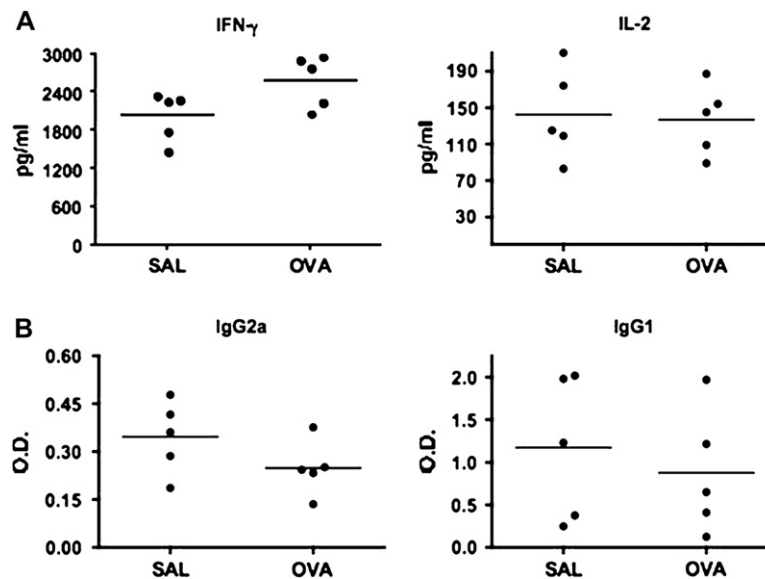


**FIG 5.** T<sub>H</sub>1 cytokine production (A) and serum anti-VV Ab levels (B) in EC sensitized mice inoculated intraperitoneally with VV. SAL, Saline.

inoculation in the peritoneal cavity. Splenocytes from mice EC sensitized with OVA and intraperitoneally inoculated with VV produced similar amounts of IFN- $\gamma$  and IL-2 in response to VV stimulation as splenocytes from similarly inoculated mice EC sensitized with saline (Fig 5, A). Furthermore, the VV specific IgG<sub>2a</sub> and IgG<sub>1</sub> Ab responses were similar in these 2 groups (Fig 5, B).

Expression of IFN- $\alpha$ , but not cathelicidin-related antimicrobial peptide (CRAMP) or IFN- $\gamma$  mRNA, is impaired after VV infection. Members of the cathelicidin family of

antimicrobial peptides exhibit antiviral activity against VV,<sup>23</sup> are downregulated by the T<sub>H</sub>2 cytokines IL-4 and IL-13 and in skin lesions of atopic dermatitis,<sup>24,25</sup> and augment T<sub>H</sub>1 responses.<sup>26</sup> Decreased cathelicidin expression at sites of OVA sensitization could contribute to the decreased T<sub>H</sub>1 response after VV inoculation. Fig 6, A, shows that there is significantly less CRAMP mRNA expression in OVA-sensitized compared with saline-exposed skin sites before VV inoculation. VV infection, however, significantly upregulated CRAMP expression

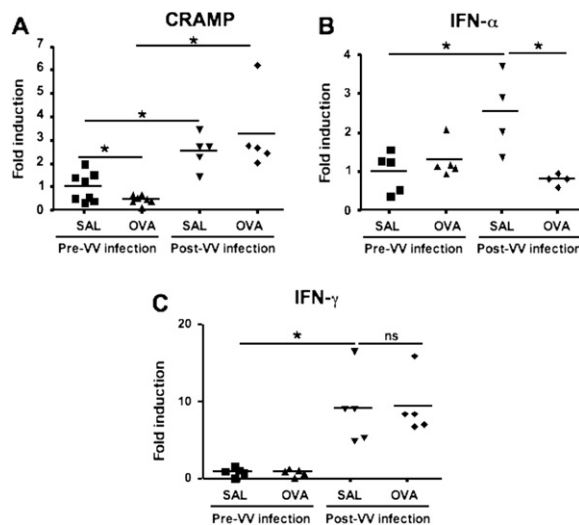


**FIG 6.** Expression of CRAMP (A), IFN- $\alpha$  (B), and IFN- $\gamma$  (C) mRNA in EC sensitized skin before and VV infection at the sensitization site. \* $P < .05$ . SAL, Saline.

in both sites to a comparable degree with no significant difference between the 2 groups. These results were confirmed at the protein level by immunoperoxidase staining (data not shown).

