

Peanut-induced intestinal allergy is mediated through a mast cell-IgE-FcεRI-IL-13 pathway

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Background: Although implicated in the disease, the specific contributions of FcεRI and IL-13 to the pathogenesis of peanut-induced intestinal allergy are not well defined.

Objectives: We sought to determine the contributions of FcεRI, IL-13, and mast cells to the development of intestinal mucosal responses in a murine model of peanut-induced intestinal allergy.

Methods: Sensitized wild-type (WT), FcεRI-deficient (FcεRI^{-/-}), and mast cell-deficient (Kit^{W-sh/W-sh}) mice received peanut orally every day for 1 week. Bone marrow-derived mast cells (BMMCs) from WT, FcεRI^{-/-}, IL-4^{-/-}, IL-13^{-/-}, and IL-4/IL-13^{-/-} mice were differentiated and transferred into WT, FcεRI^{-/-}, and Kit^{W-sh/W-sh} recipients. BMMCs from WT and UBI-GFP/BL6 mice were differentiated and transferred into WT and Kit^{W-sh/W-sh} mice. Blockade of IL-13 was achieved by using IL-13 receptor α2 (IL-13Rα2)-IgG fusion protein.

Results: FcεRI^{-/-} mice showed decreased intestinal inflammation (mast cell and eosinophil numbers) and goblet cell metaplasia and reduced levels of IL-4, IL-6, IL-13, and IL-17A mRNA expression in the jejunum. Transfer of WT BMMCs to FcεRI^{-/-} recipients restored their ability to develop intestinal allergic responses unlike transfer of FcεRI^{-/-}, IL-13^{-/-}, or IL-4/IL-13^{-/-} BMMCs. FcεRI^{-/-} mice exhibited lower IL-13 levels and treatment of WT mice with IL-13 receptor α2 prevented peanut-induced intestinal allergy and inflammation.

Conclusions: These data indicate that the development of peanut-induced intestinal allergy is mediated through a mast cell-dependent IgE-FcεRI-IL-13 pathway. Targeting IL-13 might be a potential treatment for IgE-mediated peanut-induced allergic responses in the intestine. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: Peanut, intestinal allergy, mast cell, IgE, FcεRI, IL-13

Peanut allergy is increasing in prevalence, making it one of the most common food allergies in the United States.¹ Unlike other food allergies, peanut allergy often persists throughout life and is the leading cause of fatal food-induced anaphylaxis in Western

Abbreviations used

BMMC: Bone marrow-derived mast cell
IL-13Rα2: IL-13 receptor α2
mMCP1: Murine mast cell protease 1
NIH: National Institutes of Health
OVA: Ovalbumin
PE: Peanut extract
WT: Wild-type

Europe and the United States.^{2,3} Currently, there is incomplete understanding of the molecular mechanisms underlying peanut-induced food allergy, and no effective treatment is available. Clinical and experimental analyses suggest that initiation of food-induced intestinal allergy is regulated by numerous inflammatory cells and mediators, including mast cells and T_H2 cytokines.^{4,5}

Increased numbers of activated T cells have been correlated with increased levels of T_H2 cytokines, as well as the degree of gastrointestinal inflammation and dysfunction in patients with food allergy.^{6,7} *In vitro* allergen-stimulated T cells and T-cell clones generated from patients with peanut allergy produce increased levels of T_H2 cytokines (IL-4, IL-5, and IL-13).⁸ These cytokines activate immunologic pathways associated with the initiation of allergic responses, including T_H2 cell differentiation, IgE synthesis, and mast cell and eosinophil recruitment and activation.⁵ In this study we demonstrate that FcεRI and IL-13 are central to the induction of intestinal allergy through mast cell-mediated intestinal inflammation and the imbalance in T_H1/T_H2 cytokine levels.

METHODS

Mice

Five- to 6-week-old female wild-type (WT) BALB/cByJ mice, C57BL/6 mice, and IL-4-deficient (IL-4^{-/-}) mice were purchased from the Jackson Laboratory (Bar Harbor, Me). FcεRI-deficient (FcεRI^{-/-}) mice^{9,10} were provided by Dr J. P. Kinet (Harvard Medical School, Boston, Mass), and IL-13-deficient mice (IL-13^{-/-}) and IL-4/IL-13-deficient (IL-4/IL-13^{-/-}) mice^{11,12} were provided by Dr D. Umetsu (Harvard Medical School, Boston, Mass). Mast cell-deficient (Kit^{W-sh/W-sh}) mice were provided by Dr Peter Besmer (Sloan-Kettering Institute, New York, NY),¹³ and UBI-GFP/BL6 mice were provided by Dr Philippa Marrack (National Jewish Health, Denver, Colo).¹⁴ Mice were maintained on a peanut- and soy-free diet under specific pathogen-free conditions. Experiments were performed on age- and sex-matched mice. All studies were conducted under a protocol approved by the Institutional Animal Care and Use Committee of National Jewish Health.

Preparation of peanut protein

Crude peanut extract (PE) was prepared from defatted raw flours (Golden Peanut Company, Alpharetta, Ga) by using a method previously described.¹⁵

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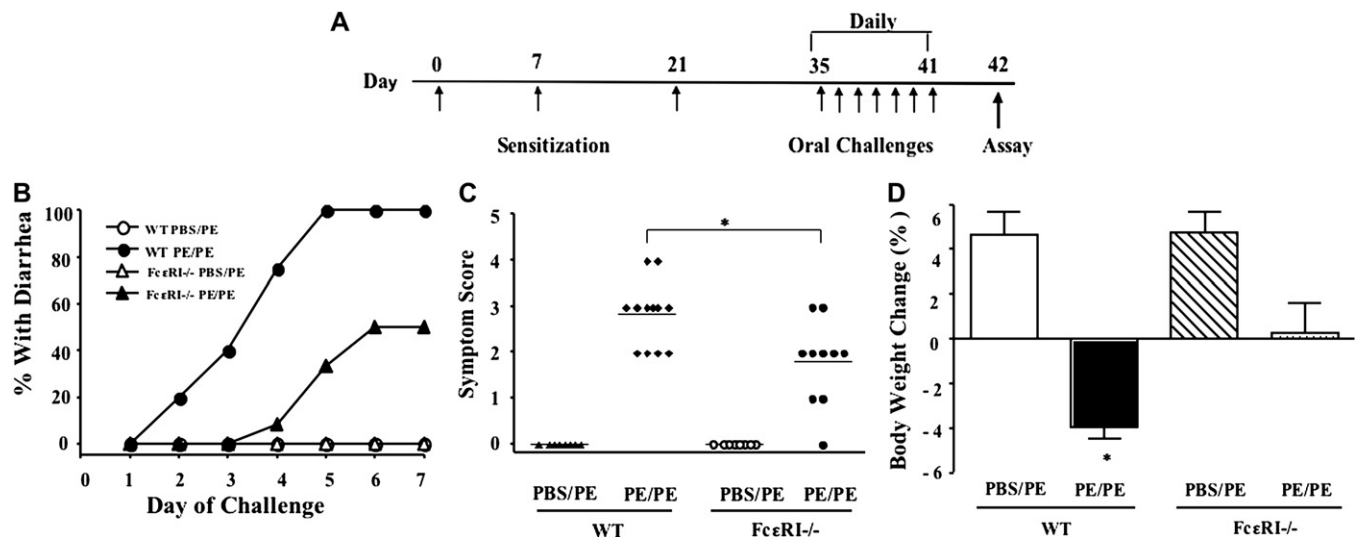


FIG 1. Decreased intestinal allergic responses in FcεRI^{-/-} mice. **A**, Protocol for induction of peanut-induced intestinal allergy. Mice were sensitized and challenged as described in the Methods section. **B**, Kinetics of development of diarrhea were assessed 30 minutes after the last challenge in WT and FcεRI^{-/-} mice. **C**, Symptom scores were assessed 30 minutes after oral challenge. **P* < .001 between mice in the sensitized and challenged with peanut (PE/PE) WT and PE/PE FcεRI^{-/-} groups. **D**, Body weight changes. Weights were obtained before challenge and after 7 days of challenge. Results were expressed as percentage weight change. Results were obtained from 3 individual experiments with 4 to 5 mice per group. **P* < .001 compared with all other groups. PBS/PE, Nonsensitized but challenged with PE group.

Endotoxin concentrations in the PE solutions were assessed by using a Chromogenic LAL endotoxin assay kit (GeneScript, Piscataway, NJ). The limit of detection for endotoxin was 0.005 EU/mL. Endotoxin depletion of PE solutions was performed with endotoxin-removing gel (Thermo Scientific, Waltham, Mass), according to the manufacturer's protocol. The final preparations of PE were depleted of endotoxin to levels of less than 0.1 EU/mL.

Sensitization and intragastric challenge

Mice were sensitized 3 times with 500 μg of PE together with 2.0 mg of alum (Pierce, Rockford, Ill) by means of intraperitoneal injection in a total volume of 100 μL on days 1, 7, and 21. Two weeks later, mice received 20 mg of PE (in 250 μL of PBS) by means of gavage with a 22-gauge feeding needle (Fisher Scientific, Pittsburgh, Pa) every day for 1 week (Fig 1, A). In some experiments C57BL/6 mice and Kit^{W-sh/W-sh} mice were passively sensitized, receiving 200 μL of serum (administered intraperitoneally) derived from peanut-sensitized and peanut-challenged BALB/c mice 1 day before oral challenge and 1 hour before the fourth oral challenge. Thirty minutes after the last challenge, plasma was collected and stored at -80°C. Twenty-four hours after the last challenge, mice were killed. Serum and jejunal tissue were collected for further analyses. Control animals were sham sensitized but challenged with peanut.

Administration of the soluble IL-13 receptor α2-IgG fusion protein

Murine soluble IL-13 receptor α2 (IL-13Rα2)-human IgG fusion protein (sIL-13Rα2-Fc) was prepared as previously described.¹⁶ IL-13Rα2-human IgG fusion protein was administered by means of intraperitoneal injection (300 μg per mouse) 1 hour before PE challenge on days 1, 3, 5, and 7. Control animals were administered the same amount of human IgG at identical time points.

Transfer of bone marrow-derived mast cells

Mast cells were derived from bone marrow, as previously described.¹⁷ After 4 to 5 weeks of culture, more than 98% expressed c-kit and FcεRIα on their surfaces. Bone marrow-derived mast cells (BMMCs; 5 × 10⁶) were injected

into WT, FcεRI^{-/-}, and Kit^{W-sh/W-sh} mice by means of intravenous injection 1 week and 1 day before starting the intestinal challenge with PE.

Assessment of hypersensitivity reactions

Anaphylactic symptoms were evaluated 30 minutes after the oral challenge, as previously reported.¹⁸ Scoring of symptoms was performed in a blinded manner by an independent observer.

Histology

Jejunum was fixed in 10% formalin and processed into paraffin blocks. Five-micrometer-thick tissue sections were cut and stained with periodic acid-Schiff for detection of mucus-containing cells. The number of mucus-containing cells from one side of the villus and the total number of villus cells on the same side were counted. The number of mucus-containing cells was expressed as the percentage of mucus-containing cells divided by the total number of cells counted, as previously described.¹⁹ At least 10 randomly selected villi were counted on each slide.

Mucosal mast cells were identified by chloroacetate esterase staining, as previously described,²⁰ and lightly counterstained with hematoxylin. Cells containing eosinophilic major basic protein were identified by means of immunohistochemical staining, as previously described, by using rabbit anti-mouse major basic protein antibody (kindly provided by Dr J. J. Lee, Mayo Clinic, Scottsdale, Ariz).²¹ At least 4 random fields per slide were examined and analyzed in a blinded manner. Quantification of stained mast cells or eosinophils per square millimeter of lamina propria was performed with an Olympus microscope linked to the National Institutes of Health (NIH) Image Analysis Program (NIH, Bethesda, Md).

