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Peanut-Induced Intestinal Allergy is Mediated Through a Mast Cell-IgE-FcεRI-IL-13 Pathway

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Abstract

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—To determine the contributions of FcεRI, IL-13, and mast cells to the development of intestinal mucosal responses in a mouse model of peanut-induced intestinal allergy.

METHODS—Sensitized wild-type (WT), FcεRI-deficient (FcεRI^{−/−}), and mast cell-deficient (KitW-sh/W-sh) mice received peanut orally every day for 1 week. Bone marrow-derived mast cells (BMMC) from WT, FcεRI^{−/−}, IL-4^{−/−}, IL-13^{−/−}, and IL-4/IL-13^{−/−} mice were differentiated and transferred into WT, FcεRI^{−/−}, and KitW-sh/W-sh recipients. BMMC from WT and UBI-GFP/BL6 mice were differentiated and transferred into WT and KitW-sh/W-sh mice. Blockade of IL-13 was achieved using IL-13Ra2-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 BMMC to FcεRI^{−/−} recipients restored their ability to develop intestinal allergic responses compared to transfer of FcεRI^{−/−}, IL-13^{−/−}, or IL-4/IL-13^{−/−} BMMC. FcεRI^{−/−} mice exhibited lower IL-13 levels and treatment of WT mice with IL-13Ra2 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 may be a potential treatment for IgE-mediated peanut allergic responses in the intestine.

Keywords

Peanut; intestinal allergy; mast cell; IgE; FcεRI; IL-13

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

INTRODUCTION

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 Europe and the US (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 Th2-cytokines (4,5).

Increased numbers of activated T cells have been correlated with elevated levels of Th2 cytokines as well as the degree of gastrointestinal (GI) inflammation and dysfunction in patients with food allergy (6,7). In vitro, allergen-stimulated T cells and T cell clones generated from peanut allergic patients produce elevated levels of Th2-cytokines (IL-4, IL-5, and IL-13) (8). These cytokines activate immunological pathways associated with the initiation of allergic responses, including Th2 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 Th1/Th2 cytokine levels.

MATERIALS AND 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, MA), 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, MA). Mast cell-deficient (KitW-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, CO) (14). Mice were maintained on a peanut- and soy-free diet under specific pathogen-free conditions. Experiments were performed on age- and gender-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) using a method previously described (15). Endotoxin concentrations in the PE solutions were assessed by Chromogenic LAL endotoxin assay kit (GeneScript, Piscataway, NJ). The limit of detection for endotoxin was 0.005EU/ml. Endotoxin depletion of PE solutions was performed using endotoxin removing gel (Thermo Scientific) according to the manufacturer's protocol. The final preparations of PE were depleted of endotoxin to levels <0.1 EU/ml.

Sensitization and intragastric challenge

Mice were sensitized 3 times with 500 µg PE together with 2.0 mg of alum (Pierce, Rockford, IL) by i.p. 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 PBS) by gavage using a 22-gauge feeding needle (Fisher Scientific, Pittsburgh, PA) every day for 1 week (Fig. 1A). In some experiments, C57BL/6 mice and KitW-sh/W-sh mice were passively sensitized, receiving 200 µl serum (i.p.) derived from peanut sensitized and challenged BALB/c mice one day

before oral challenge and one hour before the 4th 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 sacrificed. Serum and jejunal tissue were collected for further analyses. Controls were sham-sensitized but challenged with peanut.

Administration of the soluble IL- 13 α 2-IgGFc fusion protein

Murine soluble IL- 13 α 2-hIgG fusion protein (sIL- 13 α 2-Fc) was prepared as previously described (16). IL-13 α 2-hIgG fusion protein was administered by i.p. injection (300 μg /mouse) 1 hour prior to PE challenges on days 1, 3, 5, and 7. Controls were administered the same amount of human IgG at the identical time points.

Transfer of bone marrow-derived mast cells (BMMC)

Mast cells were derived from bone marrow as previously described (17). After 4–5 weeks of culture, >98% expressed c-kit and Fc ϵ RI α on their surface. BMMC (5×10^6) were injected into WT, Fc ϵ RI $^{-/-}$, and KitW-sh/W-sh mice by i.v. 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. 5- μm thick tissue sections were cut and stained with periodic acid-Schiff (PAS) 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 in each slide.

