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Exposure to food allergens through inflamed skin promotes intestinal food allergy via the TSLP-basophil axis

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Abstract

Background—Exposure to food allergens through a disrupted skin barrier has been recognized as a potential factor in the increasing prevalence of food allergy.

Objective—To test the immunological mechanisms by which epicutaneous sensitization to food allergens predisposes to intestinal food allergy.

Methods—Mice were epicutaneously sensitized with ovalbumin (OVA) or peanut on an atopic dermatitis-like skin lesion followed by intragastric antigen challenge. Antigen-specific serum IgE levels and Th2 cytokine responses were measured by ELISA. Expression of type-2 cytokines and mast cell proteases in the intestine were measured by real-time PCR. Accumulation of basophils in

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AUTHOR CONTRIBUTIONS

M.N., B.S.K., M.C.S., G.D.R, and D.A. designed and performed experiments. M.K. provided Baso-DTR mice and M.R.C. provided TSLPR-deficient mice, murine rTSLP and cDNA TSLP plasmid. M.N., B.S.K., M.C.S., M.R.C., J.M.S and D.A. analyzed the data. M.N. and D.A. wrote the manuscript and all authors critically reviewed the manuscript.

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the skin and mast cells in the intestine was examined by flow cytometry. *In vivo* basophil depletion was achieved by diphtheria toxin treatment of Baso-DTR mice. For cell transfer studies, the basophil population was expanded *in vivo* by hydrodynamic tail vein injection of thymic stromal lymphopoietin cDNA plasmid.

Results—Sensitization to food allergens through an atopic dermatitis-like skin lesion is associated with an expansion of TSLP-elicited basophils in the skin that promote antigen-specific Th2 cytokine responses, elevated antigen-specific serum IgE levels and the accumulation of mast cells in the intestine promoting the development of intestinal food allergy. Critically, disruption of TSLP responses or depletion of basophils reduced the susceptibility to intestinal food allergy while transfer of TSLP-elicited basophils into intact skin promoted disease.

Conclusion—Epicutaneous sensitization on a disrupted skin barrier is associated with the accumulation of TSLP-elicited basophils that are necessary and sufficient to promote antigen-induced intestinal food allergy.

Keywords

Food allergy; atopic dermatitis; epicutaneous sensitization; TSLP; basophils; mast cells; IgE

INTRODUCTION

The prevalence of food allergies has increased in the past several decades with an estimated 5% of children and 3–4% of adults in industrialized countries living with the daily concern that exposure to certain foods may trigger a life-threatening allergic reaction. [1,2] Food allergies are defined by an adverse immune response that occurs repeatedly following exposure to a given food and can manifest in symptoms ranging from itching, hives and diarrhea to acute anaphylaxis with a life-threatening drop in blood pressure and airway constriction. [3,4,5] As a result, individuals must avoid certain foods to guard against accidental exposures. [6] Currently, there is no treatment to prevent or cure food allergies, and available medications only treat symptoms after the allergic reaction occurs. [4,7–9] As the public health and economic impact of food allergies continue to grow, there is an urgent need to develop new intervention strategies to prevent and treat this debilitating condition.

Cross-linking of allergen-specific IgE on mast cells has been shown to play a critical role in the pathogenesis of food allergy and therapeutic strategies to target the IgE-mast cell axis in patients have shown their potential for the prevention of anaphylactic responses. [10–12] Although studies in animal models and patients have demonstrated a clear role for the IgE-mast cell axis in the pathogenesis of food allergies, little is known about the early immunological events that initiate these effector responses. Epidemiological studies have demonstrated that cutaneous inflammation associated with atopic dermatitis (AD) is a significant risk factor for the development of food allergies. [8,13] The concept of skin barrier defects contributing to allergic sensitization is further supported by animal studies demonstrating that epicutaneous sensitization can predispose to antigen-induced allergic inflammation at other barrier sites. [14–17] These data indicate that the skin may be a highly relevant site of food allergen sensitization. [18–22] However, the immunological mechanisms

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through which antigen sensitization in the skin can predispose to allergic inflammation in the intestine are incompletely understood.

Employing a new model of food allergy, we demonstrate that epicutaneous sensitization to food antigens on an AD-like skin lesion is associated with the infiltration of thymic stromal lymphopoietin (TSLP)-elicited basophils that promote the development of IgE-mediated intestinal food allergy in response to oral antigen exposure. In this model, TSLP-elicited basophils were necessary and sufficient for the development of antigen-induced intestinal food allergy indicating that the TSLP-basophil axis may offer a new therapeutic target in the treatment and prevention of food allergy.

RESULTS

Antigen sensitization in the context of AD-like skin inflammation promotes allergic intestinal inflammation

To study the potential mechanisms by which intestinal food allergy develops following epicutaneous sensitization, we established a mouse model of food allergy in which mice were sensitized with food antigens on a developing AD-like skin lesion induced by topical treatment with the vitamin D analog, MC903. [24,25] WT BALB/c mice were epicutaneously sensitized to a model antigen, ovalbumin (OVA), on a developing AD-like skin lesion associated with elevated skin TSLP production (Fig 1. *A,B*). Following epicutaneous sensitization, mice were challenged intra-gastrically (i.g) with OVA resulting in the manifestation of allergic reactions and inflammation characterized by an elevated clinical allergy score (Fig 1. *C*), antigen-specific IgE responses (Fig. 1*D*), type-2 inflammation in the intestine (Fig 1. *E,F*), the accumulation of intestinal mast cells (Fig 1. *G*) and expression of mast cell-specific proteases (Fig 1. *H*) in the small intestinal lamina propria. Control vehicle treated mice or mice that were sensitized to OVA and challenged i.g. with a control antigen (bovine serum albumin, BSA) exhibited minimal or no signs of intestinal food allergy (Fig 1. *C–H*, Fig E1). A similar pattern of disease was observed in mice that were epicutaneously sensitized to peanut, another clinically relevant food allergen (Fig E2). Clinical symptoms including episodes of scratching, piloerection and reduced activity resolved within 60 min after each oral antigen exposure.

