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IL-25 and CD4^{+}T$_{H}$2 cells enhance ILC2-derived IL-13 production that promotes IgE-mediated experimental food allergy

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Abstract

Background—Food-mediated allergic reactions have emerged as a major health problem. The underlying mechanisms that promote uncontrolled type-2 immune response to dietary allergens in the gastrointestinal tract remain elusive.

Objective—We investigated whether altering IL-25 signaling enhances or attenuates allergic responses to food allergens.

Methods—iIL-25Tg mice, which constitutively overexpress intestinal IL-25, and *Il17rb*$^{-/-}$ mice, in which the *Il17rb* gene expression is disrupted, were sensitized and gavage fed with ovalbumin (OVA). We assessed symptomatic characteristics of experimental food allergy, including incidence of diarrhea, incidence of hypothermia, intestinal T$_{H}$2 immune response, and serum OVA-specific IgE and mast cell protease (MCPt)-1 production.

Results—Rapid induction of *Il25* expression in the intestinal epithelium preceded the onset of anaphylactic response to ingested OVA antigen. iIL-25Tg mice were more prone, and *Il17rb*$^{-/-}$ were mice more resistant, to developing experimental food allergy. Resident intestinal type-2 innate lymphoid cells (ILC2s) were identified as the major producers of IL-5 and IL-13 in

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response to IL-25. Reconstituting irradiated wild-type mice with *Rora*$^{-/-}$ or *Il17rb*$^{-/-}$ bone marrow (BM) resulted in a deficiency or dysfunction of the ILC2 compartment, respectively, and resistance to developing experimental food allergy. Repeated intragastric antigen challenge induced a significant increase of CD4^{+}T$_{H}$2 cells, which enhance IL-25-stimulated IL-13 production by ILC2 *ex vivo* and *in vivo*. Finally, reconstituted IL-13–deficient ILC2s had reduced capability to promote allergic inflammation, resulting in the increased resistance to experimental food allergy.

Conclusion—IL-25 and CD4^{+}T$_{H}$2 cells induced by ingested antigens enhance ILC2-derived IL-13 production, thereby promoting IgE-mediated experimental food allergy.

Keywords

IL-25; ILC2s; CD4^{+}T$_{H}$2 cells; IL-13; food allergy

Introduction

The gastrointestinal (GI) mucosa is the largest immunological site in the body and constantly encounters a myriad of commensal microbes and dietary proteins. The epithelial lumen and GI-associated lymphoid tissues function to combat invading microbes while developing immune tolerance to food antigens. The loss of mucosal tolerance to foods and the associated induction of adverse immune-mediated reactions can lead to the development of food-induced allergic disorders. Studies in both humans and animals have demonstrated that the immediate hypersensitivity response to ingested food arises from the activation of intestinal mast cells by food-specific IgE antibodies[1]. Although the prevalence of food-induced allergic disorders has increased significantly in industrialized countries over the past decade[2], our knowledge of the underlying mechanisms that potentiate the induction of cell-mediated allergic immune responses to food allergens in the GI tract remains limited.

IL-25 (IL-17E), a distinct IL-17 inflammatory cytokine member, is a key factor that functions to promote allergic inflammation[3–6]. Systemic administration or overexpression of IL-25 induce elevated T$_{H}$2 cytokine and eotaxin production, which result in eosinophilia, increased serum IgE, mucus hyperplasia, and pathological changes in lung and GI tissues[7–10]. In contrast, administration of IL-25–neutralizing antibody significantly attenuates pulmonary allergic inflammation and preventing airway hyperresponsiveness[11]. In addition to airway and skin IL-25, endogenous intestinal IL-25 can limit T$_{H}$1 and T$_{H}$17-mediated GI inflammation induced by commensal flora[12, 13] and promote protective type-2 immunity to combat parasitic infection[3, 14]. Indeed, IL-25–deficient mice infected with *Trichuris muris*, the GI parasite, fail to develop a lymphocyte-dependent protective type-2 immunity to expel chronic parasitic infection[14]. These studies suggest that intestinal epithelium–derived IL-25 may regulate the balance of the immune response triggered by commensal microbes and dietary proteins in the GI tract.

Recent studies demonstrate that the type-2 cytokine-producing innate lymphoid cells (ILC2s) are the early cellular source of T$_{H}$2 cytokine IL-5 and IL-13[3, 15–19]. Although lacking antigen-specific receptors, ILC2s express an array of cytokine receptors; including IL-17RB, ST-2, IL-7Rα, and IL-2Rα[15–18], and respond to IL-25 and IL-33 stimulation in the

presence of IL-7 and/or IL-2 by producing large amounts of IL-5 and IL-13[15–18]. Subsequent studies reveal that ILC2s originate from common lymphoid progenitors (CLP)[20, 21] and express *Id2* and *Rorα* [15, 20, 22], the signature transcription factors for ILC lineages[23–25]. Similar to $CD4^+T_H2$ cells, ILC2s require transcription factor *Gata3* for differentiation and maintenance[26]. Parallel to their critical role in protective immunity against helminthic infection, ILC2s can promote allergic asthma[19, 27, 28]. Whether ILC2s promote the induction of experimental food allergy has not been determined. Herein, we show that mice overexpressing intestinal IL-25 or lacking a component of IL-25 receptor, IL-17RB, were prone or resistant to developing experimental food allergy, respectively. Our studies suggest that IL-25 and ingested antigen-induced $CD4^+T_H2$ cells can enhance ILC2-derived IL-13 production that promotes the development of experimental food allergy.

