The role of basophils and proallergic cytokines, TSLP and IL-33, in cutaneously sensitized food allergy

Taichiro Muto1, Ayumi Fukuoka2, Kenji Kabashima3, Steven F. Ziegler4, Kenji Nakanishi1, Kazufumi Matsushita2 and Tomohiro Yoshimoto1,2

1Department of Immunology and Medical Zoology, Hyogo College of Medicine, Nishinomiya, Hyogo 663-8501, Japan
2Laboratory of Allergic Diseases, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Nishinomiya, Hyogo 663-8501, Japan
3Department of Dermatology, Kyoto University, Graduate School of Medicine, Kyoto 606-8507, Japan
4Immunology Program, Benaroya Research Institute at Virginia Mason, Seattle, WA 98101, USA

Correspondence to: T. Yoshimoto; E-mail: tomo@hyo-med.ac.jp

Received 25 March 2014, accepted 19 May 2014

Abstract

Cutaneous sensitization with a food antigen before its consumption elicits the development of food allergy. Here, we report the site- and stage-dependent roles of basophils and proallergic cytokines, thymic stromal lymphopoietin (TSLP) and IL-33, in a mouse model of food allergy initially sensitized cutaneously with the food antigen. Mice were epicutaneously sensitized with the food antigen ovalbumin (OVA) followed by oral challenge with OVA. Epicutaneously sensitized mice produced OVA-specific IgE and developed IgE-dependent anaphylaxis after oral challenge. Basophil-depleted or TSLP–receptor-deficient mice did not produce OVA-specific IgE and were protected from oral challenge-induced anaphylaxis. IL-33-deficient mice produced normal levels of OVA-specific IgE. However, IL-33-deficient mice and mice treated with recombinant soluble IL-33 receptor were protected from anaphylaxis. Thus, basophils and TSLP have pivotal roles in T2 development in the skin during the sensitization phase of food allergy. In contrast, while IL-33 is dispensable for promoting cutaneous antigen sensitization, the cytokine is essential for inducing IgE-dependent anaphylaxis in the gut.

Keywords: anaphylaxis, gut, IgE, ovalbumin, skin

Introduction

Food allergy is a growing public health problem that compromises patient quality of life and is potentially fatal. Although the prevalence of food allergy varies study by study, it is estimated that ~5% of adults and 8% of children are affected by the disease (1). Moreover, several reports indicate an increasing trend of food allergy, especially in westernized countries (1–3). The gastrointestinal tract digests and absorbs nutrition from ingested foods and is protected from inducing inappropriate immune responses by a mechanism termed ‘oral-tolerance’ (4). However, when tolerance is compromised and T2-type immune responses are induced to food antigens, IgE-mediated anaphylaxis, namely food allergy, can be evoked (1, 2). Currently, the management of food allergy is achieved by allergen avoidance and prompt use of self-injectable epinephrine in emergency cases (1, 2, 5). These measures significantly compromise patient quality of life and cannot completely avoid the threat of food allergy. Thus, it is essential to identify immune pathways that can be targeted to prevent food allergy development.

A growing body of evidence indicates that the route of antigen sensitization is an essential determinant for inducing either food allergy or oral tolerance (6–8). This is now termed the ‘dual-allergen-exposure hypothesis’ (7, 8). According to this theory, epicutaneous sensitization of a food allergen before its oral encounter elicits food allergy, while early consumption of the food antigen induces oral tolerance (7, 8). Previously, avoidance of consumption of highly allergic foods, such as peanuts, milk or eggs, in infants and their mothers was considered a preventative measure against the development of food allergy (9). However, some children developed anaphylaxis after the first consumption of a food, even if they and their mothers had avoided its consumption previously (10, 11). Thus, the efficacy of food avoidance in preventing food allergy has been questioned (12, 13) and tissues other than the gut have been considered as initiation sites for food antigen sensitization (6, 10). Du Toit et al. (14) compared the prevalence of peanut allergy in Jewish children in the UK (infants are forbidden to eat peanuts) and Israel (infants regularly eat peanuts)
and demonstrated that the risk for peanut allergy was 9.8-fold higher in the UK. Furthermore, early onset of severe eczema is associated with the later development of food allergy (15). Filaggrin gene mutation, which is a risk factor for atopic dermatitis (AD) (16), is also linked to the prevalence of peanut allergy (17), even though the protein is expressed exclusively in the skin but not in the gastrointestinal tract (18), further suggesting the skin as an important site for food antigen sensitization. In addition to epidemiologic studies, animal models showed that skin-mediated antigen sensitization evoked systemic IgE responses and induced subsequent anaphylactic responses when the antigen was orally challenged (19, 20). These observations clearly demonstrate the importance of skin-mediated sensitization in the later development of food allergy. However, the cellular or molecular mechanisms underlying the process are poorly understood.

