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C3a receptor promotes viral containment in mice inoculated with vaccinia virus at sites of allergic skin inflammation

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To the Editor

Eczema vaccinatum (EV) is a life-threatening complication of exposure to smallpox vaccination in patients with atopic dermatitis (AD).¹ The reason patients with AD are at risk for EV is not known. We showed that inoculation of vaccinia virus (VV) in sites of T_H2-dominated allergic skin inflammation elicited by repeated epicutaneous (EC) sensitization with ovalbumin (OVA) promotes local and systemic VV dissemination in mice.² We previously showed that the C3a receptor (C3aR) downregulates the T_H2 response and promotes the T_H1 response locally and systemically in mice epicutaneously sensitized with OVA.³ We investigated whether signaling via C3aR confers resistance to cutaneous VV infection in a mouse model of allergic skin inflammation that shares characteristics with AD.

Wild-type (WT) and C3aR^{-/-} mice on BALB/c background were epicutaneously sensitized with OVA or saline over 7 weeks by using 3 1-week sensitization cycles separated by 2-week rest intervals.² At the end of the third cycle of sensitization, mice were inoculated at the site of EC sensitization with VV Western Reserve strain (ATCC, VR-1354) by skin scarification. Unsensitized mice were inoculated with VV as additional controls. Mice were sacrificed on day 8 postinfection.

OVA-, but not saline-sensitized or unsensitized, mice exhibited weight loss. Weight loss was significantly greater in OVA-sensitized C3aR^{-/-} mice than in WT controls inoculated with 10⁶ pfu VV, indicating that C3aR signaling is important in protecting against the VV infection (Fig 1, A). The difference in weight loss was not significant when the 2 groups were inoculated with 10⁷ pfu VV, suggesting that the higher dose inoculum may mask the protective effect of C3aR. Inoculation of OVA-sensitized WT and C3aR^{-/-} mice with both VV doses resulted in significantly larger primary skin lesions as determined by measuring their longest diameters compared with VV inoculation of unsensitized or saline-sensitized mice (Fig 1, B). The size of the primary lesions was significantly larger in OVA-sensitized C3aR^{-/-} mice than in WT controls. Satellite skin lesions were observed only with the higher

VV inoculum. Their number was higher in OVA-sensitized mice than in saline-sensitized mice but was comparable between strains (see Fig E1 in this article's Online Repository at www.jacionline.org). No satellite lesions were observed in VV-inoculated unsensitized skin.

Quantitative PCR analysis of VV genomes demonstrated that C3aR^{-/-} mice inoculated in OVA-sensitized skin with both doses of VV had significantly higher viral loads in the skin and ovaries than did WT controls (Fig 1, *C* and *D*). Very few VV genomes were detected in the skin and ovaries of WT and C3aR^{-/-} mice inoculated with VV in unsensitized or saline-sensitized skin.

As in WT mice,² VV inoculation of OVA-sensitized skin of C3aR^{-/-} mice caused significantly higher eosinophil and neutrophil infiltration than did VV inoculation of saline-sensitized or unsensitized skin (see Fig E2 in this article's Online Repository at www.jacionline.org and Fig 2, *A*). With both VV doses, eosinophil, but not neutrophil, infiltration was significantly greater in VV-inoculated OVA-sensitized skin of C3aR^{-/-} mice than in WT controls (Fig 2, *A*, and data not shown).

T_H2 and T_H17 cytokines promote VV replication *in vivo* and *in vitro*,^{2,4} whereas IFN- γ inhibits VV dissemination.⁵ As in WT mice,² VV inoculation of C3aR^{-/-} mice in OVA-sensitized skin elicited significantly higher levels of T_H1, T_H2, and T_H17 cytokines than did VV inoculation in saline-sensitized or unsensitized skin. With both VV doses, IL-4 and IL-13 mRNA expression levels in VV-inoculated OVA-sensitized skin were significantly higher in C3aR^{-/-} mice than in WT controls (Fig 2, *B*, and data not shown). In contrast, IFN- γ and IL-12 mRNA expression levels were significantly lower (Fig 2, *B* and *C*). IL-17 mRNA levels in VV-inoculated skin were comparable between the 2 strains. Because IL-17 drives neutrophil accumulation in VV-infected skin,^{2,6} the latter finding is consistent with the comparable neutrophil skin infiltrates in the 2 strains.