Immunofluorescence staining for the detection of mast cells and basophils

The jejunums of peanut-sensitized and peanut-challenged mice treated with or without sIL-13Rα2 or control IgG and the jejunums of PBS- or PE-sensitized and PE-challenged mice receiving BMMCs from UBI-GFP/BL6 mice were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek,

Torrance, Calif). Sections of tissue blocks (8- μ m thickness) were mounted on microscopic slides, air dried, and fixed with cold acetone. After blocking with 2% normal goat serum, sections were stained with Armenian hamster anti-mouse Fc ϵ R1 α and rat anti-mouse c-Kit antibody (eBioscience, San Diego, Calif) as primary antibodies and rhodamine red-x-goat anti-rat IgG and fluorescein isothiocyanate goat anti-Armenian hamster IgG (Jackson ImmunoResearch Laboratories, West Grove, Pa) as a secondary antibody. In sections prepared after transfer of BMMCs derived from UBI-GFP/BL6, only nuclei were stained with Vectashield mounting medium with 4'-6-diamidino-2-phenylindole dihydrochloride (DAPI; Vector Laboratories, Burlingame, Calif). The immunofluorescent images were captured with a Leica microscope camera system and Slidebook 5 digital microscopy software (Intelligent Imaging Innovations, Denver, Colo). Quantification of stained mast cells (Fc ϵ R1 α ⁺ and c-Kit⁺) and basophils (Fc ϵ R1 α ⁺ but c-Kit⁻) per square millimeter of lamina propria was performed with an Olympus microscope linked to the NIH Image Analysis Program (available at <http://rsb.info.nih.gov/ij/index.html>).

Assessment of T_H1/T_H2 cytokine levels in intestine homogenates

Jejunum was weighed and completely homogenized with a tissue homogenizer (Kinematica AG, Lucerne, Switzerland) in dilution buffer (PBS and 0.1% Triton X; Sigma-Aldrich, St Louis, Mo) containing proteinase inhibitor (Roche Diagnostics, Indianapolis, Ind) and then centrifuged at 14,000 rpm for 30 minutes. Supernatants were collected and frozen at -80°C until further analyses. Total protein content was measured by using the bicinchoninic acid (BCA) method, and levels of IL-4, IL-6, IL-13, and IFN- γ were assessed by means of ELISA (eBioscience), according to the manufacturer's directions. The limit of detection was 4 pg/mL for IL-4, IL-6, and IL-13 and 15 pg/mL for IFN- γ .

Measurement of peanut-specific antibody

Serum PE-specific IgE, IgG1, and IgG2a levels were measured by means of ELISA, as described previously.^{15,18}

Measurement of histamine

Plasma was collected 30 minutes after the last challenge and stored at -80°C. Plasma levels of histamine were measured using an enzyme immunoassay kit (Beckman Coulter, Fullerton, Calif), as described by the manufacturer. The concentration of histamine was calculated from a standard curve provided by the manufacturer.

Quantitative real-time PCR

RNA was extracted from jejunal tissue using the RNeasy mini kit (Qiagen, Hilden, Germany) and was transcribed into cDNA (cDNA synthesis kit; Bio-Rad Laboratories, Hercules, Calif). All primers and probes for murine *IL-4*, *IL-6*, *IL-13*, *IL-17A*, *IFN- γ* , murine mast cell protease (mMCP-1), and glyceraldehyde-3-phosphate dehydrogenase were obtained from Applied Biosystems (Foster City, Calif), and real-time PCR was performed with an ABI Prism 7300 sequence detector (Applied Biosystems).²² The $\Delta\Delta$ cycle threshold method was performed for relative quantification of mRNA expression.

Statistical analysis

ANOVA was used to determine the levels of difference between all groups. Comparisons for all pairs used the Tukey-Kramer highest significance difference test. *P* values for significance were set at .05. All results were expressed as means $\pm$ SEMs.

RESULTS

Decreased intestinal responses in Fc ϵ R1^{-/-} mice

Oral administration of PE to PE-sensitized WT mice triggered the onset of acute diarrhea on the second day of challenge, and

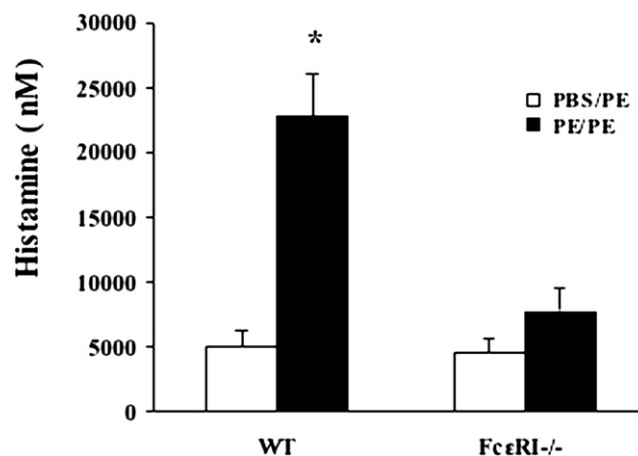


FIG 2. Levels of plasma histamine. Plasma levels of histamine were assessed within 30 minutes after the last challenge. Results were obtained from 3 individual experiments with 4 mice per group. **P* < .01 compared with all other groups. PBS/PE, Nonsensitized but challenged with peanut group; PE/PE, sensitized and challenged with PE group.

100% of the mice had diarrhea by the seventh day of challenge. In contrast, sensitized and challenged Fc ϵ R1^{-/-} mice showed a delay in onset of diarrhea and an overall lower incidence of diarrhea (Fig 1, B). Fc ϵ R1^{-/-} mice exhibited lower symptom scores compared with similarly sensitized and challenged WT mice (Fig 1, C). There were no significant body weight changes during the sensitization period (data not shown). Between 1 day before the first challenge and the last challenge, PE-sensitized and PE-challenged WT mice showed significant weight loss ($-4.29\% \pm 1.34\%$), whereas similarly sensitized and challenged Fc ϵ R1^{-/-} mice maintained their body weight ($0.26\% \pm 1.34\%$; Fig 1, D).

Increased peanut-specific IgE, IgG1, and IgG2a levels in Fc ϵ R1^{-/-} mice after sensitization and challenge with peanut

Levels of serum peanut-specific IgE were significantly increased in PE-sensitized and PE-challenged Fc ϵ R1^{-/-} mice, but the levels were lower than those in peanut-sensitized and peanut-challenged WT mice. Levels of PE-specific IgG1 and IgG2a were similarly increased in Fc ϵ R1^{-/-} and WT mice (see Fig E1 in this article's Online Repository at www.jacionline.org).

Levels of plasma histamine in Fc ϵ R1^{-/-} mice

Plasma levels of histamine were measured within 30 minutes after the last challenge to assess the extent of mast cell degranulation. Levels of histamine in Fc ϵ R1^{-/-} mice were not increased after sensitization and challenge, whereas PE-sensitized and PE-challenged WT mice showed significantly increased plasma levels of histamine (Fig 2).

Intestinal inflammation after sensitization and challenge with peanut

It has been reported that leukocytes are recruited to the gastrointestinal tract in patients with food-induced allergic diarrhea.²³ We found that mast cells and eosinophils infiltrated the small, but not the large intestine. Numbers of mast cells and eosinophils

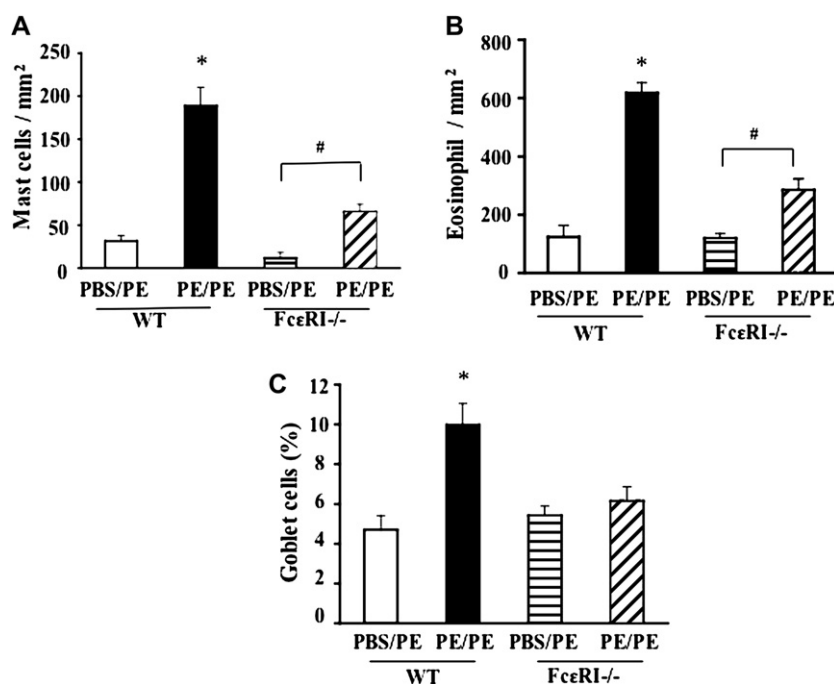


FIG 3. Decreased inflammatory cell accumulation in the intestinal walls of $Fc\epsilon RI^{-/-}$ mice. **A**, Quantitation of mucosal mast cell numbers in the jejunum. **B**, Quantitation of mucosal eosinophil numbers in the jejunum. **C**, Quantitation of mucosal goblet cells in the jejunal epithelium. For mast cells and eosinophils, results are expressed as the number of chloroacetate esterase- or anti-major basic protein-stained cells per square millimeter of lamina propria, respectively. For goblet cells, the number of periodic acid-Schiff-positive cells were divided by the total number of epithelial cells in the villi. Results were obtained from 3 independent experiments, and each experiment included 3 to 4 mice per group. * $P < .001$ compared with all other groups. # $P < .01$ compared with PBS-sensitized control $Fc\epsilon RI^{-/-}$ mice. PBS/PE, Nonsensitized but challenged with peanut group; PE/PE, sensitized and challenged with PE group.

were significantly increased in the jejunum of PE-sensitized and PE-challenged WT mice. PE-sensitized and peanut-challenged $Fc\epsilon RI^{-/-}$ mice also exhibited increased mast cell and eosinophil accumulation in the mucosa of the jejunum, but the levels were significantly lower (Fig 3, A and B, and see Figs E2 and E3 in this article's Online Repository at www.jacionline.org).

Goblet cell numbers in the intestinal walls of $Fc\epsilon RI^{-/-}$ mice

Increased mucus production is characteristic of conditions in which there is inflammation or irritation of the epithelial lining. Peanut-sensitized and PE-challenged WT mice demonstrated a large number of goblet cells ($9.97\% \pm 0.48\%$ goblet cells) in the intestinal epithelium. PE-sensitized and PE-challenged $Fc\epsilon RI^{-/-}$ mice showed a lower number of goblet cells ($6.14\% \pm 0.51\%$) in the intestinal epithelium compared with WT mice (Fig 3, C, and see Fig E4 in this article's Online Repository at www.jacionline.org).

IL-13 protein levels in intestinal homogenates are significantly decreased in $Fc\epsilon RI^{-/-}$ mice

To determine whether IL-13 is regulated in peanut-induced intestinal allergy, we measured levels of IL-13 in intestinal homogenates adjusted to the weight of each tissue sample 24 hours after the last challenge. Increased levels of IL-13 were detected in jejunal homogenates from PE-sensitized and PE-challenged WT mice but not in PE-sensitized and PE-challenged $Fc\epsilon RI^{-/-}$ mice (Fig 4).