The role of basophils in the development of T2-type immune responses is highly controversial (21, 22). Basophils express MHC class II and costimulatory molecules and secrete large amounts of IL-4 that promote T-cell differentiation to T2, thus basophils might function as antigen-presenting cells (APCs). (23–25). Some groups have challenged this theory by reporting basophils did not present antigen to T cells, and that dendritic cells (DCs) had a pivotal role in initiating T2 responses in lung house dust mite instillation (26) or helminthic infection (27) models. More recently, other reports demonstrated the unique role of basophils in cutaneously initiated T2 cell differentiation (28, 29). In the skin, basophils promote T2 cell differentiation cooperatively with DCs as IL-4 producers (29, 30), or as APCs for hapten or peptide antigens (29). These studies also demonstrated the important role of thymic stromal lymphopoietin (TSLP) in basophil-mediated T2 responses in the skin (28–30). TSLP activates DCs to promote subsequent DC-T-basophil associations (29, 30). Thus, basophils might contribute to T2 immune responses in a context-dependent manner, and skin might be a tissue that requires basophils to induce optimal T2 responses. However, although previous reports showed the precise molecular mechanisms of basophil- and TSLP-mediated induction of T2 cell differentiation in the skin (29, 30), the importance of the pathways in initiating food antigen sensitization and following gastrointestinal responses in the context of food allergy have not been demonstrated (21).

Recent studies demonstrated the important role of IL-33 in T2-type immune-mediated diseases (31). Importantly, IL-33 may participate in both skin (32, 33)- and gut (34–36)-mediated T2-type immune responses. Furthermore, IL-33 can amplify FceRI cross-linking-mediated mast cell degranulation (37, 38) and exacerbate anaphylaxis (38). Therefore, IL-33 could be a therapeutic target for food allergy initiated though cutaneous antigen sensitization.

In this study, we established an experimental food allergy mouse model, in which mice were epicutaneously sensitized with the food antigen ovalbumin (OVA) followed by oral challenge with OVA to induce anaphylactic responses. We demonstrate the essential roles of skin basophil-mediated initiation of T2 responses in the development of food allergy. Moreover, we identify the tissue- and phase-specific roles of proallergic cytokines, TSLP and IL-33, in a cutaneously sensitized food allergy model.

Methods

Mice

Wild-type (WT) BALB/c mice were purchased from Oriental yeast (Osaka, Japan). Fcer1γ−/− mice (BALB/c-background) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Kit−/− mice were purchased from the Japan SLC (Hamamatsu, Japan). Crl2−/− mice (BALB/c-background) were generated as previously described (39, 40). Il33−/− mice (BALB/c background) (37, 41) and Il4−/− (BALB/c background) (23, 42) were bred at the animal facilities of Hyogo College of Medicine. Three to eight female mice, 6–10 weeks of age, were used in each study as experimental and control groups. All mice were kept under specific pathogen-free conditions and received humane care. All animal experiments were performed in accordance with guidelines of the Institutional Animal Care Committee of Hyogo College of Medicine (Hyogo, Japan).

Antibodies and reagents

PE-Cy5-anti-CD3ε (145-2C11), FITC-anti-CD45.2 (104) and allophycocyanin-anti-CD49b (DX5) antibodies were purchased from BD Biosciences (San Jose, CA, USA). Anti-FcεRI antibody (MAR-1) was from eBiosciences (San Diego, CA, USA). Anti-CD16/32 (93), PE-Cy5-anti-CD45R/B220 (RA3-6B2) and purified-anti-MCP-8 (TUG8) antibodies were from BioLegend (San Diego, CA, USA). PE-anti-IgE (23G3) antibody was from Southern Biotechnology Associates (Birmingham, AL, USA). Anti-CD200 receptor-like-3 (Ba103) and RAT IgG2b antibodies were from Hycult Biotech (Plymouth Meeting, PA, USA). Anti-DNP IgE mAb (SPE-7), OVA (grade V) and sodium dodecyl sulfate (SDS) were from Sigma-Aldrich Japan (Tokyo, Japan). Soluble ST2 (recombinant human ST2/IL-1R4 Fc chimera) was from R&D SYSTEMS (Minneapolis, MN, USA).