To test whether epicutaneous sensitization predisposing to intestinal food allergy may also occur through a mechanically disrupted skin barrier, mice were sensitized by means of repeated application of OVA to tape-stripped skin (Fig E3A). Similar to the MC903 based model, oral antigen exposure resulted in an increased allergy score (Fig E3B), elevated antigen-specific serum IgE (Fig E3C) and the accumulation of mast cells in the small intestinal lamina propria (Fig. E3D). Collectively, employing two different models of epicutaneous sensitization, we demonstrate that antigen sensitization through a compromised skin barrier can promote the development of antigen-induced intestinal food allergy.

Intestinal food allergy in this model was associated with systemic antigen-specific IgE responses (Fig 1. *D*) and the accumulation of IgE-bearing mast cells in the intestine (Fig 1. *G*), suggesting that the IgE-mast cell axis may play a critical role. To test the role of IgE in

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this model, mice deficient in IgE (*Igh-7*$^{-/-}$) were epicutaneously sensitized on an AD-like skin lesion and subsequently challenged i.g. with OVA. While oral antigen challenge of *Igh-7*$^{+/+}$ BALB/c mice resulted in an elevated clinical allergy score and antigen-specific serum IgE levels, *Igh-7*$^{-/-}$ mice did not manifest allergic symptoms (Fig E4). Consistent with other models of food allergy and data from studies in patients, these findings demonstrate IgE as an important effector molecule in the pathogenesis of food allergy. [26–28]

Antigen-induced food allergy is dependent on TSLP-TSLPR interactions

Skin barrier dysfunction is associated with elevated expression of TSLP in the skin and the development of type-2 cytokine-associated allergic inflammation. [14,25,29–31] Further, gain-of-function polymorphisms in *TSLP* and elevated TSLP responses have been associated with increased susceptibility to multiple allergic diseases in humans and mice. [25,32–37] Recent studies demonstrated that mice with AD-like skin lesions are more susceptible to the development of airway inflammation following aero-antigen challenge in a TSLP-dependent manner. [38] Taken together, these studies provoked the hypothesis that TSLP may be a key mechanism by which cutaneous sensitization to food antigens promotes the development of intestinal food allergy. To test the role of TSLP in this model of epicutaneous sensitization to intestinal food allergy, WT BALB/c (*Tslpr*$^{+/+}$) mice or mice deficient in the receptor for TSLP (*Tslpr*$^{-/-}$) were epicutaneously sensitized and subsequently challenged i.g. with OVA (Fig 2. *A*). Following oral antigen challenge, *Tslpr*$^{+/+}$ mice exhibited an elevated clinical allergy score (Fig 2. *B*), enhanced antigen-specific serum IgE levels (Fig 2. *C*), increased intestinal inflammation associated with type-2 cytokine responses (Fig 2. *D,E*), accumulation of mast cells in the intestinal lamina propria (Fig 2. *F*) and elevated expression of mast cell-specific proteases (Fig 2. *G*). In striking contrast, *Tslpr*$^{-/-}$ mice exhibited limited antigen-induced food allergy by all parameters examined (Fig 2. *B–G*). Collectively, these data indicate that TSLP-TSLPR interactions are necessary for the development of intestinal food allergy in this model.

TSLP is sufficient to promote antigen-induced intestinal food allergy

To test whether TSLP alone is sufficient to promote antigen-induced food allergy, WT BALB/c mice were treated intradermally (i.d.) with recombinant TSLP (rTSLP) in the presence of OVA or either PBS or OVA alone (Fig 3. *A*).[14] Oral antigen challenge of mice sensitized to OVA in the skin in the presence of rTSLP exhibited an increased allergy score (Fig 3. *B*), elevated antigen-specific serum IgE levels (Fig 3. *C*), increased intestinal inflammation associated with type-2 cytokine responses (Fig 3. *D,E*) and the accumulation of mast cells in the intestine (Fig 3. *F*) that was associated with elevated expression levels of mast cell-specific proteases (Fig 3. *G*) compared to controls (Fig 3. *B–G*). Of note, i.d. injection of OVA alone resulted in episodes of scratching illustrated in an elevated allergy score, but no differences in all other parameters assessed. Collectively, these data demonstrate that TSLP-TSLPR interactions are necessary (Fig 2) and sufficient (Fig 3) to promote allergic responses in the intestine upon oral antigen exposure.

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TSLP-elicited basophils promote antigen-specific Th2 cytokine responses in the skin

TSLP is known to act on multiple cellular targets including T cells, dendritic cells, mast cells and basophils [33,39]. We recently demonstrated that AD-like skin lesions are associated with the infiltration of TSLP-dependent basophils that promote Th2 cytokine-associated inflammation in the skin. [40] We therefore hypothesized that TSLP-elicited basophils may promote cutaneous antigen-specific Th2 cytokine responses that contribute to the activation of IgE-mast cell responses and the subsequent development of intestinal food allergy.

Consistent with previous studies, [21,40] AD-like skin lesions in this model were associated with the accumulation of TSLP-dependent basophils in the skin, but not the intestine (Fig E5, and data not shown). To test whether TSLP-elicited basophils influenced the development of antigen-specific Th2 cytokine responses and associated skin inflammation, we employed a genetic approach in which basophils are selectively depleted by diphtheria toxin due to the expression of the DT receptor (DTR) on their surface. [41] Using this strategy, Baso-DTR^{pos} mice and Baso-DTR^{neg} littermate controls were epicutaneously sensitized with MC903 + OVA while simultaneously being treated with DT (Fig 4. *A*). DT treatment resulted in efficient depletion (~90 %) of basophils in Baso-DTR^{pos} mice (Fig 4. *B*) that was associated with a reduction in skin inflammation (Fig 4. *C,D*). To test whether basophil depletion affected Th2 cytokine responses, skin-draining lymph node cells were re-stimulated *ex vivo* with antigen. While Baso-DTR^{neg} mice treated with MC903 + OVA in the presence of DT exhibited elevated antigen-specific Th2 cytokine responses, this response was significantly reduced in mice depleted of basophils (Fig 4. *E*). Taken together, these data demonstrate that TSLP-elicited basophils in the context of an AD-like skin lesion promote skin inflammation and antigen-specific Th2-cytokine responses.