Type I IFNs are important for innate immunity to viral infection<sup>27</sup> and may promote  $T_H1$  responses.<sup>28</sup> Fig 6, B, shows that there was no significant difference in IFN- $\alpha$  mRNA expression between OVA versus saline-exposed skin sites before VV inoculation. VV infection of saline-sensitized skin, but not of OVA-sensitized skin, resulted in significant upregulation of IFN- $\alpha$  expression. These results suggest that allergic skin inflammation may interfere with local induction of IFN- $\alpha$  expression by VV. Plasmacytoid dendritic cells (pDCs) are a major source of type I IFN production and have been reported in some studies, but not others, to be decreased in AD skin lesions.<sup>29,30</sup> Ears of EC sensitized mice were challenged with the sensitizing agent (OVA or saline) and evaluated 48 hours later for infiltration by dendritic cells (DCs). The total number of cells recovered from skin were comparable between the 2 groups. The percentage of ClassII<sup>lo</sup>CD19<sup>+</sup>B220<sup>+</sup>CD11b<sup>+</sup>CD11c<sup>low</sup> pDCs was significantly increased in the dermis, but not epidermis, of OVA-challenged skin compared with saline-challenged skin. The percentages of ClassII<sup>+</sup>CD19<sup>+</sup>B220<sup>+</sup>CD11b<sup>+</sup>CD11c<sup>high</sup> myeloid DCs in dermis and epidermis were significantly higher in OVA-challenged skin (see this article's Fig E1 in the Online Repository at [www.jaci.org](http://www.jaci.org)). We also investigated the possibility that increased  $T_H2$  cytokine expression in OVA-sensitized sites inhibits IFN- $\alpha$  production. To test this hypothesis, we examined the effect of IL-4 on IFN- $\alpha$  production by human blood mononuclear cells stimulated with the Toll-like receptor (TLR) 3 ligand PolyI:C and TLR9 ligand CpG. In 2 experiments, IL-4 caused a modest (~25%) inhibition of TLR-triggered IFN- $\alpha$  production (see this article's Fig E2 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)). We also examined IFN- $\gamma$  expression in the skin after VV inoculation.

IFN- $\gamma$  expression was comparable in VV-infected OVA-sensitized sites and saline-sensitized sites (Fig 6, C).

## DISCUSSION

Our results demonstrate that inoculation of VV at the site of cutaneous allergic inflammation results in an impaired immune response to the virus characterized by decreased production of  $T_H1$  cytokines, decreased IgG<sub>2a</sub> antibody response, and development of satellite pox lesions.

Mice inoculated with VV at sites of skin allergic inflammation induced by EC sensitization with OVA were impaired in their capacity to secrete the  $T_H1$  cytokines IL-2 and IFN- $\gamma$  in response to VV (Fig 2, A). IFN- $\gamma$  inhibits VV replication in mouse cells<sup>31</sup> and promotes the development of VV-specific cytotoxic T cells and of VV-specific IgG<sub>2a</sub>.<sup>9</sup> Consistent with previous observations that IFN- $\gamma$  promotes the IgG<sub>2a</sub> response and downregulates the IgG<sub>1</sub> response,<sup>22</sup> mice inoculated with VV at sites of allergic skin inflammation had decreased IgG<sub>2a</sub> and increased IgG<sub>1</sub> antibody levels against VV. Surprisingly, these mice expressed IFN- $\gamma$  in their VV-infected skin sites at levels comparable to those found in VV-infected saline-sensitized sites of controls (Fig 6, C). This finding suggests that local factors in OVA-sensitized skin promote the expression of IFN- $\gamma$ . A potential candidate is increased numbers of myeloid DCs, which are a potent source of IL-12.<sup>32</sup>

The impaired  $T_H1$  response of mice inoculated with VV at sites of allergic skin inflammation was associated with the appearance of satellite pox lesions (Fig 1, B and C). However, these mice exhibited neither weight loss nor increased viral loads in skin or internal organs examined. This may be a result of the attenuated nature of the virus strain used and of intact innate immunity, which is known to play a key role in viral clearance. Mice EC sensitized with OVA mounted a normal  $T_H1$  response to intraperitoneal inoculation with VV and, more importantly, a virtually normal  $T_H1$  response to VV inoculation at distant skin sites (Figs 4 and 5). We have recently observed that mice deficient in the nuclear factor- $\kappa$ B family member RelB, which exhibit skin lesions that share features with chronic AD,<sup>33</sup> have impaired IFN- $\gamma$  production in response to VV inoculated cutaneously, but not intraperitoneally.<sup>18</sup> However, RelB deficiency is associated with dysregulated function of multiple cell types, which could have contributed to this impairment. Our current results demonstrate that in otherwise normal EC sensitized mice, local allergic skin inflammation, rather than a global dysregulation of the systemic immune response, is responsible for the impaired  $T_H1$  response to VV.

Several factors may have caused the impaired  $T_H1$  response to VV introduction at sites of allergic inflammation. One possibility is that skin inflammation caused rapid local elimination of the virus and/or interfered with carrying the virus to regional draining lymph nodes (LNs). This is unlikely because mice inoculated with VV at sites of OVA versus saline sensitization had comparable viral

loads in skin and draining LNs and mounted comparable  $T_H2$  responses to the virus (Fig 2, B). An alternative and more likely possibility is that DCs in LNs that drain sites of allergic sensitization skew the response of naive T cells away from  $T_H1$ . In support of this notion, DCs isolated from LNs that drain skin sensitized with *Aspergillus fumigatus* skew the  $T_H$  response of naive T-cell receptor-OVA transgenic T cells away from  $T_H1$  and toward  $T_H2$  (Oyoshi, unpublished data, December 2006). Finally, we found decreased local expression of CRAMP at the time of VV inoculation. Therefore, failure to upregulate local expression of IFN- $\alpha$  after infection, in part a result of increased local expression of IL-4 and IL-13, may have contributed to the decreased  $T_H1$  response to VV inoculation at OVA-sensitized sites because both CRAMP and IFN- $\alpha$  promote  $T_H1$  responses.<sup>26,28</sup> Although it is possible that host cell (CV1)-derived contaminants may contribute to the inflammatory reaction in response to VV inoculation, this would not account for the differences between mice inoculated at OVA-sensitized sites versus saline-sensitized sites.