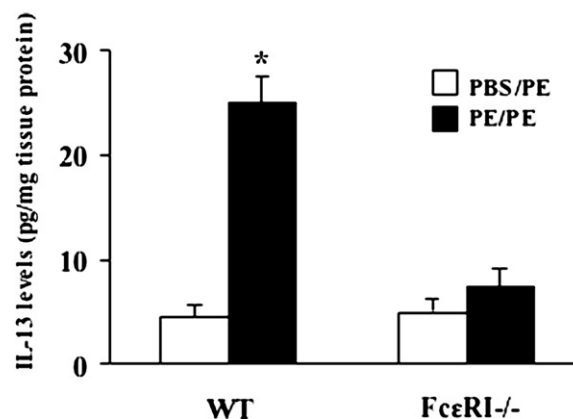


FIG 4. IL-13 protein levels in intestinal homogenates. Levels of IL-13 were assessed in jejunal homogenates 24 hours after the last challenge. Results were obtained from 3 independent experiments with 4 mice per group. * $P < .01$ compared with all other groups. PBS/PE, Nonsensitized but challenged with peanut group; PE/PE, sensitized and challenged with PE group.

Levels of T_H1/T_H2 cytokines and IL-17 mRNA expression in jejunum

After 7 days of PE challenge, sensitized WT mice demonstrated significant increases in *IL-4*, *IL-6*, *IL-13*, and *IL-17A* mRNA expression in the intestine. There were no increases in mRNA expression of these cytokines in the intestines of

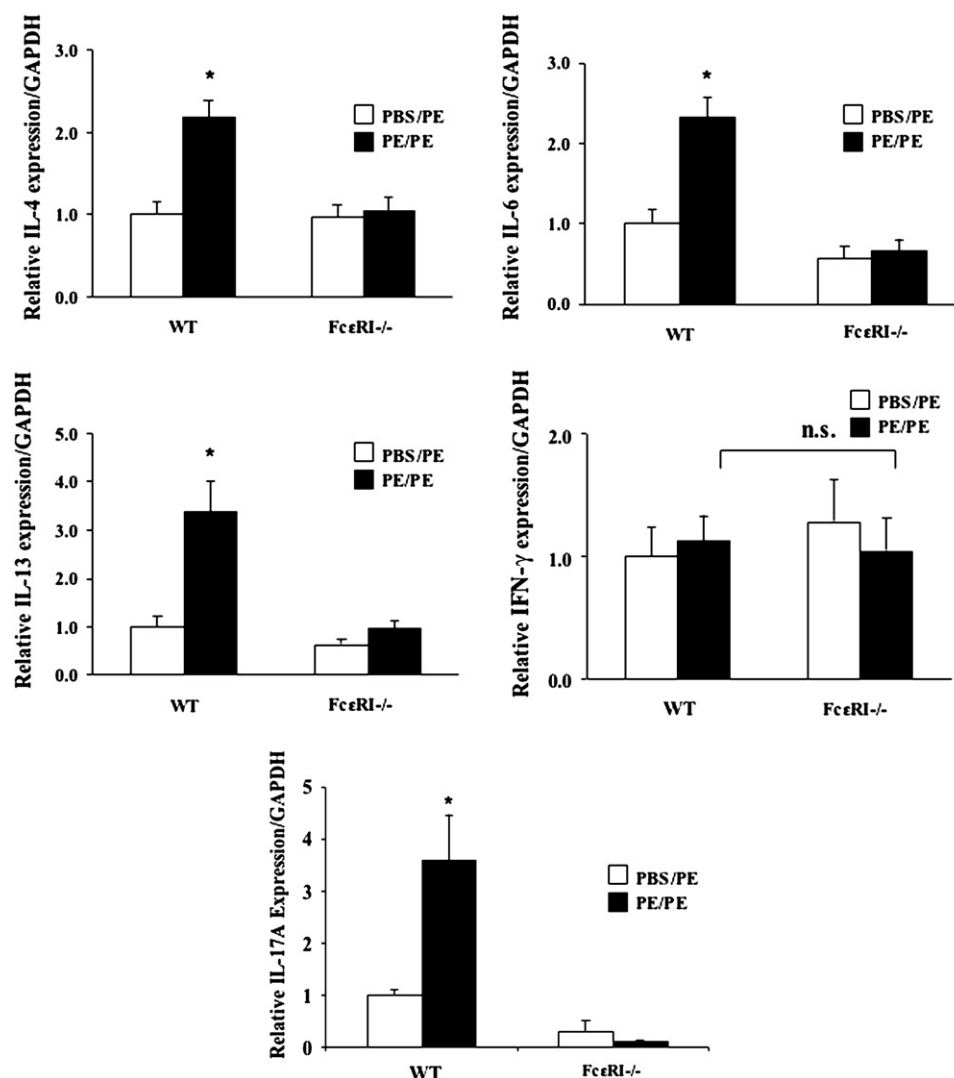


FIG 5. Cytokine mRNA expression in jejunum of sensitized and challenged mice. Relative expression levels of *IL-4*, *IL-6*, *IL-13*, *IFN-γ*, and *IL-17A* normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) in jejunum were determined by means of quantitative RT-PCR, as described in the Methods section. The values obtained in the WT nonsensitized but challenged with peanut group (*PBS/PE*) were assigned a value of 1 in all assays. Results were representative of 3 independent experiments with 4 mice per group. **P* < .001 compared with all other groups. *n.s.*, Not significant; *PE/PE*, sensitized and challenged with PE group.

PE-sensitized and PE-challenged *FcεRI*^{-/-} mice, which were similar to those in PBS-sensitized and PE-challenged WT mice. *IFN-γ* mRNA levels were not altered in peanut-sensitized and peanut-challenged WT or *FcεRI*^{-/-} mice (Fig 5).

mMCP1 mRNA expression in jejunum

Activated mast cells release mediators, such as histamine and mMCP, which might play a critical role in the induction of allergic responses.⁵ We measured mMCP1 levels in intestinal homogenates 24 hours after the last challenge using quantitative RT-PCR. Indeed, PE-sensitized and PE-challenged WT mice exhibited significantly increased levels (about 6-fold) of mMCP1 mRNA compared with levels seen in PBS-sensitized and peanut-challenged WT control animals. Levels in PE-sensitized and PE-challenged *FcεRI*^{-/-} mice were not increased (see Fig E5 in this article's Online Repository at www.jacionline.org).

Transfer of BMMCs from WT to sensitized *FcεRI*^{-/-} mice restores their ability to respond to peanut challenge

We next determined whether the attenuated intestinal allergy in *FcεRI*^{-/-} mice could be overcome by transfer of mast cells from WT, *FcεRI*^{-/-}, *IL-4*^{-/-}, *IL-13*^{-/-}, or *IL-4/IL-13*^{-/-} mice. Mast cells were differentiated from bone marrow of WT, *FcεRI*^{-/-}, *IL-4*^{-/-}, *IL-13*^{-/-}, or *IL-4/IL-13*^{-/-} mice. After 4 weeks of culture, 98% of cultured cells were identified as mast cells based on c-kit and FcεRI surface staining. BMMCs were injected intravenously into recipient mice 1 week and 1 day before starting the oral challenges. After transfer of BMMCs from WT and *IL-4*^{-/-} mice, the numbers of mast cells in the intestinal mucosa of *FcεRI*^{-/-} recipients were significantly increased (see Fig E6 in this article's Online Repository at www.jacionline.org). Recipients of *FcεRI*^{-/-} BMMCs or BMMCs from *IL-13*^{-/-} or *IL-4/IL-13*^{-/-} mice accumulated fewer mast cells in the intestinal mucosa.

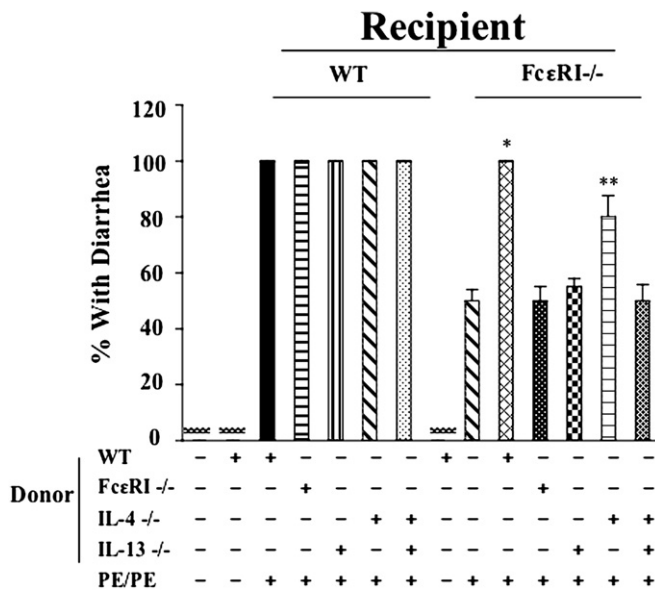


FIG 6. Transfer of BMMCs from WT and IL-4^{-/-}, but not from FcεRI^{-/-}, IL-13^{-/-}, and IL-4/IL-13^{-/-} mice into FcεRI^{-/-} mice restores their ability to develop diarrhea. Sensitized and challenged WT and FcεRI^{-/-} mice received BMMCs from WT, FcεRI^{-/-}, IL-4^{-/-}, IL-13^{-/-}, or IL-4/IL-13^{-/-} mice on 2 occasions before challenge. Results are representative of 2 independent experiments with 4 mice per group and expressed as percentage with diarrhea during challenge. **P* < .01 comparing recipients of WT mast cells with recipients of IL-13^{-/-}, IL-4/IL-13^{-/-}, or FcεRI^{-/-} mast cells. ***P* < .05 comparing recipients of IL-4^{-/-} mast cells with recipients of IL-13^{-/-}, IL-4/IL-13^{-/-}, or FcεRI^{-/-} mast cells. PE/PE, Sensitized and challenged with PE group.

Transfer of BMMCs from WT to PE-sensitized and PE-challenged FcεRI^{-/-} mice restored the ability of 100% of the mice to develop diarrhea, as seen in sensitized and challenged WT recipients. Transfer of BMMCs from IL-4^{-/-} mice to PE-sensitized and PE-challenged FcεRI^{-/-} mice restored the ability of 80% of the mice to develop diarrhea. However, transfer of mast cells from FcεRI^{-/-}, IL-13^{-/-}, and IL-4/IL-13^{-/-} mice failed to reconstitute PE-induced diarrhea in sensitized and challenged FcεRI^{-/-} recipients (Fig 6).

Transferred BMMCs can be detected in jejunal tissue of both Kit^{W-sh/W-sh} mice and C57BL/6 mice

BMMCs from C57BL/6 mice were transferred to Kit^{W-sh/W-sh} recipients 1 week and 1 day before starting the intestinal challenge with PE to assess whether transferred BMMCs migrate to the jejunum. After 7 days of peanut challenge, transferred BMMCs were found in the mucosa of the jejunum and colon in Kit^{W-sh/W-sh} mice by chloroacetate esterase staining. In contrast, without BMMC transfer, we did not detect mast cells in the jejunum and colon in Kit^{W-sh/W-sh} mice (Fig 7, A-D). Green fluorescent BMMCs from UBI-GFP/BL6 mice were transferred into C57BL/6 recipients 1 week and 1 day before initiating intestinal challenges to further confirm that transferred BMMCs circulate and become resident in jejunal tissue after sensitization and challenge. After the 7-day challenge with peanut, transferred green fluorescent mast cells could be detected in the jejunal tissue of PE-sensitized and PE-challenged mice 24 hours after the last challenge; only a rare green fluorescent positive mast cell could be detected in the nonsensitized but challenged recipients (Fig 7, E and F).