TSLP-elicited basophils regulate antigen-induced food allergy

Given that TSLP-dependent basophils promoted local antigen-specific Th2 cytokine responses (Fig 4. *E*), we sought to test the role of basophils in mediating intestinal food allergy. Using the same approach described above to deplete basophils, Baso-DTR^{pos} or Baso-DTR^{neg} mice were epicutaneously sensitized and orally challenged while simultaneously being treated with DT (Fig 5. *A*). Strikingly, while skin sensitized control Baso-DTR^{neg} mice exhibited intestinal food allergy in response to oral antigen exposure (Fig 5. *B–G*), depletion of basophils in Baso-DTR^{pos} mice limited antigen-induced food allergy by all parameters examined (Fig 5. *B–G*). Collectively, these findings demonstrate that the TSLP-basophil axis regulates antigen-specific Th2 cytokine responses, systemic antigen-specific IgE levels and the accumulation of intestinal mast cells promoting the development of intestinal food allergy.

To test whether basophils are sufficient to promote antigen-induced intestinal food allergy, gain-of-function studies employing adoptive transfer of basophils were performed. To expand the basophil population *in vivo*, BALB/c mice received hydrodynamic tail vein injections of cDNA plasmid encoding TSLP. [40] Three weeks after TSLP plasmid injections, basophils were sort-purified from the periphery and injected i.d. into the ears of naive BALB/c mice in the presence of OVA on various days prior to oral antigen exposure (Fig 6. *A*). While control mice treated with PBS or OVA alone did not manifest signs of intestinal

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food allergy, mice adoptively transferred with TSLP-elicited basophils in the presence of OVA exhibited signs of antigen-induced intestinal food allergy by all parameters assessed (Fig 6. B–G). Collectively, these data demonstrate that TSLP-elicited basophils are both necessary and sufficient to promote IgE-mediated intestinal food allergy in this model.

DISCUSSSION

AD in infancy is a risk factor for developing food allergies later in life. [8,13] However, the immunological mechanisms through which antigen sensitization in the skin can predispose to allergic inflammation in the intestine are incompletely understood. Employing a new model of intestinal food allergy initiated by epicutaneous antigen sensitization, we demonstrate that TSLP-elicited basophils orchestrate the development of IgE-mediated intestinal food allergy. We show that in the context of an AD-like skin lesion, basophils infiltrate the skin in a TSLP-dependent manner and promote antigen-specific Th2 cytokine responses. Further, we demonstrate that TSLP-elicited basophils are both necessary and sufficient for the development of IgE-mediated intestinal food allergy. These findings support the hypothesis that antigen sensitization through a disrupted skin barrier is a risk factor for the development of food allergy and provide a cellular mechanism by which TSLP-elicited basophils promote allergic inflammation in the intestine.

Accurately modeling the progression of allergic disease in patients using murine model systems presents numerous challenges, particularly regarding the time scale of the events that lead from sensitization to an allergen at one mucosal site to allergic disease at multiple mucosal barrier surfaces. Despite these challenges, previously described murine model systems have provided significant insight into the mechanisms that mediate the pathogenesis of intestinal food allergy. Consistent with other food allergy models, [16,42] antigen-induced allergic reactions in this model were mediated by the IgE-mast cell axis, as mice deficient in IgE did not manifest allergic symptoms in response to oral antigen exposure. While other models of epicutaneous sensitization reported anaphylactic reactions in response to oral allergen exposure [16], no drop in temperature after oral antigen exposure was observed in the described model (data not shown). Further, epicutaneous sensitization in absence of antigen did not result in increased mucosal mast cell frequencies as compared to other studies. [16] Differences in the schedules of sensitization of the various models may account for the observed discrepancies in models of epicutaneous sensitization. Importantly, epicutaneous antigen sensitization in the model described here resulted in the simultaneous development of intestinal food allergy and, as previously reported, eosinophilic esophagitis (EoE)-like disease, two commonly co-existing food-induced allergic disorders. [25] Eosinophil accumulation in this model was not only restricted to the esophagus, but was evident in the skin, the stomach, intestine and the periphery of mice with intestinal food allergy/EoE-like disease. [25] Taken together, the described model may provide a new research tool that can be used to test new hypotheses and potential treatment protocols in the context of food allergy/EoE.

While the effector mechanisms in the pathogenesis of food allergies are well known, the sites of sensitization to food allergens and the upstream immunological mechanisms that initiate and regulate subsequent effector responses in the intestine are poorly understood. We

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previously demonstrated that TSLP promotes the population expansion of basophils and that gain-of function polymorphisms in the gene encoding for TSLP are associated with atopy and peripheral basophilia. [25,40] In the present model system, AD-like skin lesions were associated with a significant infiltration of TSLP-elicited basophils that were necessary and sufficient to promote the development of antigen-induced intestinal food allergy. While topical treatment with MC903 results in continuous TSLP expression and a high clinical allergy score upon oral antigen exposure of previously sensitized mice, adoptive transfer of basophils into an environment with low TSLP levels may affect survival and activation status of transferred basophils resulting in a lower, but still significant clinical allergy score compared to controls. Importantly, similar to the model system used, increased expression of TSLP and infiltration of basophils into lesional skin has been reported previously in AD patients. (Kim et al., personal communication and [43]) Together, these data indicate that TSLP-elicited basophil responses may be a key upstream mechanism that precedes the induction of effector antigen-specific IgE responses and the accumulation of mast cells in the intestine promoting allergic intestinal inflammation.