Mucosal mast cells were identified using chloroacetate esterase staining as previously described (20) and lightly counterstained with hematoxylin. Cells containing eosinophilic major basic protein (MBP) were identified by immunohistochemical staining as previously described using rabbit anti-mouse MBP antibody (kindly provided by Dr. J. J. Lee, Mayo Clinic, Scottsdale, AZ) (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 using an Olympus microscope linked to the NIH Image Analysis Program (National Institutes of Health, Bethesda, MD).

Immunofluorescence staining for the detection of mast cells and basophils

The jejunums of peanut sensitized and challenged mice treated with or without sIL-13 α 2 or control IgG, and the jejunums of PBS or peanut sensitized and challenged mice receiving BMMCs from UBI-GFP/BL6 mice were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek, Torrance, CA). Sections of tissue blocks (8- μm 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 RI α and rat anti-mouse c-Kit Ab (eBioscience) as primary Abs and rhodamine red-x- goat anti-rat IgG and fluorescein (FITC) goat anti-armenian hamster IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) as secondary antibody. In sections prepared after transfer of BMMCs derived from UBI-GPF/BL6, only nuclei were stained using Vectashield mounting medium

with DAPI (Vector Laboratories, Burlingame, CA). The immunofluorescent images were captured with a Leica microscope camera system and Slidebook 5 digital microscopy software (Intelligent Imaging Innovations, Denver, CO). Quantification of stained mast cells (FcεRIα+ and c-Kit+) and basophils (FcεRIα+ but c-Kit-) per square millimeter of lamina propria was performed using an Olympus microscope linked to the NIH Image Analysis Program (National Institute of Mental Health, Bethesda, MD, and available at <http://rsb.info.nih.gov/ij/index.html>).

Assessment of Th1/Th2 cytokine levels in intestine homogenates

Jejunum was weighed and completely homogenized using a tissue homogenizer (Kinematica AG, Lucerne, Switzerland) in dilution buffer (PBS, 0.1% Triton X) (Sigma-Aldrich, St. Louis, MO) containing proteinase inhibitor (Roche Diagnostics, Indianapolis, IN) 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 the BCA method and levels of IL-4, IL-6, IL-13, and IFN-γ were assessed by 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 Ab

Serum peanut-specific IgE, IgG1, and IgG2a levels were measured by 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 by enzyme immunoassay kit (Beckman Coulter Company) 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) and was transcribed into cDNA (cDNA synthesis kit, Bio-Rad Laboratories, Hercules, CA). All primers and probes for murine IL-4, IL-6, IL-13, IL-17A, IFN-γ, mast cell protease (mMCP-1), and GAPDH were obtained from Applied Biosystems (Foster City, CA), and real-time PCR utilized 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 utilized the Tukey Kramer highest significance difference test. P values for significance were set at 0.05. All results were expressed as the mean±SEM.

RESULTS

Decreased intestinal responses in FcεRI-/- mice

Oral administration of PE to PE-sensitized WT mice triggered the onset of acute diarrhea on the second day of challenge and 100% of the mice had diarrhea by the seventh day of challenge. In contrast, sensitized and challenged FcεRI-/- mice showed a delay in onset of diarrhea and an overall lower incidence of diarrhea (Fig. 1B). FcεRI-/- mice exhibited lower symptom scores compared with similarly sensitized and challenged WT mice (Fig. 1C). 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 challenged WT mice showed significant weight loss ($-4.29 \pm 1.34\%$), whereas similarly sensitized and challenged FcεRI^{-/-} mice maintained their body weight ($0.26 \pm 1.34\%$) (Fig. 1D).

Increased peanut-specific IgE, IgG1, and IgG2a in FcεRI^{-/-} mice following sensitization and challenge with peanut

Levels of serum peanut-specific IgE were significantly increased in peanut sensitized and challenged FcεRI^{-/-} mice, but the levels were lower than those in peanut sensitized and challenged WT mice. Levels of PE-specific IgG1 and IgG2a, were similarly increased in FcεRI^{-/-} and WT mice (Fig. E1 in the Online Repository).

Levels of plasma histamine in FcεRI^{-/-} mice

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

Intestinal inflammation following sensitization and challenge with peanut

It has been reported that leukocytes are recruited to the gastrointestinal tract in food-induced allergic diarrhea (23). We found that mast cells and eosinophils only infiltrated the small but not the large intestine. The number of mast cells and eosinophils were significantly increased in the jejunum of peanut sensitized and challenged WT mice. Peanut sensitized and challenged FcεRI^{-/-} mice also exhibited increased mast cell and eosinophil accumulation in the mucosa of the jejunum, but the levels were significantly lower (Figs. 3A, 3B and Figs. E2 and E3 in the Online Repository).

