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IL-4 Production by Group 2 Innate Lymphoid cells Promotes Food Allergy by Blocking Regulatory T cell Function

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Abstract

Background—Food allergy is a major health issue, but its pathogenesis remains obscure. Group 2 innate lymphoid cells (ILC2) promote allergic inflammation. However their role in food allergy is largely unknown.

Objective—We sought to investigate the role of ILC2 in food allergy.

Methods—Food allergy-prone mice with a gain of function mutation in the IL-4 receptor α chain (*Il4raF709*) were orally sensitized with food allergens and the ILC2 compartment was analyzed. The requirement for ILC2 in food allergy was investigated using *Il4raF709*, IL-33 receptor deficient (*Il1rl1^{-/-}*), IL-13 (*Il13^{-/-}*) and IL-4 (*Il4^{-/-}*) deficient mice and by adoptive transfers of *in vitro*-expanded ILC2. Direct effects of ILC2 on regulatory T (Treg) cells and mast cells were analyzed in co-culture experiments. Treg cell control of ILC2 was assessed *in vitro* and *in vivo*.

Results—Food allergic *Il4raF709* mice exhibit increased numbers of ILC2. IL-4 secretion by ILC2 contributes to the allergic response by reducing allergen-specific Treg cells and activating mast cells. IL-33 receptor deficiency in *Il4raF709Il1rl1^{-/-}* mice protects against allergen sensitization and anaphylaxis while reducing ILC2 induction. Adoptive transfer of WT and *Il13^{-/-}* but not *Il4^{-/-}* ILC2 restored sensitization in *Il4raF709 Il1rl1^{-/-}* mice. Treg cells suppress ILC2 *in vitro* and *in vivo*.

Conclusion—IL-4 production by IL-33-stimulated ILC2 blocks the generation of allergen-specific Treg cells and favors food allergy. Strategies to block ILC2 activation or the IL-33:IL-33R pathway may lead to innovative therapies in food allergy.

Graphical Abstract

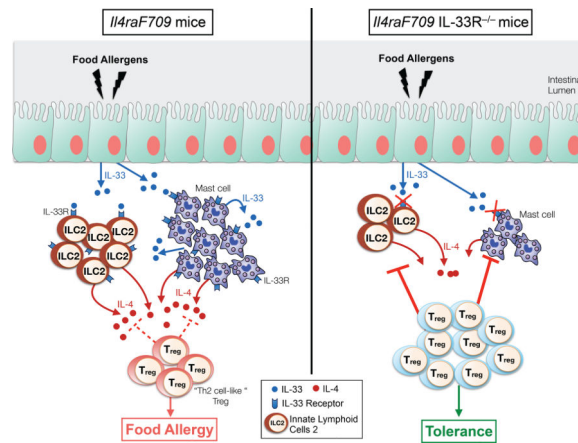
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Capsule summary

IL-4 production by ILC2 plays a crucial role in enabling sensitization to food allergens.

Keywords

Anaphylaxis; Food Allergy; IL-4; IL-13; IL-33; ILC2; Innate Lymphoid Cells; Nuocytes; Mast Cells; Regulatory T Cells; Oral Tolerance

Introduction

Food allergy is a major health issue in westernized countries with increasing prevalence over the last decades and a lack of curative treatment¹. Several major food staples, including milk, soy and wheat can induce food allergic reactions². Food allergies reflect a failure of oral tolerance of innocuous food allergens, resulting in the development of a dysregulated Th2-immune response, the secretion of allergen specific-IgE and the recruitment of effector cells to the gastrointestinal (GI) tract. By their production of Th2 cytokines, mostly IL-4 and IL-13, mast cells, basophils and Type 2 helper CD4⁺ T (Th2) cells are key effector cells of the allergic reaction. Their Th2 cytokine production amplifies the allergic immune response by blocking the induction of allergen-specific regulatory T cells (Treg), directing further Th2 cell differentiation and IgE-driven mast cell activation^{3,4}. The sequence of early events in food allergen sensitization and the involvement of innate immune cells in triggering disease in susceptible individuals remain to be fully elucidated.

A population of innate mucosal Th2 cytokine-secreting cells, called group 2 innate lymphoid cells (ILC2), has emerged as central to a broad collection of allergic disorders such as asthma, chronic rhinosinusitis, and atopic dermatitis^{5–11}. ILC2 are characterized by the expression of CD90 (Thy1), CD25 (IL-2R α), IL-25R (IL-17RB), IL-33R (ST2) and CD127 (IL-7R α) in the absence of specific markers that define other leukocyte lineages (Lin⁻; non-T/non-B cells)¹². They share morphological characteristics with lymphocytes, but do not express the rearranged antigen receptors characteristic of adaptive immune cells^{13,14}. ILC2 emerge from a common lymphoid progenitor present in the bone marrow and their development is critically dependent on the transcription factors Id2^{12,15}, ROR α ^{16,17} and

the Th2-specific transcription factor GATA-3^{8,18}. ILC2 are mostly found at the barrier surfaces, including skin, lung and the gastrointestinal tract¹³. In response to cytokines produced by innate immune sources, such as TSLP, IL-25 and IL-33, ILC2 rapidly expand at the mucosal surfaces in an innate, antigen-independent manner and produce large quantities of Th2 cytokines especially IL-5 and IL-13 but also IL-4 and IL-9^{8,19–22}.

One of the key issues in food allergy is the impaired induction of allergen-specific Treg cell and their subversion into non-functional Th2 cell-like Treg cells that further promote disease³. The cellular and molecular processes linking Th2 effector cells to the defective regulatory immune response during food allergy are still not well understood. The role of ILC2 in disrupting tolerance in food allergy remains similarly obscure, with conflicting reports on the requirement for ILC2 in food allergen sensitization^{23,24}.

In this study, we have made use of a well characterized murine transgenic model of food allergy involving a gain of function in the IL-4R α chain to investigate the role of ILC2 during food allergy^{3,4,25,26}. We found that ILC2 play a critical role in the induction of food allergy by virtue of their secretion of IL-4, which acts to enhance mucosal mast cell activation and disrupt allergen-specific Treg cell induction and suppressive functions.

Methods

Sensitization and challenge protocol

Mice were treated intragastrically once a week over 4 weeks with either PBS or with 23 mg of Peanut (PE) butter (Skippy, Hormel Foods), corresponding to 5 mg of peanut protein, suspended in 250 μ l of 0.1 M sodium bicarbonate (pH 8.0), as previously described⁴. For ovalbumin (OVA) sensitization, OVA (250 μ g) together with 10 μ g of staphylococcal enterotoxin B (SEB, Toxin Technology) was gavaged once weekly for 8 weeks. On the ninth week, mice were challenged intragastrically with either 450 mg peanut butter (~ 100 mg of protein) or 150 mg of OVA as previously described^{3,26}. WT and *DO11.10⁺ Rag2^{-/-} Il4ra^{F709} Foxp3^{EGFP}* mice were fed with 1% OVA in drinking water for 7 days followed by oral gavage every 2 days with 250 μ g OVA the following week. Anaphylaxis was assessed by measuring changes in total body core temperature with transponders placed subcutaneously 2 days before challenge (IPTT-300; Bio Medic Data Systems) and a DAS-6001 console (Bio Medic Data Systems).

In vitro ILC2 and ILC3 culture and cell purification

Lamina propria lymphocytes from *IL-13YFP^{Cre}* mice were isolated as previously described and incubated at 37°C in complete RPMI medium supplemented with IL-33 (10ng/ml, Biolegend), IL-25 (10ng/ml, Shenandoah Biotechnology) and IL-2 (5ng/ml, Shenandoah Biotechnology) for a week²⁷. The ILC were then sorted on a BD FACSAria II based on IL-13YFP expression and Lin⁻ Thy1⁺ Sca-1⁺ c-Kit^{low} for ILC2 and the lack of IL-13YFP expression and Lin⁻ Thy1⁺ Sca-1⁻ c-Kit⁺ for ILC3. Cells were subsequently maintained in cultured with IL-2, IL-25 and IL-33 for 3–4 weeks.

ILC adoptive transfer

ILC were expanded *in vitro* from the lamina propria of WT, *Il13*^{-/-} or *Il4*^{-/-} mice and cell-sorted as mentioned above. *Il4raF709 Il1r1*^{-/-} mice were injected intravenously through the retro-orbital sinus with 2×10^5 ILC each week the day prior to sensitization.

