IL-33 promotes food anaphylaxis in epicutaneously sensitized mice by targeting mast cells

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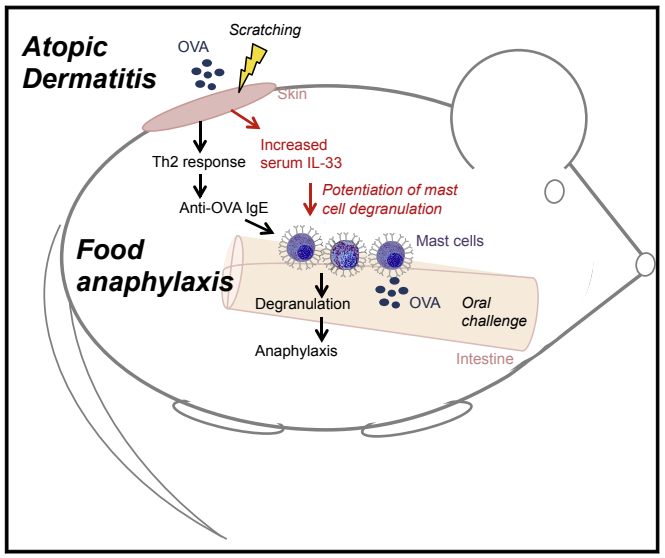
GRAPHICAL ABSTRACT



Background: Cutaneous exposure to food allergens predisposes to food allergy, which is commonly associated with atopic dermatitis (AD). Levels of the epithelial cytokine IL-33 are increased in skin lesions and serum of patients with AD. Mast cells (MCs) play a critical role in food-induced anaphylaxis and express the IL-33 receptor ST2. The role of IL-33 in patients with MC-dependent food anaphylaxis is unknown.

Objective: We sought to determine the role and mechanism of action of IL-33 in patients with food-induced anaphylaxis in a model of IgE-dependent food anaphylaxis elicited by oral challenge of epicutaneously sensitized mice.

Methods: Wild-type, ST2-deficient, and MC-deficient *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice were epicutaneously sensitized with ovalbumin (OVA) and then challenged orally with OVA. Body temperature was measured by means of telemetry, *Il33* mRNA by means of quantitative PCR, and IL-33, OVA-specific IgE, and mouse mast cell protease 1 by means of ELISA. Bone marrow–derived mast cell (BMMC) degranulation was assessed by using flow cytometry.

Results: *Il33* mRNA expression was upregulated in tape-stripped mouse skin and scratched human skin. Tape stripping caused local and systemic IL-33 release in mice. ST2 deficiency, as well as ST2 blockade before oral challenge, significantly reduced the severity of oral anaphylaxis without affecting the systemic T_H2 response to the allergen. Oral anaphylaxis was abrogated in *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice and restored by means of reconstitution with wild-type but not ST2-deficient BMMCs. IL-33 significantly enhanced IgE-mediated degranulation of BMMCs *in vitro*.

Conclusion: IL-33 is released after mechanical skin injury, enhances IgE-mediated MC degranulation, and promotes oral anaphylaxis after epicutaneous sensitization by targeting MCs. IL-33 neutralization might be useful in treating food-induced anaphylaxis in patients with AD. (J Allergy Clin Immunol 2016;138:1356-66.)

Key words: *IL-33, ST2, food allergy, atopic dermatitis, mast cells*

The global incidence of food allergy has increased in the last decade. In the United States 28% of children are sensitized to food allergens,[1] and 5% to 7% have food allergy.[2] Food allergy symptoms range from skin rashes to severe anaphylaxis and even death.[3] Atopic dermatitis (AD) is a chronic pruritic inflammatory skin disease characterized by a defect in skin barrier function, associated in many cases with filaggrin deficiency, and made worse by mechanical injury inflicted by scratching the dry inflamed skin. Allergen introduction through the disrupted skin barrier in patients with AD elicits T_H2-dominated systemic and local immune response with increased levels of total and antigen-specific serum IgE and increased local and systemic production of T_H2 cytokines.[4] IgE-mediated food allergy and AD often coexist, with one to two thirds of patients with AD having documented food allergy.[5] Epidemiologic data suggest

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Abbreviations used
AD: Atopic dermatitis
BMMC: Bone marrow–derived mast cell
DNP: Dinitrophenyl
DNP-HSA: Dinitrophenyl-conjugated human serum albumin
IL-33R: IL-33 receptor
LAMP-1: Lysosomal-associated membrane protein 1
MC: Mast cell
mMCP-1: Mouse mast cell protease 1
OVA: Ovalbumin
sLAMP-1: Surface LAMP-1
TNP: Trinitrophenyl
TNP-BSA: Trinitrophenyl-conjugated BSA
TSLP: Thymic stromal lymphopoietin
WT: Wild-type

that sensitization to peanut protein can occur in children through application of peanut oil to inflamed skin.[6] In addition, early-life environmental peanut exposure is associated with an increased risk of peanut sensitization and allergy in children who carry a filaggrin mutation.[7] These observations support the hypothesis that food allergy develops through transcutaneous sensitization in children with an impaired skin barrier.

