Filaggrin deficiency promotes the dissemination of cutaneously inoculated vaccinia virus

Michiko K. Oyoshi, PhD, a Jacqueline Beaupré, BA, a Nicholas Venturelli, BS, a Christopher N. Lewis, BA, a Yoichiro Iwakura, DSc, b and Raif S. Geha, MD a
Boston, Mass, and Chiba, Japan

Background: Eczema vaccinatum is a life-threatening complication of smallpox vaccination in patients with atopic dermatitis (AD) characterized by dissemination of vaccinia virus (VV) in the skin and internal organs. Mutations in the filaggrin (FLG) gene, the most common genetic risk factor for AD, confer a greater risk for eczema herpeticum in patients with AD, suggesting that it impairs the response to cutaneous viral infections.

Objective: We sought to determine the effects of FLG deficiency on the response of mice to cutaneous VV inoculation.

Methods: VV was inoculated by means of scarification of unsensitized skin or skin topically sensitized with ovalbumin in FLG-deficient flaky tail (ft/ft) mice or wild-type (WT) control mice. The sizes of primary and satellite skin lesions were measured, and hematoxylin and eosin staining was performed. VV genome copy numbers and cytokine mRNA levels were measured by using quantitative PCR.

Results: VV inoculation in unsensitized skin of ft/ft mice, independent of the matted hair mutation, resulted in larger primary lesions, more abundant satellite lesions, heavier viral loads in internal organs, greater epidermal thickness, dermal cellular infiltration, and higher local Il17a, Il4, Il13, and Ifng mRNA levels than in WT control mice. VV inoculation at sites of topical ovalbumin application amplified all of these features in ft/ft mice but had no detectable effect in WT control mice. The number of satellite lesions and the viral loads in internal organs after cutaneous VV inoculation were significantly reduced in both unsensitized and topically sensitized ft/ftIl17a−/− mice.

Conclusion: FLG deficiency predisposes to eczema vaccinatum. This is mediated primarily through production of IL-17A. (J Allergy Clin Immunol 2015;135:1511-8.)

Key words: Vaccinia virus, filaggrin, IL-17A

Atopic dermatitis (AD) is a chronic pruritic inflammatory skin disorder that affects more than 15% of children in the United States. AD is characterized by a defect in skin barrier function that results in dry itchy skin and is aggravated by skin injury inflicted by scratching. Patients with AD are prone to Th12-dominated immune responses, as well as bacterial and viral infections of the skin. In particular, they are susceptible to dissemination of viruses, such as vaccinia virus (VV) and herpes simplex virus, resulting in eczema vaccinatum (EV) and eczema herpeticum (EH), respectively. In patients with EV, VV spreads through the skin, resulting in large primary lesions surrounded by satellite lesions, and infects internal organs. Although smallpox was declared eradicated in 1979, recent fears that variola virus might be used as a biological weapon, along with concerns regarding the EV susceptibility of unimmunized atopic populations, have prompted the development of new safer vaccines. Predicting the efficacy of such vaccines in the absence of human smallpox depends on understanding the mechanisms of the disease. The reasons why patients with AD are at risk for EV are not known.

Strong genetic associations exist between mutations in the filaggrin (FLG) gene and AD. The FLG gene encodes for profilaggrin, the major component of keratohyalin granules. Patients with AD who carry FLG mutations have more persistent disease, a higher incidence of skin infections with herpes virus that result in EH, and a greater risk of multiple allergies, including asthma, allergic rhinitis, and peanut allergy. Whether FLG deficiency predisposes to EV has not been studied.

Flaky tail (ft/ft) mice carry a 5303delA frameshift mutation in the FLG gene, which is in linkage disequilibrium with a second mutation at the matted (ma) locus that confers hair matting. ft/ft mice have skin barrier dysfunction. We and others have previously reported that the homozygous ft mutation on an outbred genetic background is associated with spontaneous development of eczematous skin lesions; increased mRNA expression of Il17a, Il4, Il13, and Ifng in the skin; and increased serum IgE levels. After topical cutaneous exposure to ovalbumin (OVA), ft/ft mice on a mixed background have allergic skin inflammation evidenced by epidermal thickening, dermal CD4+ cell infiltration, and exaggerated local and systemic Th17 and Th12 responses. In contrast, topical application of OVA elicited no detectable local or systemic immune responses in wild-type (WT) BALB/c and C57BL/6 mice. In WT mice
tape stripping, a surrogate for human scratching, is a prerequisite for induction of allergic skin inflammation and systemic immune responses to epicutaneous (EC) application of antigen. Genetic and environmental factors favoring induction of a cutaneous T_{H}2 response lead to impaired effector immune responses and ineffective elimination of VV and herpes simplex virus in mice, resulting in phenotypes analogous to human EV and EH. Animal studies support the hypothesis that skin barrier dysfunction caused by FLG mutations or mechanical injury secondary to scratching plays a key role in antigen sensitization, which leads to the development of AD.