Goblet cell numbers in the intestinal wall of FcεRI^{-/-} mice

Increased mucus production is characteristic of conditions in which there is inflammation or irritation of the epithelial lining. Peanut sensitized and challenged WT mice demonstrated a large number of goblet cells ($9.97 \pm 0.48\%$ goblet cells) in the intestinal epithelium. Peanut sensitized and challenged FcεRI^{-/-} mice showed a lower number of goblet cells ($6.14 \pm 0.51\%$) in the intestinal epithelium compared with WT mice (Fig. 3C and Fig. E4 in the Online Repository).

IL-13 protein levels in intestinal homogenates are significantly decreased in FcεRI^{-/-} 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 peanut sensitized and challenged WT mice but not in peanut sensitized and challenged FcεRI^{-/-} mice (Fig. 4).

Levels of Th1/Th2 cytokines and IL-17 mRNA expression in jejunum

After 7 days of PE challenges, 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 intestine of peanut sensitized and challenged FcεRI^{-/-} mice, which were similar to those in PBS sensitized and peanut challenged WT mice. IFN-γ mRNA levels were not altered in peanut sensitized and challenged WT or FcεRI^{-/-} mice (Fig. 5).

MMCP-1 mRNA expression in jejunum

Activated mast cells release mediators such as histamine and mMCP which may play a critical role in the induction of allergic responses (5). We measured mMCP-1 levels in intestinal homogenates 24 hours after the last challenge by quantitative RT-PCR. Indeed, peanut sensitized and challenged WT mice exhibited significantly increased levels (about 6-fold) of mMCP-1 mRNA compared with levels in PBS sensitized and peanut challenged WT controls. Levels in peanut sensitized and challenged FcεRI^{-/-} mice were not increased (Fig. E5 in the Online Repository).

Transfer of BMMC 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 by c-kit and FcεRI surface staining. BMMC were injected i.v. into recipient mice 1 week and 1 day before starting the oral challenges. Following transfer of WT and BMMC from IL-4^{-/-} mice, the numbers of mast cells in the intestinal mucosa of FcεRI^{-/-} recipients were significantly increased (Fig. E6 in the Online Repository). Recipients of FcεRI^{-/-} BMMC, or BMMCs from IL-13^{-/-} or IL-4/IL-13^{-/-} mice accumulated fewer mast cells in the intestinal mucosa.

Transfer of BMMC from WT to peanut sensitized and challenged FcεRI^{-/-} mice restored the ability of 100% of the mice to develop diarrhea, as seen in sensitized and challenged WT recipients. Transfer of BMMC from IL-4^{-/-} mice to peanut sensitized and 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 peanut-induced diarrhea in sensitized and challenged FcεRI^{-/-} recipients (Fig. 6).

Transferred BMMCs can be detected in jejunal tissue of both KitW-sh/W-sh mice and C57BL/6 mice

To assess whether transferred BMMCs migrate and to the jejunum, BMMCs from C57BL/6 mice were transferred to KitW-sh/W-sh recipients 1 week and 1 day before starting the intestinal challenge with peanut. After seven days of peanut challenge, transferred BMMCs were found in the mucosa of the jejunum and colon in KitW-sh/W-sh mice by CAE staining. In contrast, without BMMC transfer we did not detect mast cells in the jejunum and colon in KitW-sh/W-sh mice (Figs. 8A–D). To further confirm that transferred BMMCs circulate and become resident in jejunal tissue after sensitization and challenge, green-fluorescent BMMCs from UBI-GFP/BL6 mice were transferred into C57BL/6 recipients 1 week and 1 day before initiating the intestinal challenges. After the 7-day challenge with peanut, transferred green-fluorescent mast cells could be detected in the jejunal tissue of peanut sensitized and challenged mice 24 hrs after the last challenge; only a rare GFP⁺ mast cell could be detected in the non-sensitized but challenged recipients (Figs. 8E, F).

