HHS Public Access

Author manuscript

Allergy. Author manuscript; available in PMC 2017 November 01.

Published in final edited form as:

Allergy. 2016 November ; 71(11): 1561–1574. doi:10.1111/all.12904.

Combined Blockade of the Histamine H1 and H4 Receptor Suppresses Peanut-Induced Intestinal Anaphylaxis by Regulating Dendritic Cell Function

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Abstract

Background—Signaling through histamine receptors on dendritic cells (DCs) may be involved in the effector phase of peanut-induced intestinal anaphylaxis.

Objectives—To determine the role of histamine H1 (H1R) and H4 receptors (H4R) in intestinal allergic responses in a model of peanut allergy.

Methods—Balb/c mice were sensitized and challenged to peanut. During the challenge phase, mice were treated orally with the H1R antagonist, loratadine, and/or the H4R antagonist, JNJ7777120. Bone marrow-derived DCs (BMDCs) were adoptively transferred to non-sensitized WT mice. Symptoms, intestinal inflammation, mesenteric lymph node and intestine mucosal DCs were assessed. Effects of the drugs on DC chemotaxis, calcium mobilization, and antigen-presenting cell function were measured.

Results—Treatment with loratadine or JNJ7777120 individually partially suppressed development of diarrhea and intestinal inflammation and decreased the numbers of DCs in the mesenteric lymph nodes and lamina propria. Combined treatment with both drugs prevented development of diarrhea and intestinal inflammation. In vitro, the combination suppressed DC antigen presenting cell function to T helper cells and DC calcium mobilization and chemotaxis to histamine.

Conclusion—Blockade of both H1R and H4R in the challenge phase had additive effects in preventing the intestinal consequences of peanut sensitization and challenge. These effects were mediated through limitation of mesenteric lymph node and intestinal DC accumulation and function. Identification of this histamine-H1R/H4R-DC-$CD4^+$ T cell axis provides new insights into the development of peanut-induced intestinal allergic responses and for prevention and treatment of peanut allergy.

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Conflicts of Interest: The authors have no conflicts of interest to declare.

Authors M. Wang, J. Han, J. Domenico, Y.S. Shin, and Y. Jia all contributed to the design and performance of the experiments. E.W.G. supervised the experiments. All authors contributed to the preparation and review of the manuscript.

Keywords

H4R; H1R; peanut allergy; dendritic cells; $CD4^+$ T cells

Introduction

Peanut allergy is increasing in children in many countries (1, 2). The early feeding of peanut to non-sensitized infants decreased the development of peanut allergy in children over the ensuing 4-5 years (3). For known or confirmed peanut allergy patients the only effective therapy to date remains peanut avoidance. Many cells (mast cells, T cells, dendritic cells (DCs), and eosinophils) are involved in the pathogenesis of food allergy (4-6). Histamine is a key mediator of allergic disease with many physiological and pathological effects with new roles still being elucidated mediated through four receptor subtypes (7). The histamine H1 receptor (H1R) is mainly expressed on T cells, mast cells, DCs, smooth muscle cells, and endothelial cells; the histamine H4 receptor (H4R) is primarily expressed on T cells, DCs, mast cells, basophils, and eosinophils (8, 9). The H1R antagonist, loratadine, although effective in allergic rhinitis, has had limited, if any benefit, in the treatment of asthma (10). The H4R has been identified recently (11) and, in experimental models, has been shown to be involved in the development of allergic rhinitis (12), asthma (9) and atopic dermatitis (13). Additionally, the H4R plays an important regulatory role in autoimmune disease (14).

In many tissues, DCs serve as important antigen-presenting cells (APC) and act as messengers linking the innate and adaptive immune systems. DCs capture allergens and process and present antigen to T lymphocytes, inducing T-cell differentiation and cytokine production (15). DCs play a key role in the effector phase of allergic disease through the release of numerous cytokines and chemokines which are involved in recruiting effector Th2 cells to local immune sites and enhancing inflammation during the allergen challenge phase (16). DCs were found in the small intestine including lamina propria (LP) and mesenteric lymph nodes (MLN), and the number of myeloid dendritic cells was increased in the small intestines of peanut allergic mice (6). DCs from food allergic (milk allergy) patients cocultured with CD4+ T cells produced higher levels of Th2 cytokines (IL-5, IL-13) than DCs from non-allergic patients (17). Histamine receptors are expressed on human DCs (18) and regulate DC and T lymphocyte immune functions (19-21).

In this study, we investigated the roles of H1R and H4R signaling in the development and regulation of intestinal inflammation in an experimental model of peanut-induced intestinal allergy. We focused on the challenge phase of previously sensitized mice and demonstrated that targeting both the H1R and H4R attenuated the characteristic features of peanut-induced intestinal allergy by regulating DC accumulation and function, leading to impaired $CD4^+$ T cell function.

Methods

Mice

Five- to 6-week old female BALB/c wild-type (WT) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were maintained on a peanut- and soy-free diet under specific pathogen-free conditions. All studies were conducted under a protocol approved by the Institutional Animal Care and Use Committee of National Jewish Health (Denver, CO).

Preparation of peanut protein

Crude peanut extract (PE) was prepared from defatted raw flours (Golden Peanut Company, Alpharetta, GA) as previously described (22). Endotoxin levels in PE solutions were less than 0.1 EU/ml as assessed by a Chromogenic LAL endotoxin assay kit (GeneScript, Piscataway, NJ).

Sensitization and intragastric challenge

The experimental protocol for sensitization and challenge to peanut was previously described (22). Briefly, mice were sensitized 3 times with 500 μg of PE together with 2.0 mg of alum (Pierce) by intraperitoneal (i.p.) injection in a total volume of 100 μL on days 1, 7, and 21. Two weeks later, in the challenge phase, mice received 20 mg of PE (in 250 μL of PBS) by gavage with a 22-gauge feeding needle (Fisher Scientific, Pittsburgh, PA) every day for 1 week. Thirty minutes after the last challenge, plasma was collected and stored at −80°C. Twenty-four hours after the last challenge, serum, MLN, and jejunal tissues were collected. Control animals were sham sensitized and challenged with peanut.

H1R and H4R antagonist treatment in vivo and in vitro

The selective H4R antagonist JNJ7777120 and selective H1R antagonist loratadine were obtained from Sigma-Aldrich (St. Louis, MO). The drugs were dissolved in 0.5% methycellulose (Spectrum Chemical, Gardena, CA) and diluted with saline for *in vivo* studies or dissolved in RPMI medium for *in vitro* studies. PE sensitized and challenged WT mice received different doses (5, 10, 20 mg/kg) of the antagonists by gavage using a 22-gauge feeding needle (Fisher Scientific) twice a day during the peanut challenge phase. The doses of the antagonists were chosen based on the effects of these antagonists in allergic diseases (9, 12, 23). Controls included PE sensitized and challenged but vehicle (saline)-treated (PE/PE/vehicle) or sham sensitized but PE challenged and vehicle-treated (PBS/PE/vehicle) mice.

Assessment of hypersensitivity reactions

Allergic symptoms were evaluated 30 minutes after the oral challenge, as previously reported (24) Scoring of symptoms was performed in a blinded manner by an independent observer.

