

Letter to the Editor

Thymic stromal lymphopoietin and IL-33 promote skin inflammation and vaccinia virus replication in a mouse model of atopic dermatitis

To the Editor:

Eczema vaccinatum (EV) is a life-threatening complication of exposure to smallpox vaccination in patients with atopic dermatitis (AD) characterized by dissemination of vaccinia virus

(VV) in the skin and internal organs.¹ We have shown that BALB/c mice inoculated with VV at sites of allergic skin inflammation elicited by epicutaneous sensitization with ovalbumin (OVA) exhibit features of EV.² They include satellite lesions and VV dissemination to internal organs. EV features were absent in mice inoculated with VV in control skin epicutaneously sensitized with saline, suggesting that allergic skin inflammation predisposes to VV dissemination,² which is consistent with the known role of the T_H2 cytokine IL-4 in promoting VV

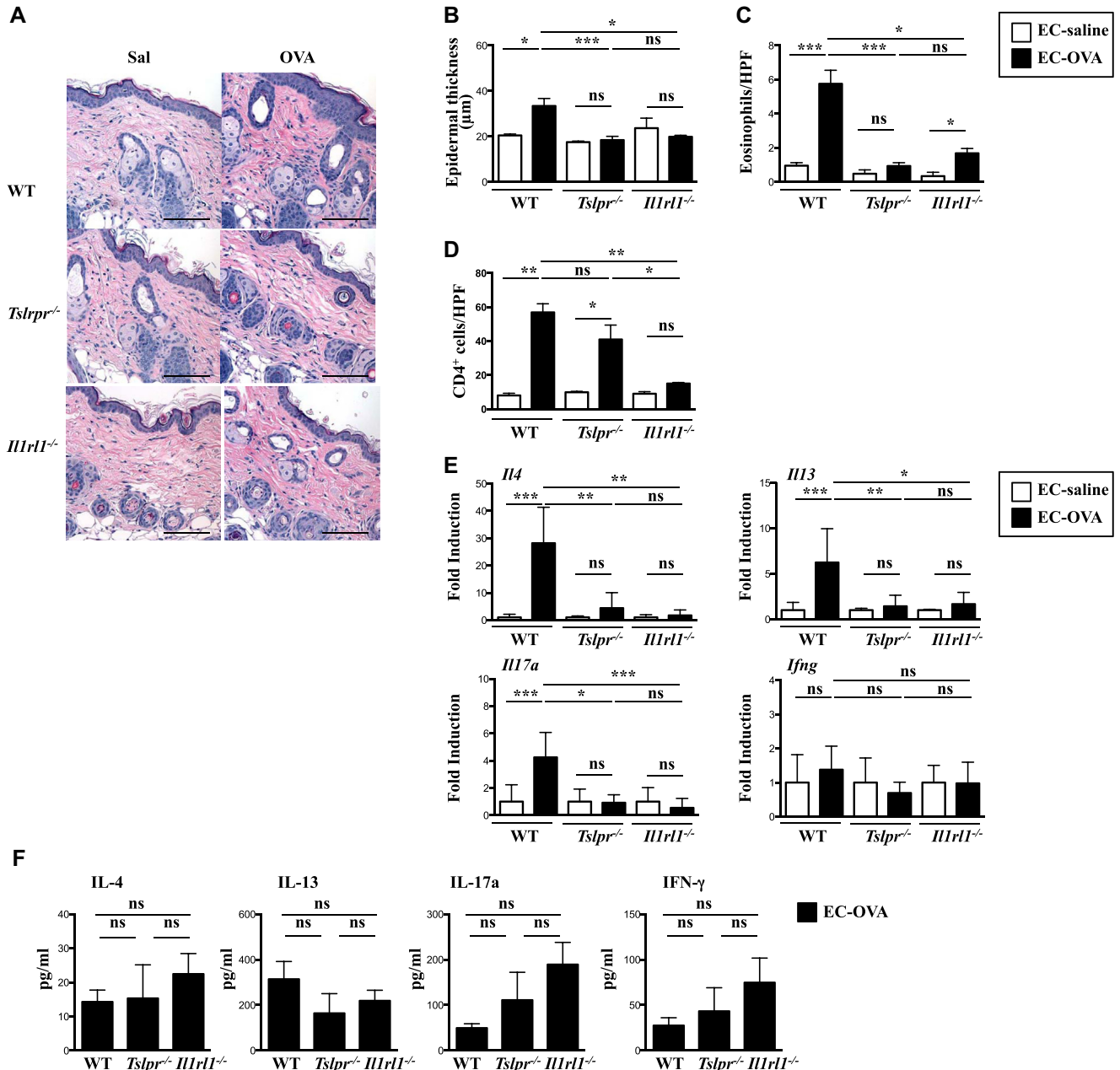
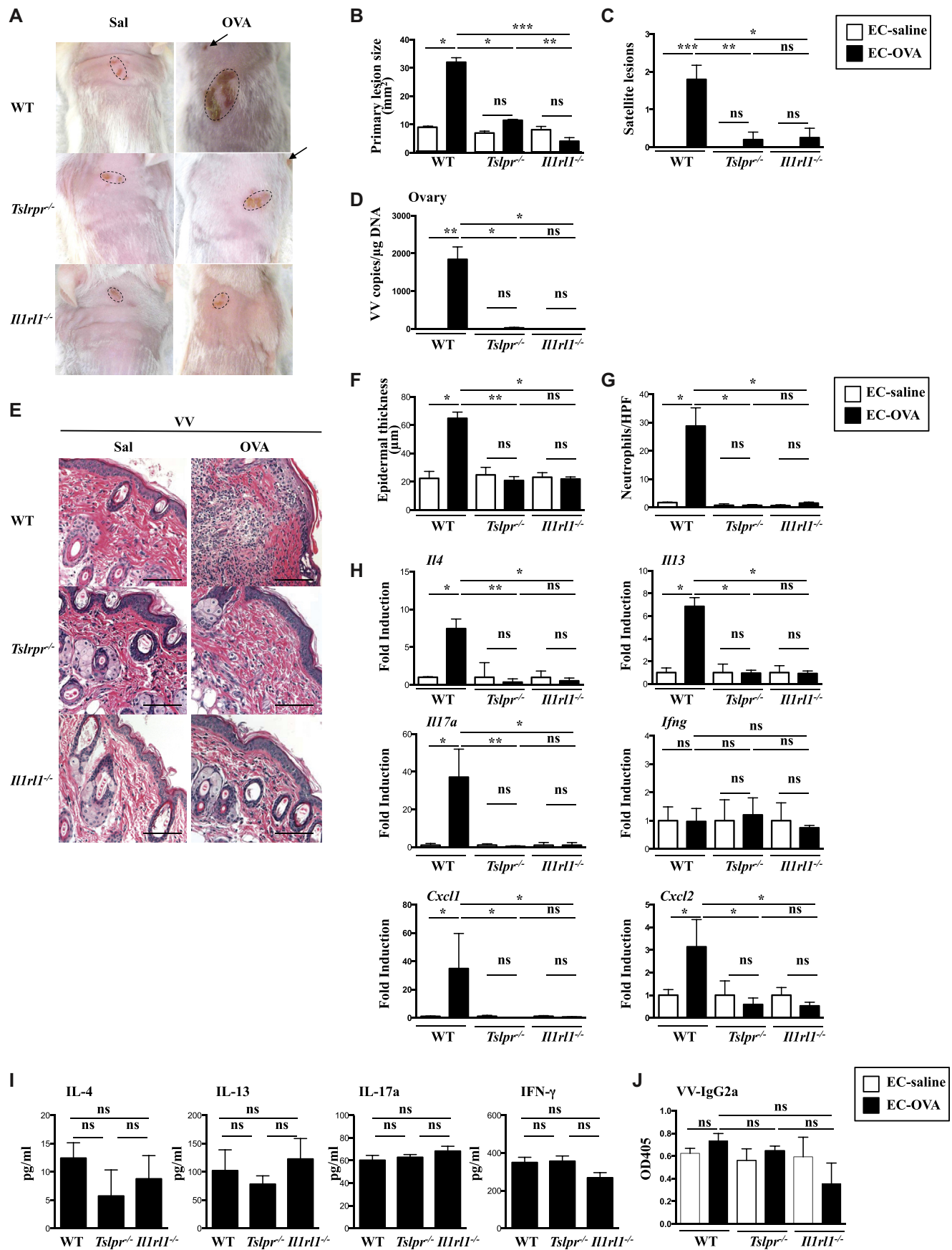