The mechanisms by which TSLP-elicited basophils promote efficient antigen sensitization and $CD4^+$ Th2 cell polarization in this model remain to be elucidated. Previous studies have shown that in some settings major histocompatibility complex class II (MHC-II)–dependent interactions between basophils and $CD4^+$ T cells can augment Th2-cytokine responses. [44–46] However, it is likely that dendritic cells (DCs) and other antigen presenting cells contribute in antigen sampling, processing and presentation. Basophil-derived mediators may upregulate OX40L on DCs creating a Th2-permissive environment. [39,44,46] Further studies are necessary to investigate the complex interplay between TSLP-elicited basophils, DCs and T cells in mediating local antigen sensitization and Th2 polarization.

Recent studies suggest that several cardinal features of food allergy including Th2 sensitization to allergens, IgE responses and the expansion of intestinal mast cells result from enhanced IL-4 signaling in humans and mice. [42,47] The critical role of IL-4 in mediating intestinal-food allergy is further substantiated by the observation that IL-4 deficient mice are protected from antigen-induced food allergy in this model (data not shown). Importantly, TSLP-elicited basophils have been attributed a critical role in the induction and propagation of type-2 immune responses by providing an early innate source of IL-4. [40,48] Our findings that TSLP-elicited basophils are recruited early in the context of AD-like inflammation and are critical for the development of subsequent intestinal food allergy provoke the hypothesis that basophil-derived IL-4 may be a key mechanism by which allergic inflammation at one epithelial barrier surface can promote the development of allergic inflammation at another barrier site. Given the short life-time of a mature basophil, it is likely that basophils accumulate in inflamed skin in response to elevated TSLP levels, and prior to degranulation, release IL-4 into the periphery that may account for optimal Th2 polarization, IgE class switching, the expansion of intestinal mast cells and subsequent intestinal allergic inflammation. In this context, adoptive transfer of IL-4 $eGFP^+$ basophils into skin of WT recipients was not associated with an accumulation of IL-4 $eGFP^+$ basophils in the periphery 24 h after transfer, indicating that transferred cells may not leave the skin (data not shown). However, further studies are necessary to interrogate fate of TSLP-elicited basophils recruited to the skin and the role of basophil-derived IL-4 that may

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regulate the susceptibility to intestinal food allergy. Importantly, recent clinical trials using anti-IL4 antibodies (dupilomab) in patients with AD demonstrated promising results in the improvement of eczema severity [49]. Given that AD patients are at a higher risk to suffer from food allergies, targeting IL-4 responses in patients with AD may be a previously unrecognized therapeutic pathway to limit the development of intestinal food allergy.

In conclusion, we establish an experimental model of food allergy that recapitulates many characteristics of human disease in that the skin may be a relevant source of allergen sensitization. AD is a known risk factor for food allergy and human AD skin lesions are associated with elevated TSLP expression and basophil infiltration. These responses, coupled with systemic allergen-specific IgE responses and the accumulation of IgE-bearing mast cells in the intestine likely act to fuel intestinal allergic inflammation. Employing this model we provide a previously unrecognized cellular mechanism by which the TSLP-basophil axis promotes early phase local antigen-specific Th2 cytokine responses, systemic antigen-specific serum IgE levels and the accumulation of IgE-bearing intestinal mast cells promoting the development of IgE-mediated intestinal food allergy. Targeting the TSLP-basophil axis in the context of AD may offer a novel therapeutic approach for early intervention and therapeutic strategies to prevent the development of intestinal allergic inflammation.

METHODS

Allergy scoring

Starting 20 min after oral antigen challenge, mice were individually observed for a period of 30 min and a clinical allergy score was quantified as follows: 0: no symptoms; 1: less than 4 episodes of scratching and rubbing around nose and head; 2: 4–10 episodes of scratching and rubbing around nose and head; 3: more than 10 episodes of scratching and rubbing around nose and head; 4: hunching and piloerection; 5: immobility (unresponsive to non-harmful, tactile disturbance); 6: death). Investigators were blinded to the treatment group conditions when scoring for allergy symptoms.

Flow cytometry

Small intestinal lamina propria cells or cells from the skin were isolated as described previously. [27,28] Single cell suspensions were incubated with Aqua Live/Dead Fixable Dye (Invitrogen) for dead cell exclusion and stained with anti-mouse fluorochrome-conjugated monoclonal antibodies against B220, CD3e, CD4, CD5, CD8, CD11c, NK1.1, CD19, IgE, CD45, CD49b or CD117 (c-kit) (BD Bioscience, BioLegend, eBioscience). Mast cells were identified as live, lin^- (CD3,CD5,CD19,CD11c,NK1.1), $CD45^+$, $c\text{-}kit^+$, IgE^+ cells and basophils as live, lin^-, $CD45^+$, $c\text{-}kit^-$, $CD49b^+$, IgE^+ cells.

Adoptive basophil transfer

WT BALB/c mice were injected with a cDNA plasmid encoding TSLP as previously described. [29] Three weeks after TSLP cDNA injection, basophils were sort purified, and 5–10×10^4 cells were transferred i.d (ears) in the presence of 100 μg OVA on various days. As control, mice were injected with PBS or 100 μg OVA alone.

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ELISA, histology and real-time PCR

To measure skin TSLP levels, ears were incubated overnight in culture media, and cell-free supernatants were stored. Measurement of TSLP was performed using a commercially available Duoset ELISA kit (eBioscience). Antigen-specific serum IgE responses were measured as described before. [30] For histological analysis, tissues were fixed in 4% paraformaldehyde and embedded in paraffin, and sections were stained with hematoxylin and eosin (H&E). For real-time PCR analysis, RNA was isolated using an RNeasy mini kit (QIAGEN) according to the manufacturer's instructions. cDNA was generated using a SuperscriptII reverse transcription kit (Invitrogen). Real-time quantitative PCR was performed on cDNA using SYBR green master mix (Applied Biosystems) and commercially available primer sets from Qiagen (Quantitect primer assays). Samples were normalized to β-actin and displayed as fold induction over controls.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

AD	atopic dermatitis
CPE	crude peanut extract
DT	diphtheria toxin
IgE	immunoglobulin E
IL	interleukin
i.d	intradermal
i.g	intragastrically
i.p.	intraperitoneally
lin$^-$	lineage negative
Mcpt	mouse mast cell protease
OVA	ovalbumin

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Th2	T-helper type 2
TSLP	thymic stromal lymphopoietin
WT	wild-type

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Clinical implications

Targeting the TSLP-basophil axis may offer a novel therapeutic approach for early intervention and therapeutic strategies to prevent the development of food allergy.