Histology

Jejunal tissue was fixed in 10% formalin and processed into paraffin blocks. The tissue sections were stained with periodic acid-Schiff (PAS). The number of mucus-containing

cells was quantitated as previously described (22). Mucosal mast cells were identified by means of chloroacetate esterase staining (4). Cells containing eosinophilic major basic protein were identified by immunohistochemical staining with rabbit anti-mouse major basic protein antibody (kindly provided by Dr. J.J. Lee, Mayo Clinic, Scottsdale, AZ) (4). At least 4 random fields per slide were examined and analyzed in a blinded manner. Quantification of stained mast cells and eosinophils per square millimeter of lamina propria was performed with an Olympus microscope linked to the National Institutes of Health Image Analysis Program (NIH, Bethesda, MD).

Cytokines levels in cell culture

IL-4, IL-13, IL-17A, and IFN-γ levels in $CD4^+$ T cell/DC coculture supernatants were measured by ELISA (eBioscience, San Diego, CA), as described by the manufacturer. The limits of detection were 4 pg/ml for IL-4, IL-13, IL-17A, and 15 pg/ml for IFN-γ.

Measurement of peanut-specific antibody

Serum peanut-specific IgE, IgG1, and IgG2a levels were measured by ELISA as described previously (24).

Histamine levels in plasma

Levels of histamine in plasma were measured using an enzyme immunoassay kit (Beckman Coulter, Fullerton, CA), as described by the manufacturer. The concentration of histamine was calculated from a standard curve provided by the manufacturer.

BMDC generation and differentiation in vitro

Bone marrow-derived DCs (BMDCs) were generated from bone marrow cells of naive BALB/c mice as previously described (25). After 8 days of culture, more than 95% of the cells expressed characteristic DC-specific markers ($CD11c^+$) as determined by flow cytometry.

Adoptive transfer of PE-pulsed BMDCs and experimental protocols

BMDCs were pulsed with PE (200 μg/ml) for 24 hrs and washed three times with PBS. BMDCs (5×10^6 cells in 100 μL of PBS) were administered by i.p. injection into naive BALB/c mice on days 0 and 10. Ten days later, mice received 20 mg of PE (in 250 μL of PBS) by gavage with a 22-gauge feeding needle every day for 1 week. Mice received 20 mg/kg of the H4R antagonist JNJ7777120 and/or H1R antagonist loratadine by gavage using a 22-gauge feeding needle twice a day during the peanut challenge phase. Controls included DC sensitized and PE challenged but vehicle (saline)-treated (DC/PE/vehicle) or sham sensitized but PE challenged and vehicle-treated (PBS/PE/vehicle) mice. Thirty minutes after the last challenge, plasma was collected and stored at −80°C. Twenty-four hrs after the last challenge, serum, MLN, and jejunal tissues were collected.

Intracellular $Ca2^+$ mobilization

BMDCs (4×10^6 cells/mL) were loaded for 45 minutes at 37°C with 5 μM indo-1 acetoxymethyl ester (Invitrogen, Carlsbad, CA) and preincubated with loratadine and/or

JNJ7777120 in RPMI 1640 medium containing 2% FCS. Histamine-induced intracellular $Ca2^{+}$ mobilization was determined as previously described (26).

In vitro chemotaxis assay

Histamine-induced DC chemotaxis was assessed using a 8-μm pore size 96-well chemotaxis system (NeuroProbe, Gaithersburg, MD) as described by the manufacturer and as previously described (26). BMDCs were preincubated with loratadine and/or JNJ7777120 for 1 hr at 37°C before assay.

Generation of CD4+ T cells and co-culture with BMDCs

$CD4^{+}CD45RB^{+}$ T cells were isolated from naïve, ovalbumin (OVA)-specific T-cell receptor transgenic mouse (OT-II mice) spleens by cell sorting (MoFlo XDP, Beckman Coulter) as previously described (22). BMDCs were loaded with 2 μg/mL of $OVA_{323-339}$ peptide (AnaSpec, Fremont, CA) in the presence or absence of histamine (10 μM), and loratadine (10 μM) and/or JNJ7777120 (10 μM) for 24 hrs at 37°C before the addition of OT-II $CD4^{+}CD45RB^{+}$ T cells to the culture. After 4 days of co-culture (27), $CD4^{+}CD45RB^{+}$ T cells were recovered and restimulated with anti-CD3 and anti-CD28 antibodies (2 μg/ml, R&D Systems) for 24 hrs. Supernatants were collected and cytokine levels measured by ELISA.

Isolation of mononuclear cells from the lamina propria and mesenteric lymph nodes

Lamina propria and mesenteric lymph node mononuclear cells were isolated after collagenase digestion and Percoll (Sigma-Aldrich) gradient centrifugation as previously described (28).

Flow cytometry

Cells from the lamina propria and MLNs were labeled with anti-CD11b, anti-CD11c, anti-MHC II, anti-CD80 and anti-CD86 antibodies (eBiosciences) and analyzed on a FACSCalibur (BD Biosciences) using FlowJo software (Tree Star, Ashland, OR).

Quantitative real-time PCR

RNA was extracted from jejunal tissue homogenates using Trizol (Invitrogen) according to the manufacturer's protocol. cDNA was generated using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). Quantitative real-time PCR was performed on the ABI Prism 7300 sequence detection system (Applied Biosystems, Foster City, CA). Primers and probes for mouse *IL4, IL13, IL17A, IFNG, GATA3, RORγt, T-bet,* and *GAPDH* were purchased as Taqman gene expression assays from Applied Biosystems. Fold changes were calculated using the delta delta cycle threshold ($\Delta\Delta C_T$) method.

Cell viability

Cell viability was determined using a trypan blue dye exclusion assay.

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 means±SEM.

Results

Blockade of H1R and H4R attenuates PE-induced allergic responses in vivo

DCs play a key role in both the sensitization and challenge phases of allergic disease (16, 29). To focus on the challenge phase, mice were sensitized and treatment with the H1R and/or H4R antagonists was restricted to the PE challenge phase. Peanut-sensitized WT mice were treated orally, twice daily (20 mg/kg) with the H1R antagonist loratadine, or a selective H4R antagonist, JNJ7777120, during the peanut challenge phase (from days 35 to 41) (Fig. 1A). The doses used were based on previous studies in experimental models of allergic disease (9, 12, 23). Treatment with loratadine or JNJ7777120 individually partially suppressed the development of diarrhea and decreased the severity of symptom scores (Figs. 1B, 1C). The symptom scores were evaluated as previously described (24). To examine whether combined treatment with both loratadine and JNJ7777120 was more effective, sensitized mice were administered different doses (0-20 mg/kg) of the H1R and H4R antagonists. This resulted in dose-dependent inhibition of intestinal allergy induction; 20 mg/kg of both drugs completely prevented the development of diarrhea and significantly diminished clinical symptom scores in PE sensitized and challenged mice (Figs. 1D, 1E). Lower doses of the drugs (10 mg/kg) were partially effective, whereas 5 mg/kg of the drugs had no observed inhibitory effects on diarrhea or clinical symptoms.

In parallel to the clinical assessment, we examined whether the drugs inhibited intestinal inflammation in peanut sensitized and challenged mice. Increased numbers of mast cells, eosinophils, and mucus-producing goblet cells were detected in the small intestines of PE sensitized and challenged mice compared with sham sensitized and PE challenged mice (Figs. 1F, 1G, and 1H, and Figs. E1, E2, and E3 in this article's Online Repository). PE sensitized and challenged mice treated with the H1R or H4R antagonist individually at a dose of 20 mg/kg exhibited reduced numbers of mast cells and mucus-producing goblet cells but not eosinophils, in the mucosa of the small intestine. Combining treatment with both drugs (20 mg/kg) markedly reduced numbers of mast cells, mucus-producing goblet cells, as well as eosinophils in the mucosa of the small intestine, compared with peanut sensitized and challenged mice treated with vehicle (Figs. 1F, 1G, and 1H, and Figs. E1, E2, and E3 in this article's Online Repository).