KitW-sh/W-sh mice develop significantly lower and less severe allergic diarrhea after systemic and passive sensitization and challenge

To determine whether the deficiency of mast cell affects the development of peanut-induced allergic diarrhea, KitW-sh/W-sh and C57BL/6 control mice were systemically sensitized with or without peanut and challenged with peanut. Since C57BL/6 mice fail to develop peanut or OVA-induced intestinal allergy to the extent of BALB/c mice due to their failure

to develop IgE antibody responses, KitW-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 developed diarrhea by the seventh day of challenge. In contrast, only 25% of sensitized and challenged KitW-sh/W-sh mice developed diarrhea. After transfer of BMMCs from C57BL/6 mice, KitW-sh/W-sh mice demonstrated an increase in the development of diarrhea (75%). KitW-sh/W-sh mice exhibited lower symptom scores compared with similarly sensitized and challenged C57BL/6 mice (Fig. 9).

Effect of treatment with IL- 13R α 2 protein on peanut-induced intestinal allergy

Based on the data showing increased levels of IL-13 mRNA and protein after peanut 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 impact of neutralizing IL-13. Peanut sensitized mice were given 300 μ g IL- 13R α 2 protein i.p. every other day during the 7 days of PE challenge (Fig. 7A). Peanut sensitized and challenged WT mice treated with IL- 13R α 2 failed to develop diarrhea (Fig. 7B). The numbers of mast cells in the intestinal mucosa were significantly decreased after treatment with IL- 13R α 2 (Figs. 7C and E7 in the Online Repository) as were the number of eosinophils (Figs. 7D and E7 in the Online Repository). As well, the number of goblet cells in the jejunal epithelium (Figs. E8 and E9 in the Online Repository). In none of the tissue sections were basophils (Fc ϵ RI+c-kit-) identified in the intestinal mucosa by immunofluorescence staining (data not shown). In parallel, IL-4, IL-6, IL-13, and mMCP-1 mRNA expression in the intestine were decreased in mice treated with IL-13R α 2 compared with mice treated with control antibody (Figs. 7E and E10 in the Online Repository). IL-4, IL-6, and IL-13 protein levels in intestinal homogenates were also significantly decreased in mice after treatment with sIL- 13R α 2 while IFN- γ protein levels in intestinal homogenates were not altered (Fig. E11 in the Online Repository). Levels of plasma histamine were also significantly decreased in mice treated with sIL- 13R α 2 compared with mice treated with control antibody (Fig. E12 in the Online Repository). Serum levels of peanut-specific IgE, IgG1, and IgG2a were not altered in peanut sensitized and challenged WT mice after treatment with IL- 13R α 2 (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.

DISCUSSION

In the present study, we examined the role of Fc ϵ RI in the development of peanut-induced intestinal allergy, using Fc ϵ RI $^{-/-}$ mice. Mice deficient in Fc ϵ RI 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 Fc ϵ RI $^{-/-}$ mice were similar to those in WT mice. Allergen-specific IgE levels were significantly increased but were nonetheless lower in Fc ϵ RI $^{-/-}$ mice than in WT mice. In all other aspects of intestinal allergy, the sensitized and challenged Fc ϵ RI $^{-/-}$ mice exhibited lower clinical manifestations, including a delayed onset of diarrhea, lower incidence of diarrhea, and lower symptom scores compared with similarly sensitized and challenged WT mice. Whereas sensitization or challenge alone did not affect body weight, peanut sensitized and challenged WT mice exhibited significant weight loss, associated with water loss due to severe diarrhea. In contrast, peanut sensitized and challenged Fc ϵ RI $^{-/-}$ mice maintained their body weight. Together, these results indicated that Fc ϵ RI expression played a critical and essential role in IgE-dependent intestinal allergy. In various acute allergic models, the role of IgE and Fc ϵ RI has been somewhat variable. Similar to the results reported here, 2,4,6-tri-nitrobenzenesulfonic acid (TNBS)-induced colitis was more pronounced in Fc ϵ RI transgenic mice, whereas Fc ϵ RI $^{-/-}$ mice were protected from developing 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 al. (25) found that attenuation of IgE affinity for FcεRI could significantly reduce mast cell degranulation and passive cutaneous anaphylaxis following allergen challenge.