Co-culture experiments

Bone marrow mast cells (BMMC) were prepared as described and cultured overnight at 10^5 cells/well with ILC2 at 20:1 ratio in conical 96 well plates in the presence of IL-2 (500 pg/ml), IL-3 (2 ng/ml), and IgE anti-DNP (5 ng/ml, SPE-7, Sigma)⁴. IL-4 (10 ng/ml, Shenandoah Biotech) or anti-IL-4 (40 µg/ml, 11B11) was supplemented in some cases. BMMC were stimulated for 10 min with 50 ng/ml DNP-albumin (Sigma Aldrich) in the presence of anti-LAMP-1-eFluor 660 (eBio1D4B, eBioscience), anti-c-Kit-PE-Cy7 and fixable viability dye eFluor 780 to detect degranulation. CD4⁺ T cells (10^5) from WT DO11.10⁺ Rag2^{-/-} Foxp3^{EGFP} mice were sorted to >98% purity using Miltenyi CD4 microbeads and labeled with the CellTrace Violet dye (Life Technologies). Naïve T cells were then cultured with ILC2 at a 20:1 ratio in round bottom 96 well plates coated with 1 µg/ml anti-CD3 and 5 µg/ml anti-CD28. Recombinant human TGF-β1 (2 ng/ml) and IL-2 (1 ng/ml) were added to promote Foxp3 induction.

Statistical analysis

Anaphylaxis temperature curves were analyzed using 2-way ANOVA. Student's unpaired two-tailed *t* tests were used for 2 groups comparisons. For more than 2 groups, 1-way ANOVA with Bonferroni post-test analysis was used. Results are presented as means and SEM where each point represents one sample. In cases where values were spread across multiple orders of magnitude, data were log-transformed for analysis with parametric tests.

Study approval

All animal studies were approved by the Boston Children's Hospital Institutional Animal Care and Use Committee.

Other Methods

Information on mouse strains employed, flow cytometry and related antibodies, reagents and methods, real time PCR analysis, IgE and cytokine ELISAs, tissue histology and TGF-β-mediated *in vitro* induced Treg (iTreg) cell induction is provided in the Methods section in this article's Online Repository at www.jacionline.org.

Results

ILC2 enrichment in orally sensitized *Il4raF709* mice

Il4raF709 mice carry an IL-4Rα chain mutation that inactivates the receptor's immunotyrosine inhibitory motif. This mutation results in augmented signal transducer and activator of transcription 6 (STAT6) activation by IL-4 and IL-13, and renders the mice prone to oral allergic sensitization^{25,26}. *Il4raF709*, but not WT mice, are particularly susceptible to oral sensitization with food allergens, such as peanut or chicken egg

ovalbumin (OVA) and respond to oral challenge by a mast cell- and IgE-dependent anaphylactic response^{3,4,28}. Peanut or OVA-sensitized *I4raF709* mice, but not WT controls, developed an anaphylactic reaction after oral challenge as evidenced by a significant drop in their core body temperature (Fig 1, *A* and Fig E1, *A* in this article's Online Repository). Food allergic *I4raF709* mice produced peanut- and OVA-specific IgE antibodies, and they released mouse mast cell protease-1 (MMCP-1), a marker of mast cell degranulation, upon allergen-sensitization and challenge (Fig 1, *B* and Fig E1, *B* and *C* in this article's Online Repository). mRNA transcripts of *IL33*, whose production by epithelial cells promotes ILC2 differentiation, were significantly higher in the small intestine (SI) of *I4raF709* mice following allergen sensitization and challenge (Fig 1, *C* and Fig E1, *D* in this article's Online Repository). Accordingly, we analyzed the presence of ILC2 and ILC3 by flow cytometry in allergen-sensitized WT and *I4raF709* mice. ILC2 and ILC3 identification in the draining mesenteric lymph nodes (MLN) of orally sensitized mice was performed by analysis of their specific patterns of cell-surface markers: Lin⁻ CD45⁺ CD25⁺ CD127⁺ and either Sca-1^{high} c-Kit^{low} (ILC2) or Sca-1⁻ c-Kit⁺ (ILC3) as previously described (Fig 1, *D* and Fig E1, *E* in this article's Online Repository)^{16,29}. Compared to PBS-treated mice, the frequencies and absolute numbers of ILC2 and ILC3 populations were higher in the MLN of allergen-sensitized *I4raF709* mice (Fig 1, *E* and Fig E1, *F* in this article's Online Repository). Compared to OVA-sensitized WT mice, increased ILC2 frequencies and absolute numbers were also observed in the SI lamina propria of OVA-sensitized *I4raF709* mice (Fig E1, *G* and *H* in this article's Online Repository). Similar data were obtained using intracellular cytokine staining to define ILC2 as CD45⁺ Lin⁻ IL-13⁺ and ILC3 as CD45⁺ Lin⁻ IL-17⁺ (Fig 1, *E*).

IL-33 receptor (IL-33R) signaling regulates ILC2 expansion and allergic sensitization

IL-33 is a key cytokine that promotes the growth and maturation of ILC2 and their consequent Th2 cytokine production²⁹. IL-33 signaling through IL-33R, encoded by *Il1rl1*, and its associated IL-1 receptor accessory protein is implicated in airway inflammation after influenza virus infection and in several murine models of asthma^{7,30,31}. Because *IL33* mRNA levels are increased in the SI of allergen-sensitized *I4raF709* (Fig 1, *C* and Fig E1, *D* in this article's Online Repository), we investigated the dependence of allergic-sensitization driven by ILC2 expansion on IL-33R signaling. Surprisingly, *I4raF709* *Il1rl1*^{-/-} mice failed to sensitize to peanut, exhibiting no decrease in core body temperature, a lack of peanut-IgE production and minimal release of MMCP-1 after oral challenge (Fig 2, *A* and *B*). Flow cytometric analysis of ILC populations by means of the cellular markers Sca-1 and c-Kit revealed reduced ILC2 frequencies and absolute numbers in the MLN and SI lamina propria of allergen-sensitized *I4raF709* *Il1rl1*^{-/-} mice (Fig 2, *C* and *D*).

GATA-3 is an essential transcription factor for ILC2 and its expression can be used to identify those cells by flow cytometry, as previously published^{8,32}. By using the following markers, Lin⁻ CD45⁺ Thy1.2⁺ GATA-3⁺ and Lin⁻ CD45⁺ Thy1.2⁺ RORγt⁺ to identify ILC2 and ILC3 respectively, we confirmed that ILC2 frequencies and absolute numbers are increased in the MLN and SI lamina propria of allergen-sensitized *I4raF709* mice and that *Il1rl1* deficiency significantly reduced those numbers (Fig E2, *A* to *C* in this article's Online Repository).

ILC2-derived IL-4 is indispensable for allergic sensitization

Following activation with IL-2, IL-25 or IL-33, ILC2 secrete large amounts of Th2 cytokines, such as IL-13 and IL-5, and it has become dogma that ILC2 produce minimal quantities of IL-4^{15,33,34}. Flow cytometric analysis of *in vitro* expanded ILC2 derived from SI lamina propria of unsensitized WT, *Il13*^{-/-}, *Il4raF709*, *Il4raF709 Il4*^{-/-} and *Il4raF709 Il1r1*^{-/-} mice demonstrates that ILC2 identified by the expression of the transcription factor GATA-3 (Lin⁻ Thy1.2⁺ CD45⁺ GATA-3⁺) express IL-5, IL-13 and copious amounts of IL-4 (Fig 3, A to C). In contrast, IL-9 expression by ILC2 was minimal (data not shown). IL-4 expression by MLN and SI ILC2 was further confirmed by using reporter mice expressing the enhanced green fluorescent protein *under control of the Il4 locus* (4Get mice) (Fig E3, A and B in this article's Online Repository)³⁵. *Il1r1* deficiency strongly reduced the production of IL-4, IL-13 and IL-5 by intestinal ILC2, even after peanut oral sensitization (Fig E4, A to C in this article's Online Repository). *Il4* deletion in *Il4raF709* mice did not affect IL-13 production by intestinal ILC2 whereas it completely abolished their IL-4 production (Fig 3, B and C). IL-13Rα1 was not expressed by CD4⁺ T cells, mast cells (FcεR1α⁺ c-Kit⁺), Treg (CD4⁺ Foxp3⁺) or B cells (CD19⁺ B220⁺), ruling out an effect by ILC2-derived IL-13 on these cell types (Fig E3, C in this article's Online Repository).

We reasoned that enforced restoration of the ILC2 compartment by adoptive transfer of *in vitro*-derived ILC2 might rescue allergic sensitization in *Il4raF709 Il1r1*^{-/-} mice by acting as an early source of IL-4. *Il4raF709 Il1r1*^{-/-} mice reconstituted with either *in vitro* expanded WT or IL-13 deficient ILC2 (*Il13*^{-/-} ILC2), and orally-sensitized with peanut developed food allergy as evidenced by a decrease in their core body temperature, higher levels of peanut-specific IgE and the release of MMCP-1 in the serum after oral challenge with peanut (Fig 4, A and B and Fig E5, A and B in this article's Online Repository). The recovery from the drop in core body temperature in mice reconstituted with *Il13*^{-/-} ILC2 was faster, suggestive of a role for ILC2-derived IL-13 in potentiating the food allergic response (Fig E5, A in this article's Online Repository). In contrast, adoptive transfer of *in vitro* generated ILC3 into *Il4raF709 Il1r1*^{-/-} mice subsequently orally sensitized and challenged with peanut did not restore the allergic reaction, thereby demonstrating the specific requirement for ILC2 in food allergy (Fig E5, A to C in this article's Online Repository). Critically, adoptive transfer of IL-4 deficient ILC2 (*Il4*^{-/-} ILC2) failed to restore oral allergic sensitization in *Il4raF709 Il1r1*^{-/-} mice (Fig 4, A and B). ILC2 frequencies and absolute numbers were similarly restored in the MLN and SI of mice receiving either WT, *Il4*^{-/-} ILC2 or *Il13*^{-/-} ILC2 (Fig 4, C and D and Fig E5, A to C in this article's Online Repository). These results revealed the importance of ILC2-secreted IL-4 in favoring allergic oral sensitization.