Materials and Methods

Further information can be found in the Methods section in this article's Online Repository at www.jacionline.org

IgE-mediated experimental food allergy

Mice were sensitized twice within a two-week interval by intraperitoneal injection with 100 μg OVA and 1 mg alum. Two weeks after the second sensitization, mice were orally gavaged with 50 mg OVA in 250 μl saline for a total of six times within two weeks and subsequently examined for the symptomatic features in experimental food allergy[2, 3]. The manifestations of systemic symptoms begin with diarrhea (profuse liquid stool), airway hyperreactivity, and then hyperthermia (rectal temperature drop > 2°C)[4, 5], 30 to 45 minutes after the last challenge. Blood samples and intestine tissues were collected from mice euthanized immediately after the measurement of rectal temperature.

Measuring parameters of food allergy

To measure intestinal mast cell number and levels of goblet cell hyperplasia, duodenal tissue was fixed in 10% formalin and processed by standard histological techniques. 5–8–μm tissue sections were stained with Leder stain for chloroacetate esterase (CAE) activity in intestinal mast cells or periodic acid-Schiff (PAS) for mucins in goblet cells. Stained cells were quantified as previously described[3]. To measure secreted mediators, serum samples were analyzed using ELISA kits of OVA-specific IgE (MD Bioproducts), MCPt-1 (eBioscience), and OVA-specific IgG1 (Alpha Diagnostic International). Diarrhea assessments (profuse liquid stool) and hyperthermia measurements (rectal temperature drop > 2°C) are performed as previously described[4].

Statistical analysis

For comparisons between experimental groups, statistical significance was determined using unpaired Student's *t* test. For the measurement of food allergy parameters, 3 independent experiments (n=4, total 12 mice per group) were performed in blinded fashion for Figure 1B–1E, Figure 3, 4, 5, and 6A–6B. 2 independent experiments were performed for Figure 1A and Figure 6C–6D (n=4, total 8 mice per group). Results were considered significant at P

≤ 0.05. Error bars denote mean ± S.D. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001. ns, not significant. ND, not detected. All data were analyzed using Prism (Graphpad Software).

Results

Early induction of intestinal IL-25 promotes susceptibility to experimental food allergy

To determine the involvements of IL-25 in regulating food allergy, intestinal *Il25* expression was examined. Compared to naïve mice, sensitized mice received only two intragastric OVA challenges rapidly upregulated *Il25* expression (> 5 fold) in the duodenal epithelium; this *Il25* expression remained elevated until the onset of anaphylactic response to ingested OVA (Fig. 1A). Concomitantly, the expression of *Il13* (> 5 fold) and chemokine genes, including *Tarc* (> 7 fold), *Cxcl1* (> 7 fold), and *Ccl11* (> 20 fold) (eotaxin 1), but not *Ccl24* (eotaxin 2), were also upregulated, primarily in the small intestinal epithelium prior to the onset of experimental food allergy. To address whether dysregulated IL-25 signaling contributes to susceptibility to developing experimental food allergy, we generated IL-25 transgenic mice lines (iIL-25Tg) that constitutively overexpress murine IL-25 driven by the promoter of intestinal fatty acid–binding protein (iFABP) in the small intestinal epithelium and *Il17rb*$^{-/-}$ mice that had disrupted *Il17rb* gene expression. Although intestinal IL-25 overexpression induced increases of intestinal *Il5* (> 20,000 fold) and *Il13* (> 75 fold) gene expression, and mast cell (MC) numbers and levels of goblet cell hyperplasia in naïve or sensitized iIL-25Tg mice (Fig. 1B and data not shown), these mice did not develop symptomatic features of IgE-mediated experimental food allergy, including allergic diarrhea, hypothermia, intestinal mastocytosis, or increased serum OVA-specific IgE and/or mast cell protease 1 (MCPt-1), the latter of which indicates MC degranulation (Fig. 1B). Notably, a regimen of only four intragastric OVA antigen challenges was sufficient to induce sensitized iIL-25Tg mice, but not their littermate controls, to manifest systemic symptoms beginning with diarrhea (>84%, profuse liquid stool) and then hyperthermia (>60%) within 45 min (Fig. 1B). These iIL-25Tg mice also produced higher titers of serum MCPt-1 and OVA-specific IgE and exhibited more pronounced goblet cell (GC) hyperplasia, but not intestinal mastocytosis, compared to those of littermate controls (Fig. 1B and C). In contrast, sensitized mice lacking IL-17RB were more resistant to manifest allergic diarrhea and hypothermia than wild-type BALB/c mice after six times of intragastric OVA challenge (Fig. 1D). These *Il17rb*$^{-/-}$ mice produced less MCPt-1 and displayed less intestinal mastocytosis and GC hyperplasia, while producing comparable amounts of OVA-specific IgE (Fig. 1D and E). These results suggest that intestinal epithelium *Il25* expression increases at the early phase of experimental food allergy and the alterations in IL-25 signaling can positively or negatively regulate the susceptibility to developing experimental food allergy.

Intestinal ILC2s produce IL-5 and IL-13 in response to IL-25

Next, we search for intestinal IL-17RB-expressing immune cells during the development of experimental food allergy. Two dominant IL-17RB–expressing cell populations were identified in the laminar propria (LP) of the small intestine of mice with active allergic diarrhea: (i) $CD3^+CD4^+$IL-$17RB^+$ cells and (ii) $CD3^-CD4^-$IL-$17RB^+$c-KIT^- cells, both of which expressed hematopoietic cell lineage marker CD45, but not other known cell lineage markers (Lin^-) (Fig. 2A and data not shown). $CD3^+CD4^+$IL-$17RB^+$ cells expressed surface