Kit^{W-sh/W-sh} mice have significantly less and less severe allergic diarrhea after systemic and passive sensitization and challenge

Kit^{W-sh/W-sh} and C57BL/6 control mice were systemically sensitized with or without PE and challenged with PE to determine whether the deficiency of mast cells affects the development of PE-induced allergic diarrhea. C57BL/6 mice do not have PE- or ovalbumin (OVA)-induced intestinal allergy to the extent of BALB/c mice because of their failure to have IgE antibody responses. Kit^{W-sh/W-sh} and C57BL/6 mice were passively sensitized with serum containing peanut-specific IgE. All systemically and passively sensitized and challenged C57BL/6 mice had diarrhea by the seventh day of challenge. In contrast, only 25% of sensitized and challenged Kit^{W-sh/W-sh} mice had diarrhea. After transfer of BMMCs from C57BL/6 mice, Kit^{W-sh/W-sh} mice demonstrated an increase in the development of diarrhea (75%). Kit^{W-sh/W-sh} mice exhibited lower symptom scores compared to similarly sensitized and challenged C57BL/6 mice (Fig 8).

Effect of treatment with IL-13Rα2 protein on peanut-induced intestinal allergy

Based on the data showing increased levels of IL-13 mRNA and protein after PE sensitization and challenge in WT mice, IL-13 appeared to play an essential role in the development of intestinal allergy. To directly test this hypothesis, we determined the effect of neutralizing IL-13. Peanut-sensitized mice were administered 300 μg of IL-13Rα2 protein intraperitoneally every other day during the 7 days of PE challenge (Fig 9, A). Peanut-sensitized and PE-challenged WT mice treated with IL-13Rα2 did not have diarrhea (Fig 9, B). Numbers of mast cells in the intestinal mucosa were significantly decreased after treatment with IL-13Rα2 (Fig 9, C, and see Fig E7 in this article's Online Repository at www.jacionline.org), as were numbers of eosinophils (Fig 9, D, and see Fig E7) and numbers of goblet cells in the jejunal epithelium (see Figs E8 and E9 in this article's Online Repository at www.jacionline.org). In none of the tissue sections were basophils (FcεRI⁺c-kit⁻) identified in the intestinal mucosa by means of immunofluorescence staining (data not shown). In parallel, IL-4, IL-6, IL-13, and m MCP-1 mRNA expression in the intestine was decreased in mice treated with IL-13Rα2 compared with that seen in mice treated with control antibody (Fig 9, E, and see Fig E10 in this article's Online Repository at www.jacionline.org). IL-4, IL-6, and IL-13 protein levels in intestinal homogenates were also significantly decreased in mice after treatment with sIL-13Rα2, whereas IFN-γ protein levels in intestinal homogenates were not altered (see Fig E11 in this article's Online Repository at www.jacionline.org). Levels of plasma histamine were also significantly decreased in mice treated with sIL-13Rα2 compared with those seen in mice treated with control antibody (see Fig E12 in this article's Online Repository at www.jacionline.org). Serum levels of PE-specific IgE, IgG1, and IgG2a were not altered in PE-sensitized and PE-challenged WT mice after treatment with IL-13Rα2 (data not shown). These results suggested that inhibition of IL-13 suppresses allergic diarrhea and intestinal inflammation through inhibition of mast cell recruitment into the intestine but not through inhibition of immunoglobulin production.

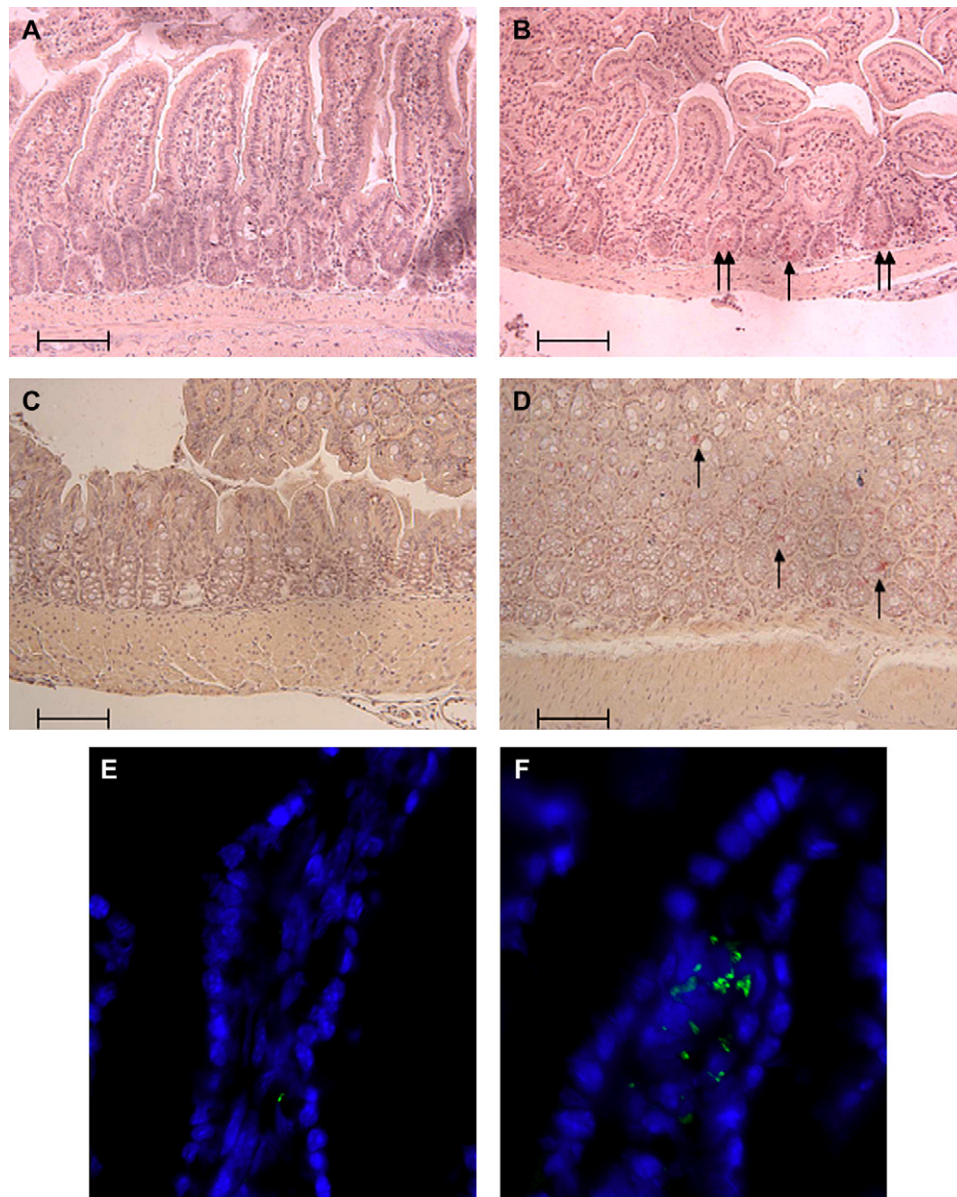


FIG 7. Transferred BMMCs can be detected in intestinal tissue. Sections of jejunum and colon from sensitized and challenged with PE Kit^{W-sh/W-sh} mice with or without BMMC transfer were stained with chloroacetate esterase. Mast cells were detected (arrows) in the mucosa of the jejunum and colon after BMMC transfer (B and D). Without BMMC transfer (A and C) mast cells were not detected in the mucosa of the jejunum and colon. Sections of jejunal tissue from PBS- or PE-sensitized and challenged C57BL/6 mice after transfer of BMMCs from UBI-GFP/BL6 mice were assessed by immunofluorescence. Mast cells from UBI-GFP/BL6 mice were found in the lamina propria of jejunal mucosa of PE-sensitized C57BL/6 mice. Green fluorescent signals identify BMMCs from UBI-GFP/BL6 mice, and blue fluorescent signals identify nuclei stained with 4'-6-diamidino-2-phenylindole dihydrochloride (E and F).

DISCUSSION

In the present study we examined the role of FcεRI in the development of PE-induced intestinal allergy using FcεRI^{-/-} mice. Mice deficient in FcεRI were able to mount an immune response to allergen exposure, as shown by increased serum levels of allergen-specific IgE, IgG1, and IgG2a. Levels of allergen-specific IgG1 and IgG2a in FcεRI^{-/-} mice were similar to those seen in WT mice. Allergen-specific IgE levels were significantly increased but were nonetheless lower in FcεRI^{-/-} mice than in WT mice. In all other aspects of intestinal allergy, the sensitized

and challenged FcεRI^{-/-} mice exhibited lower clinical manifestations, including a delayed onset of diarrhea, lower incidence of diarrhea, and lower symptom scores compared with those seen in similarly sensitized and challenged WT mice. Whereas sensitization or challenge alone did not affect body weight, PE-sensitized and PE-challenged WT mice exhibited significant weight loss associated with water loss caused by severe diarrhea. In contrast, PE-sensitized and PE-challenged FcεRI^{-/-} mice maintained their body weight. Together, these results indicated that FcεRI expression played a critical and essential role in IgE-dependent

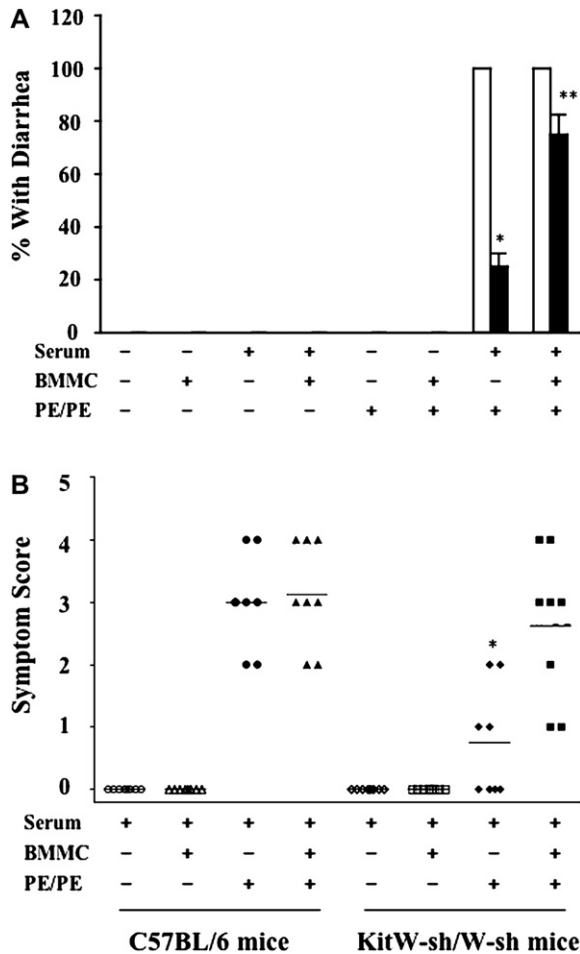


FIG 8. Decreased intestinal allergic responses in Kit^{W-sh/W-sh} mice. **A**, The development of diarrhea was assessed 30 minutes after the last challenge in WT (white bar) and Kit^{W-sh/W-sh} mice (black bar). **B**, symptom scores were assessed 30 minutes after oral challenge. * $P < .001$ between sensitized and challenged with peanut (PE/PE) WT and PE/PE Kit^{W-sh/W-sh} mice after passive sensitization. ** $P < .01$ between PE/PE Kit^{W-sh/W-sh} mice after passive sensitization with and without BMMC transfer from WT mice. Results were obtained from 2 individual experiments with 4 to 5 mice per group.

intestinal allergy. In various acute allergic models, the role of IgE and FcεRI has been somewhat variable. Similar to the results reported here, 2,4,6-tri-nitrobenzenesulfonic acid–induced colitis was more pronounced in FcεRI transgenic mice, whereas FcεRI^{-/-} mice were protected from colitis, indicating that FcεRI can affect the onset of intestinal inflammation.²⁴ In an OVA-induced model FcεRI^{-/-} mice exhibited only a partial reduction in OVA-induced diarrhea.⁴ Recently, Hunt et al²⁵ found that attenuation of IgE affinity for FcεRI could significantly reduce mast cell degranulation and passive cutaneous anaphylaxis after allergen challenge.