Treg cells suppress ILC2 expansion and activation

Establishment of oral tolerance and Treg cells suppressive functions are critical to prevent the development of oral sensitization and are involved in the resolution process of food allergy^{3,36,37}. Failure of Treg cell-mediated oral tolerance or Treg cells absence exacerbates food allergy^{3,38,39}. To demonstrate that Treg cells can directly suppress ILC2 expansion during food allergy we used transgenic DO11.10⁺ Rag2^{-/-} Foxp3^{EGFP} mice, which express the DO11.10⁺ TCR specific to OVA₃₂₃₋₃₃₉ peptide on a RAG2-deficient genetic

background. Those mice do not carry DO11.10⁺ CD4⁺ Foxp3⁺ T cells at steady-state, however supplying them with OVA in the drinking water triggers the conversion of naïve DO11.10⁺ CD4⁺ Foxp3⁻ T cells into induced DO11.10⁺ CD4⁺ Foxp3^{EGFP} iTreg cells³. As previously established, the presence of the *I4raF709* allele markedly impaired iTreg cell conversion in the MLN and SI of DO11.10⁺ Rag2^{-/-} *I4raF709* Foxp3^{EGFP} mice (Fig 5, A and B)³. Impaired allergen-specific iTreg cell formation was associated with a marked increase of the Lin⁻ CD45⁺ CD4⁻ TCR-β⁻ Thy1.2⁺ GATA-3⁺ ILC2 but not RORγt⁺ ILC3 population in the MLN and SI of DO11.10⁺ Rag2^{-/-} *I4raF709* Foxp3^{EGFP} mice (Fig 5, C and D and Fig E6, A to B in this article's Online Repository). These results established that defective iTreg cell induction and impaired oral tolerance are correlated with a significant expansion of the intestinal ILC2 population.

To further demonstrate the regulation of the ILC2 compartment by Treg cells, we employed Foxp3-deficient weanling mice (*Foxp3*^{K276X}), which lack functional Treg cells because of a nonsense mutation in *Foxp3*^{40,41}. Compared to age-matched WT mice, 20 days old *Foxp3*^{K276X} exhibited marked increase in the frequency and absolute numbers of ILC2 in the SI and MLN (Figure E7, A to D in this article's Online Repository). ILC3 were also increased, but to a lesser extent. Since *Foxp3*^{K276X} mice succumb to early death due to an uncontrolled lymphoproliferative disease, we examined the impact of acute transient ablation of Treg cells on ILC2 expansion by using adult Balb/c *Foxp3*^{EGFP/DTR+} mice⁴¹. Those mice express the diphtheria toxin receptor (DTR) under the control of the *Foxp3* promoter. Compared to age-matched WT *Foxp3*^{EGFP} mice, two weeks of diphtheria toxin (DT) treatment resulted in significant weight loss and a 2-fold decrease of MLN and SI Treg cell population in *Foxp3*^{EGFP/DTR+} (Fig E8, A to C in this article's Online Repository). Treg cell depletion with DT treatment resulted in increased ILC2 frequencies and absolute numbers in the SI and draining MLN of *Foxp3*^{EGFP/DTR+} (Fig 6, A to C). These results indicate that Treg cell deficiency, either congenital or inducible, results in dysregulated ILC2 expansion.

We further analyzed the capacity of Treg cells to regulate ILC2 Th2 cytokines production using an *in vitro* co-culture system. Cell-sorted iTreg cells *in vitro* derived from either WT or *I4raF709* naïve CD4⁺ T cells were used in a suppressive assay and cultured in the presence of an increased gradient of cell-sorted WT or *I4raF709* ILC2 (Fig 6, D and E). In the absence of iTreg cells and under IL-2 and IL-33 stimulation, purified ILC2 derived from either WT or *I4raF709* mice secrete substantial quantities of IL-4, IL-13 and IL-5 (Fig 6, D and E). Addition of iTreg cells resulted in a dose-dependent reduction in the production of IL-4 and IL-13, with IL-4 being more amenable to suppression (Fig 6, D and E). IL-33R expression on Treg cells plays a critical role in the suppression of intestinal inflammation⁴². When co-cultured with *in vitro* derived ILC2, *I4raF709* *Il1r1*^{-/-} iTreg cells displayed significantly less efficient suppressive function than their *I4raF709* counterparts, being only half as potent in reducing IL-4 and completely failing to reduce IL-13 secretion by ILC2 (Fig 6, F). In summary, Treg cells restrain ILC2 expansion in the gastrointestinal mucosa, and directly suppress ILC2 activation in an IL-33R-dependent manner.

IL-4 secretion by ILC2 antagonizes allergen-specific Treg cells induction and impairs their suppressive function

In vitro differentiation of naïve CD4⁺ T cells into CD4⁺ Foxp3⁺ iTreg cells is disrupted by the presence of IL-4^{3,43}. Accordingly, we examined the capacity of ILC2-derived IL-4 to disrupt oral tolerance in food allergy by impairing allergen-specific Treg cell formation and function. When naïve CD4⁺ Foxp3^{EGFP-} T cells are cultured in Treg cell-inducing conditions, addition of ILC2 to the culture resulted in a significant reduction of CD4⁺ Foxp3^{EGFP+} iTreg cell conversion in an IL-4-dependent fashion (Fig 7, A to D). Proliferation was not markedly altered, indicating a specific effect on Treg cell lineage commitment rather than outgrowth of the Foxp3⁻ compartment. IL-4 is a potent modulator of mast cell activation⁴⁴. Co-culture of IgE-sensitized mast cells in the presence of ILC2 resulted by upwards of a 50% increase of degranulating Lysosomal-associated membrane protein 1 (LAMP-1)⁺ mast cells which was largely abrogated when ILC2 were derived from IL-4-deficient mice (Fig 7, E).

We further examined the impact of ILC2 on allergen-specific Treg cells *in vivo* using the ILC2 reconstitution approach described in Figure 4. MLN cells isolated from peanut-sensitized *I4raF709 Il1r1l1*^{-/-} that received either WT or *I4*^{-/-} ILC2 were labeled with a proliferative cell dye and cultured in the presence of peanut. Analysis revealed significantly higher frequencies of peanut-specific Treg cells in recipients treated with *I4*^{-/-} ILC2 (Fig 7 F and G). Together, these results established that ILC2-derived IL-4 may act to promote food allergy by antagonizing the induction of allergen-specific Treg cells and inhibiting their regulatory function, which feeds into dysregulated mast cell activation.

Discussion

In this report, we demonstrate that ILC2 play a requisite role in mediating oral sensitization to food allergens and delineate the mechanisms involved in such action. *I4raF709* mice, which are genetically prone to develop food allergy, fail to do so when their ILC2 differentiation is compromised by concurrent IL-33R deficiency. Furthermore, we have established for the first time, using several genetic approaches, that Treg cells regulate ILC2 expansion and suppress their Th2 cell cytokine secretion. In turn, ILC2 activation and expansion promote food allergy by secreting IL-4, which compromises Treg cell function and augments mast cell activation. Our results define a cellular network involving ILC2, Treg cells and mast cells that is normally poised towards tolerance to food allergens but whose perturbation upon ILC2 activation may tip the balance towards food allergy.

A key finding in our studies relevant to food allergy is that Treg cells and ILC2 are here shown to engage in reciprocal regulation centered on ILC2-derived IL-4. While some previous reports have documented IL-4 secretion by ILC2, most have focused on the production by ILC2 of IL-5 and IL-13^{8,15,33,45}. Our present investigations reveal that ILC2 produce copious amounts of IL-4 that are sufficient to suppress allergen-specific iTreg cell differentiation and promote food allergy. Dysregulated ILC2 expansion and activation may well be an initiating step in the pathogenesis of food allergy. The ensuing impairment in the mucosal regulatory compartment contributes to a Th2-skewed, food allergen-specific adaptive immune response and disease persistence.

The function of ILC2 in the mucosal innate immune response and their localization at the environmental interface indicates that they may play a critical role in fueling food allergy in response to alarmin signals emanating from the intestinal epithelium, such as IL-33⁴⁶. Of note, production of the alarmin IL-33 is augmented in the intestinal tract of food allergic mice. Increased expression of alarmins by intestinal epithelial cells could be induced by an altered intestinal microbial flora^{47,48}. We have demonstrated that food allergy is associated with intestinal microbial dysbiosis, which in turn further promotes oral allergic sensitization²⁶. A dysbiotic flora may upregulate the expression of alarmins in the intestinal epithelium, creating an environment conducive to ILC2 activation and expansion following oral exposure to allergen. Some allergens, including peanut, have been shown to activate alarmins secretion by epithelial cells and provide one trigger leading to Th2 cytokines production and food allergy induction⁴⁹.