In mice, FcεRI is expressed only on mast cells and basophils, whereas in humans, it may also be expressed on eosinophils, monocytes, Langerhans cells, and platelets (26). IgE bound to FcεRI on mast cells and basophils following crosslinking 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 induces 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 to their WT littermates. Peanut sensitized and challenged FcεRI^{-/-} mice demonstrated lower levels of Th2-type cytokine (IL-4, IL-6, and IL-13) mRNA expression and IL-17A mRNA expression. In contrast, Th1-type cytokine production such as IFN-γ was not different among all study groups. These results indicated that peanut-induced intestinal allergy is associated with predominantly Th2-type cytokine expression and FcεRI contributed to the pathogenesis of intestinal allergy by enhancing Th2 responses and inflammatory cell accumulation.

It has been reported that the Th2-type cytokines IL-4 and IL-13 were increased in the GI tract of food-allergic mice (23,30). Coëffier et al. found that Th2 cytokine mRNA expression (IL-4 mRNA) was elevated in the intestinal mucosa of patients with food allergy compared with controls whereas mRNA expression for a Th1 cytokine (IFN-γ) was not altered in food-allergic patients (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 individuals who had outgrown their peanut allergy, the cytokine profile shifted to a Th1 response (8).

IL-17 mRNA and protein expression were increased in the inflamed mucosa from patients with inflammatory bowel disease (IBD) (32,33) and from asthma patients (34). In the absence of IL-17 receptors, mice were resistant to developing airway inflammation (35). IL-6 drives Th17 differentiation and IL-17 production (36–38). The mRNA expression levels of IL-6 were significantly increased in the jejunum of the food-allergic animals (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 to WT animals, FcεRI^{-/-} mice failed to exhibit increases in IL-13 protein levels in the small intestinal homogenates, 24 hours after 7 oral peanut challenges. In addition, IL-13 mRNA expression was significantly decreased in the small intestine 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 soluble IL-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 Th2 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 AHR 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 targeting IL-13 would be beneficial in peanut-induced food allergy. As histamine levels in the plasma and protease mMCP-1 in jejunal tissue were elevated in WT animals after peanut sensitization and challenge, this confirmed the activation of mast cells whereas 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 KitW-sh/W-sh mice. Since KitW-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 KitW-sh/W-sh mice using serum containing peanut-specific IgE. Sensitization and challenged KitW-sh/W-sh mice exhibited lower and weaker intestinal allergic responsiveness compared to their littermates. Transfer of mast cells from WT mice to these passively and actively sensitized KitW-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 KitW-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 BMDCs 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 via 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 failed to develop 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, where IL-13^{-/-} mast cells were capable of restoring AHR in FcεRI^{-/-} mice (42). In this model, mast cell IL-13 production appeared insufficient but mast cells were essential for releasing leukotriene B4 (LTB4), a major chemoattractant for effector memory CD8+IL-13+ T cell accumulation in the lung (43,47). IL-4 and IL-13 are important Th2 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, where 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 peanut-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 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 peanut-induced intestinal allergy.

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ABBREVIATIONS

AHR	Airway hyperresponsiveness
BMMC	Bone marrow-derived mast cells
CAE	Chloroacetate esterase
FcεRI	High affinity IgE receptor
GI	Gastrointestinal
IF	Immunofluorescence
i.p.	Intraperitoneal
LTB4	Leukotriene B4
MBP	Major basic protein
mMCP-1	Mouse mast cell protease-1
OVA	Ovalbumin
PAS	Periodic acid-Schiff
PBS	Phosphate-buffered saline
PBS/PE	Non-sensitized but challenged with peanut
PE	Peanut
PE/PE	Sensitization and challenge with peanut
Th1	Type 1 T helper
Th2	Type 2 T helper
TNBS	2,4,6-tri-nitrobenzenesulfonic acid
WT	Wild-type

REFERENCES

1. 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 dial telephone survey: a 5-year follow-up study. *J Allergy Clin Immunol* 2003;112:1203–1207. [PubMed: 14657884]
2. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001;107:191–193. [PubMed: 11150011]