In mice FcεRI is expressed only on mast cells and basophils, whereas in human subjects it can also be expressed on eosinophils, monocytes, Langerhans cells, and platelets.²⁶ IgE bound to FcεRI on mast cells and basophils after cross-linking by allergen leads to degranulation and the release of diverse preformed mediators, such as histamine, and newly synthesized mediators, such as IL-13, which directly induce allergic symptoms and promotes the recruitment and activation of leukocytes into local sites of

allergen challenge.²⁷⁻²⁹ Mice deficient in FcεRI accumulated significantly lower numbers of mast cells and eosinophils in the small intestine after sensitization and challenge with peanut compared with their WT littermates. Peanut-sensitized and PE-challenged FcεRI^{-/-} mice demonstrated lower levels of T_H2-type cytokine (*IL-4*, *IL-6*, and *IL-13*) mRNA expression and *IL-17A* mRNA expression. In contrast, T_H1-type cytokine production, such as IFN-γ, was not different among all study groups. These results indicated that PE-induced intestinal allergy is associated with predominantly T_H2-type cytokine expression and that FcεRI contributed to the pathogenesis of intestinal allergy by enhancing T_H2 responses and inflammatory cell accumulation.

It has been reported that levels of the T_H2-type cytokines IL-4 and IL-13 were increased in the gastrointestinal tract of mice with food allergy.^{23,30} Coëffier et al³¹ found that T_H2 cytokine mRNA expression (*IL-4* mRNA) was increased in the intestinal mucosa of patients with food allergy compared with that seen in control subjects, whereas mRNA expression for a T_H1 cytokine (*IFN-γ*) was not altered in patients with food allergy.³¹ Peanut-specific lymphocytes from allergic patients were shown to release high levels of IL-4 and IL-13 and lower levels of IFN-γ. In the subjects who had outgrown their PE allergy, the cytokine profile shifted to a T_H1 response.⁸

IL-17 mRNA and protein expression were increased in the inflamed mucosa from patients with inflammatory bowel disease^{32,33} and from asthmatic patients.³⁴ In the absence of IL-17 receptors, mice were resistant to airway inflammation.³⁵ IL-6 drives T_H17 differentiation and IL-17 production.³⁶⁻³⁸ The mRNA expression levels of IL-6 were significantly increased in the jejunum of the animals with food allergy,³⁹ as confirmed here along with increased expression of *IL-17* mRNA. Decreased IL-6 production by mast cells might indirectly account for decreased *IL-17* expression in FcεRI^{-/-} mice.

IL-13 is a central regulator of many allergic diseases and is primarily responsible for mucus hyperproduction.^{40,41} Compared with WT animals, FcεRI^{-/-} mice did not exhibit increases in IL-13 protein levels in the small intestinal homogenates 24 hours after 7 oral PE challenges. In addition, *IL-13* mRNA expression was significantly decreased in the small intestines from sensitized and challenged FcεRI^{-/-} mice. These results suggested that FcεRI modulated mucosal inflammation through IL-13. To directly evaluate the regulatory role of IL-13 in the development of peanut allergy, we neutralized IL-13 *in vivo* by administering sIL-13Rα2-IgGfC fusion protein, a decoy receptor known to prevent IL-13–mediated effects during the challenge period.^{40,42,43} Inhibition of IL-13 almost completely abolished allergic diarrhea and significantly suppressed intestinal inflammation and goblet cell metaplasia. Plasma histamine, mMCP-1 expression, and T_H2 cytokine mRNA and protein levels were similarly reduced in the treated animals. Thus, IL-13 appeared necessary for the full expression of peanut-induced intestinal allergy in this model. Interestingly, treatment with sIL-13Rα2 did not alter antibody production in the serum, confirming that IL-13 does not regulate serum antibody production.^{42,44,45} Immunoneutralization of IL-13 in this way attenuated airway hyperresponsiveness and goblet cell metaplasia in the airways.^{42,46} Our results indicate that FcεRI is required for the production of IL-13 *in vivo* and that targeting IL-13 would be beneficial in PE-induced food allergy. Because histamine levels in the plasma and protease mMCP-1 levels in jejunal tissue were increased in WT animals after PE sensitization and challenge, this confirmed the activation of mast cells, whereas

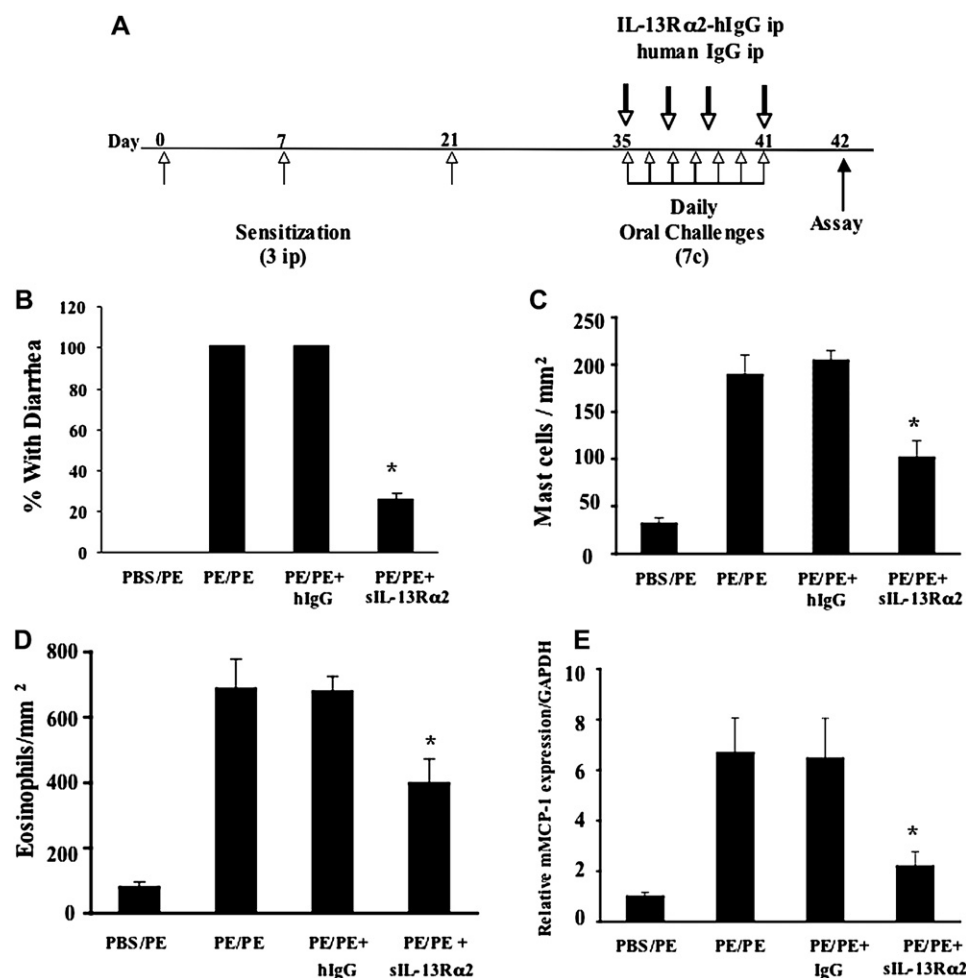


FIG 9. Effect of treatment with IL-13Rα2 protein on PE-induced intestinal allergy. Sensitized and challenged WT mice received IL-13Rα2 every other day during PE challenge. Control mice received human IgG (*hIgG*) at the same dose and time points. **A**, Protocol for treatment with IL-13Rα2 protein. *ip*, Intraperitoneal. **B**, Treatment with IL-13Rα2 prevented the development of peanut-induced diarrhea. **C**, Effect of treatment with IL-13Rα2 on mast cell numbers in the jejunal mucosa. **D**, Effect of treatment with IL-13Rα2 on eosinophil numbers in the jejunal mucosa. **E**, Relative mMCP-1 mRNA expression in jejunum from WT mice that received IL-13Rα2 or human IgG. **P* < .01 compared with hIgG treatment. *PBS/PE*, Nonsensitized but challenged with peanut group; *PE/PE*, sensitized and challenged with PE group.

these levels were significantly lower in sensitized and challenged *FcεRI*^{-/-} mice. Together, these results implicate mucosal mast cell degranulation in the responses and further demonstrate the essential role for *FcεRI* in the pathogenesis of food allergy.

Mast cells are key effector cells and play an important role in the development of allergic responses. To directly determine the role of mast cells in this model, we used mast cell-deficient *Kit*^{W-sh/W-sh} mice. Because *Kit*^{W-sh/W-sh} mice are on the C57B6 background, previous studies and this study have demonstrated that C57B6 mice are resistant to peanut- or OVA-induced intestinal allergy, in large part because of the poor IgE antibody response.⁴ To circumvent this, we passively sensitized *Kit*^{W-sh/W-sh} mice using serum containing peanut-specific IgE. Sensitized and challenged *Kit*^{W-sh/W-sh} mice exhibited lower and weaker intestinal allergic responsiveness compared with that seen in their littermates. Transfer of mast cells from WT mice to these passively and actively sensitized *Kit*^{W-sh/W-sh} mice largely restored their ability to develop diarrhea. These results demonstrated that mast cells

are important in promoting the development of peanut-induced intestinal responses.

In this study we assessed the response to peanut challenge 2 weeks after transfer of mast cells from different strains of mice. Mast cells were found in the jejunum and colon in sensitized and challenged *Kit*^{W-sh/W-sh} mice after transfer of mast cells from WT mice. Green fluorescent mast cells derived from UBI-GFP mice were also detected in the jejunum of sensitized and challenged WT mice. These results confirmed that transferred mast cells do migrate to the gastrointestinal mucosa after intravenous injection, as seen previously with accumulation of transferred BMMCs in the airways of sensitized and airway challenged mice.⁴²

In an attempt to directly link mast cells, *FcεRI*, and IL-13 in this model, we adoptively transferred mast cells from WT, *FcεRI*^{-/-}, *IL-4*^{-/-}, *IL-13*^{-/-}, or *IL-4/IL-13*^{-/-} mice into *FcεRI*^{-/-} recipients. Transferring mast cells from WT mice into *FcεRI*^{-/-} recipients fully restored their ability to develop diarrhea and inflammatory cell infiltration in the intestine. These findings

indicated that triggering mast cells through FcεRI is important in this model and that mast cells alone can reconstitute these allergic responses in the FcεRI^{-/-} recipients. The transfer of mast cells from IL-4^{-/-} mice into FcεRI^{-/-} recipients was able to partially restore their ability to develop diarrhea and inflammatory cell infiltration in the intestine. Interestingly, these same FcεRI^{-/-} recipients did not have any allergic diarrhea after transfer of mast cells from IL-13^{-/-} or IL-4/IL-13^{-/-} mice, suggesting that a primary source of IL-13 in this model was the mast cell. These results differ from findings in a mast cell-dependent model of asthma in which IL-13^{-/-} mast cells were capable of restoring airway hyperresponsiveness in FcεRI^{-/-} mice.⁴² In this model mast cell IL-13 production appeared insufficient, but mast cells were essential for releasing leukotriene B₄, a major chemoattractant for effector memory CD8⁺IL-13⁺ T-cell accumulation in the lung.^{43,47} IL-4 and IL-13 are important T_H2 cytokines and exhibit both common and distinct roles in allergic diseases. In this model IL-13 seems to play a major role in the development of intestinal allergic responses. This finding is somewhat in contrast to the results obtained with OVA-induced intestinal anaphylaxis, in which inhibition of IL-4 prevented intestinal allergic responses, whereas IL-13^{-/-} mice were only partially impaired.⁴⁸

In summary, these studies provide new information regarding the cellular and molecular mechanisms underlying PE-induced intestinal allergy. The initiation of food-induced intestinal inflammation and anaphylaxis appears to be mediated by an IgE–FcεRI–IL-13 pathway involving the activation of mast cells and leading to the release of IL-13. In the absence of IL-13, inflammatory cell accumulation was markedly reduced. These findings identify the potential for cytokine-modulating immunotherapy, targeting IL-13 as a potential treatment for PE-induced intestinal allergy.