markers of T_H2 effector/memory cells, including; CD44, CD69, IL-7Rα, ST-2, and Thy1.2, and produced significant amounts of T_H2 cytokines, IL-4, IL-5, and IL-13, not IL-17 or IFN-γ upon CD3/CD28 re-stimulation (Fig. 2B and see Fig. E1A and E1B in this article's Online Repository at www.jacionline.org). Notably, the $Lin^-CD3^-CD4^-IL\text{-}17RB^+c\text{-}KIT^-$ cells expressed Sca-I, KLRG1, IL-7Rα (CD127), ST-2, Thy1.2, ICOS, IL-2Rα (CD25), and MHCII, the signature markers of recently described ILC2s[30], but not FcεR, IL-3R (CD123), and Siglec-F (Fig. 2B and see Fig. E2A in this article's Online Repository at www.jacionline.org). On the contrary, $Lin^-CD3^-CD4^-IL\text{-}17RB^-c\text{-}KIT^+$ cells expressed surface FcεRαI, not IL-2Rα (CD25), IL-3R (CD123), or ICOS, suggesting that these cells are of MC lineage (see Fig. E2A in this article's Online Repository at www.jacionline.org). Thus, systemic administrations of IL-25 proteins in mice resulted in the selective expansion of $Lin^-CD3^-CD4^-IL\text{-}17RB^+c\text{-}KIT^-$ cells, not $Lin^-CD3^-CD4^-IL\text{-}17RB^-c\text{-}KIT^+$ cells in their mesenteric lymph nodes (MLN) (see Fig. E2B in this article's Online Repository at www.jacionline.org). Comparative gene expression analyses among examined hematopoietic cell lineages revealed that both IL-17RB-expressing cell subsets expressed high levels of *Gata3* (>5 $\times 10^3$ fold), and T_H2 cytokine genes *Il4* (>5 $\times 10^3$ fold), *Il5* (>1 $\times 10^4$ fold), and *Il13* (>5 $\times 10^4$ fold); however, only $CD3^-CD4^-IL\text{-}17RB^+c\text{-}KIT^-$ cells expressed ILC2 signature transcripts; *Id2* (>50 fold), and *Rora* (>3$\times 10^3$ fold) (see Fig. E1C in this article's Online Repository at www.jacionline.org). Thus, $Lin^-CD3^+CD4^+IL\text{-}17RB^+$ and $Lin^-CD3^-CD4^-IL\text{-}17RB^+c\text{-}KIT^-$ cell subsets represent the intestinal $CD4^+T_H2$ cells and ILC2s, respectively.

Both IL-17RB–expressing $CD4^+T_H2$ cells (~5.0%) and ILC2s (~3.0%) accumulated preferentially in the small intestine of mice developed experimental food allergy (Fig. 2C), the primary anatomical site that expressed induced *Il25* transcript (Fig. 1A). IL-25 alone induced very few T_H2 cytokine production by purified intestinal $CD4^+T_H2$ cells and ILC2s; however, the addition of IL-2 and/or IL-7 potentiated ILC2s, but not $CD4^+T_H2$ cells, to respond to IL-25 stimulation by producing large amounts of IL-5 (>10 ng/ml per 10^4 cells) and IL-13 (>10 ng/ml per 10^4 cells), and very little IL-4 (<0.05 ng/ml per 10^4 cells) and IFN-γ (<0.1 ng/ml per 10^4 cells) (Fig. 2D). These results suggest that both $CD4^+T_H2$ cells and ILCs are intestinal IL-17RB–expressing cells and that ILC2s are the primary IL-5 and IL-13 producers in response to IL-25 stimulation in experimental food allergy.

IL-25-responsive ILC2s promote susceptibility to experimental food allergy

To determine the contributions of ILC2s to the development of food allergy, we develop a reconstitution model of experimental food allergy, adoptively transferring BM cells from 4GET (*Il4* expression–driven GFP reporter) BALB/c mice into OVA-sensitized BALB/c mice one day after sub-lethal irradiation (protocol diagramed in Fig. E3A in this article's Online Repository at www.jacionline.org). After the second sensitization and repeated intragastric OVA challenge, transferred BM cells replenished the $IL\text{-}17RB^+c\text{-}KIT^-IL\text{-}7R\alpha^+KLRG^+$ ILC2 compartment with GFP-marked cells in the irradiated recipients, whereas most of the $CD4^+ST\text{-}2^+IL\text{-}17RB^+$ T_H2 cells lacked GFP expression and were therefore derived from the sensitized recipients (Fig. 3A). Consistently, most of replenished ILC2s were $Thy1.1^{high/low}$ (>96%) in the irradiated $Thy1.2^+$ recipients that generated primarily Thy1.2-expressing $CD4^+$ T cell compartment (>96%) after reconstitution with

congenic Thy1.1$^+$BM cells. Furthermore, ILC2 frequencies were comparable (~2.0%) between reconstituted wild type (WT) and *STAT6*$^{-/-}$ Thy1.2$^+$ recipients (see Fig. E3B and E3C in this article's Online Repository at www.jacionline.org). Thus, BM transplants will reconstitute innate ILC2 cell lineage within 2 weeks, whereas *de novo* T cell generation after thymus engraftment from donor-derived hematopoietic progenitors require >2 months[31]. Having established a reconstitution model of experimental food allergy, we showed that irradiated mice reconstituted with BM cells that lacked transcription factor ROR-α, a transcription factor for ILC2 development[20], failed to develop ILC2s, while CD4$^+$T$_H$2 cells developed normally (Fig. 3B and C). Consequently, irradiated mice reconstituted with WT, not ROR-α-deficient, BM cells exhibited pronounced intestinal mastocytosis, produced increased MCPt-1 and OVA-specific IgE, and developed allergic diarrhea after repeated antigen challenge (Fig. 3D). While IL-17RB–deficient BM cells developed into ILC2s normally, these reconstituted IL-17RB–deficient ILC2s did not promote experimental food allergy in the irradiated recipients that produced OVA-specific IgE normally (Fig. 3B–D). Notably, LP cells from the small intestine of WT BM reconstituted mice produced significant higher amounts of IL-5 and IL-13 than those by LP cells from *Rora*$^{-/-}$ or *Il17rb*$^{-/-}$ BM reconstituted recipients which lacked ILC2s or generated dysfunctional ILC2s, respectively, in response to IL-25 stimulation *ex vivo* (Fig. 3E). These results demonstrate that intestinal ILC2s are the primary IL-5 and IL-13 producers in response to IL-25 stimulation and play a key role in promoting experimental food allergy *in vivo*.