FIG 1. Impaired allergic skin inflammation in *Tslpr*^{-/-} and *Il1rl1*^{-/-} mice. **A**, Representative hematoxylin and eosin-stained sections. **B**, Epidermal thickness. **C** and **D**, Numbers of eosinophils/high-power field (HPF; Fig 1, C) and CD4⁺ cells/HPF (Fig 1, D). **E**, Cytokine mRNA expression relative to saline-exposed skin of the same genotype. **F**, OVA-specific splenocyte cytokine production. Scale bars = 100 μm (n = 5 per group). **P* < .05, ***P* < .01, and ****P* < .001. ns, Not significant. Results were analyzed by using nonparametric 1-way ANOVA.



dissemination.³ Levels of the T_H2-promoting epithelial cell–derived cytokines thymic stromal lymphopoietin (TSLP) and IL-33 are increased in skin lesions and serum of patients with AD.⁴ We have used our mouse model of AD to examine the potential role of these cytokines in patients with EV.

Wild-type (WT), TSLP receptor–deficient (*Tslpr*^{−/−}), and IL-33 receptor–deficient (*Il1rl1*^{−/−}) mice on a BALB/c background were epicutaneously sensitized with OVA or saline over 7 weeks.⁵ As previously reported,⁵ WT mice epicutaneously sensitized with OVA exhibited an increase in epidermal thickening, numbers of dermal eosinophils and CD4⁺ cells, and levels of mRNA expression for *Il4*, *Il13*, and *Il17a*, but not *Ifng*, compared with values in saline-sensitized control mice (Fig 1, A–E). In contrast, *Tslpr*^{−/−} and *Il1rl1*^{−/−} mice epicutaneously sensitized with OVA did not have skin inflammation, except for comparable numbers of dermal CD4⁺ cells in *Tslpr*^{−/−} mice, as previously reported,⁵ and a small increase in the number of dermal eosinophils in *Il1rl1*^{−/−} mice (Fig 1, A–E). This was not due to an impaired systemic cytokine response to OVA. In response to *in vitro* restimulation with OVA, splenocytes from *Tslpr*^{−/−} and *Il1rl1*^{−/−} mice secreted comparable amounts of IL-4, IL-13, and IL-17A, as well as IFN-γ, as splenocytes from WT control mice (Fig 1, F), which is consistent with previous observations in *Tslpr*^{−/−} and *Il33*^{−/−} mice.^{5,6} Thus in contrast to other modes of immunization,⁷ the systemic T_H2 response to epicutaneous sensitization is not dependent on TSLP or IL-33. Importantly, our findings indicate that IL-33 and TSLP play nonredundant roles in promoting the expression of T_H2 and IL-17A cytokines in epicutaneously sensitized skin. Intradermal injection of anti-TSLP neutralizing antibody inhibits the development of allergic skin inflammation after cutaneous antigen challenge of previously sensitized WT mice,⁵ suggesting that local expression of TSLP and possibly IL-33 might be important for the upregulation of T_H2 and IL-17A cytokine expression by immune cells in epicutaneously sensitized skin.

Tslpr^{−/−} and *Il1rl1*^{−/−} mice were inoculated in epicutaneously sensitized skin sites with VV Western Reserve strain (American Type Culture Collection, VR-1454) by means of skin scarification using 10⁷ plaque-forming units per mouse and killed 7 days later to examine the role of TSLP and IL-33 in the response to cutaneous VV inoculation. As previously reported,² VV inoculation in OVA-sensitized skin of WT mice resulted in significantly larger and erosive primary lesions, significantly more satellite lesions, and significantly greater recovery of VV genomes from the ovaries compared with VV inoculation in saline-sensitized skin (Fig 2, A–D). In contrast, VV inoculation in OVA-sensitized skin of *Tslpr*^{−/−} and *Il1rl1*^{−/−} mice resulted in no significant increase in the size of the primary lesion, number of satellite lesions, or recovery of VV genomes from the ovaries compared with VV inoculation in saline-sensitized skin (Fig 2, A–D). All 3 parameters were comparable in saline-sensitized skin of *Tslpr*^{−/−}, *Il1rl1*^{−/−}, and WT mice. No VV genomes could be detected in inoculated skin epicutaneously sensitized with saline

or OVA 7 days after VV inoculation. In previous studies no or small numbers of VV genomes were detected in VV-inoculated sensitized skin epicutaneously sensitized with saline or OVA.^{2,8}