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;123:689–693. [PubMed: 19217654]
4. 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;112:1666–1677. [PubMed: 14660743]
5. 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. [PubMed: 18378796]
6. Eigenmann PA. T lymphocytes in food allergy: overview of an intricate network of circulating and organ-resident cells. *Pediatr Allergy Immunol* 2002;13:162–171. [PubMed: 12144637]
7. 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. [PubMed: 9095244]
8. 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;111:1065–1072. [PubMed: 12671056]
9. 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;75:969–976. [PubMed: 8252632]
10. Dombrowicz D, Flamand V, Miyajima I, Ravetch JV, Galli SJ, Kinet JP. Absence of FcRI chain results in upregulation of FcRIII-dependent mast cell degranulation and anaphylaxis: evidence of competition between FcRI and FcRIII for limiting amounts of FcR and chains. *J Clin Invest* 1997;99:915–925. [PubMed: 9062349]
11. McKenzie GJ, Emson CL, Bell SE, Anderson S, Fallon P, Zurawski G. Impaired development of Th2 cells in IL-13-deficient mice. *Immunity* 1998;19:423–432. [PubMed: 9768762]
12. 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;189:1565–1572. [PubMed: 10330435]
13. Lyon MF, Glenister PH. A new allele sash (Wsh) at the W-locus and a spontaneous recessive lethal in mice. *Genet Res* 1982;39:315–322. [PubMed: 7117838]
14. 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;214:110–122. [PubMed: 12088410]
15. 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;114:915–921. [PubMed: 15480335]
16. 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;161:2317–2324. [PubMed: 9725226]
17. Ishizuka T, Chayama K, Takeda K, Hamelmann E, Terada N, Keller GM, et al. Mitogen-activated protein kinase activation through Fc epsilon 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;162:2087–2094. [PubMed: 9973482]
18. 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;106:150–158. [PubMed: 10887318]
19. Hernandez L, Pereira LC, Alvares EP. Goblet cell number in the ileum of rats denervated during suckling and weaning. *Biocell* 2003;27:347–351. [PubMed: 15002751]
20. 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;135:279–290. [PubMed: 8858180]

21. Tomkinson A, Cieslewicz G, Duez C, Larson KA, Lee JJ, Gelfand EW. Temporal association between airway hyperresponsiveness and airway eosinophilia in ovalbuminsensitized mice. *Am J Respir Crit Care Med* 2001;163:721–730. [PubMed: 11254531]
22. 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;205:1087–1097. [PubMed: 18426985]
23. 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. [PubMed: 10903335]
24. Dombrowicz D, Nutten 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. [PubMed: 11136818]
25. Hunt J, Bracher MG, Shi J, Fleury S, Dombrowicz D, Gould HJ, et al. Attenuation of IgE affinity for FcεpsilonRI radically reduces the allergic response in vitro and in vivo. *J Biol Chem* 2008;283:29882–29887. [PubMed: 18703499]
26. Kinet JP. The high-affinity IgE receptor (Fc εpsilon RI): from physiology to pathology. *Annu Rev Immunol* 1999;17:931–972. [PubMed: 10358778]
27. Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nature Rev Immunol* 2008;8:205–217. [PubMed: 18301424]
28. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;454:445–454. [PubMed: 18650915]
29. Metz M, Grimaldeston MA, Nakae S, Piliponsky AM, Tsai M, Galli SJ. Mast cells in the promotion and limitation of chronic inflammation. *Immunol Rev* 2007;217:304–328. [PubMed: 17498068]
30. 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;38:338–349. [PubMed: 18005184]
31. 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;60:822–827. [PubMed: 15876314]
32. 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. [PubMed: 12477762]
33. 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;38:180–185. [PubMed: 12678335]
34. 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;108:430–438. [PubMed: 11544464]
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;203:2715–2725. [PubMed: 17101734]
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;181:7420–7427. [PubMed: 18981165]
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. *Nature Immunol* 2007;8:967–974. [PubMed: 17581537]
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. *Nature Immunol* 2007;8:1390–1397. [PubMed: 17994024]
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. [PubMed: 19450258]

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;282:2261–2263. [PubMed: 9856950]
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;282:2258–2261. [PubMed: 9856949]
42. Taube C, Wei X, Swasey CH, Joetham A, Zarini S, Lively T, et al. Mast Cells, FcεRI, and IL-13 are required for development of airway hyperresponsiveness after aerosolized allergen exposure in the absence of adjuvant. *J Immunol* 2004;172:6398–6406. [PubMed: 15128831]
43. Taube C, Miyahara N, Ott V, Swanson B, Takeda K, JE, et al. The leukotriene B4 receptor (BLT1) is required for effector CD8+ T cell-mediated, mast cell-dependent airway hyperresponsiveness. *J Immunol* 2006;176:3157–3164. [PubMed: 16493075]
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 hyperreactivity. *J Immunol* 2001;167:4668–4675. [PubMed: 11591797]
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;169:6482–6489. [PubMed: 12444158]
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;166:5219–5224. [PubMed: 11290806]
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;40:672–682. [PubMed: 19029019]
48. Brandt EB, Munitz A, Orekov T, Mingler MK, McBride M, Finkelman FD, Rothenberg ME. Targeting IL-4/IL-13 signaling to alleviate oral allergen-induced diarrhea. *J Allergy Clin Immunol* 2009;123:53–58. [PubMed: 18996576]