In addition to disabling Treg cells, IL-4 production by ILC2 amplified mast cell activation. We have previously shown that that Treg cell inhibition of mast cell degranulation is critical to forestalling food allergy^{3,4}. In turn, dysregulated IgE-mast cell interaction profoundly inhibits iTreg cells induction and mediates the latter Th2 cell-like reprogramming by an IL-4-dependent mechanism, providing a requisite step in food allergy pathogenesis³. Allergen re-exposure of intestinal IgE-stimulated mast cells could also lead to robust IL-33 production and consequently create an ILC2 activation and expansion loop that positively feeds back on mast cell activation and negatively regulates allergen-specific iTreg cells induction^{50,51}. Thus ILC2 may also sustain the immune dysregulation during the adaptive phase of the allergic immune response to food allergens.

While oral immunotherapy has been effective in inducing a desensitized state with short-lived unresponsiveness to food allergens, it has had more limited success in conferring true long-term tolerance following cessation of therapy⁵². Our previous results and those reported herein emphasize the importance of interrupting the ILC2-Treg cell-mast cell circuit of immune dysregulation that acts to suppress oral tolerance by blocking effective allergen-specific iTreg cell responses in food allergy. Resetting this circuit either pharmacological and/or by the use of immunomodulators, such as anti-IL-4R antibody, therapy may be essential in conferring long-term tolerance to food allergens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

GI Gastrointestinal

ILC2	innate lymphoid cell type 2
iTreg	induced regulatory T
ILC3	innate lymphoid cell type 3
MLN	mesenteric lymph nodes
SI	small intestine
Treg	regulatory T

References

1. Sicherer SH, Sampson HA. Food allergy. *J Allergy Clin Immunol.* 2010; 125:S116–S125. [PubMed: 20042231]
2. Sicherer SH, Sampson HA. Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol.* 2014; 133:291–295. [PubMed: 24388012]
3. Noval Rivas M, Burton OT, Wise P, Charbonnier L-M, Georgiev P, Oettgen HC, et al. Regulatory T Cell Reprogramming toward a Th2-Cell- like Lineage Impairs Oral Tolerance and Promotes Food Allergy. *Immunity.* 2015; 42:512–523. [PubMed: 25769611]
4. Burton OT, Noval Rivas M, Zhou JS, Logsdon SL, Darling AR, Koleoglou KJ, et al. Immunoglobulin E Signal Inhibition during Allergen Ingestion Leads to Reversal of Established Food Allergy and Induction of Regulatory T Cells. *Immunity.* 2014; 41:141–151. [PubMed: 25017467]
5. Doherty TA, Khorram N, Lund S, Mehta AK, Croft M, Broide DH. Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production. *J Allergy Clin Immunol.* 2013; 132:205–213. [PubMed: 23688412]
6. Kim HY, Chang Y-J, Subramanian S, Lee H-H, Albacker LA, Matangkasombut P, et al. Innate lymphoid cells responding to IL-33 mediate airway hyperreactivity independently of adaptive immunity. *J Allergy Clin Immunol.* 2012; 129:216–227. e1–e6. [PubMed: 22119406]
7. Chang Y-J, Kim HY, Albacker LA, Baumgarth N, McKenzie ANJ, Smith DE, et al. Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity. *Nat Immunol.* 2011; 12:631–638. [PubMed: 21623379]
8. Mjösberg J, Bernink J, Golebski K, Karrich JJ, Peters CP, Blom B, et al. The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. *Immunity.* 2012; 37:649–659. [PubMed: 23063330]
9. Shaw JL, Fakhri S, Citardi MJ, Porter PC, Corry DB, Kheradmand F, et al. IL-33-responsive innate lymphoid cells are an important source of IL-13 in chronic rhinosinusitis with nasal polyps. *Am J Respir Crit Care Med.* 2013; 188:432–439. [PubMed: 23805875]
10. Roediger B, Kyle R, Yip KH, Sumaria N, Guy TV, Kim BS, et al. Cutaneous immunosurveillance and regulation of inflammation by group 2 innate lymphoid cells. *Nat Immunol.* 2013
11. Kim BS, Siracusa MC, Saenz SA, Noti M, Monticelli LA, Sonnenberg GF, et al. TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med.* 2013; 5 170ra16–6.
12. Walker JA, Barlow JL, McKenzie ANJ. Innate lymphoid cells - how did we miss them? *Nat Rev Immunol.* 2013
13. Spits H, Cupedo T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. *Annu Rev Immunol.* 2012; 30:647–675. [PubMed: 22224763]
14. Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol.* 2011; 12:21–27. [PubMed: 21113163]
15. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)/Sca-1(+) lymphoid cells. *Nature.* 2010; 463:540–544. [PubMed: 20023630]

16. Halim TYF, MacLaren A, Romanish MT, Gold MJ, McNagny KM, Takei F. Retinoic-Acid-Receptor-Related Orphan Nuclear Receptor Alpha Is Required for Natural Helper Cell Development and Allergic Inflammation. *Immunity*. 2012; 37:463–474. [PubMed: 22981535]
17. Wong SH, Walker JA, Jolin HE, Drynan LF, Hams E, Camelo A, et al. Transcription factor ROR α is critical for nuocyte development. *Nat Immunol*. 2012; 13:229–236. [PubMed: 22267218]
18. Hoyler T, Klose CSN, Souabni A, Turqueti-Neves A, Pfeifer D, Rawlins EL, et al. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity*. 2012; 37:634–648. [PubMed: 23063333]
19. Saenz SA, Siracusa MC, Monticelli LA, Ziegler CGK, Kim BS, Brestoff JR, et al. IL-25 simultaneously elicits distinct populations of innate lymphoid cells and multipotent progenitor type 2 (MP2) cells. *J Exp Med*. 2013; 210:1823–1837. [PubMed: 23960191]
20. Salimi M, Barlow JL, Saunders SP, Xue L, Gutowska-Owsiak D, Wang X, et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med*. 2013; 210:2939–2950. [PubMed: 24323357]
21. Halim TYF, Steer CA, Mathä L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity*. 2014; 40:425–435. [PubMed: 24613091]
22. Turner J-E, Morrison PJ, Wilhelm C, Wilson M, Ahlfors H, Renauld J-C, et al. IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. *J Exp Med*. 2013; 210:2951–2965. [PubMed: 24249111]
23. Chu DK, Llop-Guevara A, Walker TD, Flader K, Goncharova S, Boudreau JE, et al. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization. *J Allergy Clin Immunol*. 2013; 131:187–200. e1–e8. [PubMed: 23006545]
24. Lee J-B, Chen C-Y, Liu B, Mugge L, Angkasekwinai P, Facchinetti V, et al. IL-25 and CD4(+) TH2 cells enhance type 2 innate lymphoid cell-derived IL-13 production, which promotes IgE-mediated experimental food allergy. *J Allergy Clin Immunol*. 2015
25. Tachdjian R, Khatib Al S, Schwingshackl A, Kim HS, Chen A, Blasioli J, et al. In vivo regulation of the allergic response by the IL-4 receptor alpha chain immunoreceptor tyrosine-based inhibitory motif. *J Allergy Clin Immunol*. 2010; 125:1128–1128. [PubMed: 20392476]
26. Noval Rivas M, Burton OT, Wise P, Zhang Y-Q, Hobson SA, Garcia Lloret M, et al. A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *J Allergy Clin Immunol*. 2013; 131:201–212. [PubMed: 23201093]
27. Noval Rivas M, Koh YT, Chen A, Nguyen A, Lee YH, Lawson G, et al. MyD88 is critically involved in immune tolerance breakdown at environmental interfaces of Foxp3-deficient mice. *J Clin Invest*. 2012; 122:1933–1947. [PubMed: 22466646]
28. Mathias CB, Hobson SA, Garcia Lloret M, Lawson G, Poddighe D, Freyschmidt E-J, et al. IgE-mediated systemic anaphylaxis and impaired tolerance to food antigens in mice with enhanced IL-4 receptor signaling. *J Allergy Clin Immunol*. 2011; 127:795–805. e1–e6. [PubMed: 21167580]
29. Halim TYF, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity*. 2012; 36:451–463. [PubMed: 22425247]
30. Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, et al. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J Allergy Clin Immunol*. 2013; 132:933–941. [PubMed: 23810766]
31. Oczypok EA, Milutinovic PS, Alcorn JF, Khare A, Crum LT, Manni ML, et al. Pulmonary receptor for advanced glycation end-products promotes asthma pathogenesis through IL-33 and accumulation of group 2 innate lymphoid cells. *J Allergy Clin Immunol*. 2015
32. Spencer SP, Wilhelm C, Yang Q, Hall JA, Bouladoux N, Boyd A, et al. Adaptation of Innate Lymphoid Cells to a Micronutrient Deficiency Promotes Type 2 Barrier Immunity. *Science*. 2014; 343:432–437. [PubMed: 24458645]
33. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TKA, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature*. 2010; 464:1367–1370. [PubMed: 20200518]

34. Mjösberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat Immunol.* 2011; 12:1055–1062. [PubMed: 21909091]
35. Mohrs M, Shinkai K, Mohrs K, Locksley RM. Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. *Immunity.* 2001; 15:303–311. [PubMed: 11520464]
36. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med.* 2004; 199:1679–1688. [PubMed: 15197226]
37. Syed A, Garcia MA, Lyu S-C, Bucayu R, Kohli A, Ishida S, et al. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). *J Allergy Clin Immunol.* 2014; 133:500–510. [PubMed: 24636474]
38. Lin W, Truong N, Grossman WJ, Haribhai D, Williams CB, Wang J, et al. Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. *J Allergy Clin Immunol.* 2005; 116:1106–1115. [PubMed: 16275384]
39. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, et al. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. *J Clin Invest.* 2000; 106:R75–R81. [PubMed: 11120765]
40. Lin W, Haribhai D, Relland LM, Truong N, Carlson MR, Williams CB, et al. Regulatory T cell development in the absence of functional Foxp3. *Nat Immunol.* 2007; 8:359–368. [PubMed: 17273171]
41. Haribhai D, Williams JB, Jia S, Nickerson D, Schmitt EG, Edwards B, et al. A requisite role for induced regulatory T cells in tolerance based on expanding antigen receptor diversity. *Immunity.* 2011; 35:109–122. [PubMed: 21723159]
42. Schiering C, Krausgruber T, Chomka A, Fröhlich A, Adelmann K, Wohlfert EA, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature.* 2014; 513:564–568. [PubMed: 25043027]
43. Veldhoen M, Uyttenhove C, Van Snick J, Helmby H, Westendorf A, Buer J, et al. Transforming growth factor-beta “reprograms” the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. *Nat Immunol.* 2008; 9:1341–1346. [PubMed: 18931678]
44. Burton OT, Darling AR, Zhou JS, Noval-Rivas M, Jones TG, Gurish MF, et al. Direct effects of IL-4 on mast cells drive their intestinal expansion and increase susceptibility to anaphylaxis in a murine model of food allergy. *Mucosal Immunol.* 2013; 6:740–750. [PubMed: 23149659]
45. Price AE, Liang H-E, Sullivan BM, Reinhardt RL, Eislely CJ, Erle DJ, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc Natl Acad Sci USA.* 2010; 107:11489–11494. [PubMed: 20534524]
46. Palmer G, Gabay C. Interleukin-33 biology with potential insights into human diseases. *Nat Rev Rheumatol.* 2011; 7:321–329. [PubMed: 21519352]
47. Sawa S, Lochner M, Satoh-Takayama N, Dulauroy S, Bérard M, Kleinschek M, et al. ROR γ t+ innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota. *Nat Immunol.* 2011; 12:320–326. [PubMed: 21336274]
48. Mosconi I, Geuking MB, Zaiss MM, Massacand JC, Aschwanden C, Kwong Chung CKC, et al. Intestinal bacteria induce TSLP to promote mutualistic T-cell responses. *Mucosal Immunol.* 2013; 6:1157–1167. [PubMed: 23515135]
49. Li J, Wang Y, Tang L, de Villiers WJS, Cohen D, Woodward J, et al. Dietary medium-chain triglycerides promote oral allergic sensitization and orally induced anaphylaxis to peanut protein in mice. *J Allergy Clin Immunol.* 2013; 131:442–450. [PubMed: 23182172]
50. Lefrançois E, Duval A, Mirey E, Roga S, Espinosa E, Cayrol C, et al. Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells. *Proc Natl Acad Sci USA.* 2014; 111:15502–15507. [PubMed: 25313073]
51. Hsu C-L, Neilsen CV, Bryce PJ. IL-33 is produced by mast cells and regulates IgE-dependent inflammation. *PLoS ONE.* 2010; 5:e11944. [PubMed: 20689814]
52. Jones SM, Burks AW, Dupont C. State of the art on food allergen immunotherapy: oral, sublingual, and epicutaneous. *J Allergy Clin Immunol.* 2014; 133:318–323. [PubMed: 24636471]

Key Messages

- Food allergy is accompanied by ILC2 expansion and activation in the gastrointestinal tract.
- Treg cells control ILC2 expansion and activation.
- IL-4 secretion by ILC2 blocks allergen-specific Treg cell induction.

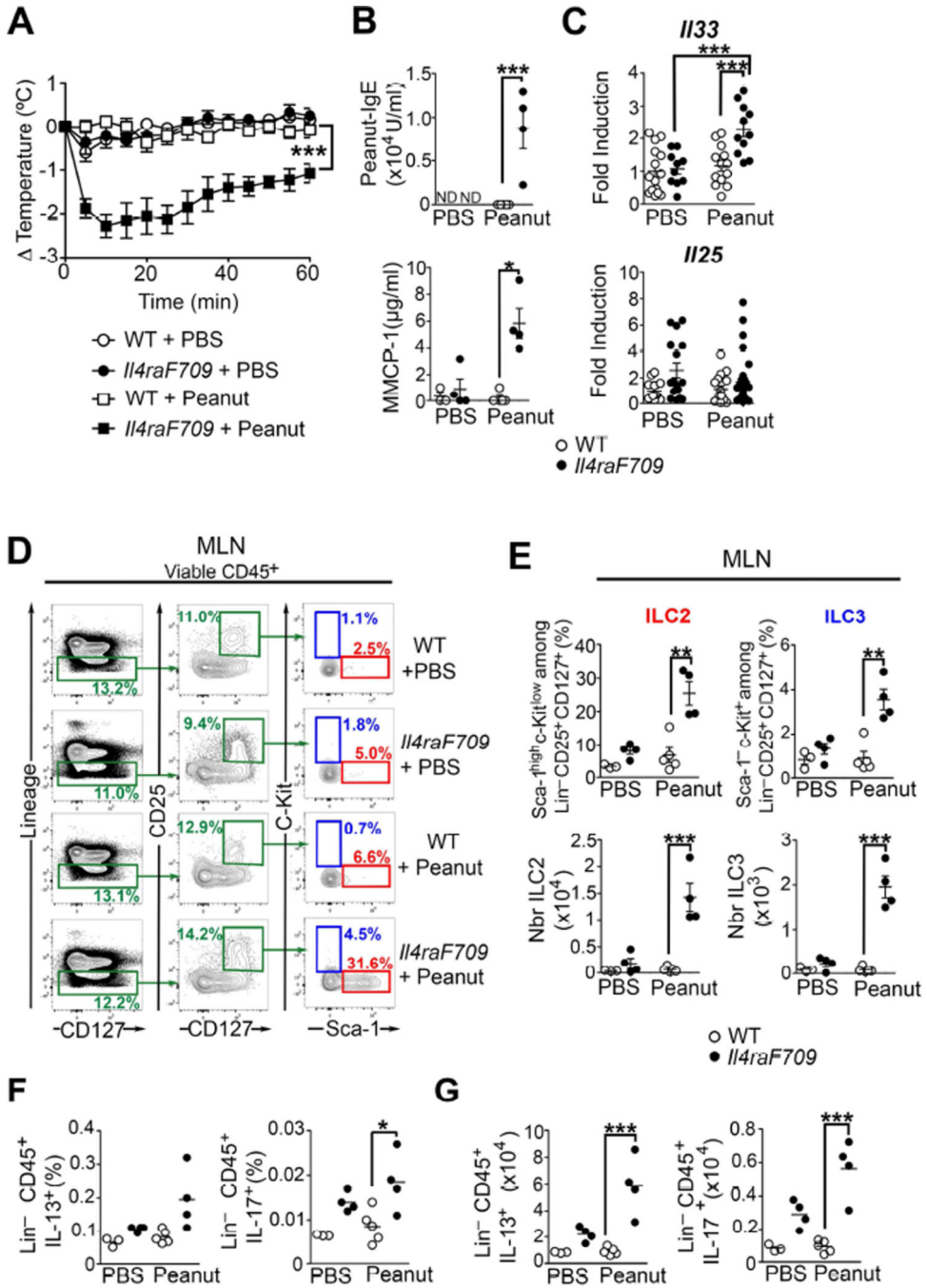


Figure 1. ILC2 are enriched in peanut-sensitized *Il4raF709* mice

(A) Core body temperature changes in PBS or peanut-sensitized WT and *Il4raF709* mice after oral peanut challenge. (B) Serum concentrations of peanut-specific IgE and MMCP-1 after anaphylaxis. (C) *I133* and *I125* mRNA levels in the SI of PBS and Peanut-sensitized WT and *Il4raF709* mice. (D – E) Flow cytometric analysis (D), frequencies and absolute numbers (E) of ILC2 (Lin⁻ CD25⁺ CD127⁺ Sca-1^{high} c-Kit^{low}) and ILC3 (Lin⁻ CD25⁺ CD127⁺ Sca-1⁻ c-Kit⁺) in the MLN. (F and G), Frequencies and absolute numbers of IL-13⁺ ILC2 and IL-17⁺ ILC3. Results represent data on 3 to 15 mice per group derived

from two independent experiments. *P < .05, **P < 0.01 and ***P < 0.001 by two-way ANOVA and one-way ANOVA with post-test analysis.