Mouse model of food allergy

For epicutaneous sensitization, mice were treated with 4% SDS in sterile distilled water on their shaved back skin, and 10 min later 300 µg of OVA in 60 µl of PBS or PBS alone (control) were applied to the skin. The mice were epicutaneously sensitized with OVA three times a week, for the first 2 weeks. To induce systemic anaphylaxis, mice were intra-gastrically challenged on day 21 with 5 mg of OVA using a ball-ended mouse feeding needle. In some experiments, mice were given 1 mg of OVA intra-gastrically before epicutaneous sensitization. To block IL-33 signaling, mice were given 50 µg of soluble ST2 intra-peritoneally 24 and 2 h before oral challenge with OVA.

Measurement of systemic anaphylaxis

The changes in rectal temperature of mice were measured using a Microprobe Thermometer BAT-12 (Physitemap, NJ, USA), at 0, 5, 10, 15, 20, 30, 45 and 60 min after OVA challenge.

Vascular permeability measurements

Vascular permeability was evaluated by measuring the leakage of Evans blue dye into the skin and intestine. Evans
blue dye (20mg kg⁻¹) was injected in the tail vein just before challenge. At 15min after intra-gastric challenge with OVA, mice were sacrificed and systemic circulation was perfused with saline to remove intravascular dye. The skin and intestine were dissected from the mice, blotted dry and weighed. Evans blue dye was extracted in formamide at 37°C for 24h and the concentration was determined with a spectrophotometer at the absorbance maximum of 620 nm wavelength. The tissue content of the Evans blue dye (ng g⁻¹ wet weight tissue) was calculated from a standard curve of Evans blue dye concentration.

**OVA-specific IgE measurement**

OVA-specific IgE levels were measured by DS Mouse IgE ELISA (OVA) (DS Pharma Biomedical, Osaka, Japan).

**RT–PCR**

Total RNA was isolated from tissues or CD4⁺ T cells isolated from inguinal lymph nodes (LN) using an RNaseasy Mini Kit (Qiagen, Valencia, CA, USA), and cDNA was synthesized using superscript III (Invitrogen, Carlsbad, CA, USA). For quantitative PCR, DNA fragments were amplified using Premix Ex Taq (Takara Bio, Otsu, Japan) and gene-specific TaqMan probe (Applied Biosystems, Carlsbad, CA, USA). Gene-specific PCR products were measured using the Thermal Cycler Dice Real Time System II (Takara Bio). The levels of target gene expression were normalized to β-actin expression using the 2⁻ΔΔCt method. The following primers and probes were purchased from Applied Biosystems: mouse: Actb (Mm00607939_s1), Foxp3 (Mm00475162_m1), Il4 (Mm00445259_m1), Il13 (Mm00434204_m1), Il23 (Mm00505403_m1), Mcp1 (Mm00484933_m1), Tsip (Mm01157588_m1).

**Immunohistochemistry**

Paraffin-embedded sections (4 μm thick) of the skins were deparaffinized, and heated in citrate buffer (pH 6.0) for epitope retrieval and then cooled at room temperature for 50min before blocking. The sections were incubated in PBS containing 1.0% BSA and 0.05% Tween 20 for blocking. The sections were incubated with a primary antibody for basophils, anti-mMCP-8 mAb (TUG8) (BioLegend), at 4°C overnight, followed by incubation with a secondary antibody, biotin-conjugated goat antibody against rat IgG (Vector Laboratories, Burlingame, CA, USA) at room temperature for 30min and staining with Alexa Fluor 594-conjugated streptavidin (Invitrogen), at room temperature for 30min. The sections were cover-slipped with mounting medium containing 4',6-diamidino-2-phenylindole (DAPI) (Invitrogen). The sections were washed with PBS containing 0.05% Tween 20 before each step. Sections were examined under a Zeiss LSM 510 microscope (Carl Zeiss, Thornwood, NY, USA). Computer software, ZEN 2011 (Carl Zeiss), was used for image processing and analysis.

**Hematoxylin and eosin staining of skin**

Skin from the backs of mice was dissected from mice. The specimens were fixed in 4% (w/v) paraformaldehyde, embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin.