To monitor mast cell degranulation, we measured plasma levels of histamine within 30 minutes of the last antigen challenge. Challenge of sensitized mice resulted in increased levels of histamine in plasma; after treatment with the H1R or H4R antagonist (20 mg/kg), significantly lower plasma histamine levels were detected (Fig. 1I); plasma histamine levels were reduced almost to baseline levels in PE sensitized and challenged mice treated with both drugs. Under these conditions, no effects on peanut-specific IgE, IgG1, and IgG2a serum levels were seen (Fig. E4 in this article's Online Repository), likely since sensitization

was completed before any intervention. Together, these data indicated that both H1R and H4R played a role in the development of peanut-induced intestinal allergy and that targeting both the H1R and H4R had additive benefits. .

Effect of H1R and H4R antagonist treatment on mucosal DCs in vivo

Dendritic cells are involved in development of peanut allergy (6) and play an important role both in the induction and maintenance of immune responses in the challenge phase of allergic disease (16, 29). To determine the role of H1R and H4R on DC accumulation in the challenge phase of peanut-induced intestinal allergy, we quantitated the number of $CD11c^+MHCII^+$, $CD11c^+CD11b^+$, and $CD11c^+CD80^+$ in the MLN and the number of $CD11c^+CD11b^+$ DCs in lamina propria of the small intestine of WT mice. After PE sensitization and challenge, the percentage of conventional DCs ($CD11c^+MHCII^+$) and $CD11c^+CD11b^+$ DCs in the MLN increased significantly from 0.42±0.05% to 1.33±0.14%, and from 0.24±0.04% to 0.64±0.06% respectively in PE sensitized and challenged mice compared with sham sensitized and PE challenged mice (Fig. 2B and Tables 1 and 2). Numbers of $CD11c^+CD80^+$ DCs were markedly increased in the MLN from PE sensitized and challenged mice compared to sham sensitized and PE challenged mice (Fig. 2B and Table 2). Administration of either the H1R or H4R antagonist to sensitized mice during the PE challenge phase resulted in decreases in the percentages of conventional DCs ($CD11c^+MHCII^+$), $CD11c^+CD11b^+$ DCs, and $CD11c^+CD80^+$ DCs in the MLN. Moreover, combined treatment with the drugs further decreased these numbers. In parallel, the percentages of $CD11c^+CD11b^+$ DCs in the lamina propria of the jejunum increased significantly from 0.12+0.02% to 2.23+0.34% in PE sensitized and challenged mice compared with sham sensitized and PE challenged mice (Fig. 2C and Table 1). In mice treated with the H1R and/or H4R antagonist, the percentages of $CD11c^+CD11b^+$ DCs in the lamina propria of the jejunum decreased almost to baseline levels (Fig. 2C and Table 1).

Inhibition of H1R and H4R suppresses Th2 and Th17 cytokine production without affecting the expression of lineage-specific transcription factors in vivo

To determine the mechanisms underlying the effects of preventing H1R and H4R signaling on development of intestinal allergy, we measured levels of cytokine and transcription factor mRNA in jejunal tissue. PE sensitization and challenge increased *IL4*, *IL13*, and *IL17A,* but not *IFNG* mRNA expression in the small intestine compared to the sham sensitized group (Fig. 3A). In parallel, mRNA expression levels of the lineage-specific transcription factor *GATA3* and retinoic acid-related orphan receptor γt (*RORγt*) were significantly increased in sensitized and challenged mice, whereas levels of *IFNG* and *T-bet* mRNA were not altered (Figs. 3A, 3B). Treatment with loratadine alone significantly reduced *IL13*, but not *IL4* and *IL17A* levels; treatment with JNJ7777120 alone decreased *IL4*, *IL13,* and *IL17A* levels but did not reach statistical significance. After treatment with both loratadine and JNJ7777120, *IL4*, *IL13*, and *IL17A* mRNA expression levels were significantly reduced to baseline levels, but *IFNG, T-bet*, *GATA3*, or *RORγt* mRNA expression was not affected (Fig. 3B). These data indicated that treatment with both H1R and H4R antagonists altered Th2 and Th17, but not Th1 cytokine expression. Further, the effects on cytokine transcription appeared to be mediated downstream of transcription factor expression as no changes in levels of expression of the lineage-specific transcription factors *T-bet*, *GATA3*, or *RORγt* mRNA

were seen. These findings indicated that combination treatment with both the H1R and H4R antagonists had additive effects in modulating Th2 and Th17 cytokine expression following peanut sensitization and challenge.

Effect of H1R and H4R antagonists on DC chemotaxis and intracellular calcium mobilization

In light of the effects of the H1R and H4R antagonists on DC accumulation in the MLN and lamina propria, we determined whether additive effects could be demonstrated on DC function. To assess the role of H1R and H4R signaling on DC function, BMDCs were differentiated in vitro and histamine-induced DC chemotaxis assays were carried out. The dose of histamine chosen was based on the effects of histamine-induced DC migration (Fig. 4A). As shown in Figure 4B, 10 μM of either loratadine or JNJ7777120 significantly inhibited DC migration, and combination of both drugs further suppressed migration. Lower concentrations (3 μM) of loratadine and/or JNJ7777120 had little to no effect on DC migration (data not shown).

Next, we monitored the effects of loratadine and/or JNJ7777120 on DC calcium mobilization. Histamine triggered increases in intracellular calcium concentrations in a dose-dependent manner (Fig. 4D) and doses of 10 and 30 μM of either loratadine (but not 3 μM) or JNJ7777120 reduced the histamine-induced effects (Fig. 4E). Changes in intracellular calcium concentrations triggered by ionomycin were unaffected by the drugs. Thus, two aspects of DC function, migration in response to a histamine gradient and histamine-induced calcium mobilization were altered by interfering with H1R and H4R signaling.

Effect of H1R and H4R antagonists on DC antigen presenting cell function

To assess the effects of the H1R and H4R antagonists on DC antigen presenting cell function, we carried out coculture experiments with antigen-pulsed DCs and antigen-specific $CD4^+$ T cells. $OVA_{323-339}$-loaded BMDCs were pre-treated with loratadine or JNJ7777120 in the presence or absence of histamine before being added to $CD4^+$ T cells from spleens of OT-II mice and cultured for 4 days. The doses of histamine, loratadine or JNJ7777120 were chosen based on the effects of histamine and the antagonists in the DC chemotaxis experiments (Figs. 4A, 4B). Histamine alone enhanced IL-4 and IL-13 cytokine production in coculture experiments of DCs and $CD4^+$ T cells; IFN-γ and IL-17A cytokine levels were unaffected. Treatment of DCs with loratadine or JNJ7777120 significantly reduced the production of IL-4, IL-13, and IL-17A by TCR-activated $CD4^+$ T cells (Figs. 5A, 5B, 5C) The combination of both drugs further reduced the levels of IL-4, IL-13, and IL-17A (Figs. 5A, 5B, 5C). Levels of IFN-γ were unaffected by loratadine and/or JNJ7777120 treatment. Treatment of DCs with loratadine and/or JNJ7777120 had no significant effects on DCs viability (Fig. E5 in this article's Online Repository). These results indicated that signaling through H1R and H4R affected DC antigen presentation, reducing Th2 and Th17 cytokine production from stimulated $CD4^+$ T cells.