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Clinical implications: Peanut-induced intestinal allergy is dependent on a mast cell–IgE–FcεRI–IL-13 pathway. Targeting IL-13 might serve as a potential treatment for peanut allergy.

REFERENCES

- Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit telephone survey: a 5-year follow-up study. *J Allergy Clin Immunol* 2003;112:1203-7.
- Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001;107:191-3.
- Mullins RJ, Dear KB, Tang ML. Characteristics of childhood peanut allergy in the Australian Capital Territory, 1995 to 2007. *J Allergy Clin Immunol* 2009;123:689-93.
- Brandt EB, Strait RT, Hershko D, Wang Q, Muntel EE, Scribner TA, et al. Mast cells are required for experimental oral allergen-induced diarrhea. *J Clin Invest* 2003;112:1666-77.
- Forbes EE, Groschwitz K, Abonia JP, Brandt EB, Cohen E, Blanchard C, et al. IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. *J Exp Med* 2008;205:897-913.
- Eigenmann PA. T lymphocytes in food allergy: overview of an intricate network of circulating and organ-resident cells. *Pediatr Allergy Immunol* 2002;13:162-71.
- Eigenmann PA, Huang SK, Ho DG, Sampson HA. Human T cell clones and cell lines specific to ovomucoid recognize different domains and consistently express IL-5. *Adv Exp Med Biol* 1996;409:217.
- Turcanu V, Maleki SJ, Lack G. Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children, and allergic children who acquired tolerance to peanuts. *J Clin Invest* 2003;111:1065-72.
- Dombrowicz D, Flamand V, Brigman KK, Koller BH, Kinet JP. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor chain gene. *Cell* 1993;75:969-76.
- Dombrowicz D, Flamand V, Miyajima I, Ravetch JV, Galli SJ, Kinet JP. Absence of FcεRI chain results in upregulation of FcγRIII-dependent mast cell degranulation and anaphylaxis: evidence of competition between FcεRI and FcγRIII for limiting amounts of FcεR and chains. *J Clin Invest* 1997;99:915-25.
- McKenzie GJ, Emson CL, Bell SE, Anderson S, Fallon P, Zurawski G. Impaired development of Th2 cells in IL-13-deficient mice. *Immunity* 1998;19:423-32.
- McKenzie GJ, Fallon PG, Emson CL, Grecis RK, McKenzie AN. Simultaneous disruption of interleukin (IL)-4 and IL-13 defines individual roles in T helper cell type 2-mediated responses. *J Exp Med* 1999;189:1565-72.
- Lyon MF, Glenister PH. A new allele sash (Wsh) at the W-locus and a spontaneous recessive lethal in mice. *Genet Res* 1982;39:315-22.
- Schaefer BC, Schaefer ML, Kappler JW, Marrack P, Kedla RM. Observation of antigen-dependent CD8⁺ T-cell/dendritic cell interactions in vivo. *Cell Immunol* 2001;214:110-22.
- Pons L, Ponnappan U, Hall RA, Simpson P, Cockrell G, West CM, et al. Soy immunotherapy for peanut-allergic mice: modulation of the peanut-allergic response. *J Allergy Clin Immunol* 2004;114:915-21.
- Donaldson DD, Whitters MJ, Fitz LJ, Neben TY, Finnerty H, Henderson SL, et al. The murine IL-13 receptor alpha 2: molecular cloning, characterization, and comparison with murine IL-13 receptor alpha 1. *J Immunol* 1998;161:2317-24.
- Ishizuka T, Chayama K, Takeda K, Hamelmann E, Terada N, Keller GM, et al. Mitogen-activated protein kinase activation through Fcε receptor I and stem cell factor receptor is differentially regulated by phosphatidylinositol 3-kinase and calcineurin in mouse bone marrow-derived mast cells. *J Immunol* 1999;162:2087-94.
- Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH, et al. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *J Allergy Clin Immunol* 2000;106:150-8.
- Hernandes L, Pereira LC, Alvares EP. Goblet cell number in the ileum of rats denervated during suckling and weaning. *Biocell* 2003;27:347-51.
- Friend DS, Ghildyal N, Austen KF, Gurish MF, Matsumoto R, Stevens RL. Mast cells that reside at different locations in the jejunum of mice infected with *Trichinella spiralis* exhibit sequential changes in their granule ultrastructure and chymase phenotype. *J Cell Biol* 1996;135:279-90.
- Tomkinson A, Cieslewicz G, Duez C, Larson KA, Lee JJ, Gelfand EW. Temporal association between airway hyperresponsiveness and airway eosinophilia in ovalbumin-sensitized mice. *Am J Respir Crit Care Med* 2001;163:721-30.
- Okamoto M, Takeda K, Joetham A, Ohnishi H, Matsuda H, Swasey CH, et al. Essential role of Notch signaling in effector memory CD8⁺ T cell-mediated airway hyperresponsiveness and inflammation. *J Exp Med* 2008;205:1087-97.
- Kweon MN, Yamamoto M, Kajiki M, Takahashi I, Kiyono H. Systemically derived large intestinal CD4(+) Th2 cells play a central role in STAT6-mediated allergic diarrhea. *J Clin Invest* 2000;106:199-206.
- Dombrowicz D, Nutton S, Desreumaux P, Neut C, Torpier G, Peeters M, et al. Role of the high affinity immunoglobulin E receptor in bacterial translocation and intestinal inflammation. *J Exp Med* 2001;193:25-34.
- Hunt J, Bracher MG, Shi J, Fleury S, Dombrowicz D, Gould HJ, et al. Attenuation of IgE affinity for FcεRI radically reduces the allergic response in vitro and in vivo. *J Biol Chem* 2008;283:29882-7.
- Kinet JP. The high-affinity IgE receptor (FcεRI): from physiology to pathology. *Annu Rev Immunol* 1999;17:931-72.
- Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol* 2008;8:205-17.
- Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;454:445-54.
- Metz M, Grimbaldston MA, Nakae S, Piliponsky AM, Tsai M, Galli SJ. Mast cells in the promotion and limitation of chronic inflammation. *Immunol Rev* 2007;217:304-28.
- Cardoso CR, Teixeira G, Provinciatto PR, Godoi DF, Ferreira BR, Milanezi CM, et al. Modulation of mucosal immunity in a murine model of food-induced intestinal inflammation. *Clin Exp Allergy* 2008;38:338-49.
- Coëffier M, Lorentz A, Manns MP, Bischoff SC. Epsilon germ-line and IL-4 transcripts are expressed in human intestinal mucosa and enhanced in patients with food allergy. *Allergy* 2005;60:822-7.
- Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, et al. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut* 2003;52:65-70.
- Nielsen OH, Kirman I, Rudiger N, Hendel J, Vainer B. Up-regulation of interleukin-12 and -17 in active inflammatory bowel disease. *Scand J Gastroenterol* 2003;38:180-5.
- Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Page N, et al. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J Allergy Clin Immunol* 2001;108:430-8.

35. Schnyder-Candrian S, Togbe D, Couillin I, Mercier I, Brombacher F, Quesniaux V, et al. Interleukin-17 is a negative regulator of established allergic asthma. *J Exp Med* 2006;203:2715-25.
36. Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Zaba LC, Cardinale I, Nogales KE, et al. Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. *J Immunol* 2008;181:7420-7.
37. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, et al. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 2007;8:967-74.
38. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T. TGF- β and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T-17 cell-mediated pathology. *Nat Immunol* 2007;8:1390-7.
39. Valeur J, Lappalainen J, Rita H, Lin AH, Kovanen PT, Berstad A, et al. Food allergy alters jejunal circular muscle contractility and induces local inflammatory cytokine expression in a mouse model. *BMC Gastroenterol* 2009;9:33.
40. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261-3.
41. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-61.
42. Taube C, Wei X, Swasey CH, Joetham A, Zarini S, Lively T, et al. Mast Cells, Fc ϵ RI, and IL-13 are required for development of airway hyperresponsiveness after aerosolized allergen exposure in the absence of adjuvant. *J Immunol* 2004;172:6398-406.
43. Taube C, Miyahara N, Ott V, Swanson B, Takeda K, Loader J, et al. The leukotriene B4 receptor (BLT1) is required for effector CD8+ T cell-mediated, mast cell-dependent airway hyperresponsiveness. *J Immunol* 2006;176:3157-64.
44. Walter DM, McIntire JJ, Berry G, McKenzie AN, Donaldson DD, DeKruyff RH, et al. Critical role for IL-13 in the development of allergen-induced airway hyper-reactivity. *J Immunol* 2001;167:4668-75.
45. Taube C, Duez C, Cui ZH, Takeda K, Rha YH, Park JW, et al. The role of IL-13 in established allergic airway disease. *J Immunol* 2002;169:6482-9.
46. Blease K, Jakubzick C, Westwick J, Lukacs N, Kunkel SL, Hogaboam CM. Therapeutic effect of IL-13 immunoneutralization during chronic experimental fungal asthma. *J Immunol* 2001;166:5219-24.
47. Miyahara N, Ohnishi H, Miyahara S, Takeda K, Matsubara S, Matsuda H, et al. Leukotriene B4 release from mast cells in IgE-mediated airway hyperresponsiveness and inflammation. *Am J Respir Cell Mol Biol* 2009;40:672-82.
48. Brandt EB, Munitz A, Orekov T, Mingler MK, McBride M, Finkelman FD, et al. Targeting IL-4/IL-13 signaling to alleviate oral allergen-induced diarrhea. *J Allergy Clin Immunol* 2009;123:53-8.

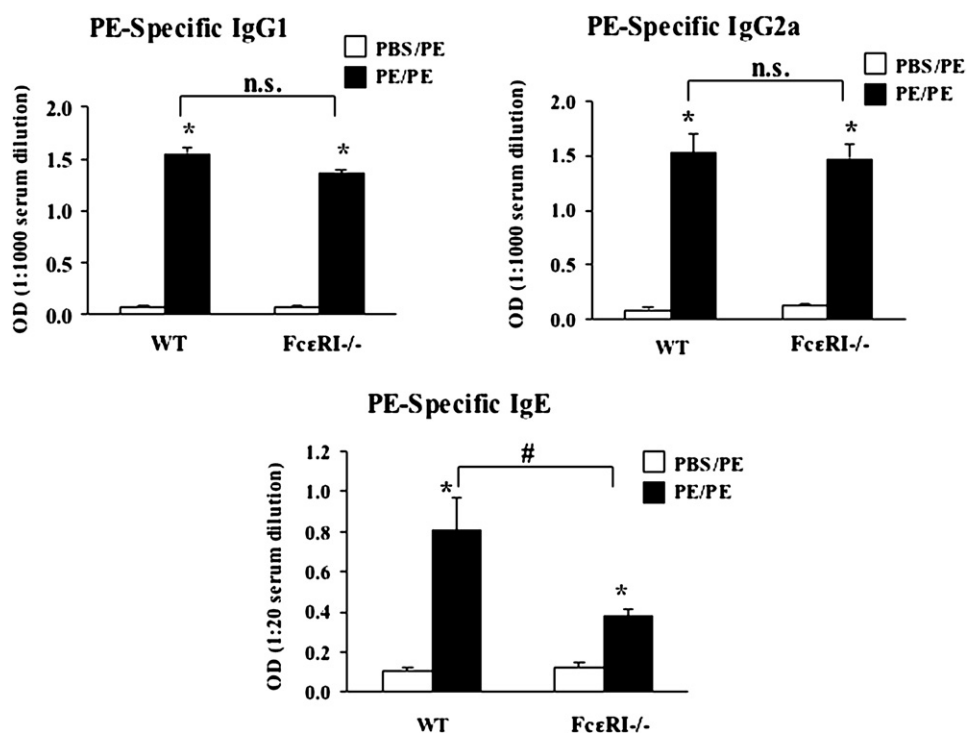


FIG E1. Levels of serum PE antibodies. Serum levels of peanut-specific IgE, IgG1, and IgG2a were assessed by means of ELISA 24 hours after the last challenge and expressed as OD of diluted serum, as described in the Methods section. Results were obtained from 3 individual experiments with 4 to 5 mice per group. # $P < .05$ between the sensitized and challenged with peanut (PE/PE) WT and PE/PE FcεRI^{-/-} groups. * $P < .001$ compared with PBS-sensitized control mice. n.s., Not significant; PBS/PE, nonsensitized but challenged with PE group.