Antigen-induced CD4$^+$T$_H$2 cells enhance ILC2 function in response to IL-25

Recent reports suggest an interplay relationship between innate ILC2s and adaptive CD4$^+$ T cells in the protective response against helminthic infection[32, 33]. To address whether ingested antigen-induced CD4$^+$T$_H$2 immune response is involved in ILC2 function, the frequencies of intestinal ILC2s and CD4$^+$T$_H$2 cells during the development of experimental food allergy were examined. While very few CD4$^+$T$_H$2 cells (<0.1%) could be detected, a considerable pool of ILC2s resided in the small intestine of naïve or sensitized mice (1.5–3.0%) constantly (Fig. 4A and 4B). Notably, repeated intragastric OVA antigen challenge induced a significant accumulation of intestinal CD4$^+$T$_H$2 cells (<0.1% to >5%), not ILC2s and an increase of peripheral eosinophils (Fig. 4A–C). Correspondingly, intestinal LP cells from re-challenged mice produced significant higher amounts of IL-4, IL-5, and IL-13 than those by LP cells from naïve mice after OVA peptide stimulation *ex vivo* (see Fig. E4A in this article's Online Repository at www.jacionline.org). Although ILC2 frequencies were comparable among naïve, sensitized, and re-challenged mice, the capability of ILC2s to produce IL-5 and IL-13 after IL-25 or PMA/ionomycin stimulation was significantly enhanced only in re-challenged mice that developed a considerable pool of CD4$^+$T$_H$2 cells (Fig. 4A, 4D, and 4E). To directly assess whether CD4$^+$T$_H$2 cells would enhance ILC2 responsiveness to IL-25 stimulation, we measured their T$_H$2 cytokine-producing capability in the presence or absence of CD4$^+$T$_H$2 cells *ex vivo*. Indeed, co-culture of *in vitro*-derived CD4$^+$T$_H$2 cells significantly enhanced the responsiveness of ILC2s to IL-25 stimulation, as these stimulated, co-culture ILC2s produced large amounts of IL-5 and IL-13 (Fig. 4F and see Fig. E4B in this article's Online Repository at www.jacionline.org). Intriguingly, the presence of OVA peptides further enhanced IL-5 production by co-cultured OVA antigen-specific CD4$^+$T$_H$2 cells and ILC2s in response to IL-25 stimulation (see Fig. E4B in this

article's Online Repository at www.jacionline.org). Treatments with antibodies against IL-2 greatly diminished the ILC2s' enhanced responsiveness to IL-25 stimulation in $CD4^+T_H2$ cell co-culture (Fig. 4F). Furthermore, ILC2s purified from the small intestine of re-challenged WT or *Il17rb*$^{-/-}$ mice failed to respond to IL-25 when cultured alone; however, purified WT $CD4^+T_H2$ cells from mice developed active allergic diarrhea greatly enhanced the IL-25–stimulated IL-5 and IL-13 production by co-cultured WT, but not IL-17RB-deficient, ILC2s (Fig. 4G). Notably, IL-17RB-deficient $CD4^+ST\text{-}2^+T_H2$ cells purified from *Il17rb*$^{-/-}$ mice, which were resistant to developing experimental food allergy, were less capable of potentiating the IL-5 and IL-13 production by WT ILC2s in response to IL-25 (Fig. 4G). Collectively, these intriguing findings suggest that IL-17RB–expressing $CD4^+T_H2$ cells induced by ingested antigens enhance the capability of intestinal ILC2 residents to respond to epithelial-derived IL-25 by producing prodigious amounts of IL-5 and IL-13, and thereby promote experimental food allergy.

ILC2s fail to promote experimental food allergy in mice that lack $CD4^+T_H2$ cells

Next, we address whether the induction of $CD4^+T_H2$ cells by ingested antigens is essential for ILC2 function in promoting experimental food allergy. Compared to mice received isotype-matched antibodies, mice ablated of $CD4^+T$ cells after anti-CD4 antibody treatments failed to develop symptomatic features of experimental food allergy, despite their intestinal ILC2 compartment remaining intact (Fig. 5A–C). Although replenished ILC2s restored the susceptibility of irradiated wild-type recipients to develop experimental food allergy after BM reconstitution, these donor-derived ILC2s failed to restore the capability of irradiated *Stat6*$^{-/-}$ recipients, which lacked the $CD4^+T_H2$ cells, to develop intestinal mastocytosis, produce MCPt-1 or OVA-specific IgE, or allergic diarrhea (Fig. 5D–F). These results suggest that ILC2s alone are insufficient to drive the development of experimental food allergy and that the induction of intestinal $CD4^+T_H2$ cells by ingested antigens is a prerequisite for ILC2 function to promote experimental food allergy.

IL-13 production by ILC2s promotes experimental food allergy

To understand the mechanisms underlying the function of ILC2s in promoting the susceptibility to experimental food allergy, we examine the role of IL-13, the major cytokine produced by ILC2s and shown to be involved in the development of IgE-mediated experimental food allergy[34]. Although ILC2s developed normally in *Il13*$^{-/-}$ mice, much fewer $CD4^+T_H2$ cells were induced in *Il13*$^{-/-}$ mice than in wild-type mice after repeated intragastric OVA antigen challenge (Fig. 6A). Consequently, *Il13*$^{-/-}$ mice exhibited reduced levels of intestinal mastocytosis, goblet cell hyperplasia, produced less MCPT-1 and OVA-specific IgE, and were more resistant to developing experimental food allergy, compared to those in wild-type mice (Fig. 6B). Consistently, the capabilities of WT and IL-13-deficient BM cells were comparable in replenishing ILC2s in irradiated recipients that developed $CD4^+T_H2$ cell normally (Fig. 6C and 6D). However, the recipients reconstituted with *Il13*$^{-/-}$ BM cells exhibited less pronounced intestinal mastocytosis and goblet cell hyperplasia, produced fewer MCPt-1 and OVA-IgE, and were thereby more resistant to developing an anaphylactic response to ingested OVA antigen, compared to those received WT BM cells (Fig. 6D). These intriguing results suggest that IL-13 elicited by ILC2s may play a key role

in promoting intestinal allergic inflammation that promote the development of IgE-mediated experimental food allergy.