Blockade of H1R and H4R attenuates PE-pulsed BMDC-induced peanut allergic response in vivo following adoptive transfer

To determine the role of DC function *in vivo* in this model of peanut allergy, we adoptively transfer PE-pulsed BMDCs into (non-sensitized) mice prior to oral peanut challenge (Fig. 6A). After PE challenge, all of the recipient mice had diarrhea by the seventh day of challenge (Figs. 6B, 6C). Further, increased numbers of mast cells, eosinophils, and mucus-producing goblet cells were detected in the small intestine of PE-challenged recipients of PE-pulsed BMDCs compared with PE-challenged recipients of a sham adoptive transfer (Figs. 6D, 6E, and 6F, and Figs. E6, E7, and E8 in this article's Online Repository). In parallel, plasma levels of histamine and serum levels of peanut-specific IgE, IgG1, and IgG2a were significantly increased compared with PE-challenged sham recipients (Fig. 6G and Fig. E9 in this article's Online Repository). Together, these data indicated that antigen-pulsed DCs played an essential role in the development of peanut-induced intestinal allergy and could replace the need for systemic sensitization.

We determined the effects of administering the H1R and/or H4R antagonist to recipients of the PE-pulsed BMDCs. Since we focused on the effects of the H1R and/or H4R antagonist in the challenge phase, the antagonists (20 mg/kg) were administered orally after the transfer of DCs. As shown in Figures 6B and 6C, treatment with loratadine or JNJ7777120 individually partially suppressed the development of diarrhea and decreased the severity of symptom scores in recipients of PE-pulsed BMDCs. Combined treatment with both loratadine and JNJ7777120 completely prevented the development of diarrhea and significantly diminished clinical symptom scores in PE- challenged, PE-pulsed BMDC recipients (Figs. 6B, 6C).

In parallel, treatment with loratadine or JNJ7777120 individually partially reduced numbers of mast cells and mucus-producing goblet cells, but not eosinophils, in the mucosa of the small intestine. Combining treatment with both drugs markedly reduced numbers of mast cells, eosinophils, as well as mucus-producing goblet cells in the mucosa of the small intestine compared to vehicle-treated mice (Figs. 6D, 6E, and 6F, and Figs. E6, E7, and E8 in this article's Online Repository). Mast cell degranulation was monitored by plasma histamine measurements. Figure 6G shows that levels of histamine were significantly lower in loratadine or JNJ7777120 individually treated mice and treatment with both drugs further reduced the levels of histamine.

Together, these data indicated that DCs were involved in the development of peanut-induced intestinal allergy and that targeting H1R and H4R had additive benefits in attenuating peanut-induced allergic responses triggered by allergen-pulsed BMDCs and allergen challenge in vivo.

Inhibition of H1R and H4R alters Th2 and Th17 responses without affecting the expression of lineage-specific transcription factors following BMDC transfer

Th2 and Th17 immunity appeared to be associated with the development of food allergy (22). We determined the effects of the H1R and/or H4R antagonist on Th2 and Th17 immune responses by measuring levels of cytokine and transcription factor mRNA

expression in jejunal tissue in the BMDC recipients. Allergen-pulsed BMDC transfer and PE challenge increased *IL4*, *IL13*, and *IL17A*, but not *IFNG* mRNA expression in the small intestine of mice compared to the sham transfer and PE challenged mice (Fig. E10A in this article's Online Repository). Levels of *GATA3* and retinoic acid-related orphan receptor γt (*RORγt*) mRNA expression were significantly increased in BMDC recipients while levels of *IFNG* and *T-bet* mRNA expression were not altered in BMDC recipients (Figs. E10A, E10B in this article's Online Repository). Treatment of recipients with loratadine alone significantly reduced *IL4* and *IL13*, but not *IL17A* expression levels; treatment with JNJ7777120 alone decreased *IL13*, but not *IL4* or *IL17A* levels. Treatment with both drugs had additive effects in decreasing Th2 and Th17 mRNA expression (Fig. E10A in this article's Online Repository). Treatment with either drug alone or both drugs failed to alter *IFNG*, *T-bet*, *GATA3,* or *RORγt* expression levels.

Discussion

Histamine is a critical mediator of the manifestations of most allergic diseases, including asthma, allergic rhinitis, and food allergy (8, 12, 22). Histamine is produced or released by a wide variety of different cell types including mast cells, basophils, dendritic cells, T cells, neutrophils, platelets, and gastric enterochromaffin-like cells (30-32). Histamine mediates allergic and inflammatory activities through its receptor subtypes. Targeting the H1R has been shown to prevent allergen-induced airway inflammation and airway hyperreactivity in mouse models of asthma, where high doses of the drug appeared necessary (33, 34). In contrast, several clinical studies have reported that treatment with H1R antagonists had little or no efficacy in patients with asthma, except possibly at very high doses (35, 36). These differing therapeutic effects might be due to insufficient dosing of H1R antagonists in human subjects or the failure to concomitantly block the H4R-mediated histamine actions by only targeting the H1R. The H4R antagonist JNJ7777120 is a high-affinity and highly-specific H4R antagonist (37). JNJ7777120 has been demonstrated to have anti-inflammatory effects with well-described efficacy in the treatment of experimental models of asthma and allergic rhinitis (9, 12, 38).

DCs play a major role not only in the sensitization phase but also in the challenge phase of allergic disease (16, 29). In the present study, we focused on the roles of H1R and H4R in allergic intestinal responses in the challenge phase of a mouse model of peanut allergy. To achieve this, mice were sensitized prior to any drug treatment and the drugs were administered only during the effector or challenge phase. We and others previously demonstrated mast cell, eosinophil and CD4+ T cell infiltration in the small intestine in association with food–induced diarrhea (4, 5, 39). Treatment of mice with loratadine or JNJ7777120 individually, partially suppressed the development of diarrhea, symptoms, and intestinal inflammation (mast cell and eosinophil accumulation, and goblet cell metaplasia) accompanied by decreases in *IL4*, *IL13* and *IL17A* mRNA levels in the intestine and histamine levels in plasma. Combined treatment with loratadine and JNJ7777120 effectively prevented the full development of diarrhea and intestinal inflammation and further reduced Th2 and Th17 cytokine expression and plasma histamine levels. However, receptor antagonism did not alter expression of the Th1, Th2, or Th17 lineage-specific transcription factors *T-bet*, *GATA3*, or *RORγt*. The data suggested that suppression of Th2 and Th17

cytokine production was not mediated through effects on lineage-specific transcription factor expression but on later cytokine producing events.

It has been reported that histamine receptors modulate DC function (20, 40). Dendritic cells are found throughout the intestine including the lamina propria of the small and large intestine and the MLN (41). Lamina propria DCs localize to intestine mucosal surfaces and are positioned for antigen uptake throughout the intestinal lumen. After capturing antigens, DCs migrate from mucosal surfaces to MLNs, present antigens to T lymphocytes, and result in the migration T cells to the intestinal mucosa, culminating in T cell immune responses to the encountered antigens (28, 42). DCs play an important role during the Th2 effector phase of allergic disease, recruiting allergen-specific Th2 effector cells and enhancing Th2 responses through the production of chemokines such as CCL17 and CCL22 (16, 29). Several subsets of DCs have been identified in tissues of the gastrointestinal tract; $CD11c^+CD11b^+$ DCs were an important functional subset in intestinal immune regulation to food allergen (43).