To understand whether FLG-deficient mice are susceptible to development of EV, we backcrossed the mutant FLG allele of the \textit{ft/ft} strain onto the T_{H}2-prone BALB/c background. This inbred strain has been well characterized with respect to models of allergic skin inflammation and EV. Transferring the \textit{ft/ft} genotype into this background permits rigorous analyses of \textit{ft/ft} phenotypes and a comparison of the responses of the mutant and WT BALB/c control mice. In this study we demonstrate that \textit{ft/ft} mice inoculated with VV in the skin have more severe skin lesions, greater viral dissemination, and more intense cutaneous inflammation than WT control mice, indicating that FLG deficiency impairs VV containment. IL-17A was shown to mediate the susceptibility of \textit{ft/ft} mice to EV because FE features were markedly attenuated in \textit{ft/ftxIl17a\textsuperscript{−/−}} mice.

**METHODS**

**Mice**

Flaky tail (\textit{ft/ft}) mice from the Jackson Laboratory (Bar Harbor, Me) were backcrossed onto BALB/c mice from Charles River Laboratories (Wilmington, Mass). \textit{ft/ft} mice on a BALB/c background were subsequently crossed with \textit{Il17a\textsuperscript{−/−}} BALB/c mice to generate \textit{ft/ftxIl17a\textsuperscript{−/−}} mice. Later in the course of the study, by extensive backcrossing, we were able to remove the matted hair mutation (\textit{ma}) present in the original mixed strain. The mice with the removed \textit{ma} mutation were designated \textit{ft/ftma\textsuperscript{−/−}} mice. The sample size in each group was 5. All mice were bred in our animal facility, kept in a specific pathogen-free environment, and fed an OVA-free diet. All procedures performed on the mice were in accordance with the Animal Care and Use Committee of Boston Children’s Hospital.

**Topical skin sensitization and VV inoculation**

The dorsal skin of anesthetized 6- to 8-week-old female mice was shaved and rested for 2 days for recovery from any injury by shaving, and then 100 \mu g of OVA (Grade V; Sigma, St Louis, Mo) in 100 \mu L of normal saline or placebo (100 \mu L of normal saline) was placed on a patch of sterile gauze (1 \times 1 cm), which was secured to dorsal skin with a transparent bio-occlusive dressing (Tegaderm; Westnet, Canton, Mass). Each mouse had a total of three 1-week exposures to the patch separated by 2-week intervals.

Shaved dorsal skin of unsensitized mice or the sensitized site of topically sensitized mice was inoculated with VV Western Reserve strain (VR-1354, American Type Culture Collection, Manassas, Va) by means of skin scarification with 10\textsuperscript{4} plaque-forming units per mouse. Skin was examined 7 days after inoculation (see Fig E1 in this article’s Online Repository at www.jacionline.org). Lesion sizes were analyzed with the National Institutes of Health’s Image software Image J (National Institutes of Health, Bethesda, Md). No mortality was observed after VV inoculation.

**Histologic analysis**

Multiple 4-\mu m sections of skin were stained with hematoxylin and eosin (H&E). Neutrophils were counted in a blind fashion in 10 to 15 high-power fields at a magnification of ×400. The epidermal thickness of 10 different randomly chosen sites was measured in each skin section from each mouse at a magnification of ×400.

**Quantitative PCR analysis of VV genomes**

Tissue samples were immediately frozen and stored at −80°C. Quantification of VV genomes was performed, as previously described. Cytokine mRNA expression was shown as fold induction relative to that seen in uninfected WT skin unless otherwise specified. Viral genome measurements obtained by using PCR correlated with viral titer measurements obtained by using a plaque assay.

**Quantitative PCR analysis of cytokines**

Total RNA was extracted from homogenized skin tissue or from cultured cells with the RNAqueous Extraction Kit (Ambion, Life Technologies, Grand Island, NY), according to the manufacturer’s instructions. cDNA was generated with the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, Calif). Quantitative real-time PCR was done with the TaqMan Gene Expression Assay, universal PCR master mix, and ABI Prism 7300 Sequence Detection System with commercial primers and probes, all from Applied Biosystems (Foster City, Calif). Fold induction of target gene expression was calculated by using the comparative method for relative quantitation by means of normalization to the internal control β2-microglobulin, as described previously.