Discussion

The immune response to allergenic dietary proteins depends on the balance of a complex interplay between immune and non-immune cell interactions in the GI tract. Mechanistically, it remains unclear how an adverse allergic reaction to ingested food can be initiated and amplified, leading to the loss of oral tolerance. TSLP, IL-33, and IL-25, the epithelial-derived cytokines, have been shown to play an important role in initiating and amplifying type-2 immune responses in allergic asthma and against parasitic infection[6, 35]. TSLP can potentiate dendritic cells to promote naïve $CD4^+$ T cells to differentiate into $CD4^+T_H2$ cells and maintain $CD4^+T_H2$ memory/effector cells[36–39], and enhance allergic sensitization in the cutaneous sensitization model of food allergy[40]. While IL-33 is responsible for the development of allergic lung diseases[41–43], this cytokine appears to be dispensable for cutaneous allergic sensitization, but essential for inducing IgE-dependent anaphylaxis[40]. Whether IL-25 is involved in regulating the dysregulated type-2 immune response to ingested food antigens has not been established. Herein, we demonstrate that repeated intragastric antigen challenge induce an increase in *Il25* gene expression by intestinal epithelium before the onset of anaphylactic response to ingested food. Studies using genetically modified mice further demonstrate that IL-25 signaling promotes the susceptibility to developing experimental food allergy. Notably, $CD4^+T_H2$ cell induction by ingested antigens appears to be a prerequisite for intestinal ILC2 residents to produce large amounts of IL-13 in response to IL-25 stimulation and to promote the susceptibility to experimental food allergy. Our studies further suggest that intestinal IL-25 signaling promotes the interplay between $CD4^+T_H2$ cells and ILC2s to elicit allergic reactions to ingested food.

The findings that IL-25 mediates protective immunity against GI helminthic infection, such as *Trichuris muris* and *Nippostrongylus braziliensis*, and *Trichinella spiralis* by promoting a T_H2 cytokine–dependent immune response[3, 14, 44, 45] suggest a potential involvement of IL-25 in the allergic reactions to ingested food antigens. Indeed, analyses of iIL-25Tg and *Il17rb*$^{-/-}$ mice reveal their susceptibility and resistance to developing IgE-mediated experimental food allergy, respectively, substantiating the role of IL-25 in promoting allergic reaction to ingested antigens. While intestinal ILC2s are identified to be the primary IL-25-responding cells, ILC2s alone are insufficient to drive intestinal anaphylaxis in naïve or sensitized transgenic mice overexpressing IL-25. Because their capability to produce IL-5 and IL-13 in response to IL-25 and to promote experimental food allergy requires $CD4^+$IL-17RB$^+T_H2$ cells that are induced after repeated intragastric OVA antigen challenge. In agreement with recent studies[32, 33], anti–IL-2 antibody treatments result in a significant reduction of IL-5 and IL-13 production by IL-25–stimulated ILC2s co-cultured with $CD4^+T_H2$ cells. Notably, IL-17RB–deficient $CD4^+$ST-2^+T_H2 cells are less capable of enhancing ILC2s to produce T_H2 cytokine production in response to IL-25, possibly that IL-17RB–deficient $CD4^+$ST-2^+T_H2 cells produce less IL-2 after IL-25 stimulation. Our studies support a view that IL-25 enhances the concerted interactions between intestinal

ILC2s and antigen-induced $CD4^+T_H2$ cells to amplify allergic reactions to ingested food antigen.

A role of ILC2s in promoting IgE-mediated experimental food allergy has been further substantiated in a reconstitution model of experimental food allergy. Donor-derived ILC2s promote irradiated recipients mice to develop experimental food allergy after wild-type BM reconstitution. However, irradiated recipients reconstituted with *Rora*$^{-/-}$ or *Il17rb*$^{-/-}$ BM that are defective in ILC2 development or function, respectively, are resistant to developing experimental food allergy. It appears that IL-13 produced primarily by ILC2s plays a key role in promoting the susceptibility to experimental food allergy, possibly by inducing goblet cell hyperplasia, increase intestinal permeability, and modulate gut barrier function[46–48]. In contrast to our findings, a recent study suggest that mice ablated of ILC2 and deficient of IL-25 or IL-13 remain capable of developing splenic T_H2 immune response and systemic anaphylactic response to antigen administered intraperitoneally after sensitization by orally gavaged cholera toxin (CT)[49, 50]. The discrepancies may be attributable to differences in the experimental approaches, including; sensitization adjuvant (alum vs. CT), murine strains (BALB/c vs C3H/HeJ or C57/B6)[34, 50, 51], and the anatomic locations where the anaphylaxis is induced (intestine vs. peritoneal)[34, 49, 50]. Importantly, previous studies demonstrate that anaphylactic response to ingested antigens administered by intragastric inoculations following alum sensitization is dependent on the classic mast cell/IgE/FcεR pathway[52, 53], whereas the anaphylaxis induced by intraperitoneal antigens following CT sensitization can be mediated by the alternative IgG/FcγR pathway[54, 55]. It remains to be determined whether the differences in the genetic predisposition to allergic sensitization between BALB/c and C57/B6 strains and/or the antigen administration routes results in this perceived discrepancy in the necessity of IL-25 signaling in experimental food allergy.