**Basophil depletion**

For basophil depletion, anti-FcεRI mAb (MAR-1) and anti-CD200 receptor like-3 mAb (Ba103) were used. For depletion with MAR-1 mAb, mice were intraperitoneally injected with 5 μg of MAR-1 or isotype control (eBio299Arm) antibodies, 3 days before starting skin sensitization. Antibodies were injected twice a day for three consecutive days every week (days −3 to −1, 4–6 and 11–13). For depletion with the Ba103 mAb, mice were intravenously injected with 10 μg of Ba103 or control Rat IgG2b antibodies from 1 day before starting skin sensitization. Antibodies were injected every 4 days (days −1, 3, 7 and 11).

**Flow cytometry**

Single-cell suspensions were pre-incubated with anti CD16/32 mAb (93) for blocking before staining. Basophils are defined as CD3ε⁻B220⁻CD49b⁺IgE⁺ cells.

**Statistical analyses**

Data are expressed as mean ± SEM and statistical significance was analyzed by analysis of variance or unpaired Student’s t-tests. Significance for all statistical tests is shown in figures as P < 0.05 (*) and P < 0.01 (**).

**Results**

**Epicutaneous sensitization and oral challenge with a food antigen induces IgE-dependent anaphylaxis**

WT Balb/c mice were epicutaneously sensitized with OVA or PBS at a shaven skin site topically treated with SDS, three times a week for the first 2 weeks, and then intra-gastrically challenged with OVA at day 21 (Fig. 1A). Il4 mRNA levels in the inguinal LNs at day 20 were ~6-fold higher in epicutaneously OVA sensitized-mice compared with control mice (Fig. 1B). Mice epicutaneously sensitized and orally challenged with OVA, but not control mice, showed significantly increased OVA-specific IgE levels in their sera over time (Fig. 1C). After oral challenge with OVA, epicutaneously OVA-sensitized mice showed a prompt and significant decrease in core-body temperature (Fig. 1D). To monitor serum leakage caused by anaphylaxis, mice epicutaneously sensitized with OVA were intravenously injected with Evans blue dye just before the oral challenge. Fifteen minutes after oral challenge with OVA, but not PBS, Evans blue leaked at the sensitized skin site, as well as the upper small intestine of sensitized mice (Fig. 1E and F), similar to the symptoms of food allergy patients including skin rashes (5). To determine if the anaphylactic responses were IgE-dependent, mice deficient for the high-affinity receptor for IgE (Fcer1⁺⁺⁺ mice) were used. Although Fcer1⁺⁺⁺ mice produced OVA-specific IgE comparable to WT mice (Fig. 1G), they were completely protected from oral challenge-induced anaphylaxis as demonstrated by a decrease in core-body temperature (Fig. 1H) and serum leakage at skin and intestine (Fig. 1I). Thus, consistent with previous studies (19, 20), cutaneous sensitization of a food...
antigen induced systemic T<sub>2</sub>-type responses, and IgE-dependent anaphylaxis after oral challenge, with the symptoms closely resembling human food allergy.

**Early oral exposure to food antigen prevents food allergy development**

Studies have suggested that early consumption of food prevents the onset of food allergy by establishing oral tolerance before sensitization, thus the site an antigen engages first might be an important determinant for food allergy development (4, 7, 8, 14). We sought to determine whether oral introduction of OVA before epicutaneous sensitization prevented food allergy development in our model. To this end, mice were administered 1 mg of OVA intra-gastrically three times a week for 2 weeks before epicutaneous sensitization to induce OVA-specific oral tolerance (Fig. 2A). Tolerance-induced mice showed Foxp3 mRNA up-regulation but did not show...
II4 mRNA up-regulation in the inguinal LNs (Fig. 2B and C) or elevation of serum OVA-specific IgE levels (Fig. 2D) after epicutaneous OVA application. As a result, tolerance-induced mice were protected completely from oral challenge-induced anaphylaxis (Fig. 2E). Thus, our mouse model clearly reflects the dual-allergen-exposure hypothesis in which allergic sensitization results from cutaneous food antigen exposure before its consumption (7, 8).