Epithelial cytokines released in response to mechanical injury include thymic stromal lymphopoietin (TSLP), IL-25, and IL-33.[8] IL-33, a member of the IL-1 cytokine family, is produced by keratinocytes, fibroblasts, endothelial cells, macrophages, and other immune cells.[9] IL-33 promotes IL-4 and IL-13 production by *in vitro* differentiated T_H2 lymphocytes,[10] polarizes skin-derived dendritic cells to drive a T_H2 response after epicutaneous sensitization to peanut extract,[11] and causes an increase in serum IgE levels and eosinophilia when injected *in* vivo.[12] IL-33 has been shown to be important for the T_H2 response to intranasal sensitization with house dust mite antigen and intragastric sensitization with peanut[13] but not to epicutaneous sensitization with ovalbumin (OVA).[14] Forced IL-33 local overexpression by keratinocytes induces AD-like symptoms,[15] and IL-33 levels are increased in the skin lesions and sera of patients with AD[16,17] and in mouse skin after epicutaneous sensitization with OVA.[16] A polymorphism in the gene coding for ST2, a chain of the IL-33 receptor (IL-33R), has been associated in human subjects with AD.[18] ST2 is a member of the IL-1 receptor family encoded by *Il1rl1* and forms a dimer with IL-1 receptor accessory protein in a ligand-dependent manner. ST2 is expressed in T_H2 lymphocytes, mast cells (MCs), eosinophils, basophils, type 2 innate lymphoid cells, smooth muscle cells, and endothelial cells.[19]

MCs play an important role in allergic diseases and particularly in food-induced anaphylaxis by virtue of their degranulation and mediator release after antigen-driven, IgE-mediated crosslinking of the high-affinity IgE receptor FcεRI expressed on their surfaces.[20,21] IL-33 induces release of proinflammatory cytokines by MCs and promotes their survival.[22-24] IL-33 has also been reported to trigger antigen-independent MC degranulation in naive mice,[12] but the role of IL-33 in IgE-dependent MC degranulation has been controversial.[25,26] A recent report found IL-33 to be essential for IgE-dependent anaphylaxis in epicutaneously sensitized mice, but the mechanism involved was not investigated.[27]

We have reported that epicutaneous sensitization of mice through application of OVA to skin mechanically injured by tape stripping results in allergic skin inflammation with many features of AD and in IgE-dependent anaphylaxis on oral challenge.[28,29] We have used this model to verify the role and determine the mechanism of action of IL-33 in patients with food-induced anaphylaxis after epicutaneous sensitization. We demonstrate that tape stripping the skin induces local and systemic release of IL-33, that IL-33 signaling potentiates antigen-driven IgE-dependent MC degranulation *in vitro*, and that IL-33 promotes food-induced anaphylaxis in epicutaneously sensitized mice by targeting its receptor on MCs.

METHODS

Mice

Wild-type (WT) BALB/c mice were purchased from Taconic (Germantown, NY). ST2-deficient *Il1rl1*$^{-/-}$ mice, obtained from Dr McKenzie, and *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, obtained from Dr Stassen, and bred for more than 9 generations on a BALB/c background have been described previously.[30,31] All mice were housed in a specific pathogen-free environment and fed an OVA-free diet. All procedures were performed in accordance with the Animal Care and Use Committee of Boston Children's Hospital.

Human subjects

After obtaining informed consent, the inner sides of the forearms of 2 healthy nonallergic adult subjects were scratched 30 times with a #11 sterile blade, with care taken not to draw blood. Six hours later, a 4-mm punch biopsy specimen was obtained from the scratched site and another one from a skin site on the contralateral forearm. RNA was extracted from the skin with the Total RNA Isolation Kit (Ambion, Austin, Tex). cDNA was prepared 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 (Applied Biosystems, Foster City, Calif). *IL33* mRNA fold induction was calculated by using ΔΔ cycle threshold with normalization to the internal control β_2-microglobulin. An arbitrary unit of 1 was assigned to the mean value of unmanipulated skin samples.

Il33 mRNA expression and its release on tape stripping

The back skin of anesthetized mice was shaved and subjected to tape stripping 6 times with a film dressing (Tegaderm; 3M, St Paul, Minn). Six hours later, RNA was extracted and *Il33* expression was measured, as described for human skin samples. For measuring IL-33 release, patches of approximately 1 cm^2 of skin were excised from unmanipulated back skin or immediately after tape stripping. Subcutaneous fat was removed, and the patches were cultured for 1 hour in complete RPMI. IL-33 levels in the supernatant and serum were measured with the Quantikine ELISA Kit (R&D Systems, Minneapolis, Minn).

Epicutaneous sensitization and oral antigen challenge

Epicutaneous sensitization was described previously.[32] Briefly, epicutaneous sensitization consists of three 1-week cycles of tape stripping, followed by application of OVA or saline. For each cycle, 6- to 8-week-old female mice were anesthetized, and their back skin was shaved and tape stripped with a film dressing (Tegaderm, 3M) 6 times at day 0 and 3 times at day 3 of each cycle. Two-week rest intervals were observed between cycles. Epicutaneous sensitization consisted of applying a 1-cm^2 gauze containing 100 μg of OVA (Sigma-Aldrich, St Louis, Mo) after each tape stripping and securing it with a film dressing. On the last day of sensitization (day 49), mice were challenged intragastrically with 100 mg of OVA in 150 μL of saline buffer. Temperature changes were measured every 5 minutes after OVA challenge by using the DAS-6001 Smart Probe and IPTT-300 transponders (Bio Medic Data Systems, Seaford, Del) injected subcutaneously. Sera were collected 60 minutes after challenge.