In our murine food allergy model, mice eventually develop systemic manifestations pertaining to some characteristics of food allergy–induced life-threatening anaphylaxis in humans, including; cutaneous and mesenteric vascular leak, airway hyperresponsiveness, and hypothermia[52, 56], despite that “food allergic” mice do not develop skin allergic disorders that are often comorbid in some of food allergy patients[57]. Although the initial triggers and genetically predisposing factors that initiate allergic sensitization in the GI tract remain unclear, our findings suggest that IL-25-mediated collaborations between ILC2s and $CD4^+T_H2$ cells can be a key step in amplifying the cascade of allergic reactions to ingested antigens at the effector phase of IgE-mediated food allergy via the mast cell/IgE pathway[52, 58]. These studies underscore the importance of understanding the mechanisms that underlie intestinal allergic response to ingested food and the knowledge gained from the basic studies may eventually serve as the rationale to design innovative approaches for the care of food allergy in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used

ILC2s	type-2 innate lymphoid cells
T_H2	T helper type 2
MCPt-1	mast cell protease-1
OVA	ovalbumin
BM	bone marrow
GI	gastrointestinal
LP	laminar propria
Lin	lineage
CT	cholera toxin
OG	oral gavage
GC	goblet cell
4GET	*Il4* expression–driven GFP reporter
WT	wild type

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Key messages

- Alteration of IL-25 signals can positively or negatively regulate the susceptibility to experimental food allergy.
- Intestinal ILC2s are the primary IL-5 and IL-13 producers in response to IL-25 stimulation and play a key role in promoting experimental food allergy.
- Ingested antigen-induced $CD4^{+}T_{H}2$ cells potentiate ILC2s' capability to respond to IL-25 by producing prodigious amounts of IL-13, that promotes experimental food allergy.

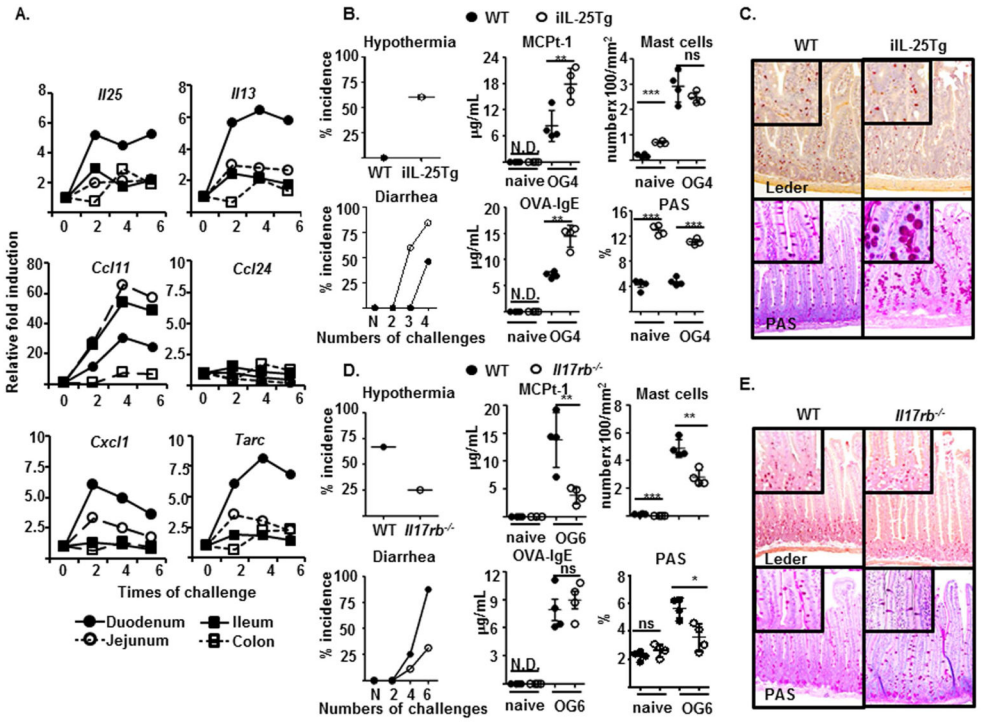

FIG 1.

(**A**) Expression levels of indicated genes by indicated tissues of sensitized BALB/c mice after indicated times of intragastric OVA challenge were examined and compared as described in the methods. (**B**–**E**) Indicated murine strains were sensitized and orally gavaged (OG) with OVA for four (**B** and **C**), six (**D** and **E**), or the indicated times (**B** and **D**) before measuring the indicated features of experimental food allergy and staining of intestinal mastocytosis and GC hyperplasia (**C** and **E**).

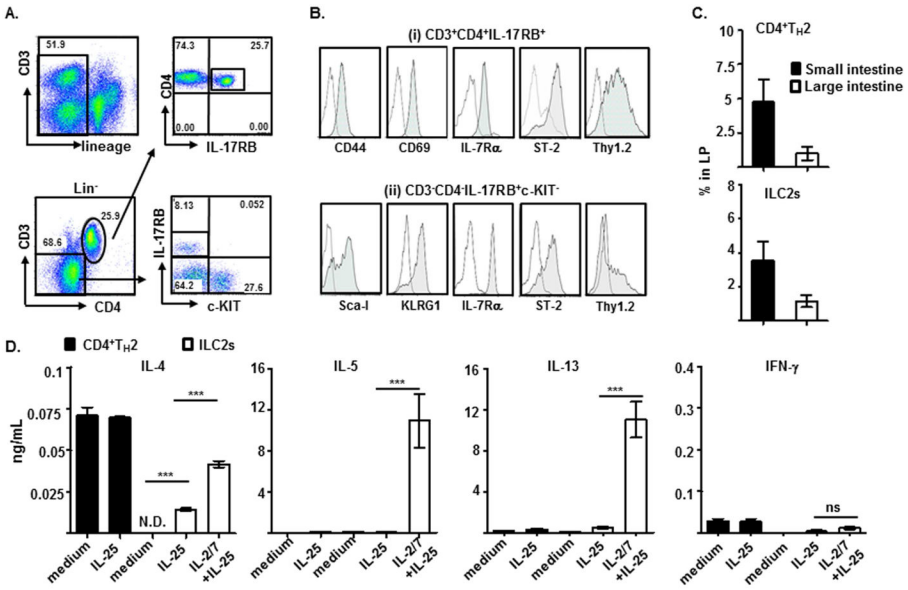

FIG 2.