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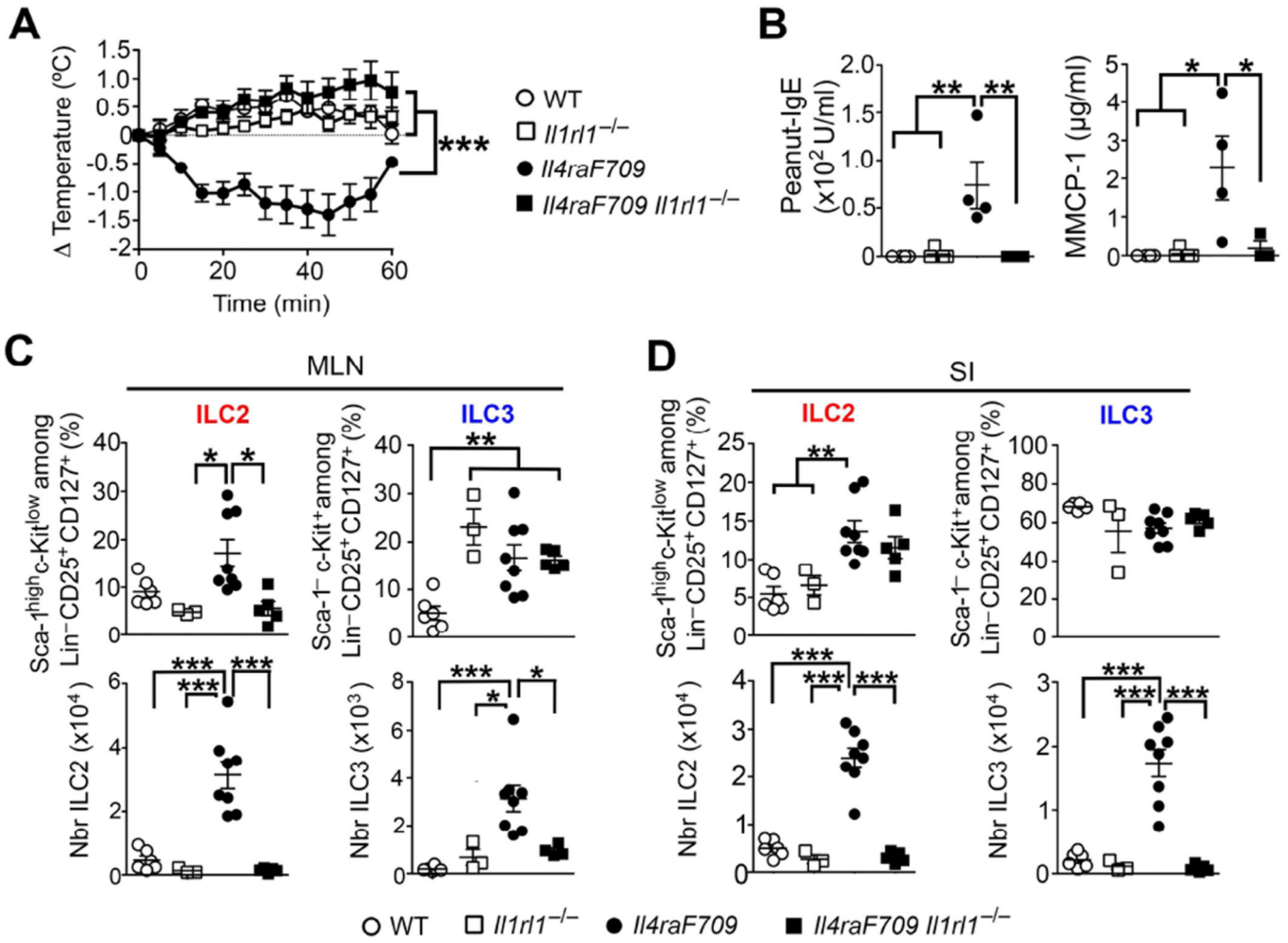


Figure 2. *Il1rl1* signaling drives ILC2 expansion and peanut allergy

(A) Core body temperature change in peanut-sensitized WT, *Il1rl1*^{-/-}, *Il4raF709* and *Il4raF709 Il1rl1*^{-/-} mice after oral challenge. (B) Serum concentrations of peanut-specific IgE and MMCP-1 after anaphylaxis. (C–D) Frequencies and absolute numbers of ILC2 and ILC3 in the MLN (C) and SI (D) from the mouse groups in (A). Results represent 3 to 8 mice per group derived from two independent experiments; *P < .05, **P < .01 and ***P < .001 by two-way ANOVA and one-way ANOVA with post-test analysis.

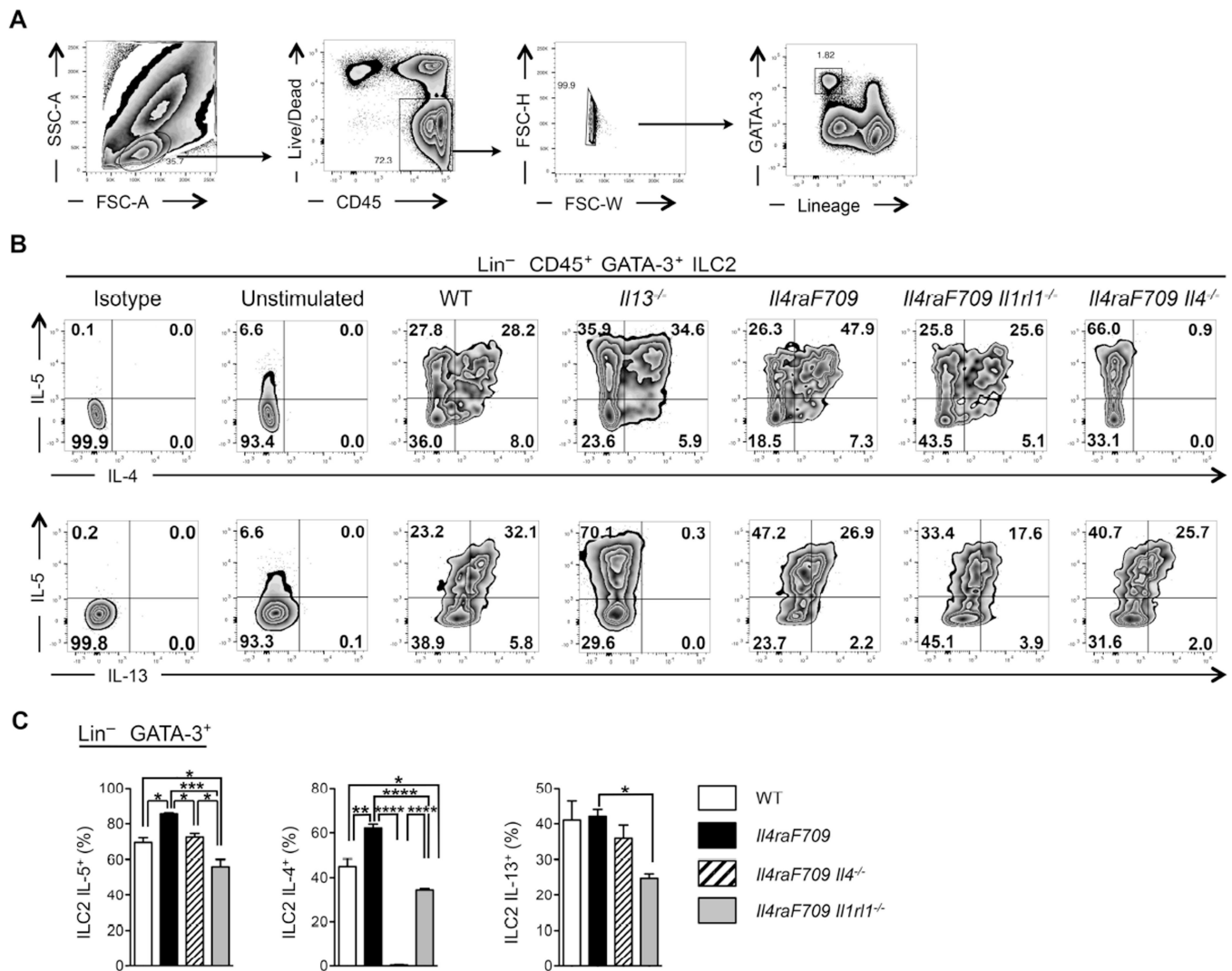


Figure 3. Th2 cytokine production by *in vitro* expanded SI ILC2

(A) Flow cytometric gating strategy used to identify *in vitro*-expanded ILC2 from the SI of naïve mice. (B) Flow cytometric analysis of IL-5, IL-4 and IL-13 expression by Lin⁻ CD45⁺ GATA-3⁺ ILC2 *in vitro*-expanded from the SI of naïve WT, *Il13*^{-/-}, *Il4raF709*, *Il4raF709 Il1rl1*^{-/-} and *Il4raF709 Il4*^{-/-} mice. (C) Frequencies of IL-5⁺, IL-4⁺ and IL-13⁺ Lin⁻ CD45⁺ GATA-3⁺ ILC2 isolated from the SI of the mouse groups from (B). Results represent 3 to 5 mice per group derived from 2 independent experiments. *P < .05, **P < .01, ***P < .001 and ****P < 0.0001 by one-way ANOVA with post-test analysis.