Measurement of \textit{in vitro} cytokine production and VV-specific IgG\textsubscript{2a} antibody ELISAs were performed, as described previously.

**Statistical analysis**

The Mann-Whitney \textit{U} test was used to compare the distribution of each outcome between 2 groups. A Bonferroni-corrected significance level of .05 was used to account for pairwise comparisons among 3 or more groups. All analyses were performed with GraphPad Prism software, version 5.0 (GraphPad Software, La Jolla, Calif).

**RESULTS**

\textit{ft/ft} mice are susceptible to cutaneous VV inoculation

We tested the hypothesis that \textit{ft/ft} mice are predisposed to the development of EV. Seven days after VV inoculation in the back skin, \textit{ft/ft} mice had significantly larger primary lesions and significantly higher numbers of satellite lesions than WT BALB/c control mice (Fig 1, A-C). Furthermore, they exhibited significantly higher viral loads in internal organs than WT control.
mice (Fig 1, D). These results suggest that ft/ft mice are more susceptible to having features of EV after inoculation with VV.

Inoculation of ft/ft mice with VV resulted in a significant increase in epidermal thickness, cellular infiltration of the dermis by mononuclear cells, and skin Il4, Il13, and Ifng mRNA levels compared with those seen in uninfected ft/ft mice or VV-inoculated WT control mice (Fig 1, E, F, and H). Inoculation of WT control mice with VV resulted in a slight but significant increase in epidermal thickness and skin Il4, Il13, and Ifng mRNA levels compared with those seen in uninfected WT control mice (Fig 1, E, F, and H). However, no increase in skin-infiltrating neutrophil counts was observed in any of the experimental groups (Fig 1, E and G). Epidermal thickness and skin Il17a mRNA expression were increased in the skin of uninfected ft/ft mice compared with that seen in uninfected WT control mice (Fig 1, F and H), as previously reported in ft/ft mice on a mixed background.18 The high basal levels of skin Il17a mRNA in ft/ft mice were significantly decreased after VV inoculation but remained higher than the Il17a mRNA levels in the skin of VV-inoculated WT mice (Fig 1, H). VV inoculation had no detectable effect on Il17a mRNA in the skin of WT control mice. Splenocytes from ft/ft and WT control mice inoculated with VV secreted comparable amounts of IL-4, IL-13, IFN-γ, and IL-17A in response to in vitro stimulation with VV (see Fig E2, A, in this article’s Online Repository at www.jacionline.org). Serum levels of VV-specific IgG2a were higher in VV-inoculated ft/ft mice than in WT control mice (see Fig E2, B). These results demonstrate that ft/ft mice mount exaggerated cutaneous T H2 and T H1 responses to VV infection.

Recently, it has become evident that FLG-null (Flg−/−) mice differ from ft/ft mice in that they do not experience spontaneous dermatitis and do not have abnormal transdermal water loss.21 However, Flg−/− mice, like ft/ft mice, mount a T H2 response to topical sensitization of antigen.11 The differences between ft/ft mice and Flg−/− mice raised the possibility that the homozygous ma mutation linked to the ft mutation might contribute to the susceptibility of ft/ft mice to cutaneous inoculation with VV. To address this issue, we backcrossed our ft/ft mice on a BALB/c background to generate ft/ft mice that lack the ma mutation, as evidence by the lack of matted hairs and based on genomic sequencing for the ma mutation (data not shown).20 These mice were indistinguishable from ft/ft mice, which harbor the ma mutation in their susceptibility to VV infection, as measured by the size of the primary lesion, the number of satellite lesions, viral loads in internal organs, epidermal thickening, and local cytokine expression profiles (see Fig E3 in this article’s Online Repository at www.jacionline.org). Differences in the aforementioned changes between ft/ft or ft/ft.ma−/− mice and WT control mice, but not between ft/ft and ft/ft.ma−/− mice, were statistically significant. These results indicate that the ma mutation does not play an important role in the susceptibility of ft/ft mice to VV inoculation.
VV inoculation of topically sensitized skin aggravates the features of EV in ft/ft mice