D **Figure 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 Materials and Methods; (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<0.001 between PE/PE WT and PE/PE FcεRI^{-/-} mice; and (D) Body weight changes. Weights were obtained before challenge and after 7 days of challenge. Results were expressed as % weight change. Results were obtained from three individual experiments with 4–5 mice per group. *P<0.001 compared to all other groups.

Figure 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<0.01 compared with all other groups.

Figure 3.

Decreased inflammatory cell accumulation in the intestinal wall of FcεRI^{-/-} 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 numbers in the jejunal epithelium. For mast cells and eosinophils, results are expressed as the number of CAE or anti-MBP staining cells/mm² of lamina propria, respectively, for goblet cells, the number of PAS+ were divided by the total number of epithelial cells in the villi. Results were obtained from 3 independent experiments and each experiment included 3–4 mice per group. *P<0.001 compared with all other groups. #P<0.01 compared with PBS sensitized control FcεRI^{-/-} mice.

Figure 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<0.01 compared to all other groups.

Figure 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 GAPDH in jejunum were determined by quantitative RT-PCR as described in Methods. The values obtained in the WT PBS/PE group were assigned “1” in all assays. Results were representative of 3 independent experiments with 4 mice per group. * $P < 0.001$ compared with all other groups.

Figure 6. Transfer of BMMC 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 BMMC from WT, FcεRI^{-/-}, IL-4^{-/-}, IL-13^{-/-}, or IL-4/IL-13^{-/-} mice on 2 occasions prior to challenge. Results are representative of 2 independent experiments with 4 mice per group and expressed as % with diarrhea during challenge. *P<0.01 comparing recipients of WT mast cells to recipients of IL-13^{-/-}, IL-4/IL-13^{-/-}, or FcεRI^{-/-} mast cells. **P<0.05 comparing recipients of IL-4^{-/-} mast cells to recipients of IL-13^{-/-}, IL-4/IL-13^{-/-}, or FcεRI^{-/-} mast cells.

Figure 7.

Effect of treatment with IL-13Rα2 protein on peanut-induced intestinal allergy. Sensitized and challenged WT mice received IL-13Rα2 every other day during PE challenge. Control mice received human IgG at the same dose and time points. (A) Protocol for treatment with IL-13Rα2 protein; (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; and (E) Relative mMCP-1 mRNA expression in jejunum from WT mice which received IL-13Rα2 or hIgG. *P<0.01 compared with hIgG treatment.

Figure 8.

Transferred BMMCs can be detected in intestinal tissue. Sections of jejunal and colon from PE/PE KitW-sh/W-sh with or without BMMC transfer were stained by CAE. Mast cells were detected in the mucosa of jejunum and colon after BMMC transfer. Without BMMC transfer, mast cells were not detected in the mucosa of jejunum and colon (Figs. 8A–D). Sections of jejunal tissue from PBS or PE sensitized and challenged C57B6 mice after transfer of BMMCs from UBI-GFP/BL6 mice were stained by IF. Mast cells from UBI-GFP/BL6 mice were found in the lamina propria of jejunal mucosa of both PBS and PE sensitized C57BL/6 mice. Green-fluorescent signals identify BMMCs from UBI-GFP/BL6 mice and the bluefluorescent signals identify nuclei stained with DAPI (Figs. 8E, F).

A

Figure 9. Decreased intestinal allergic responses in KitW-sh/W-sh mice. (A) The development of diarrhea was assessed 30 minutes after the last challenge in WT and KitW-sh/W-sh mice; (B) symptom scores were assessed 30 minutes after oral challenge. *P<0.001 between PE/PE WT and PE/PE KitW-sh/W-sh mice after passive sensitization. **P<0.01 between PE/PE KitW-sh/W-sh mice after passive sensitization with and without BMMCs transfer from WT mice. Results were obtained from two individual experiments with 4–5 mice per group.