Detection (**A**), phenotypic analysis (**B**), and frequencies (**C**) of $Lin^{-}CD3^{+}CD4^{+}IL\text{-}17RB^{+}T_{H}2$ cells and $Lin^{-}CD3^{-}CD4^{-}IL\text{-}17RB^{+}c\text{-}KIT^{-}$ILC2s in mice developed food allergy. (**D**) Indicated cytokines produced by purified $Lin^{-}CD3^{+}CD4^{+}IL\text{-}17RB^{+}$ $T_{H}2$ cells and $Lin^{-}CD3^{-}CD4^{-}IL\text{-}17RB^{+}c\text{-}KIT^{-}$ILC2s from mice developed food allergy after stimulation with IL-25 alone or IL-25 plus IL-2 and IL-7 (IL-2/7) were measured and compared.

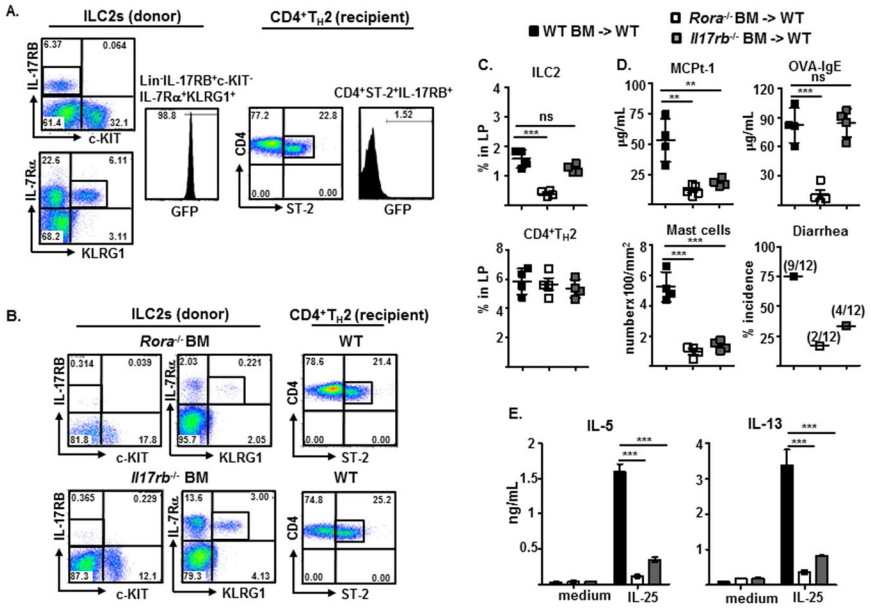

FIG 3.

Detection (**A** and **B**) and frequency (**C**) of donor-derived ILC2s ($Lin^-CD3^-CD4^-IL\text{-}17RB^+c\text{-}KIT^-IL\text{-}7R\alpha^+KLRG1^+$) and recipient-derived $CD4^+T_H2$ cells ($Lin^-CD3^+CD4^+IL\text{-}17RB^+ST\text{-}2^+$), and measurements of indicated parameters of experimental food allergy (**D**) and indicated cytokine production by IL-25-stimulated LP cells (**E**), from irradiated WT BALB/c recipients reconstituted with BM progenitors from 4GET mice (**A**), or WT BALB/c, *Rora*$^{-/-}$, or *Il17rb*$^{-/-}$ mice (**B**–**E**).

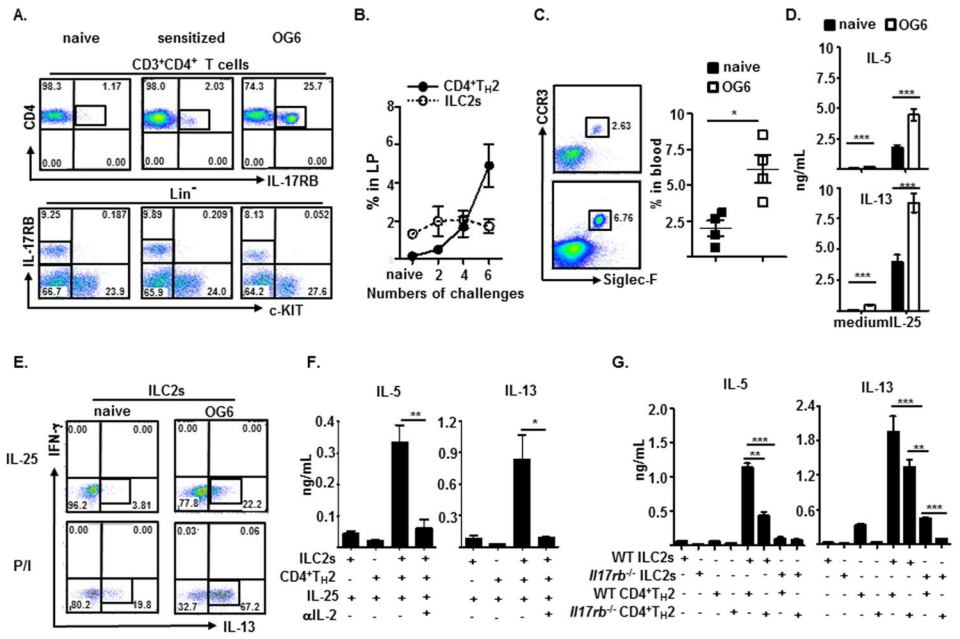

FIG 4.