Topical application of OVA to the shaved unstripped back skin elicited local skin inflammation and a systemic immune response in ft/ft mice on a BALB/c background (see Fig E4, A-D, in this article’s Online Repository at www.jacionline.org and data not shown), as previously shown in ft/ft mice on a mixed background.\(^1\) In contrast, topical application of OVA to the shaved unstripped back skin did not result in the development of local skin inflammation or a systemic response in WT control mice (see Fig E4, A-D, and data not shown). We tested the hypothesis that VV inoculation of ft/ft mice at sites of topical OVA application would result in the development of more severe features of EV. Mice were topically sensitized with OVA or saline over a 7-week period, inoculated with VV at the site of sensitization, and examined 7 days later (Fig E4, E). ft/ft mice inoculated with VV at OVA-exposed sites had significantly larger primary lesions, exhibited significantly higher numbers of satellite lesions, and had significantly greater VV loads in the ovaries and kidneys compared with ft/ft mice inoculated with VV at saline-exposed sites or WT control mice inoculated with VV at OVA-exposed sites (Fig 2, A-D). They also had significantly greater epidermal thickness, significantly denser dermal infiltration with neutrophils, and significantly higher expression of Il17a, Il4, Il13, and Ifng mRNA (Fig 2, E-H). The size of the primary lesion, epidermal thickness, and expression of Il17a, Il4, Il13, and Ifng mRNA were comparable in WT mice inoculated with VV in OVA- or saline-exposed skin and significantly reduced compared with those in ft/ft mice inoculated with VV at saline-exposed sites (Fig 2, A-C and H). WT mice inoculated with VV in OVA- or saline-exposed skin had no satellite lesions, no detectable viral loads in the internal organs, and very few neutrophils in the primary lesion (Fig 2, C-E and G). Splenocyte cytokine secretion in response to VV stimulation was similar in ft/ft and WT mice (data not shown). VV-specific serum IgG2a levels were higher in ft/ft mice than in WT mice, regardless of whether the skin was sensitized with OVA or saline (see Fig E5, A, in this article’s Online Repository at www.jacionline.org). These results

---

**FIG 2.** Exaggerated EV features in ft/ft mice inoculated with VV in OVA-sensitized skin. A-C, Gross appearance (Fig 2, A), area of primary lesions (Fig 2, B), and number of satellite lesions (Fig 2, C) of EV. Dashed circles indicate primary lesions. Arrows indicate satellite lesions. **D-E**, Viral load. F-H, H&E-stained skin sections (×200 magnification; Fig 2, E), epidermal thickness (Fig 2, F), number of neutrophils (Fig 2, G), and cytokine mRNA expression (Fig 2, H, Scale bars = 100 μm. The line in the box indicates the median, and the whiskers represent the minimum to the maximum (n = 5 per group). *P < .05, **P < .01, and ***P < .001. nd, Not detectable; ns, not significant; Sal, saline.
sugg[st] suggestion that cutaneous sensitization to topically encountered antigens in ft/ft mice aggravates their susceptibility to EV.

**IL-17A contributes to the increased susceptibility of ft/ft mice to cutaneous VV inoculation**

We have previously reported that treatment with anti–IL-17A attenuates EV features in WT mice inoculated with VV in tape-stripped OVA-sensitized skin. 30 Here we tested the hypothesis that increased IL-17A expression in the skin of ft/ft mice contributes to their increased susceptibility to VV inoculation. We bred the ft/ft genotype onto the IL-17A–null background and examined responses to VV inoculation.

There was no detectable skin inflammation in 6- to 8-week-old ft/ft x Il17a−/− mice or ft/ft mice. As expected, Il17a mRNA expression was undetectable in ft/ft x Il17a−/− mice. After VV inoculation in unsensitized skin, ft/ft x Il17a−/− mice had significantly fewer satellite lesions and significantly lower viral loads compared with ft/ft control mice, but the size of the primary lesion was comparable between the 2 groups (Fig 3, A-D). VV-inoculated ft/ft x Il17a−/− mice had significantly less epidermal thickening than VV-inoculated ft/ft control mice, with no increase in dermal infiltration with neutrophils (Fig 3, E-G). However, VV-inoculated ft/ft x Il17a−/− skin showed significantly higher levels of Il4 mRNA expression than VV-inoculated ft/ft skin. Levels of Il13 and Ifng mRNA in VV-inoculated skin were not significantly different between the 2 groups (Fig 3, H). VV-inoculated ft/ft x Il17a−/− skin exhibited comparable epidermal thickness and dermal infiltration with neutrophils but significantly higher levels of Il4, Il13, and Ifng mRNA compared with uninfected ft/ft x Il17a−/− skin (Fig 3, E-H). Epidermal thickness, dermal infiltration with neutrophils, and skin Il4, Il13, and Ifng mRNA levels were comparable between uninfected ft/ft and ft/ft x Il17a−/− mice (Fig 3, E-H). Splenocyte secretion of IL-4, IL-13, and IFN-γ in response to VV stimulation, and VV-specific serum IgG2a levels were comparable in ft/ft x Il17a−/− and ft/ft mice inoculated with VV (data not shown and see Fig E5, B).