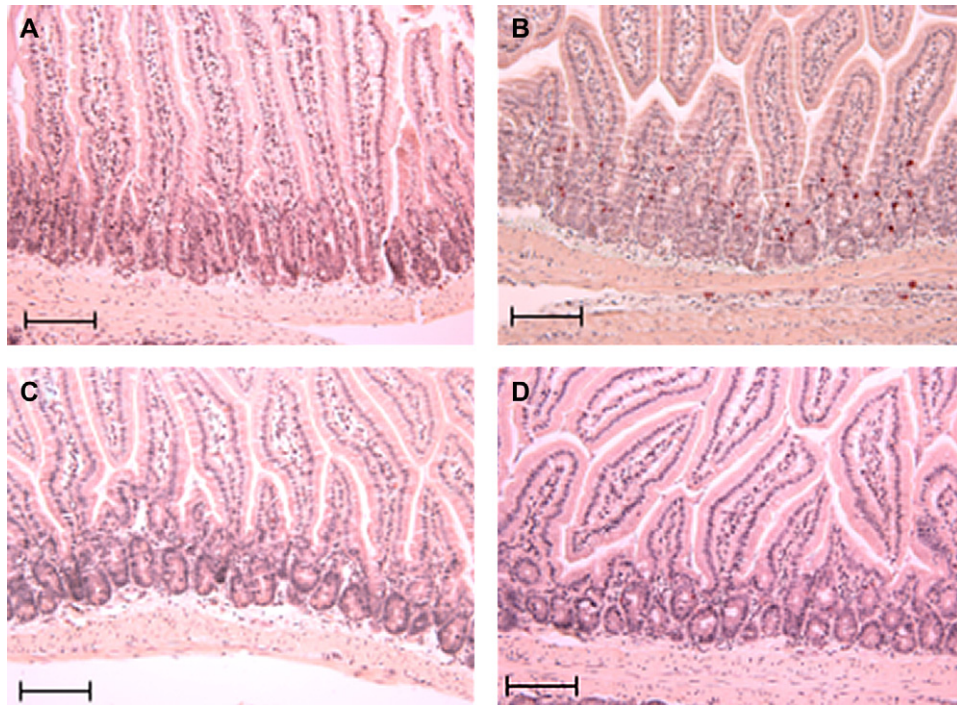


FIG E2. Decreased mast cell infiltration in the intestinal walls of $Fc\epsilon RI^{-/-}$ mice. Intestinal mucosal mast cells were quantified in jejunum using chloroacetate esterase staining. Representative sections of nonsensitized but challenged with peanut (PBS/PE) WT mice (**A**), sensitized and challenged with peanut (PE/PE) WT mice (**B**), PBS/PE $Fc\epsilon RI^{-/-}$ mice (**C**), and PE/PE $Fc\epsilon RI^{-/-}$ mice (**D**) are shown (magnification $\times 200$). Bar = 100 μm .

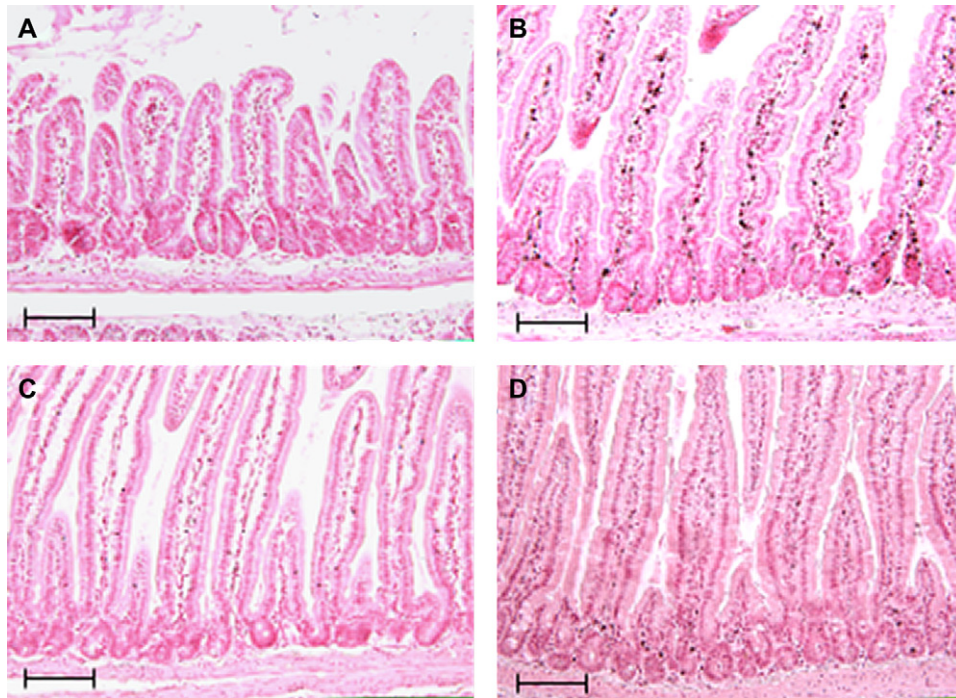


FIG E3. Decreased eosinophil accumulation in the intestines of $Fc\epsilon RI^{-/-}$ mice. Eosinophils were identified and numbers were quantitated in jejunum by immunohistochemistry with anti-major basic protein antibody. Representative sections of nonsensitized but challenged with peanut (PBS/PE) WT mice (**A**), sensitized and challenged with peanut (PE/PE) WT mice (**B**), PBS/PE $Fc\epsilon RI^{-/-}$ mice (**C**), and PE/PE $Fc\epsilon RI^{-/-}$ mice (**D**) are shown (magnification $\times 200$). *Bar* = 100 μm .

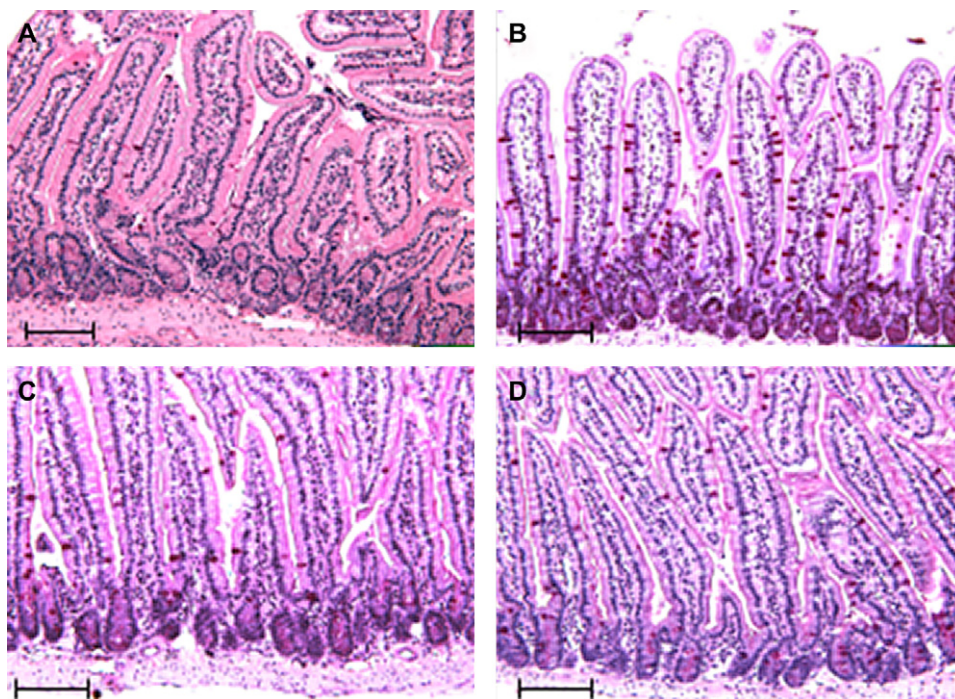


FIG E4. Decreased numbers of goblet cells in intestinal epithelium of sensitized and challenged FcεRI^{-/-} mice. Representative sections of nonsensitized but challenged with PE (PBS/PE) WT mice (**A**), sensitized and challenged with PE (PE/PE) WT mice (**B**), PBS/PE FcεRI^{-/-} mice (**C**), and PE/PE FcεRI^{-/-} mice (**D**) are shown. Goblet cells were identified by periodic acid-Schiff staining 24 hours after the last challenge. (Magnification ×200). Bar = 100 μm.

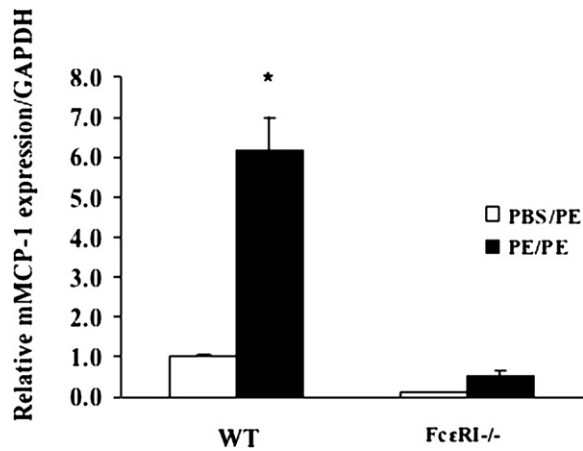


FIG E5. mMCP1 mRNA expression levels in jejunum of sensitized and challenged mice. Relative expression levels of mMCP1 normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) in jejunum were determined by means of quantitative RT-PCR. Results were representative of 3 independent experiments with 4 mice per group. * $P < .001$ compared with all other groups. *PBS/PE*, Nonsensitized but challenged with peanut group; *PE/PE*, sensitized and challenged with PE group.