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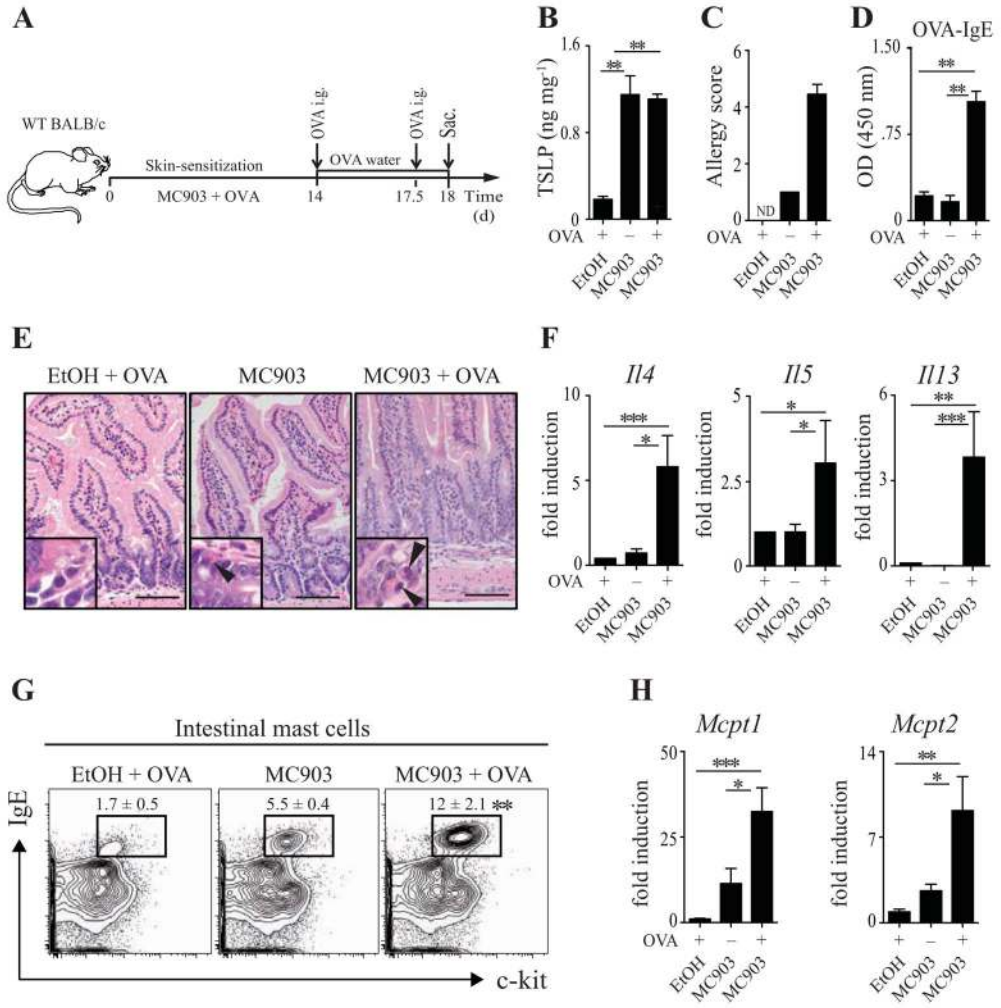

FIG. 1.

Epicutaneous sensitization promotes antigen-induced food allergy. **A,** Experimental protocol. **B,** TSLP protein in the skin. **C,** Allergy score. **D,** OVA-specific serum IgE levels. **E,** H&E staining from the jejunum. Scale bar: 100 μm. Arrows: tissue-infiltrating granulocytes. **F,** Type-2 cytokine expression in the jejunum. **G,** Frequencies of mast cells in the lamina propria. **H,** Jejunal *Mcpt1* and *Mcpt2* expression. $N = 3–4$ per group. Data are representative of three independent experiments. ND: no disease.

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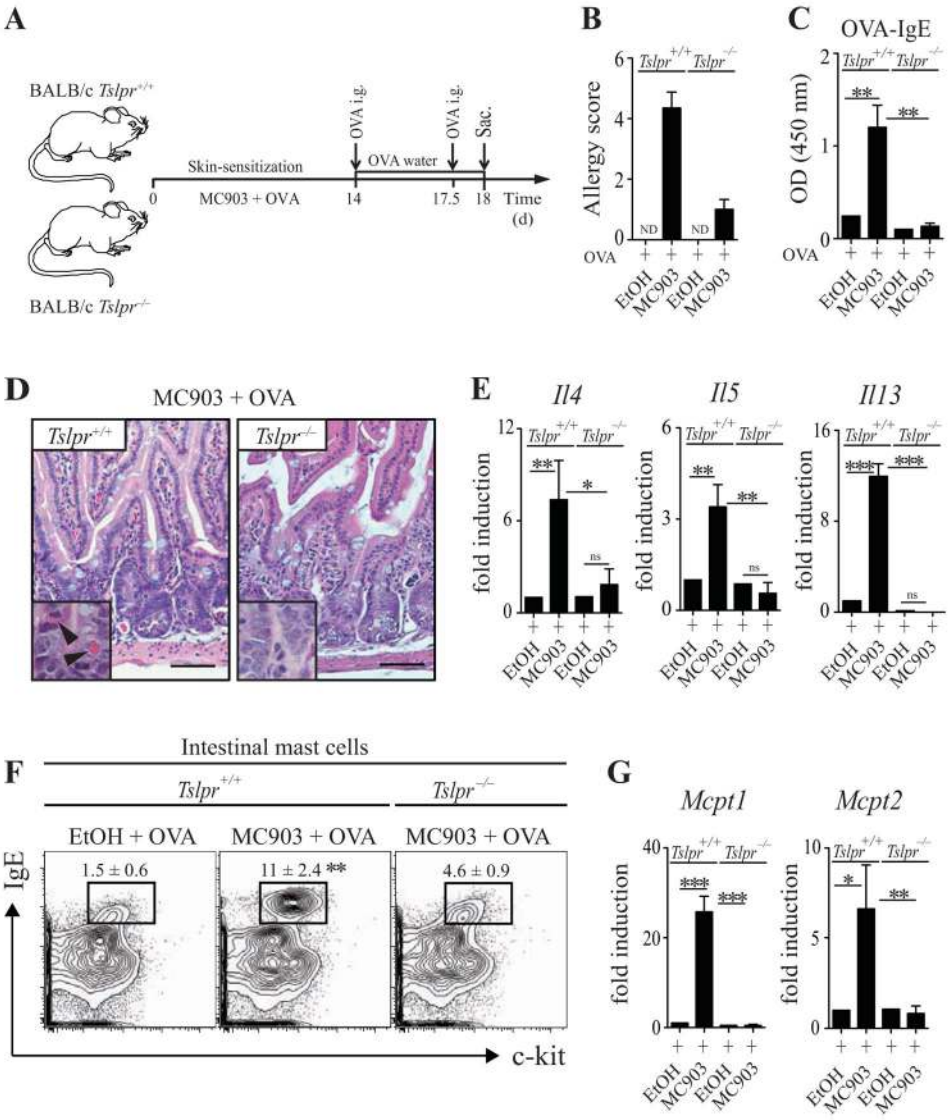