Systemic VV-driven cytokine secretion was examined by stimulating splenocytes from VV-inoculated mice with the VV-infected B cell line A20 as APCs. As in WT mice,² VV inoculation in OVA-sensitized skin caused splenocytes from C3aR^{-/-} mice to secrete significantly more IL-4, IL-13, and IFN- γ in response to VV stimulation compared with VV inoculation in saline-sensitized or unsensitized skin (see Fig E3, *A*, in this article's Online Repository at www.jacionline.org). More importantly, with both VV doses, splenocytes from C3aR^{-/-} mice inoculated with VV in OVA-sensitized skin secreted significantly more IL-4 and IL-13 but significantly less IFN- γ than did splenocytes from WT controls (Fig E3, *A*, and data not shown).

T_H2 cytokines drive IgG₁ antibody responses, while IFN- γ drives IgG_{2a} antibody responses, which protect against VV dissemination.⁷ As previously reported in WT mice,² C3aR^{-/-} mice inoculated with VV in OVA-sensitized skin mounted a significantly higher IgG₁ response and a significantly lower IgG_{2a} response to VV than did C3aR^{-/-} mice inoculated with VV in saline-sensitized or unsensitized skin (Fig E3, *B*). C3aR^{-/-} mice inoculated with both VV doses in OVA-sensitized skin mounted a significantly higher IgG₁ response to VV than did WT controls (Fig E3, *B*, and data not shown). There was no significant difference in the VV-specific IgG_{2a} response between the 2 strains.

Lack of C3aR enhanced IL-4 expression and inhibited IFN- γ expression in VV-inoculated OVA-sensitized skin and by VV-stimulated splenocytes, but did not alter the expression of these cytokines in VV-inoculated unsensitized or saline-sensitized skin. VV genomes in the skin and internal organs were significantly increased in VV-inoculated OVA-sensitized skin but not in VV-inoculated unsensitized and saline-sensitized skin of C3aR^{-/-} mice. These observations suggest that C3aR deficiency synergizes with EC antigen sensitization to promote VV dissemination by skewing the local and systemic immune response to VV further toward T_H2 and away from T_H1 responses.

Keratinocytes are a rich source of C3,⁸ and trauma to the skin results in the generation of C3a.⁹ C3a upregulates IL-12 mRNA expression by dendritic cells (DCs), and C3aR signaling promotes T_H1 responses.³ Viral DNA also upregulates IL-12 mRNA expression by DCs via TLR9 ligation and promotes T_H1 responses. C3a released by damaged keratinocytes and viral DNA in VV-inoculated OVA-sensitized skin might synergize to enhance IL-12 expression by DCs, which present VV antigens to T cells in draining lymph nodes. This would promote a systemic T_H1 response, which negatively regulates the T_H2 response to the virus. The resulting systemic T_H1:T_H2 imbalance would favor the containment of the virus. Therapies that promote C3aR signaling might be useful in treating EV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG 1.

Skin lesions and viral loads in VV-inoculated WT and C3aR^{-/-} mice. **A**, Body-weight change in mice inoculated with VV. The percentage of body-weight change relative to day 0 of VV inoculation is shown. *Error bars* represent mean and SEMs (n = 6-8 per group). **B**, Longest diameter of primary skin lesion. **C** and **D**, VV load in inoculated skin (Fig 1, C) and ovaries (Fig 1, D). Columns and *error bars* represent mean and SEM (n = 6-8 mice per group). *n.s.*, Not significant; *SAL*, saline. **P* < .05, ***P* < .01, ****P* < .005.

FIG 2.

Cells and cytokines in VV-inoculated skin of WT and C3aR^{-/-} mice. **A**, Quantitative analysis of eosinophils and neutrophils in the dermis. **B**, mRNA expression of IL-4, IL-13, IFN- γ , and IL-17. **C**, mRNA expression of IL-12p35. Data are shown for mice inoculated with 10^7 pfu of VV. Values represent fold induction relative to VV-inoculated unsensitized skin of WT mice. Columns and *error bars* represent mean and SEM (n = 6-8 mice per group). *n.s.*, Not significant; *SAL*, saline. **P* < .05, ***P* < .01, ****P* < .005.