Basophils play a pivotal role in cutaneous induction of T,2 responses and food allergy development

Basophils have essential roles in the skin-mediated development of T,2 responses (28–30). To investigate the role of basophils in an epicutaneously sensitized food allergy model, we first examined the recruitment of basophils in the affected skin and inguinal LNs. Mmcp8-expressing cells (a basophil marker) (Supplementary Figure 1, available at International Immunology Online) and Mcpt8 mRNA (encoding Mmcp8) levels (Fig. 3A) increased in the skin after sensitization with OVA and peaked at day 11. In addition, basophil-attracting chemokine mRNAs, Ccl2, Ccl3 and Ccl7, were up-regulated in the skin (Supplementary Figure 2A, available at International Immunology Online). FACS analysis of cells residing in the skin also showed increased CD49b+FceRI+ basophils (Fig. 3B). In contrast, levels of the mast cell marker Mcpt1 and eosinophil marker Prg2 were not altered in the skin (Supplementary Figure 2B, available at International Immunology Online). Consistent with skin basophils, Mcpt8 mRNA expression (Fig. 3C), and the percentage and absolute number of basophils (Fig. 3D) increased in the inguinal LNs at day 11 of OVA sensitization. Along with increased basophil numbers, II4 mRNA levels increased in the skin (Fig. 3A) and inguinal LNs (Fig. 3C), suggesting a close relationship between basophils and T,2 development. Indeed, CD4+ T cells isolated from inguinal LNs at day 11 in epicutaneously OVA-sensitized mice showed increased II4 mRNA levels (Supplementary Figure 3, available at International Immunology Online). Moreover, as basophils are considered important IL-4 producers (28–30), basophils recruited to the inguinal LNs at day 11 in epicutaneously OVA-sensitized mice produced larger amounts of IL-4 compared with naive mice (Supplementary Figure 4, available at International Immunology Online).

We next examined the functional role of basophils in an epicutaneously sensitized food allergy model. To this end, we temporally depleted basophils from mice using two different antibodies, anti-FceRI antibody (MAR-1) (23) and anti-CD200 receptor like-3 antibody (Ba103) (43), by intra-peritoneal injection during the sensitization phase (Fig. 4A and B). Both antibodies efficiently depleted basophils (Supplementary Figure 5, available at International Immunology Online).
The elevation of Il4 mRNA expression in the inguinal LNs (Fig. 4C and D) and serum OVA-specific IgE levels (Fig. 4E and F) were markedly abrogated in basophil-depleted mice. Furthermore, basophil-depleted mice did not show decreased core-body temperature after oral antigen challenge (Fig. 4G and H). Basophil-depleting antibody treatment potentially affects mast cell function. However, mast cell-deficient KitW/W-v and the control Kit+/+ mice produced comparable levels of OVA-specific IgE in response to epicutaneous OVA sensitization, indicating that basophils, but not mast cells, are responsible for skin-mediated initiation of Th2 responses (Supplementary Figure 6, available at International Immunology Online). Thus, basophils are essential for the skin-mediated induction of Th2 responses and food allergy development. However, basophil numbers were unchanged in the small intestine following the induction of food allergy (Supplementary Figure 7, available at International Immunology Online), suggesting the specific involvement of the cells in the sensitization phase in the skin.

TSLP is essential for skin-mediated food antigen sensitization

Next, we sought to explore cytokines involved in the development of cutaneously sensitized food allergy. TSLP is an epithelia-derived Th2-inducing cytokine that may have a central role in Th2 initiation in the skin-basophil pathway (28–30). Epicutaneous sensitization with OVA induced the prompt up-regulation of TSLP mRNA (Fig. 5A) and protein (Fig. 5B) levels. Basophil recruitment in the skin (Fig. 5C) and inguinal LNs (Fig. 5D) after OVA sensitization was completely abrogated in TSLP-receptor-deficient Crlf2−/− mice. Furthermore, Crlf2−/− mice did not develop anaphylaxis by oral challenge (Fig. 5G). Thus, Crlf2−/− mice showed a defect in the initiation of Th2 responses in the epicutaneously sensitized food allergy model.

IL-33 is dispensable for sensitization, but is essential for effector phase responses

IL-33 is another epithelia-derived pro-Th2 cytokine (31). As for TSLP, the mRNA (Fig. 6A) and protein (Fig. 6B) levels of IL-33 showed a prompt increase in the skin after the epicutaneous application of OVA. However, basophil numbers in the skin (Fig. 6C) and inguinal LNs (Fig. 6D) were comparable between Il33−/− and WT mice. Although Il33−/− mice showed decreased Il4 mRNA levels (Fig. 6E), serum OVA-specific IgE levels were comparable between Il33−/− and WT mice (Fig. 6F). Thus, IL-33 may be partially involved in skin-mediated Th2 development, but is dispensable for inducing systemic IgE responses. However, even though the IgE levels were high, Il33−/− mice were completely protected from oral challenge-induced anaphylaxis (Supplementary Figure 8, available at International Immunology Online). These results demonstrate that IL-33
has a more important role in the effector phase in the gut rather than the sensitization phase in the skin.