FIG. 2.

TSLP-TSLPR responses mediate antigen-induced food allergy. **A,** Experimental protocol. **B,** Allergy score. **C,** OVA-specific serum IgE levels. **D,** H&E staining from the jejunum. Scale bar: 100 μm. Arrows: tissue-infiltrating granulocytes. **E,** Type-2 cytokine expression in the jejunum. **F,** Frequencies of mast cells in the lamina propria. **G,** Jejunal *Mcpt1* and *Mcpt2* expression. $N = 3–4$ per group. Data are representative of three independent experiments. ns: not significant.

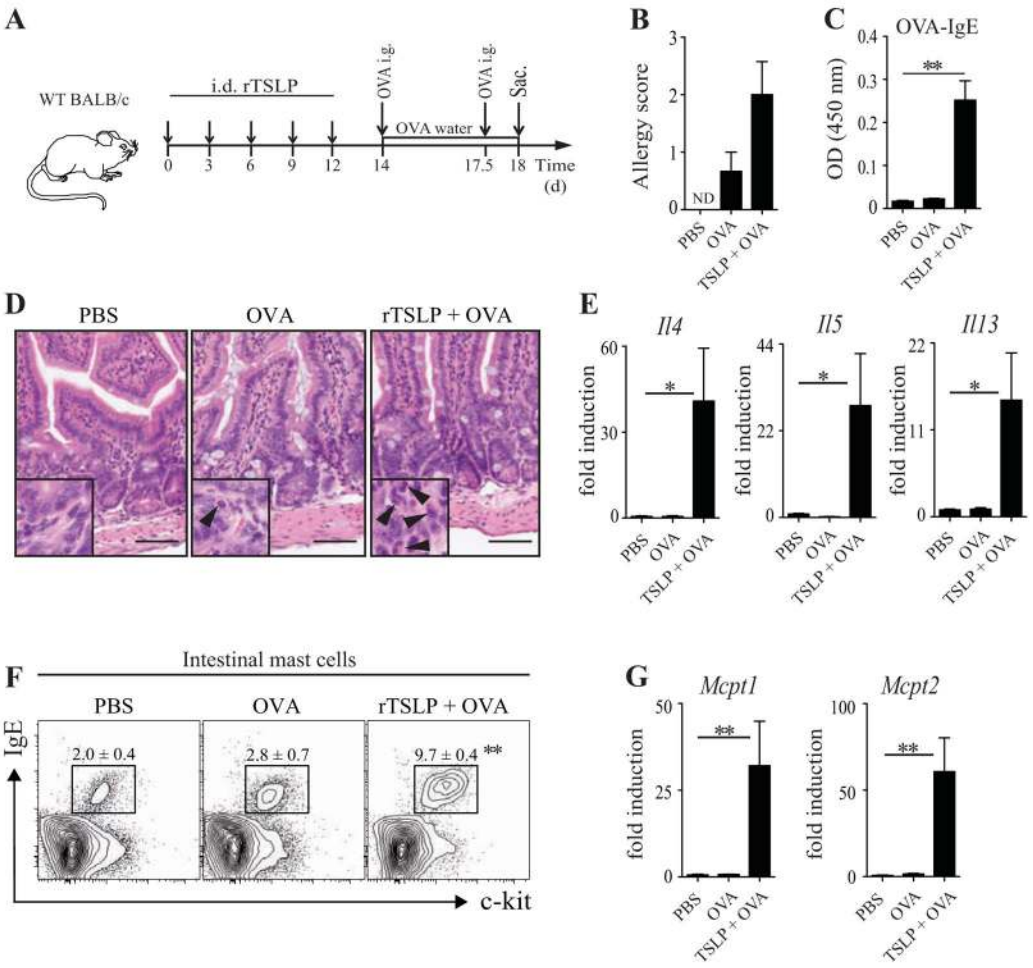

FIG. 3.

TSLP is sufficient to promote antigen-induced intestinal food allergy. **A,** Experimental protocol. **B,** Allergy score. **C,** OVA-specific serum IgE levels. **D,** H&E staining from the jejunum. Scale bar: 100 μm. Arrows: tissue-infiltrating granulocytes. **E,** Type-2 cytokine expression in the jejunum. **F,** Frequencies of mast cells in the lamina propria. **G,** Jejunal *Mcpt1* and *Mcpt2* expression. $N = 3$ mice per group. Data are representative of three independent experiments.