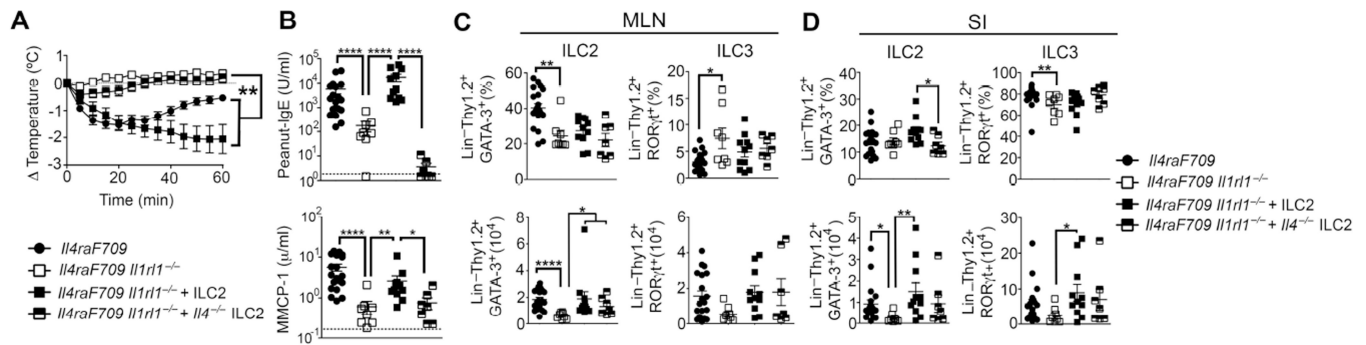


Figure 4. Oral allergic sensitization and anaphylaxis are obligately dependent on IL-4 production by ILC2

(A) Change in core body temperature after peanut oral challenge of peanut-sensitized *Il4raF709* and *Il4raF709 Il1r1^{-/-}* mice reconstituted with either WT or *Il4^{-/-}* ILC2. (B) Peanut-specific IgE concentration and MMCP-1 release after anaphylaxis. (C – D) Frequencies and absolute numbers of ILC2 (Lin⁻ Thy1.2⁺ GATA-3⁺) and ILC3 (Lin⁻ Thy1.2⁺ ROR γ t⁺) in the MLN (C) and SI (D) from the mouse group in (A). Results represent data of minimum 7 to 18 mice per group derived from two independent experiments. *P < .05, **P < .01, ***P < .001, ****P < .0001 by two-way and one-way ANOVA with post-tests analysis.

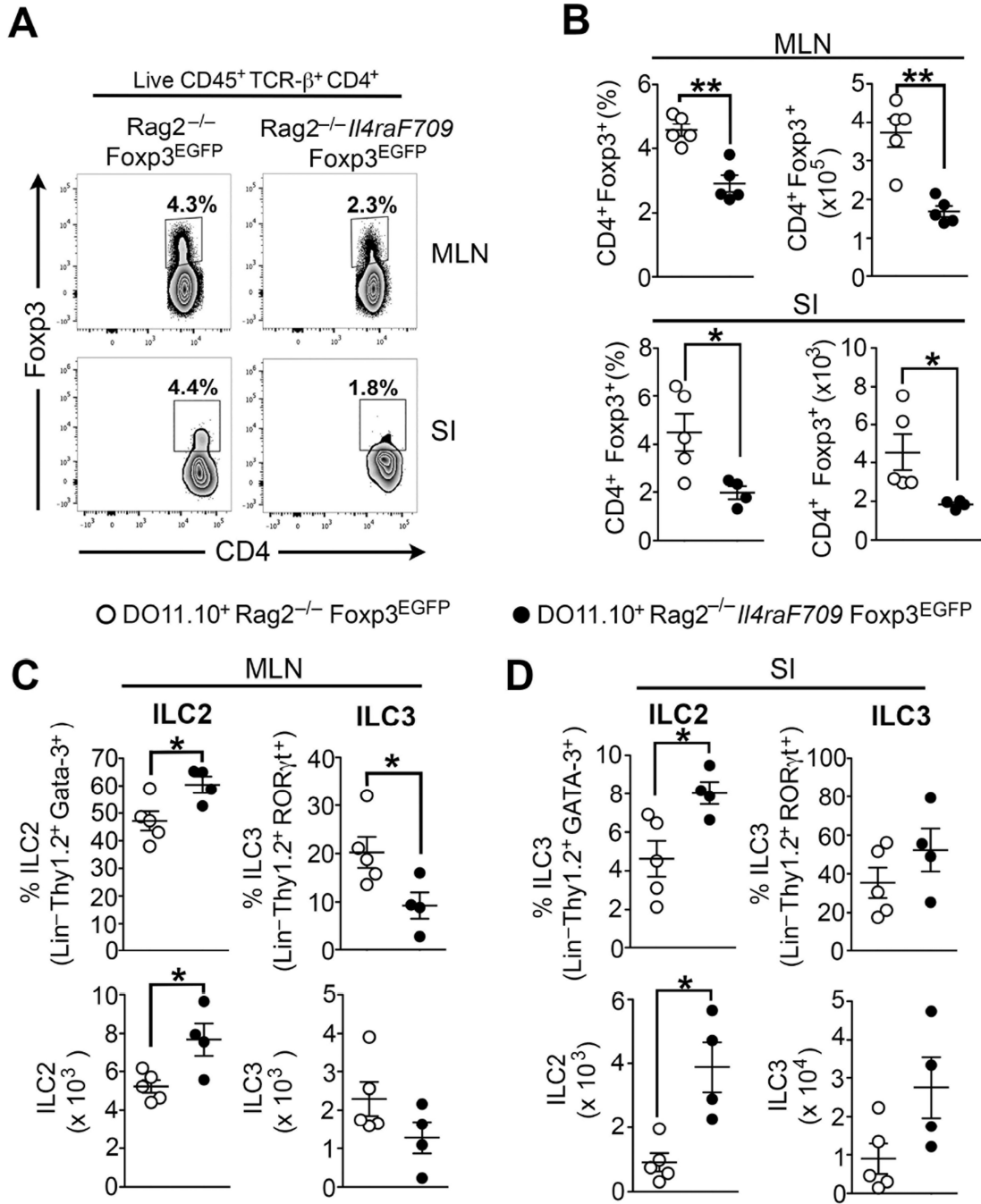


Figure 5. Defective allergen-specific Treg cell induction is associated with ILC2 expansion
(A) Flow cytometric analysis of iTreg cells (CD4⁺ Foxp3^{EGFP}⁺) in the MLN and SI of OVA-fed WT DO11.10⁺ Rag2^{-/-} Foxp3^{EGFP} and DO11.10⁺ Rag2^{-/-} Il4raF709 Foxp3^{EGFP} mice. **(B)** Frequencies and absolute numbers of CD4⁺ Foxp3⁺ iTreg cells in the MLN and SI of the mouse groups from (A). **(C – D)** Frequencies and absolute numbers of ILC2 (Lin⁻ Thy1.2⁺ GATA-3⁺) and ILC3 (Lin⁻ Thy1.2⁺ RORγt⁺) in the MLN (C) and SI (D) from the mouse groups in (A). Results represent data from 4 to 5 mice per group derived from two independent experiments. *P < .05, **P < 0.01, unpaired two-tailed Student's t test.