In the model of peanut-induced intestinal allergy described here, important interactions between histamine and DC accumulation and function were demonstrated. Treatment *in vivo* of sensitized mice with either the H1R or H4R antagonist decreased the percentages of DC subsets ($CD11c^+CD11b^+$DCs, $CD11c^+MHCII^+$DCs, and $CD11c^+CD80^+$DCs) in the MLN and $CD11c^+CD11b^+$ DCs in the lamina propria of the jejunum. Combined treatment with both drugs further reduced the numbers of these DC subsets. The results suggested that inhibition of DC accumulation in the intestine and MLN was mediated, at least in part, through H1R and H4R signaling. The antagonists did not alter the development of specific antibodies, including peanut-specific IgE, IgG1, and IgG2a, likely because sensitization was completed before treatment. H2R are expressed in the gastrointestinal tract (44) and signaling through H2R can modulate DC activation, perhaps by altering cAMP accumulation (30, 45). However, signaling through H1R also reportedly increases cAMP accumulation (46) suggesting other mechanisms may be involved. The doses of the antagonists used in these studies were based on previous studies in experimental models of allergic disease (9, 12, 23). Further, the concentrations of the antagonists needed likely reflect the recognized markedly increased metabolism of these antagonists in mice and the differences from what is seen in humans (47-49). Collectively, these *in vivo* data identified for the first time that H1R and H4R signaling played an important role in the effector phase of peanut-induced allergic responses.

In parallel to effects on accumulation of DCs in the target tissue and draining lymph nodes, the importance of signaling through the H1R and H4R on DC function was determined in different assays. In these studies, BMDCs were used as surrogates for the intestinal DCs. To assess the role of histamine receptors on DC movement, *in vitro* chemotaxis assays were carried out. The data showed that histamine stimulated the migration of DCs and this was blocked by H1R or H4R antagonism. Targeting the H4R was previously shown to affect DC migration through the skin (50). Targeting both the H1R and H4R had additive inhibitory effects on DC chemotaxis suggesting that both the H1R and H4R were involved in modulating histamine-induced DC chemotaxis. To further examine the role of histamine receptors on DC function, we monitored intracellular calcium concentrations in response to

addition of histamine. Addition of histamine to BMDCs resulted in increases in intracellular calcium concentrations. These increases were effectively blocked by the combination of both antagonists in a dose-dependent manner. Taken together, these results indicated that histamine had direct effects on DC activation and that these effects were mediated through both H1R and H4R. There is evidence for H1R and H4R signaling interactions at several levels enhancing functional activity (46), whereas the intracellular second messenger cAMP was generated mainly through the H1R, the cooperative interactions of signaling through both the H1R and H4R mediated activation of mitogen-activated protein kinases (MAPKs) and cAMP responsive element binding (CREB) in transfected cells. As shown in this study, increases in intracellular calcium triggered by histamine appeared to involve the H1R and H4R, as targeting of both receptors maximally reduced intracellular calcium concentrations.

We and others have demonstrated that $CD4^+$ T cells play a critical role in the development of allergic inflammation including food allergy (39, 51-53). $CD4^+$ T cells secrete Th2 cytokines such as IL-4 and IL-13 and the Th17 cytokine IL-17A, promoting and maintaining allergic responses in the intestine. In these responses, DCs play a major role as antigen-presenting cells in both the sensitization and challenge phases. As a means of determining the effect of histamine receptor blockade on this major DC function, we investigated the consequences of histamine receptor blockade on DCs which were cocultured with antigen-specific (OVA) $CD4^+$ T cells. Antigen-primed DCs pretreated with the H1R and/or H4R antagonist were co-cultured with $CD4^+$ T cells from the spleens of OT-II mice for 4 days followed by TCR stimulation. This coculture system resulted in increased production of IL-4, IL-13, and IL-17A. Histamine alone had effects on DC activity to promote Th2 cytokine production, and pretreatment of the DCs with either antagonist significantly reduced the production of IL-4, IL-13, and IL-17A, whereas production of IFN-γ was unaffected. Moreover, combining treatment with both drugs was even more effective than either drug alone. These coculture results were consistent with the findings shown with *in vivo* treatment where administration of the antagonists inhibited Th2 (IL-4, IL-13) and Th17 cytokine (IL-17A) production without affecting Th1 cytokine (IFN-γ) levels.

We demonstrated that, in the absence of systemic sensitization, peanut-primed DCs induced strong intestinal allergic responses, including severe diarrhea, intestinal inflammation as well as elevated serum peanut-specific antibodies on peanut challenge. These data provided direct evidence that DCs played an important role in the pathogenesis of food allergy, resulting in an expansion of adaptive immunity, including elevated Th2 and Th17 immune responses. Treatment of the antigen-challenged recipients of PE-primed DCs with either the H1R or H4R antagonist partially inhibited the development of diarrhea and allergic intestinal inflammation; the combination of both drugs further prevented the development of diarrhea and intestinal inflammation and Th2 and Th17 adaptive immune responses. Although less direct than the *in vitro* experiments, these findings suggested that the *in vivo* activities of the DCs were similarly mediated, at least in part, through H1R and H4R signaling in the challenge phase.

In summary, data gathered in both *in vivo* and *in vitro* systems identified the potent effects of histamine in the development of pro-allergic responses, here focusing on the migration and function of DCs. DC accumulation in the intestine *in vivo* or in chemotaxis assays *in vitro*

were altered by blocking H1R and H4R signaling. Similarly, *in vivo* manifestations of clinical symptoms, inflammatory cell accumulation in the jejunum, cytokine production, and plasma histamine levels triggered by allergen sensitization and challenge were significantly altered by H1R and H4R antagonism in the challenge phase. *In vitro*, calcium mobilization by histamine and DC antigen presenting functions were similarly altered by the drugs. In all cases, individual treatment with either antagonist was effective, but the combination of both drugs maximized efficacy. As these drugs are highly selective for their respective receptor, the data indicated a role for both receptors in these varied responses. Based on these experimental data, there may be benefit in treating food allergic patients with the combination of an H1R and H4R antagonist when the H4R antagonists become clinically available. Although DCs are certainly not the only cell type involved in the development and maintenance of an allergic response, they are nonetheless pivotal in the initiation and enhancement of allergic responses in many organs and thus could be a major target for preventing development or maintenance of an allergic response. Given the important effects of histamine on DC function, the involvement of both the H1R and the H4R in these activities may explain some of the limitations of H1R antagonist monotherapy clinically. The histamine-H1R/H4R-DC-$CD4^+$ T-cell axis provides a potential new target for prevention and treatment of peanut allergy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Shannon K. Oda, Greg Kirchenbaum, and Diana Nabighian for their assistance.

Grant Support: This work was supported by NIH grants HL-36577 and AI-77609 (E.W.G.), the Eugene F. and Easton M. Crawford Charitable Lead Unitrust, and the Joanne Siegel Fund. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NHLBI or the NIH.