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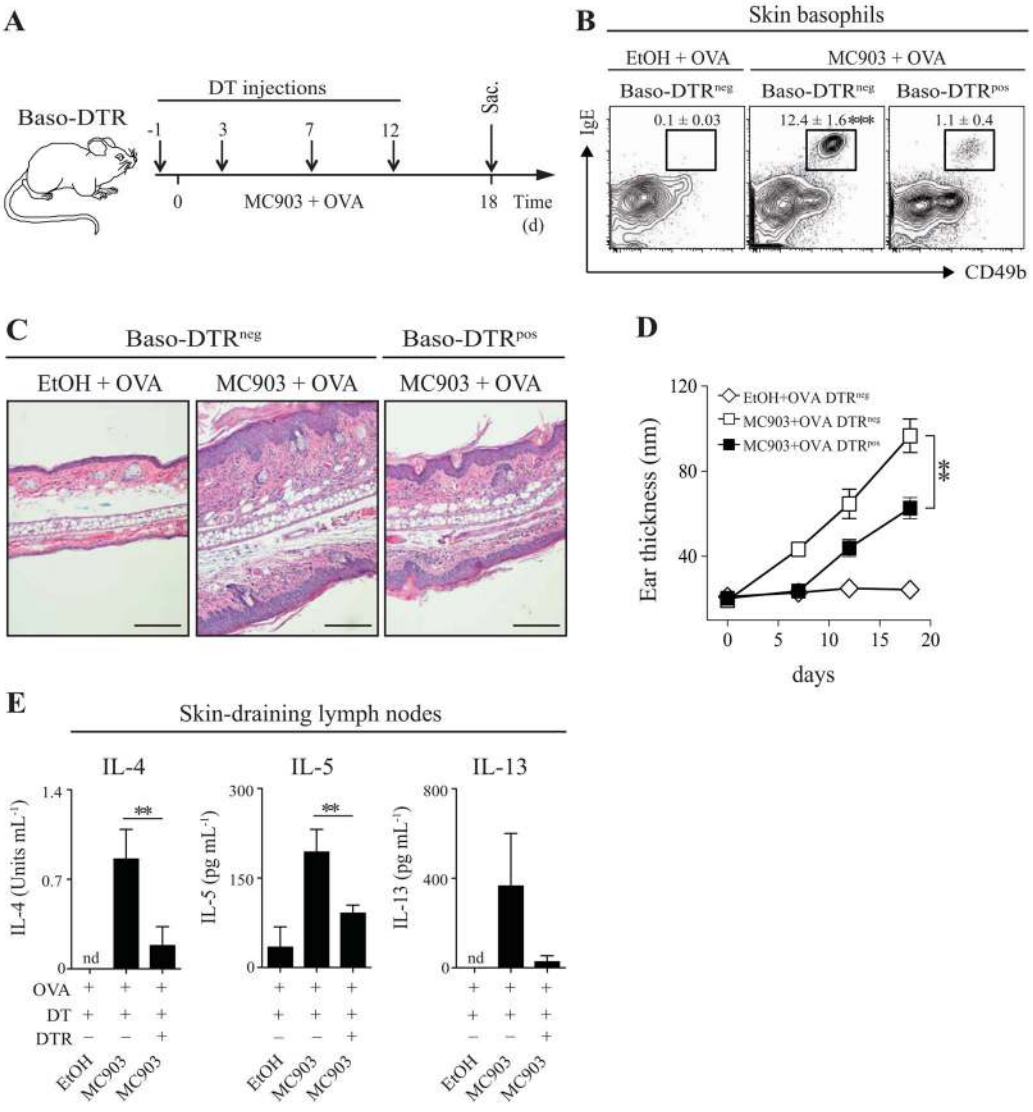

FIG. 4.

AD-like skin lesions are associated with the accumulation of TSLP-elicited basophils in the skin. **A,** Experimental protocol. **B,** Frequencies of basophils in the skin. **C,** H&E staining from the skin. Scale bar: 100 μm. **D,** Ear thickness measurement. **E,** Th2 cytokine responses in antigen re-stimulated skin-draining lymph nodes. $N = 3–4$ per group. Data are representative of three independent experiments. nd: not detected.