Taken together, TSLP is essential for local basophil recruitment in response to the application of antigens to the skin and the subsequent induction of systemic T helper 2 responses. In contrast, although IL-33 is not essential for IgE production induced by cutaneous antigen sensitization, IL-33 has a pivotal role in the effector phase of food allergy.

Discussion

Here, we studied the pathophysiology of mice with food allergy initially sensitized to the food antigen by a cutaneous route. The mice developed symptoms closely resembling human food allergy patients with an IgE-dependent reduced core-body temperature and serum leakage in both the skin and intestine. We showed that basophils and TSLP have central roles in the development of food allergy by initiating cutaneous T helper 2-type sensitization. In contrast, IL-33 was not involved in the cutaneous sensitization but was essential for inducing anaphylaxis after oral challenge.

Skin is considered a major route for antigen sensitization in food allergy (7, 8). In this study, we demonstrated that when an antigen was exposed to barrier-disrupted skin, T helper 2-type immune responses were evoked resulting in the development of food allergy. In contrast, following oral exposure to the antigen, antigen-specific oral tolerance was induced and prevented skin-mediated antigen sensitization.
and the subsequent development of food allergy. Previously, experts recommended families with high-risk food allergy infants (based on a family history of atopy) to avoid consuming common food allergens, such as peanuts, milk or eggs, during the first 3 years to prevent food antigen sensitization (9). However, recent studies question the beneficial effect of delaying the introduction of solid foods that are considered allergic (12, 13). Epidemiologic studies showed that the delayed consumption of a food increases the risk for developing food allergy (14, 44). Here, our animal study supports the theory that early consumption of a food antigen prevents future sensitization to the antigen. Thus, the introduction of tolerance and reduction of food allergy development could be achieved by early oral exposure of food antigens. Furthermore, skin is a major target site for the prevention of food antigen sensitization, especially in barrier-disrupted individuals.

The important roles of basophils and TSLP in the initiation of T\(_2\)-type immune responses, especially in the skin, are becoming clear. Recently, Otsuka et al. (28) described a potential reason for the controversial basophil APC functions. In their epicutaneous antigen sensitization model, basophils functioned as APCs and were sufficient for initiating T\(_2\) responses when the antigen was a hapten or peptide. However, DCs were essential for T\(_2\) initiation to protein antigens, while basophils augmented T\(_2\)-differentiation in an in vitro culture. Other groups also proposed a basophil-DC cooperation model and described the skin-based, basophil- and DC-mediated T\(_2\)-inducing immune cascade (29, 30). Epicutaneous application of a vitamin D analog (29) or subcutaneous injection of vitamin D might be a treatment strategy for food allergy.
papain (30) induced local TSLP production, which activated DCs to up-regulate OX40 ligand and migrate into draining LNs. In turn, the DCs activated T cells cooperatively with basophils. Basophils skew the immune response toward Th2 by secreting IL-4. In the present study, basophil-depleted or Crlf2−/− mice were completely defective in Th2 development against epicutaneously sensitized antigens. Basophils in the inguinal LNs from OVA-sensitized mice produced more IL-4 than in naive LNs. Accordingly, in the case of skin-mediated sensitization to protein antigens, although the TSLP-DC pathway may play a central role in T cell activation, basophils are essential for skewing the response toward Th2, as they are indispensable IL-4 producers. Thus, skin is a unique tissue where basophils have a pivotal role in the initiation of optimal Th2 responses. In addition to localized Th2 responses in the skin, we showed that skin basophils and TSLP pathways are essential for evoking systemic IgE responses and the development of food allergy.