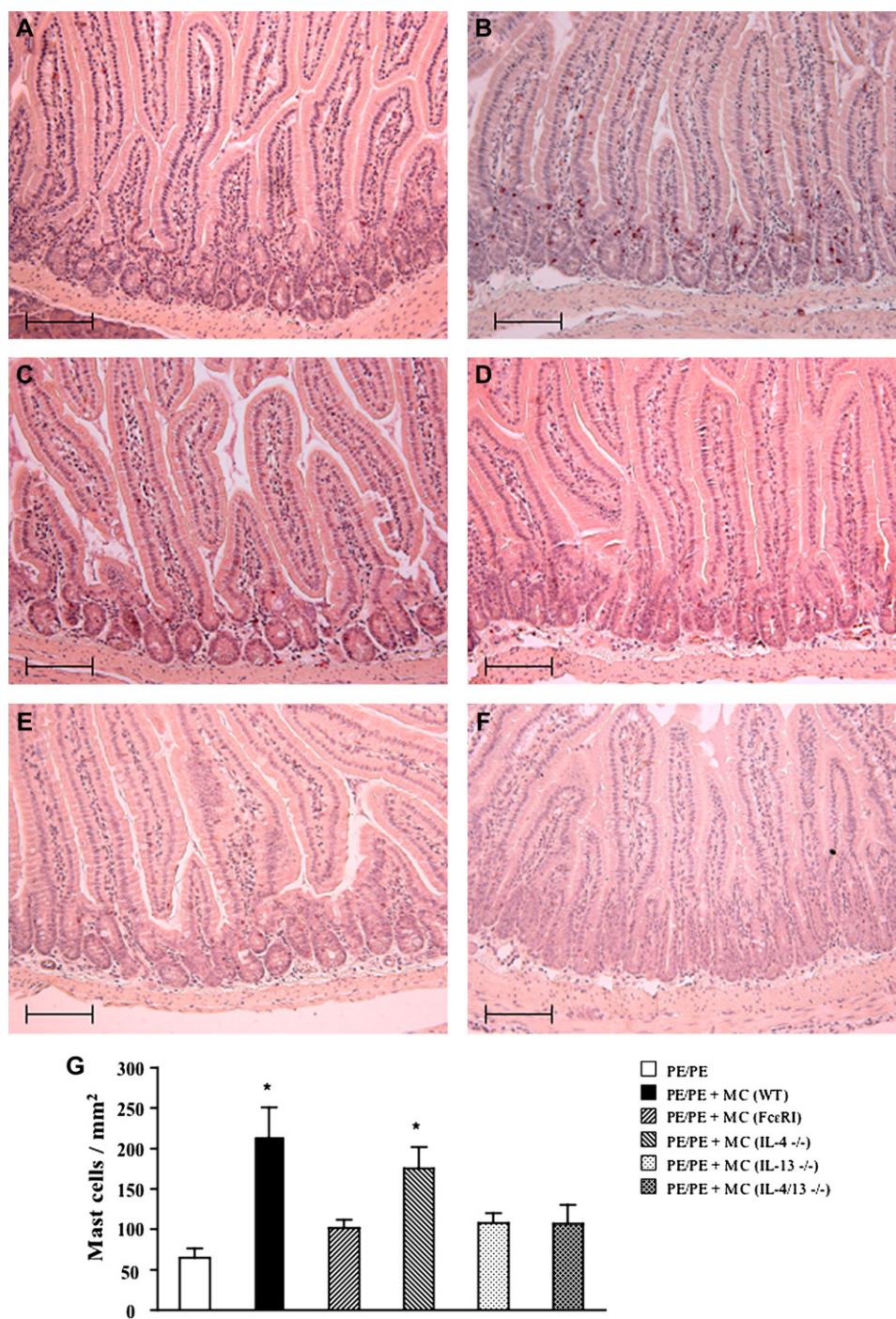


FIG E6. Transfer of BMMCs from WT mice into FcεRI^{-/-} mice increases mast cell accumulation in the jejunal mucosa. Representative jejunal sections of sensitized and challenged with PE (PE/PE) FcεRI^{-/-} mice (A), PE/PE FcεRI^{-/-} recipients of BMMCs from WT mice (B), PE/PE FcεRI^{-/-} recipients of BMMCs from FcεRI^{-/-} mice (C), and PE/PE FcεRI^{-/-} recipients of BMMCs from IL-13^{-/-} mice (D), and representative jejunal sections of PE/PE FcεRI^{-/-} recipients of BMMCs from IL-4^{-/-} mice (E), Representative jejunal sections of PE/PE FcεRI^{-/-} recipients of BMMCs from IL-4/IL-13^{-/-} mice F. Quantitation of mucosal mast cell (MC) numbers in the jejunum G, **P* < .05, PE/PE FcεRI^{-/-} recipients of BMMCs from WT mice and from IL-4^{-/-} mice compared with all other groups (magnification ×200). Bar = 100 μm.

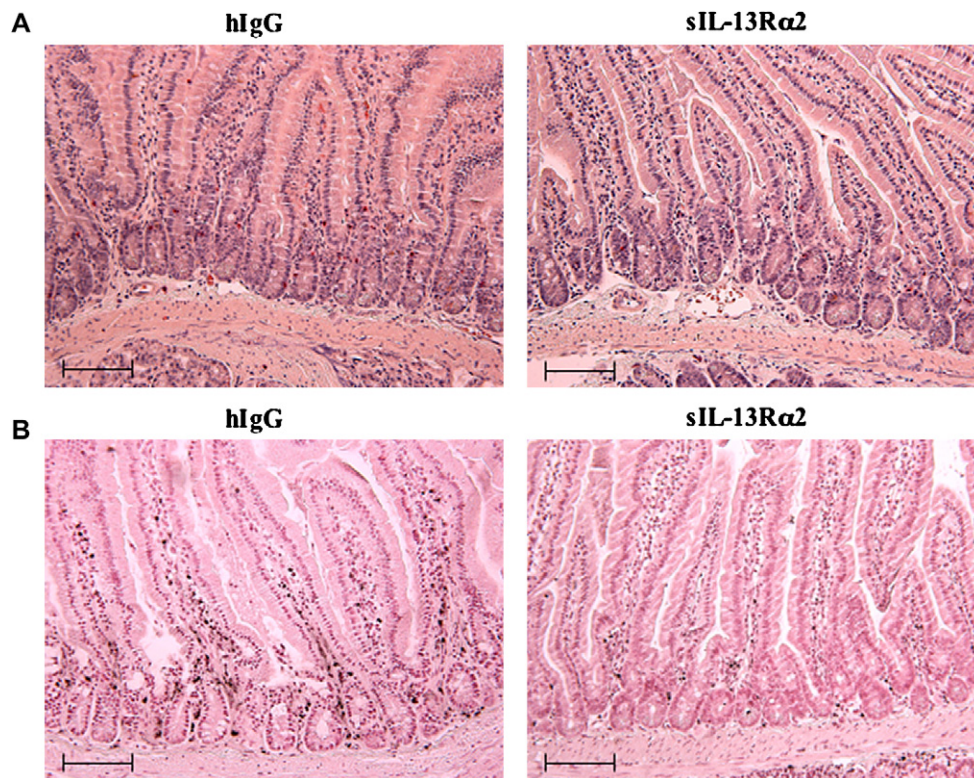


FIG E7. Effect of treatment with IL-13R α 2 protein on PE-induced intestinal allergy. Sensitized and challenged WT mice received IL-13R α 2 every other day during PE challenge. Control mice received human IgG at the same dose and time points. **A**, Chloroacetate esterase staining of representative jejunal sections in IL-13R α 2- or human IgG-treated mice. **B**, Major basic protein staining of representative jejunal sections of IL-13R α 2- or human IgG-treated mice. (Magnification $\times 200$). Bar = 100 μ m

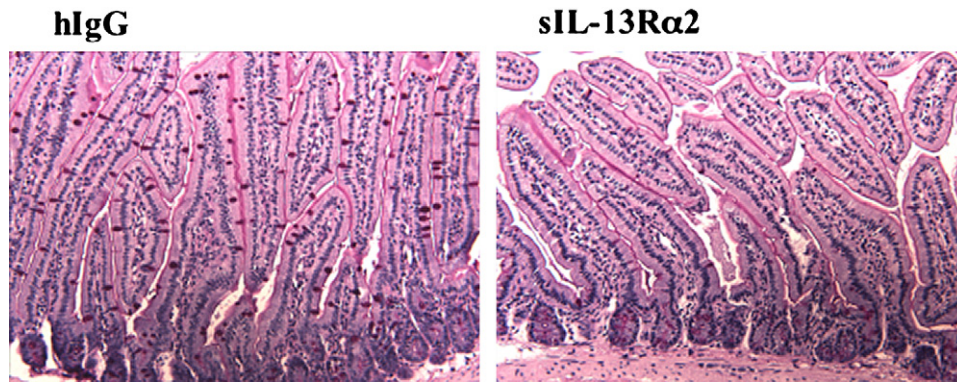


FIG E8. Effect of IL-13R α 2 on goblet cell numbers in jejunum. Representative sections of jejunum of IL-13R α 2- and human IgG-treated mice after periodic acid-Schiff staining (magnification $\times 200$).

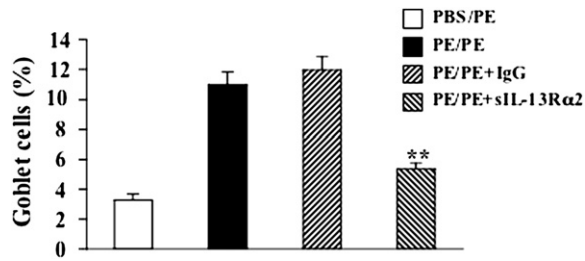


FIG E9. Quantitation of goblet cells in jejunum after treatment with IL-13Rα2. ** $P < .01$ comparing IL-13Rα2-treated mice with control animals. *PBS/PE*, Nonsensitized but challenged with PE group; *PE/PE*, sensitized and challenged with PE group.

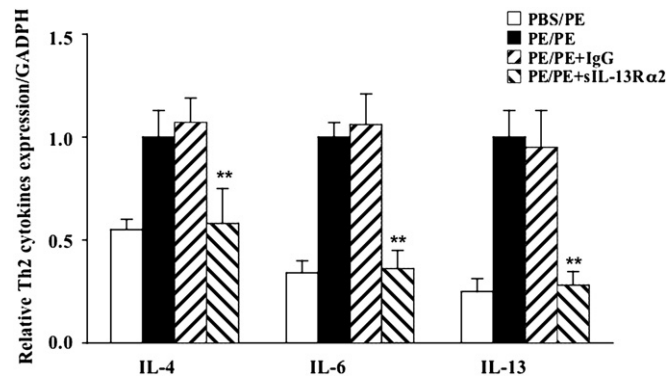


FIG E10. Effect of IL-13R α 2 treatment on cytokine expression in jejunal tissue. Relative levels of T_H2 cytokine mRNA expression in jejunum from WT mice that received IL-13R α 2 or human IgG are shown. * $P < .01$ compared with human IgG treatment. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase; *PBS/PE*, nonsensitized but challenged with peanut group; *PE/PE*, sensitized and challenged with PE group.

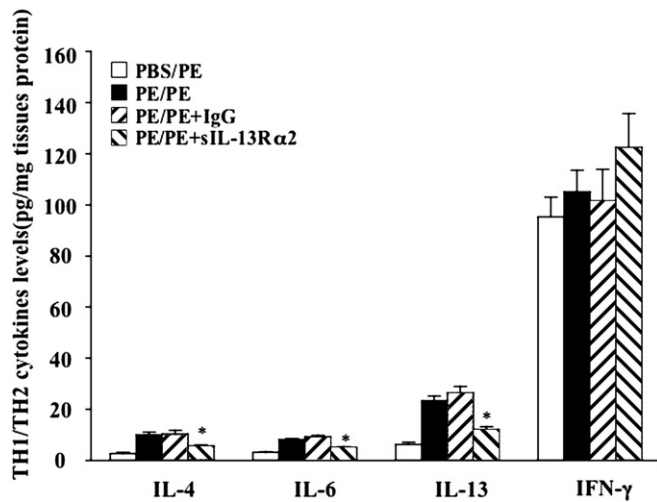


FIG E11. Effect of IL-13R α 2 treatment on cytokine protein levels in jejunal tissue. T_H1/T_H2 cytokines protein levels in intestinal homogenates from WT mice that received IL-13R α 2 or human IgG are shown. * $P < .05$ compared with human IgG treatment. *PBS/PE*, Nonsensitized but challenged with peanut group; *PE/PE*, sensitized and challenged with PE group.

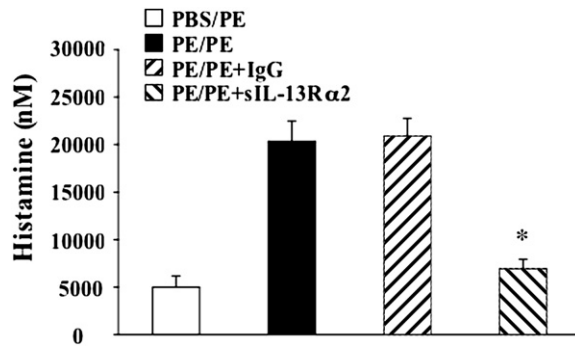


FIG E12. Effect of IL-13R α 2 treatment on plasma histamine levels. * $P < .05$ compared with human IgG treatment. *PBS/PE*, Nonsensitized but challenged with peanut group; *PE/PE*, sensitized and challenged with PE group.