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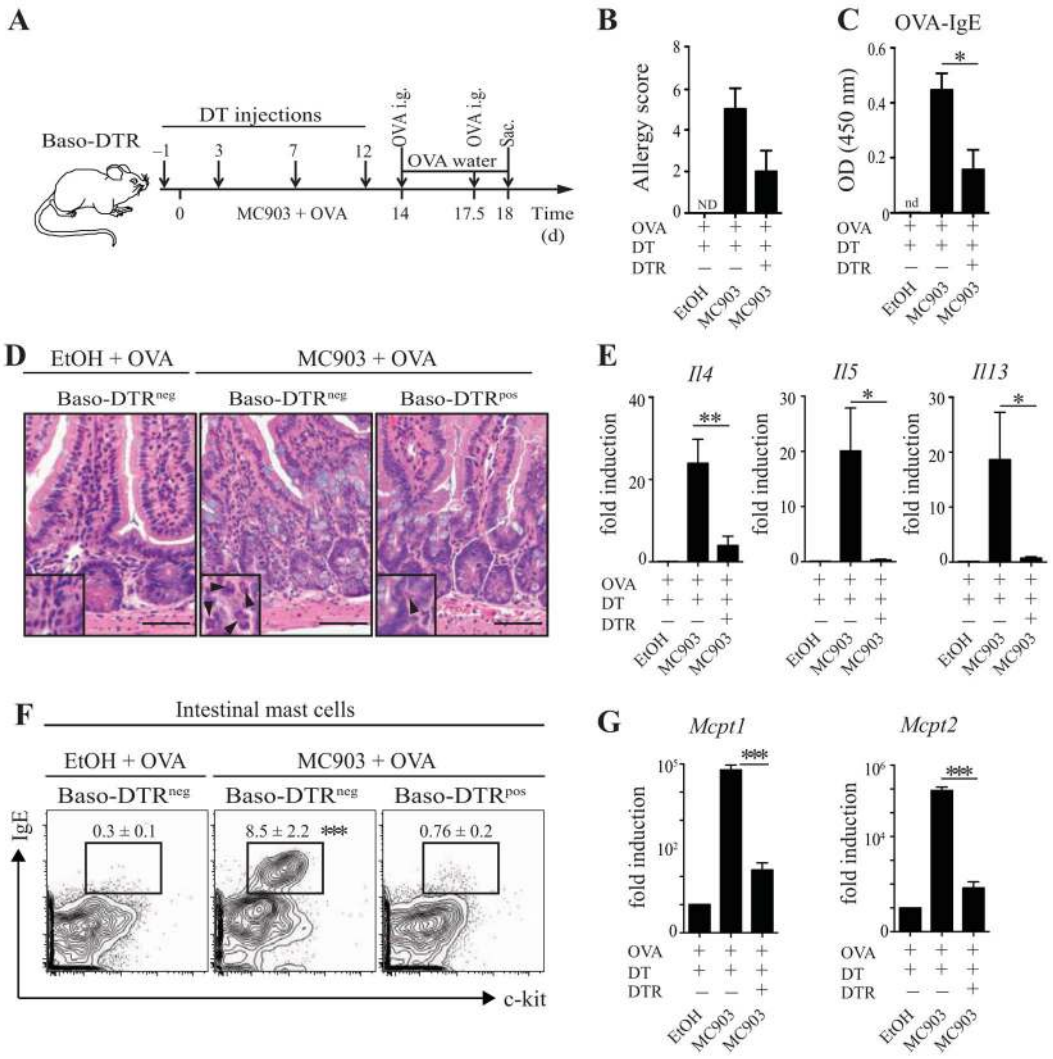

FIG. 5.

TSLP-elicited basophil responses regulate antigen-induced food allergy. **A,** Experimental protocol. **B,** Allergy score. **C,** OVA-specific serum IgE levels. **D,** H&E staining from the jejunum. Scale bar: 100 μm. Arrows: tissue-infiltrating granulocytes. **E,** Type-2 cytokine expression in the jejunum. **F,** Frequencies of mast cells in the lamina propria. **G,** Jejunal *Mcpt1* and *Mcpt2* expression. $N = 3–5$ per group. Data are representative of three independent experiments.

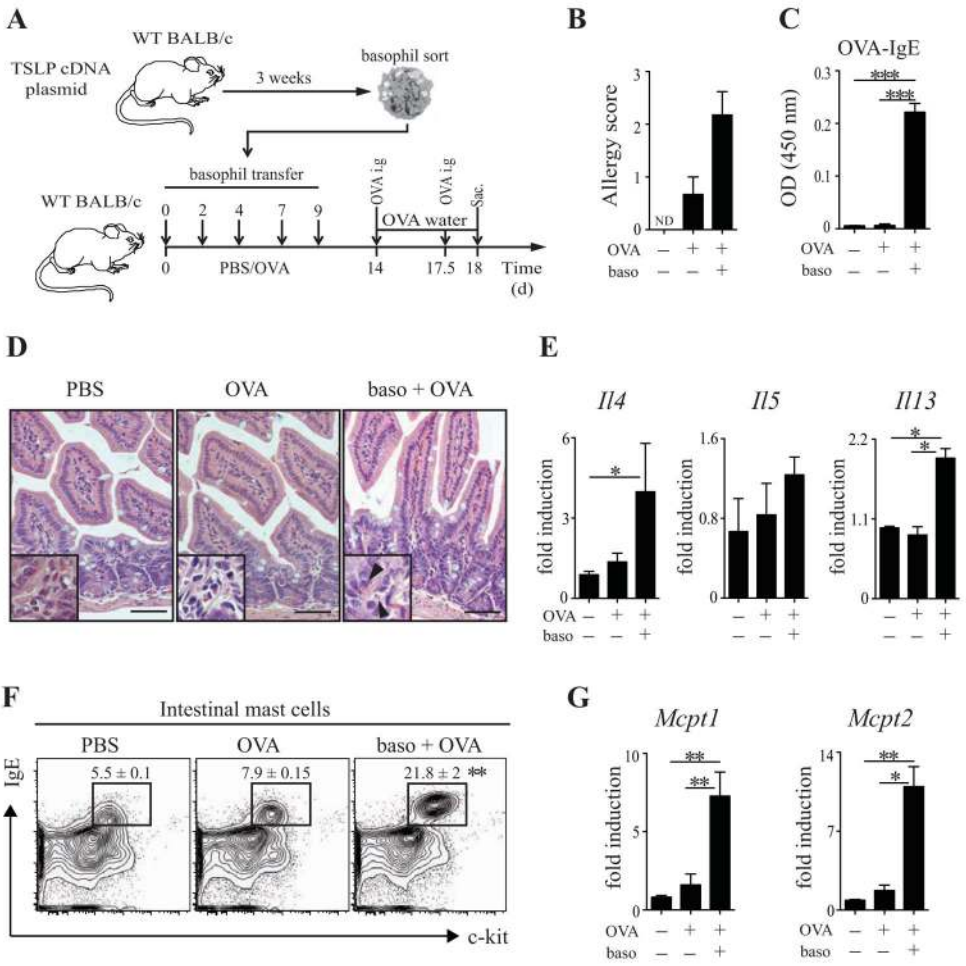

FIG. 6.

TSLP-elicited basophils are sufficient to promote intestinal food allergy. **A,** Experimental protocol. **B,** Allergy score. **C,** OVA-specific serum IgE levels. **D,** H&E staining from the jejunum. Scale bar: 100 μm. Arrows: tissue-infiltrating granulocytes. **E,** Type-2 cytokine expression in the jejunum. **F,** Frequencies of mast cells in the lamina propria. **G,** Jejunal *Mcpt1* and *Mcpt2* expression. $N = 3$ per group. Data are representative of three independent experiments.

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