Detection and frequency of intestinal $CD4^{+}IL\text{-}17RB^{+}T_{H}2$ cells and $Lin^{-}c\text{-}KIT^{-}IL\text{-}17RB^{+}ILC2s$ (**A** and **B**), blood $CCR3^{+}Siglec\text{-}F^{+}CD11b^{+}$ eosinophils (**C**), and intracellular IL-13-producing ILC2s (**E**) from naïve or sensitized mice orally gavaged (OG) with OVA for 6 (**A**, **C**, and **E**) or indicated times (**B**). IL-5 and IL-13 production by medium- or IL-25-stimulated LP cells (**D**) or by indicated cells from WT (**F** and **G**) or *Il17rb*$^{-/-}$ mice (**G**) OG with OVA for 6 times after 3 day co-culture with IL-25 only (**F** and **G**) or plus anti-IL-2 or control antibodies (**F**).

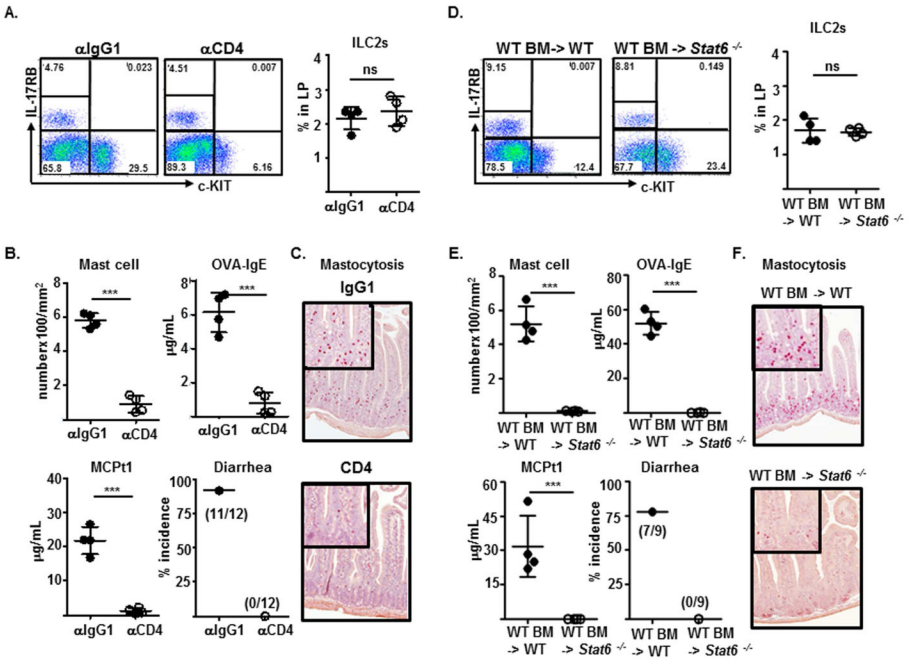

FIG 5.

Detection and frequency of ILC2s (**A** and **D**), measurement of indicated features of experimental food allergy (**B** and **E**), and staining of intestinal mastocytosis (**C** and **F**), in sensitized WT BALB/c mice treated with indicated antibodies one day before the first and fourth intragastric OVA challenges (**A**–**C**) or in irradiated recipients reconstituted with BM progenitors from WT or *Stat6*$^{-/-}$ mice (**D**–**F**).

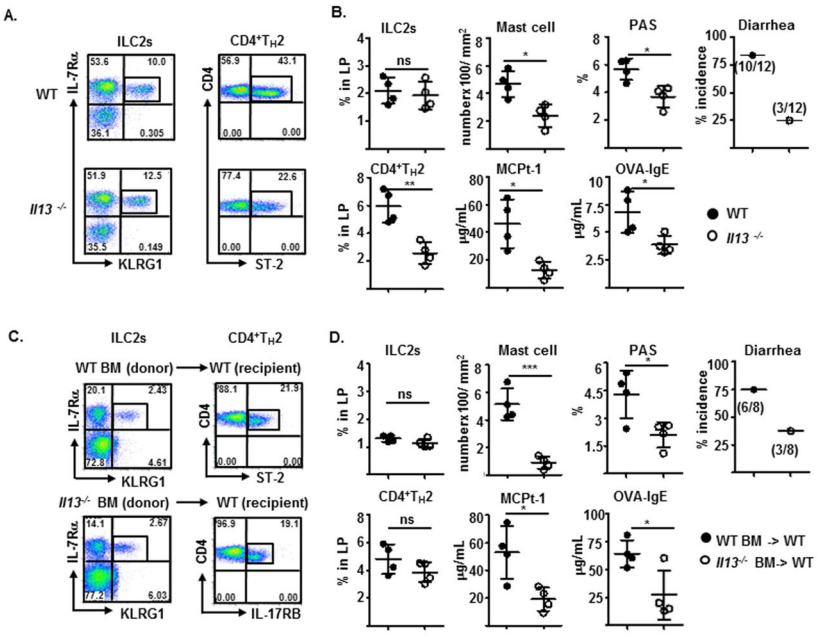

FIG 6.

Detection (**A** and **C**) and frequency of Lin^{-}IL-7Rα^{+}KLRG1^{+}ILC2s and CD4^{+}ST-2^{+}T$_{H}$2 cells in, and measurements of indicated parameters of experimental food allergy of (**B** and **D**), sensitized WT or *Il13*$^{-/-}$ mice (**A** and **B**) or irradiated WT BALB/c recipients reconstituted with BM progenitors from WT or *Il13*$^{-/-}$ mice after six intragastric OVA challenge (**C** and **D**).