We next examined the response of ft/ft x Il17a−/− mice to VV inoculation at the site of cutaneous sensitization with OVA. After VV inoculation in skin topically sensitized with OVA, ft/ft x Il17a−/− mice had significantly smaller primary lesions, and significantly lower viral loads in internal organs compared with similarly sensitized ft/ft control mice (Fig 4, A-D). They also had significantly less epidermal thickening and significantly less neutrophil infiltration in the dermis; however, they expressed significantly more Il4, Il13, and Ifng mRNA in VV-infected skin than VV-infected
OVA-sensitized skin of ft/ft control mice or saline-exposed ft/ftxIl17a−/− skin (Fig 4, E-H). Splenocyte secretion of IL-4, IL-13, and IFN-γ in response to VV stimulation and VV-specific serum IgG2a levels were comparable in topically sensitized or saline-exposed ft/ftxIl17a−/− and ft/ft mice inoculated with VV (data not shown and see Fig E5, C). The sizes of primary lesions, numbers of satellite lesions, viral loads in internal organs, epidermal thickness, and dermal neutrophil infiltration were comparable between topically sensitized or saline-exposed ft/ftxIl17a−/− mice inoculated with VV. These results suggest that increased expression of IL-17A in the skin of ft/ft mice contributes to their enhanced susceptibility to cutaneous infection with VV.

**DISCUSSION**

We demonstrate that FLG-deficient ft/ft mice are more susceptible to features of EV after VV inoculation in the skin and that FLG deficiency and allergic inflammation synergize to promote VV dissemination. The increased susceptibility of ft/ft mice to EV depends on IL-17A.

ft/ft mice, but not WT control mice, had features of EV after VV inoculation in unsensitized skin. These included larger primary lesions at the site of cutaneous VV inoculation, higher numbers of satellite skin lesions, and greater viral dissemination to internal organs compared with those seen in WT control mice. The increased susceptibility of ft/ft mice to VV dissemination, a feature of EV, is in line with the observation that FLG mutations predispose patients with AD to EH.11 Taken together, these results suggest that FLG plays a critical role in containing viral dissemination after cutaneous infection.

The lesions in ft/ft mice were characterized by epidermal thickening, which was significantly greater than in WT control mice, and massive upregulation of T_{H1} and T_{H2} cytokine expression compared with that seen in WT control mice. The T_{H1} cytokine IFN-γ and IgG2a antibody play a protective role in limiting VV...
dissemination. The increased susceptibility of \( ft/ft \) mice to having satellite lesions and viral dissemination after VV inoculation in unsensitized skin was dependent on IL-17A. The number of satellite lesions and viral loads in internal organs in \( ft/ft \times Il17a^{\text{−/−}} \) mice inoculated in unsensitized skin or in skin topically sensitized with OVA were reduced to levels seen in WT control mice. T\(_{\text{H}1}\) and T\(_{\text{H}2}\) cytokine secretion by VV-stimulated splenocytes and serum levels of VV-specific IgG\(_2\) antibody were comparable in \( ft/ft \times Il17a^{\text{−/−}} \) and \( ft/ft \) mice, indicating that IL-17A played no detectable role in the systemic T\(_{\text{H}1}\) and T\(_{\text{H}2}\) responses of \( ft/ft \) mice to cutaneous inoculation with VV. However, in the absence of IL-17A, epidermal thickening was reduced and mRNA expression of Il4 was increased in VV-inoculated skin of \( ft/ft \times Il17a^{\text{−/−}} \) mice. These findings are consistent with previous observations that IL-17A activates keratinocytes and downregulates T\(_{\text{H}2}\) cytokine expression. Neutrophil infiltration in VV-inoculated OVA-sensitized skin, which was observed in \( ft/ft \) mice, was abolished in \( ft/ft \times Il17a^{\text{−/−}} \) mice. This finding is consistent with the role of IL-17A in driving neutrophil chemotaxis through induction of CXCL2 expression in target cells. This finding is also in agreement with our previous finding that administration of anti–IL-17A neutralizing antibody inhibits neutrophil accumulation in tape-stripped EC-sensitized skin of WT mice. These findings suggest that IL-17A plays a critical role in the development of EV and in the local neutrophilic infiltration of VV-inoculated skin in \( ft/ft \) mice.