The role of TSLP in food allergy was previously unclear. Although a genetic epidemiological study predicted an association between TSLP and food antigen sensitization (45), the cytokine was dispensable in gut-mediated food antigen sensitization in mice (36, 46). Therefore, TSLP may participate in food allergy by mediating skin-, but not gut-, based antigen sensitization. During the preparation of this manuscript, the important roles of basophils and TSLP in a cutaneously sensitized food

Fig. 6. IL-33 is dispensable for cutaneous antigen sensitization but essential for the development of anaphylaxis. (A and B) Skin was obtained from mice at the indicated time points after epicutaneous application with OVA. (A) Total RNAs were extracted and subjected to quantitative PCR analysis for the expression of Il33 and Actb. (B) Total proteins were extracted and IL-33 content was determined by ELISA. (C–G) WT and Il33−/− mice were epicutaneously sensitized and orally challenged with OVA as in Fig. 1(A). (C and D) Skin (C) and nodes (iLNs) (D) were obtained from WT and Il33−/− mice at day 0 and 11 following epicutaneous sensitization with OVA. The presence of basophils (CD45+CD3−B220−CD49b+FceRI+) was examined. (E) iLNs were obtained from WT and Il33−/− mice at day 0 and 11 following epicutaneous sensitization with OVA. Total RNAs were extracted and subjected to quantitative PCR analysis for the expression of Il4 and Actb. (F) Sera from WT and Il33−/− mice were collected at indicated time points of epicutaneous sensitization. OVA-specific IgE levels were determined by ELISA. (G) Changes in rectal temperature of WT and Il33−/− mice after oral challenge were measured at the indicated time points. Data represent mean ± SEM from five to six mice of two independent experiments. **P < 0.01, *P < 0.05, ns, not significant.
allergy model were reported (47). Consistent with our results here, the depletion of basophils or TSLP in the sensitization phase protected mice from developing systemic IgE responses and oral challenge-induced anaphylaxis (47). Taken together, basophil- and TSLP-mediated immunological pathways can be targets for preventing the development of food allergy, especially for high-risk populations such as eczematous infants.

IL-33 is an epithelial cell-derived pleiotropic cytokine whose receptor is expressed on variety of cells including T cells, DCs, basophils and mast cells (31). Although our data showed the partial involvement of IL-33 in the initiation of T2 responses in the skin, Il33−/− mice developed serum OVA-specific IgE comparable to WT mice. Thus, skin-derived IL-33 is not important for inducing systemic IgE responses. However, Il33−/− mice and mice treated with soluble ST2 were completely protected from oral challenge-induced anaphylaxis. Interestingly, some individuals with high serum food allergen-specific IgE have no clinical evidence of food allergy (5). This suggests that IgE alone is not sufficient for inducing anaphylaxis and that other factor(s) are required (19), such as IL-33. IL-33 signaling might have an essential role in gut-mediated food allergen sensitization, elicited by intra-gastric application of peanut allergen together with cholera toxin (36). Moreover, IL-33 amplifies IgE cross-linking-mediated mast cell degranulation (37, 39), and thus can enhance anaphylaxis (38). In this study, because anaphylaxis was induced by a single challenge of the antigen and was an immediate reaction, amplifying mast cell degranulation rather than augmenting T2 expansion likely explains the role of IL-33 in our system.

Because polymorphisms in the gene encoding ST2/IL-33R are significantly linked to AD prevalence, the IL-33-ST2 pathway might be a risk factor for AD (48). In addition, although it is controversial, ectopic expression of IL-33 in keratinocytes could induce AD-like symptoms in mice (33, 49). As AD is closely associated with food allergy (15, 17), IL-33 could also be secondarily involved in skin-mediated food allergy development.

Here, we demonstrated the role of basophils and proallergic cytokines, TSLP and IL-33, in cutaneously sensitized food allergy. The mode of action of the cytokines in food allergy pathogenesis is not consistent with that previously reported in gut-mediated sensitization models (36, 46), thus the cytokines may have tissue- and context-specific roles. As the cause of food allergy can vary depending on the patient (1, 2), further studies are required to clarify the involvement of immune pathways in the distinct disease settings of food allergy. Our results suggest that basophil/TSLP pathways can be targeted to manage food allergy development prophylactically in presensitized high-risk individuals, such as eczematous infants. In addition, the IL-33 pathway could be a major target for postsensitized individuals to prevent anaphylaxis.

Supplementary data
Supplementary data are available at International Immunology Online.

Funding
Strategic Program Grant for Research Institute Development in Private Institute (S1001055) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in Japan; the Takeda Science Foundation.

Acknowledgements
We thank all the colleagues in our laboratories, K. Kumasako and C. Minemoto for secretarial assistance, and M. Nagata for technical assistance.

Disclosures: The authors have no financial conflicts of interest.

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