Abbreviations

BMDCs	Bone marrow-derived DCs
CCL5	Chemokine (C-C motif) ligand 5
CCL17	Chemokine (C-C motif) ligand 17
CCL22	Chemokine (C-C motif) ligand 22
CREB	cAMP responsive element binding
DC	Dendritic cell
H1R	Histamine H1 receptor
H4R	Histamine H4 receptor
IHC	Immunohistochemistry

iTregs	Induced regulatory T cells
JNJ	JNJ7777120
LOLR	Loratadine
LP	Lamina propria
MAPK	Mitogen-activated protein kinase
MCP1	Monocyte chemoattractant protein-1
MLN	Mesenteric lymph node
OT-II mouse	T-cell receptor transgenic mouse
PAS	Periodic acid-Schiff
PE	Peanut extract
RORγt	Retinoic acid-related orphan receptor γt
RT-PCR	Real-time polymerase chain reaction
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. 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]
3. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, Brough HA, Phippard D, Basting M, Feeney M, Turcanu V, Sever ML, Gomez Lorenzo M, Plaut M, Lack G, LEAP Study Team. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med. 2015; 372:803–13. [PubMed: 25705822]
4. Wang M, Takeda K, Shiraishi Y, Okamoto M, Dakhama A, Joetham A, et al. Peanut-induced intestinal allergy is mediated through a mast cell-IgE-FcepsilonRI-IL-13 pathway. J Allergy Clin Immunol. 2010; 126:306–316. [PubMed: 20624645]
5. 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]
6. Smit JJ, Bol-Schoenmakers M, Hassing I, Fiechter D, Boon L, Bleumink R, et al. The role of intestinal dendritic cells subsets in the establishment of food allergy. Clin Exp Allergy. 2011; 41:890–898. [PubMed: 21477183]
7. Thurmond RL, Gelfand EW, Dunford PJ. The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. Nat Rev Drug Discov. 2008; 7:41–53. [PubMed: 18172439]
8. Jutel M, Blaser K, Akdis CA. Histamine in chronic allergic responses. J Invest Allergol Clin Immunol. 2005; 15:1–8.
9. Dunford PJ, O'Donnell N, Riley JP, Williams KN, Karlsson L, Thurmond RL. The histamine H4 receptor mediates allergic airway inflammation by regulating the activation of CD4+ T cells. J Immunol. 2006; 176:7062–7070. [PubMed: 16709868]

10. Town GI, Holgate ST. Comparison of the effect of loratadine on the airway and skin responses to histamine, methacholine, and allergen in subjects with asthma. J Allergy Clin Immunol. 1990; 86:886–893. [PubMed: 2148176]
11. Nguyen T, Shapiro DA, George SR, Setola V, Lee DK, Cheng R, et al. Discovery of a novel member of the histamine receptor family. Mol Pharmacol. 2001; 59:427–433. [PubMed: 11179435]
12. Shiraishi Y, Jia Y, Domenico J, Joetham A, Karasuyama H, Takeda K, et al. Sequential engagement of FcεRI on mast cells and basophil histamine H(4) receptor and FcεRI in allergic rhinitis. J Immunol. 2013; 190:539–548. [PubMed: 23241885]
13. Dunford PJ, Williams KN, Desai PJ, Karlsson L, McQueen D, Thurmond RL. Histamine H4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus. J Allergy Clin Immunol. 2007; 119:176–183. [PubMed: 17208599]
14. del Rio R, Noubade R, Saligrama N, Wall EH, Krementsov DN, Poynter ME, et al. Histamine H4 receptor optimizes T regulatory cell frequency and facilitates anti-inflammatory responses within the central nervous system. J Immunol. 2012; 188:541–547. [PubMed: 22147765]
15. Ruiter B, Shreffler WG. The role of dendritic cells in food allergy. J Allergy Clin Immunol. 2012; 129:921–928. [PubMed: 22464669]
16. Lambrecht BN, Hammad H. The immunology of asthma. Nat Immunol. 2015; 16:45–56. [PubMed: 25521684]
17. Frischmeyer-Guerrerio PA, Guerrerio AL, Chichester KL, Bieneman AP, Hamilton RA, Wood RA, et al. Dendritic cell and T cell responses in children with food allergy. Clin Exp Allergy. 2011; 41:61–71. [PubMed: 20825428]
18. Idzko M, la Sala A, Ferrari D, Panther E, Herouy Y, Dichmann S, et al. Expression and function of histamine receptors in human monocyte-derived dendritic cells. J Allergy Clin Immunol. 2002; 109:839–846. [PubMed: 11994709]
19. Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OA, Malolepszy J, et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature. 2001; 413:420–425. [PubMed: 11574888]
20. Caron G, Delneste Y, Roelandts E, Duez C, Bonnefoy JY, Pestel J, et al. Histamine polarizes human dendritic cells into Th2 cell-pro- moting effector dendritic cells. J Immunol. 2001; 167:3682–3686. [PubMed: 11564782]
21. Gutzmer R, Diestel C, Mommert S, Köther B, Stark H, Wittmann M, et al. Histamine H4 receptor stimulation suppresses IL-12p70 production and mediates chemotaxis in human monocyte-derived dendritic cells. J Immunol. 2005; 174:5224–5232. [PubMed: 15843518]
22. Wang M, Ramirez J, Han J, Jia Y, Domenico J, Seibold MA, et al. The steroidogenic enzyme Cyp11a1 is essential for development of peanut-induced intestinal anaphylaxis. J Allergy Clin Immunol. 2013; 132:1174–1183. [PubMed: 23870673]
23. Cowden JM, Zhang M, Dunford PJ, Thurmond RL. The histamine H4 receptor mediates inflammation and pruritus in Th2-dependent dermal inflammation. J Invest Dermatol. 2010; 130:1023–1033. [PubMed: 19907432]
24. 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]
25. Okamoto M, Matsuda H, Joetham A, Lucas JJ, Domenico J, Yasutomo K, et al. Jagged1 on dendritic cells and Notch on CD4+ T cells initiate lung allergic responsiveness by inducing IL-4 production. J Immunol. 2009; 183:2995–3003. [PubMed: 19667086]
26. Ohnishi H, Miyahara N, Dakhama A, Takeda K, Mathis S, Haribabu B, et al. Corticosteroids enhance CD8+ T cell-mediated airway hyperresponsiveness and allergic inflammation by upregulating leukotriene B4 receptor 1. J Allergy Clin Immunol. 2008; 121:864–871. [PubMed: 18395551]
27. González-Navajas JM, Fine S, Law J, Datta SK, Nguyen KP, Yu M, et al. TLR4 signaling in effector CD4+ T cells regulates TCR activation and experimental colitis in mice. J Clin Invest. 2010; 120:570–581. [PubMed: 20051628]