The vast majority of patients with AD with FLG mutations are heterozygous. However, mice heterozygous for the \( ft \) mutation have no detectable skin abnormalities and do not mount an immune response to topical sensitization with antigen (our unpublished observations). Consistent with their lack of a cutaneous phenotype, \( ft/+ \) heterozygous mice had a response to VV inoculation comparable with that seen in WT control mice (data not shown). Altogether, these findings suggest that genetic factors, environmental factors, or both operate in human subjects to modulate the effect of heterozygous FLG mutations in human subjects on skin biology and thereby susceptibility of VV inoculation. Patients with AD can have EV, even in the absence of active skin lesions. The FLG-deficient mice we studied had no grossly visible dermatitis, yet they were susceptible to EV features after VV inoculation in the skin. This parallel with the human AD phenotype further supports the validity of using FLG-deficient mice as a model for the study of EV. The protective effect of IL-17A deficiency in these mice suggests that neutralization of IL-17A might be an effective therapy for EV in FLG-deficient patients with AD.

We thank Dr Hans Oettgen for scientific discussion and reading the manuscript. We thank Dr Denise Babineau for discussion about the statistical analysis of our experimental data.

**Clinical implications:** IL-17A blockade might prevent the development of EV.

**REFERENCES**


Fig E1. Experimental design of cutaneous VV inoculation using ft/ft mice. Back skin of mice was shaved and inoculated with $10^7$ plaque-forming units (pfu) per mouse per mouse by means of skin scarification. Mice were killed after 7 days of VV inoculation and examined for clinical responses, viral load, serum VV antibody levels, and cytokine production, as previously described.15
FIG E2. VV-specific systemic immune responses in ft/ft mice cutaneously inoculated with VV. A, VV-specific cytokine production by splenocytes after stimulation with VV-infected A20 cells. B, Serum VV-specific IgG2a antibody levels. The line in the box indicates the median, and the whiskers represent the minimum to the maximum (n = 5 per group). *P < .05. ns, Not significant.
FIG E3. EV features in ft/ft mice are not influenced by the ma mutation. A-C, Primary and satellite lesions in ft/ft mice homozygous for the ma mutation (ft/ft) or without the ma mutation (ft/ft.ma<sup>wt/wt</sup>) and WT control mice inoculated with VV in shaved back skin: gross appearance (Fig E3, A), area of primary lesions (Fig E3, B), and number of satellite lesions (Fig E3, C). Dashed circles indicate primary lesions. Arrows indicate satellite lesions. D, Viral load. E-G, H&E-stained skin sections (×200 magnification; Fig E3, E), epidermal thickness (Fig E3, F), number of neutrophils (Fig E3, G), and cytokine mRNA expression as fold induction relative to VV-inoculated WT skin (Fig E3, H). Scale bars = 100 μm. The line in the box indicates the median, and the whiskers represent the minimum to the maximum (n = 5 per group). *P < .05 and **P < .01. ns, Not significant.
FIG E4. Histology and cytokine expression of skin topically sensitized with OVA in ft/ft mice. A, H&E-stained skin sections. B, Epidermal thickness. C, Numbers of eosinophils. D, Cytokine mRNA expression expressed as fold induction relative to saline-exposed WT skin. Scale bars = 100 μm. E, Experimental plan of VV inoculation in topically sensitized skin. Mice were shaved and topically sensitized with OVA or saline for three 1-week-long cycles with 2-week rest intervals (total of 7 weeks), followed by inoculation of 10^7 plaque-forming units (pfu) per 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. The line in the box indicates the median, and the whiskers represent the minimum to the maximum (n = 5 per group). *P < .05. ns, Not significant; Sal, saline.
FIG E5. VV-specific IgG2a antibody response to cutaneous VV inoculation. A, VV-specific IgG2a levels in ft/ft and WT control mice topically sensitized with OVA or saline. B and C, VV-specific IgG2a levels in ft/ft and ft/ftIl17a−/− mice inoculated in unsensitized skin (Fig E5, B) or in skin topically sensitized with OVA or saline (Fig E5, C). The line in the box indicates the median, and the whiskers represent the minimum to the maximum (n = 5 per group). *P < .05. ns, Not significant; Sal, saline.