As previously reported,^{2,8} VV inoculation in OVA-sensitized skin of WT mice resulted in significantly increased epidermal thickness and caused significantly more dermal infiltration with neutrophils and significantly higher expression of *Il4*, *Il13*, *Il17a*, *Cxcl1*, and *Cxcl2*, but not *Ifng*, compared with VV inoculation in saline-sensitized skin (Fig 2, E–H). In contrast, VV inoculation in OVA-sensitized skin of *Tslpr*^{−/−} and *Il1rl1*^{−/−} mice resulted in no significant increase in epidermal thickness, dermal infiltration with neutrophils, or cytokine mRNA expression compared with VV inoculation in saline-sensitized skin (Fig 2, E–H). The response of *Tslpr*^{−/−} and *Il1rl1*^{−/−} mice to VV inoculation in saline-sensitized skin was comparable with that seen in WT control skin. T_H2 and T_H17 cytokines promote, whereas IFN-γ and IgG_{2a} antibody limit, VV replication.^{2,3,9} Perturbations in the systemic response to VV are unlikely to explain the failure of OVA-sensitized VV-inoculated *Tslpr*^{−/−} and *Il1rl1*^{−/−} to have skin inflammation and their resistance to VV dissemination. VV-stimulated cytokine production by splenocytes and VV-specific IgG_{2a} antibody levels were comparable in *Tslpr*^{−/−} mice, *Il1rl1*^{−/−} mice, and WT control mice (Fig 2, I and J).

IL-4 and IL-17A promote VV replication.^{2,3} The failure of *Tslpr*^{−/−} and *Il1rl1*^{−/−} mice to upregulate the expression of these cytokines in OVA-sensitized skin might have constrained local viral replication early after VV inoculation, resulting in a minimally sized primary lesion, virtual lack of satellite lesions, and undetectable viral dissemination to the ovaries, as observed after VV inoculation in saline-sensitized skin. IL-17A is highly upregulated after VV inoculation in OVA-sensitized skin sites and is critical for neutrophil infiltration and the development of erosive skin lesions in our EV model.² The failure to upregulate *Il17a*, *Cxcl1*, and *Cxcl2* mRNA expression in VV-inoculated OVA-sensitized skin of *Tslpr*^{−/−} and *Il1rl1*^{−/−} mice likely underlies the failure of neutrophils to be recruited to the VV inoculation site and thereby the absence of erosive skin lesions in these mice.

TSLP and IL-33 expression is upregulated in skin lesions of patients with AD.⁴ Genetic variants in *TSLP* are associated with AD and eczema herpeticum,¹⁰ supporting the involvement of TSLP in the control of viral skin infection in patients with AD. It has been suggested that TSLP and IL-33 drive AD through group 2 innate lymphoid cells.⁴ Comparable numbers of dermal CD4⁺ cells in *Tslpr*^{−/−} mice, but not *Il1rl1*^{−/−} mice, suggest that TSLP and IL-33 might drive the development of allergic skin inflammation through different mechanisms. Identification of the targeted cells by TSLP and IL-33 in our model, which can include group 2 innate lymphoid cells, dendritic cells, basophils, mast cells, and T_H2 cells, requires future investigations. Our results in a mouse model of AD suggest that TSLP and IL-33 might play key roles in the development of AD and

FIG 2. EV features are ameliorated in *Tslpr*^{−/−} and *Il1rl1*^{−/−} mice inoculated with VV in OVA-sensitized skin. **A**, Gross appearance. Dashed circles indicate primary lesions, and arrows indicate satellite lesions. **B**, Area of primary lesions. **C**, Number of satellite lesions. **D**, Viral load. **E**, Representative hematoxylin and eosin–stained sections. Scale bars = 100 μm. **F**, Epidermal thickness. **G**, Number of neutrophils/high-power field (HPF). **H**, Cytokine mRNA expression relative to infected saline-exposed skin. **I**, VV-specific splenocyte cytokine production.² **J**, VV-specific IgG_{2a} serum levels (n = 5 per group). *P < .05, **P < .01, and ***P < .001. ns, Not significant. Results were analyzed by using nonparametric 1-way ANOVA.

EV. The clinical relevance of our findings needs to be established by examining whether blockade of these 2 epithelial cytokines is effective in patients with AD and in preventing EV.

*Michiko K. Oyoshi, PhD
Nicholas Venturelli, BS
Raif S. Geha, MD*

From the Division of Immunology, Boston Children's Hospital and the Departments of Pediatrics, Harvard Medical School, Boston, Mass. E-mail: raif.geha@childrens.harvard.edu.

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