28. Chirdo FG, Millington OR, Beacock-Sharp H, Mowat AM. Immunomodulatory dendritic cells in intestinal lamina propria. Eur J Immunol. 2005; 35:1831–1840. [PubMed: 16010704]
29. Hammad H, Lambrecht BN. Recent progress in the biology of airway dendritic cells and implications for understanding the regulation of asthmatic inflammation. J Allergy Clin Immunol. 2006; 118:331–336. [PubMed: 16890754]
30. Smolinska S, Jutel M, Crameri R, O'Mahony L. Histamine and gut mucosal immune regulation. Allergy. 2014; 69:273–81. [PubMed: 24286351]
31. Alcañiz L, Vega A, Chacón P, El Bekay R, Ventura I, Aroca R, Blanca M, Bergstralh DT, Monteseirin J. Histamine production by human neutrophils. FASEB J. 2013; 27:2902–10. [PubMed: 23572231]
32. Michael T, Kelly R, Russell Martin, Arthur White. Mediators of histamine release from human platelets, lymphocytes, and granulocytes. J Clin Invest. 1971; 50:1044–1049. [PubMed: 4101589]
33. Bryce PJ, Geha R, Oettgen HC. Desloratadine inhibits allergen-induced airway inflammation and bronchial hyperresponsiveness and alters T-cell responses in murine models of asthma. J Allergy Clin Immunol. 2003; 112:149–158. [PubMed: 12847492]
34. Gelfand EW, Cui ZH, Takeda K, Kanehiro A, Joetham A. Fexofenadine modulates T cell function, preventing allergen-induced airway inflammation and hyperresponsiveness. J Allergy Clin Immunol. 2002; 110:85–95. [PubMed: 12110826]
35. Dirksen A, Engel T, Frølund L, Heinig JH, Svendsen UG, Weeke B. Effect of a non-sedative antihistaminic (loratadine) in moderate asthma. A double-blind controlled clinical crossover-trial. Allergy. 1989; 44:566–571. [PubMed: 2532868]
36. Town GI, Holgate ST. Comparison of the effect of loratadine on the airway and skin responses to histamine, methacholine, and allergen in subjects with asthma. J Allergy Clin Immunol. 1990; 86:886–893. [PubMed: 2148176]
37. Thurmond RL, Desai PJ, Dunford PJ, Fung-Leung WP, Hofstra CL, Jiang W, Nguyen S, Riley JP, Sun S, Williams KN, Edwards JP, Karlsson L. A potent and selective histamine H4 receptor antagonist with anti-inflammatory properties. J Pharmacol Exp Ther. 2004; 309:404–13. [PubMed: 14722321]
38. Cowden JM, Riley JP, Ma JY, Thurmond RL, Dunford PJ. Histamine H4 receptor antagonism diminishes existing airway inflammation and dysfunction via modulation of Th2 cytokines. Respir Res. 2010; 11:86–98. [PubMed: 20573261]
39. Lin XP, Magnusson J, Ahlstedt S, Dahlman-Höglund A, Hanson LLA, Magnusson O, Bengtsson U, Telemo E. Local allergic reaction in food-hypersensitive adults despite a lack of systemic food-specific IgE. J Allergy Clin Immunol. 2002; 109:879–87. [PubMed: 11994715]
40. Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. J Clin Invest. 2001; 108:1865–1873. [PubMed: 11748270]
41. Rescigno M, Di Sabatino A. Dendritic cells in intestinal homeostasis and disease. J Clin Invest. 2009; 119:2441–2450. [PubMed: 19729841]
42. Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol. 2003; 3:331–341. [PubMed: 12669023]
43. Coombes JL, Powrie F. Dendritic cells in intestinal immune regulation. Nat Rev Immunol. 2008; 8:435–446. [PubMed: 18500229]
44. Sander LE, Lorentz A, Sellge G, Coëffier M, Neipp M, Veres T, et al. Selective expression of histamine receptors H1R, H2R, and H4R, but not H3R, in the human intestinal tract. Gut. 2006; 55:498–504. [PubMed: 16299042]
45. Frei R, Ferstl R, Konieczna P, Ziegler M, Simon T, Rugeles TM, Mailand S, Watanabe T, Lauener R, Akdis CA, O'Mahony L. Histamine receptor 2 modifies dendritic cell responses to microbial ligands. J Allergy Clin Immunol. 2013; 132:194–20. [PubMed: 23465664]
46. Beermann S, Bernhardt G, Seifert R, Buschauer A, Neumann D. Histamine H(1)- and H(4)-receptor signaling cooperatively regulate MAPK activation. Biochem Pharmacol. 2015; 98:432–9. [PubMed: 26385311]

47. Ramanathan R, Alvarez N, Su AD, Chowdhury S, Alton K, Stauber K, Patrick J. Metabolism and excretion of loratadine in male and female mice, rats and monkeys. Xenobiotica. 2005; 35:155–89. [PubMed: 16019945]

48. https://en.wikipedia.org/wiki/Loratadine

49. Zhang YF, Chen XY, Zhong DF, Dong YM. Pharmacokinetics of loratadine and its active metabolite descarboethoxyloratadine in healthy Chinese subjects. Acta Pharmacol Sin. 2003; 24:715–71849. [PubMed: 12852841]

50. Bäumer W, Wendorff S, Gutzmer R, Werfel T, Dijkstra D, Chazot P, et al. Histamine H4 receptors modulate dendritic cell migration through skin--immunomodulatory role of histamine. Allergy. 2008; 63:1387–1394. [PubMed: 18782117]

51. Wang M, Okamoto M, Domenico J, Han J, Ashino S, Shin YS, et al. Inhibition of Pim1 kinase prevents peanut allergy by enhancing Runx3 expression and suppressing T(H)2 and T(H)17 T-cell differentiation. J Allergy Clin Immunol. 2012; 130:932–944. [PubMed: 22944483]

52. Knight AK, Blázquez AB, Zhang S, Mayer L, Sampson HA, Berin MC. CD4 T cells activated in the mesenteric lymph node mediate gastrointestinal food allergy in mice. Am J Physiol Gastrointest Liver Physiol. 2007; 293:G1234–1243. [PubMed: 17916645]

53. Islam SA, Luster AD. T cell homing to epithelial barriers in allergic disease. Nat Med. 2012; 18:705–715. [PubMed: 22561834]

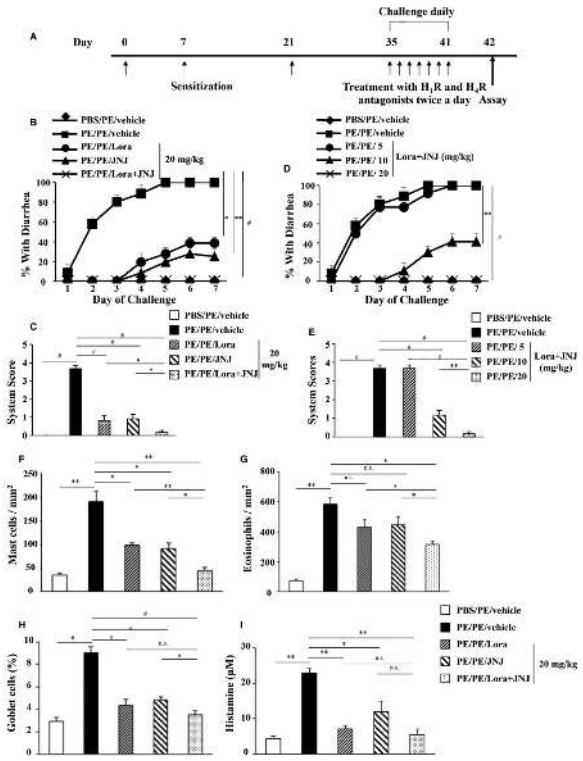

Figure 1.