Our model of skin allergic skin inflammation shares many features with AD. These include skin inflammation characterized by infiltration of  $CD4^+$  T cells and eosinophils and expression of mRNA for  $T_H2$  cytokines and a systemic  $T_H2$  response to antigen characterized by elevated antigen specific and total IgE. Given these shared features, our findings suggest that the increased susceptibility of patients with AD to EV may be related to local immune dysregulation in the skin rather than their systemic  $T_H2$ -dominated immune response. They also suggest that to achieve an optimal immune response after immunization of patients with AD with attenuated VV strains, care should be taken to avoid inoculation of the virus in inflamed skin sites.

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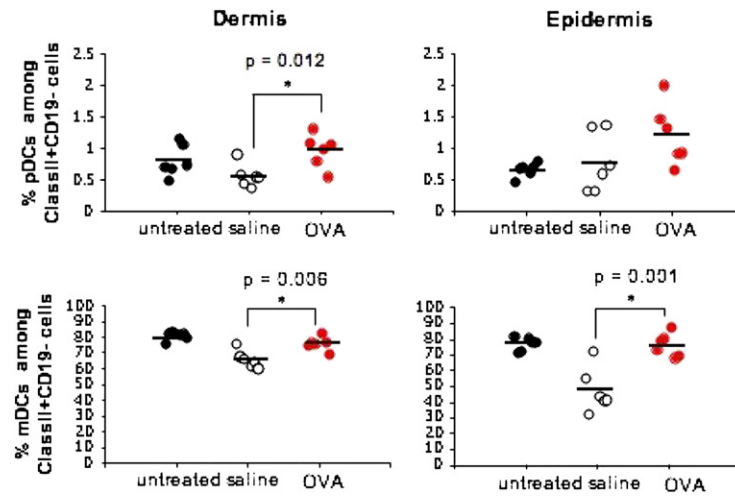


**FIG E1.** Percentages of pDCs and myeloid DCs (*mDCs*) in the skin of ears from unsensitized mice, mice EC sensitized with saline and challenged on the ears with saline, and mice EC sensitized with OVA and challenged with OVA. Ears were separated in halves and floated on trypsin/EDTA (Cellgro, Mediatech, Inc, Herndon, Va) for 25 to 45 minutes at 37°C to allow dissociation of the epidermis. Both epidermal and dermal sheets were incubated for a further 1 to 2 hours at 37°C. Cells were stained for ClassII, CD19, B220, CD11b, and CD11c and analyzed by flow cytometry. The graphs show the percentages of pDCs (ClassII<sup>low</sup>, CD19<sup>+</sup>, B220<sup>+</sup>, CD11b<sup>+</sup>, CD11c<sup>low</sup>; *top panels*) and mDCs (ClassII<sup>+</sup>, CD19<sup>+</sup>, B220<sup>+</sup>, CD11b<sup>+</sup>, CD11c<sup>high</sup>; *bottom panels*) among ClassII<sup>+</sup>CD19<sup>+</sup> cells isolated from dermis (*left panels*) and epidermis (*right panels*). Six ears per group were analyzed. \* $P < .05$  saline-treated vs OVA-treated mice.

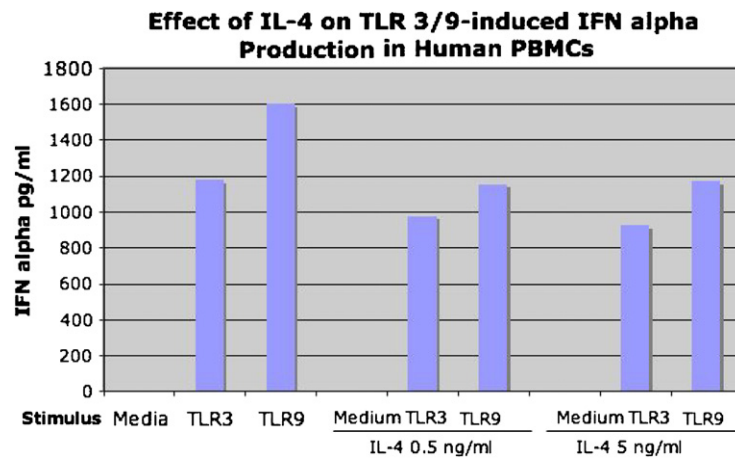


**FIG E2.** Effect of IL-4 on IFN- $\alpha$  production by normal human peripheral mononuclear cells after stimulation with polyI:C (1  $\mu$ g/mL), which engages TLR3, and CpG (1  $\mu$ g/mL), which engages TLR9. The results represent the average of 2 experiments.