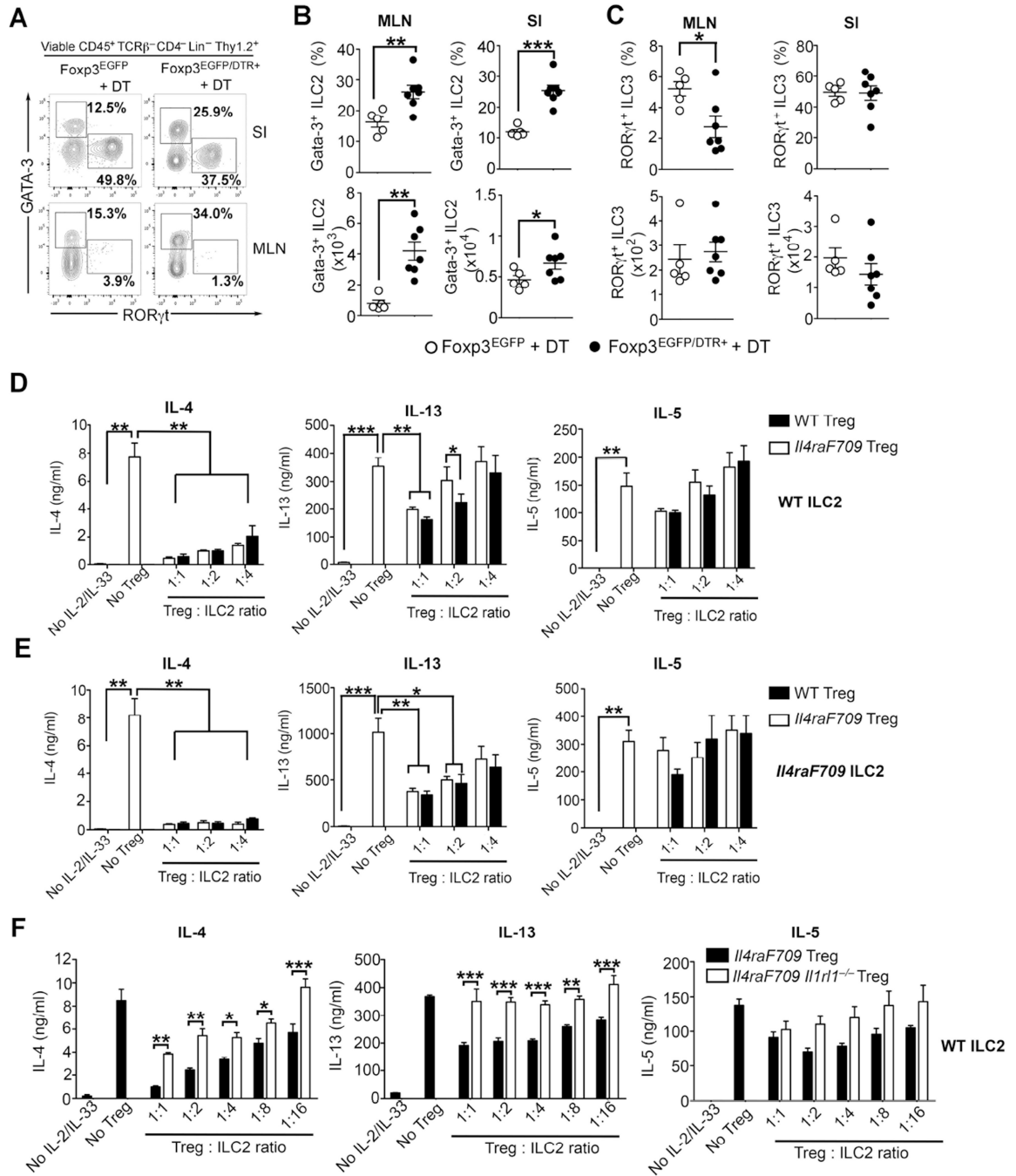


Figure 6. Treg cell inhibit ILC2 expansion and their Th2 cytokine production

(A) Flow cytometric analysis of ILC2 (Lin⁻ Thy1.2⁺ GATA-3⁺) and ILC3 (Lin⁻ Thy1.2⁺ RORγt⁺) in the SI and MLN of *Foxp3^{EGFP}* and *Foxp3^{EGFP/DTR+}* mice following DT administration. (B – C) Frequencies and absolute numbers of Lin⁻ Thy1.2⁺ GATA-3⁺ ILC2 (B) and Lin⁻ Thy1.2⁺ RORγt⁺ ILC3 (C) in the MLN and SI from the mouse groups in (A). (D – E) IL-4, IL-13 and IL-5 production by an increased gradient of cell-sorted WT ILC2 (D) or *I4raF709* ILC2 (E) cultured in the presence of either WT or *I4raF709* i Treg cells. (F) IL-4, IL-13 and IL-5 production by WT ILC2 cells co-cultured in the presence of

IL4raF709 or *IL4raF709 Il1r1^{-/-}* iTreg cells. Results represent data from 5 to 7 mice per group. *P < .05, **P < .01, and ***P < .001 by one-way ANOVA with post-tests analysis or unpaired two-tailed Student's t test.

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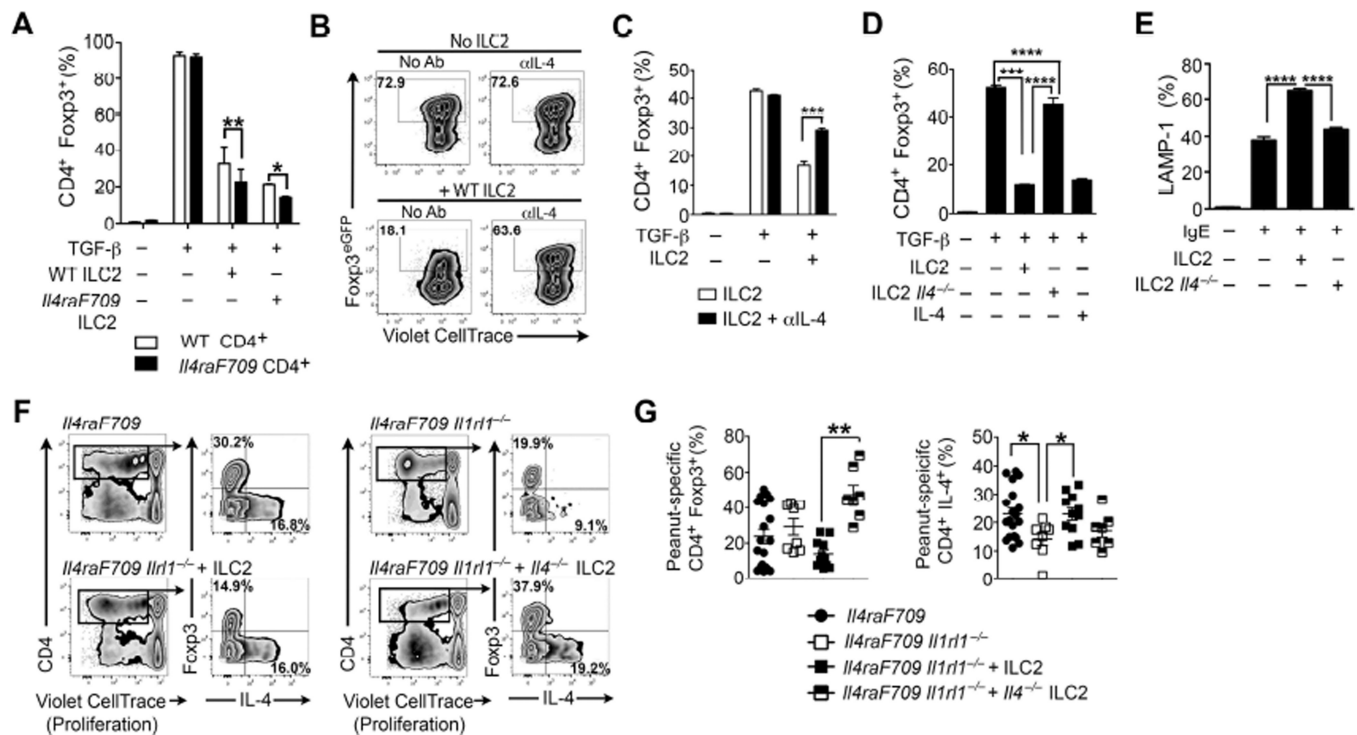


Figure 7. IL-4 secretion by ILC2 promotes food allergy by decreasing allergen-specific Treg cell induction

(A) TGF- β induction of iTreg cells from naïve CD4⁺ WT or *Il4raF709* T cells co-cultured with cell-sorted WT or *Il4raF709* ILC2. (B) Flow cytometric analysis of the effect of ILC2 and anti(α)-IL-4 treatment on TGF- β -driven *in vitro* iTreg cell induction. (C) Frequency of TGF- β -induced iTreg cells co-cultured in the presence of WT ILC2 without or with α IL4. (D) Frequency of TGF β -induced iTreg cells co-cultured with either WT ILC2, *Il4*^{-/-} ILC2 or recombinant IL-4. (E) IgE-mediated mast cell degranulation following co-culture with ILC2, ILC2 supernatants or IL-4 in the presence or absence of α IL-4. (F) IgE-mediated degranulation of mast co-cultured with WT ILC2 or *Il4*^{-/-} ILC2. (G) Flow cytometric analysis of peanut extract-induced *in vitro* proliferation of CD4⁺ Foxp3⁺ Treg cells and CD4⁺ Foxp3⁻ T conventional cells isolated from the MLN of peanut-sensitized *Il4raF709* and *Il4raF709 Il1r1*^{-/-} mice reconstituted with either WT or *Il4*^{-/-} ILC2 (F) Frequency of peanut-specific CD4⁺ Foxp3⁺ Treg cells and CD4⁺ T cells producing IL-4. Results represent data of 5 mice per group (A to F) and 7 to 18 mice (G and H) derived from two independent experiments. *P < .05, **P < .01, ***P < .001, ****P < .0001 by two-way and one-way ANOVA with post-tests analysis.