Inhibition of histamine H1 and H4 receptors *in vivo* reduces intestinal responses. (A) Protocol for induction of peanut allergy and treatment with H1R or H4R antagonists. Sensitization was performed on days 1, 7, and 21. Challenge and treatment with H1R and H4R antagonists were performed from day 35 to day 41. (B) Kinetics of the development of diarrhea after treatment with JNJ7777120 and/or loratadine. (C) Scores based on the severity of clinical signs were assessed 30 minutes after oral challenge. (D) Kinetics of the development of diarrhea after treatment with JNJ7777120 and loratadine at different doses. (E) Scores based on the severity of clinical signs were assessed 30 minutes after oral challenge. (F, G, H) Quantitation of mucosal mast cells, eosinophils, and goblet cell numbers in jejunum. (I) Plasma histamine levels were assessed within 30 minutes of the last oral challenge. Results are from 3 independent experiments, and each experiment included 3-4 mice per group. *P<0.05, **P<0.01, and #P<0.001. n.s., not significant. PBS/PE, sham sensitized and peanut challenged; PE/PE, peanut sensitized and challenged; PE/PE/Lora, peanut-sensitized and challenged and treated with loratadine; PE/PE/JNJ, peanut-sensitized and challenged and treated with JNJ7777120; PE/PE/Lora+JNJ, peanut-sensitized and challenged and treated with loratadine and JNJ7777120.

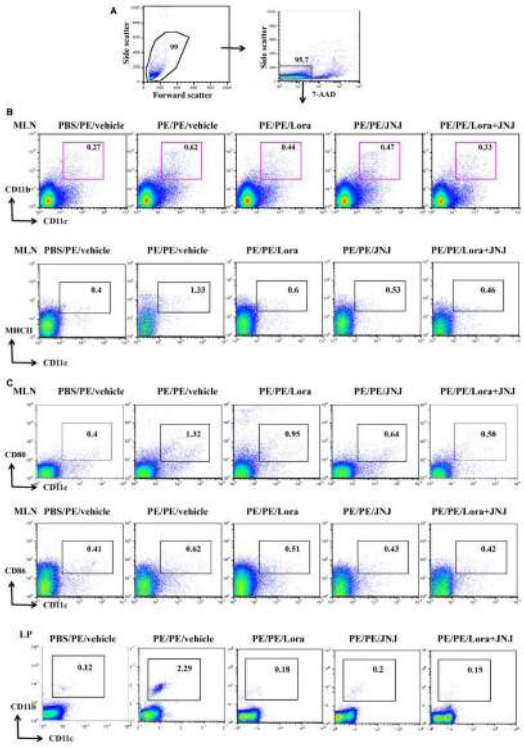

Figure 2.

Inhibition of H1R and H4R decreases DC numbers in the MLN and lamina propria. A) Cells were gated based on the FSC-SSC pattern and live cell (7-AAD negative) determination. B) Percentages of $CD11c^+CD11b^+$, $CD11c^+MHCII^+$, $CD80^+CD11c^+$, and $CD86^+CD11c^+$ DCs in the MLN. C) Percentages of $CD11c^+CD11b^+$ DCs in the lamina propria. Illustrated are representative dotplots of DC analysis in the MLN and lamina propria from PE/PE mice treated with H1R and/or H4R antagonists or vehicle.

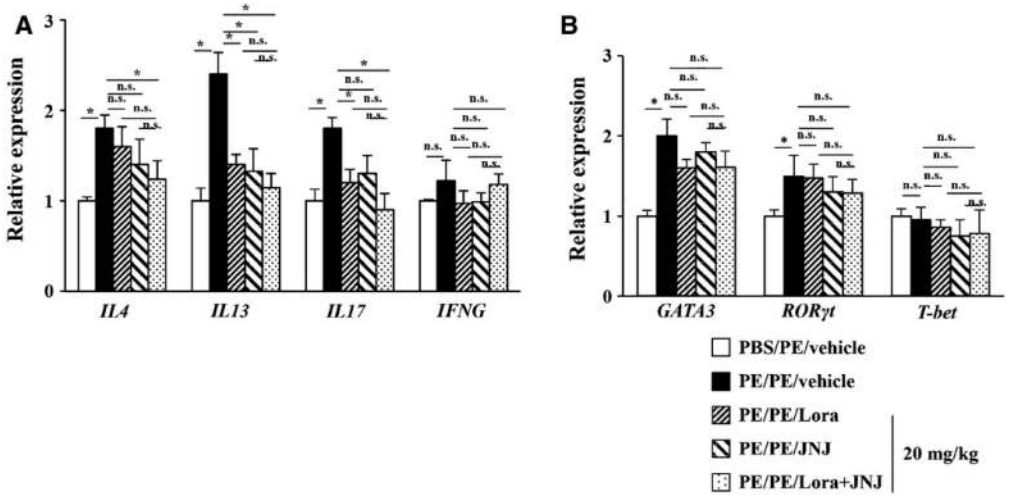

Figure 3.

Effects of H1R and H4R inhibition on cytokine and lineage-specific transcription factor expression in the mouse jejunum. (A) *IFNG, IL4, IL13,* and *IL17A* mRNA expression in jejunum of mice treated with loratadine and JNJ7777120 or vehicle. (B) Th1, Th2, and Th17 transcription factors *T-bet*, *GATA3,* and *RORγt* expression in jejunum of mice treated with loratadine and JNJ7777120 or vehicle. Results were from 3 independent experiments (n=12). *P<0.05. n.s., not significant.

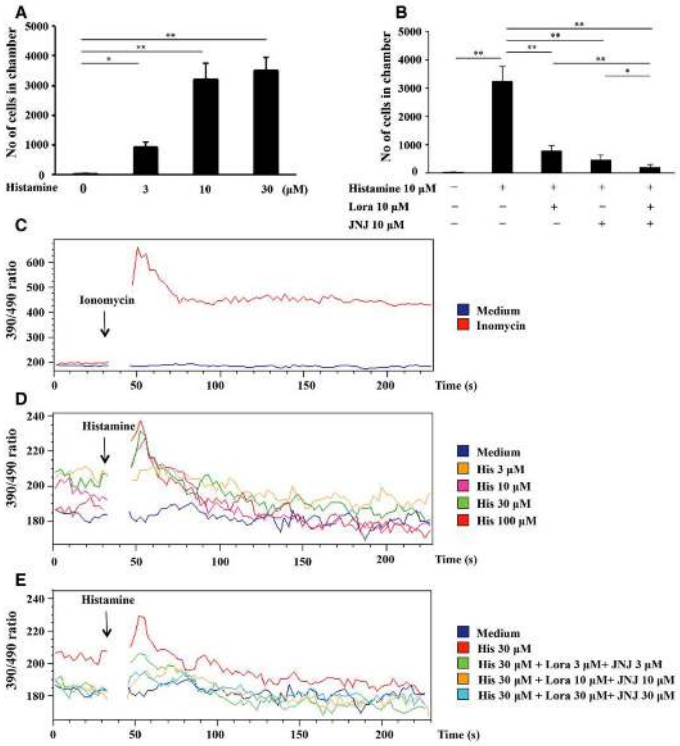

Figure 4.

In vitro histamine-induced DC chemotaxis and intracellular calcium mobilization. (A) Histamine-induced DC chemotaxis. (B) H1R antagonist loratadine and/or H4R antagonist JNJ7777120 effects on histamine-induced DC migration. (C). Ionomycin as a positive control in calcium mobilization assay. (D) The calcium mobilization effects of different doses of histamine (0, 3, 10, 30, and 100 μM) on DCs. (E) The effects of different doses of loratadine and JNJ7777120 treatment on calcium mobilization of DCs. The illustrated experiments are representative of at least 3 independent experiments performed. Data shown are the means±SEM; *p<0.05, **p<0.01.

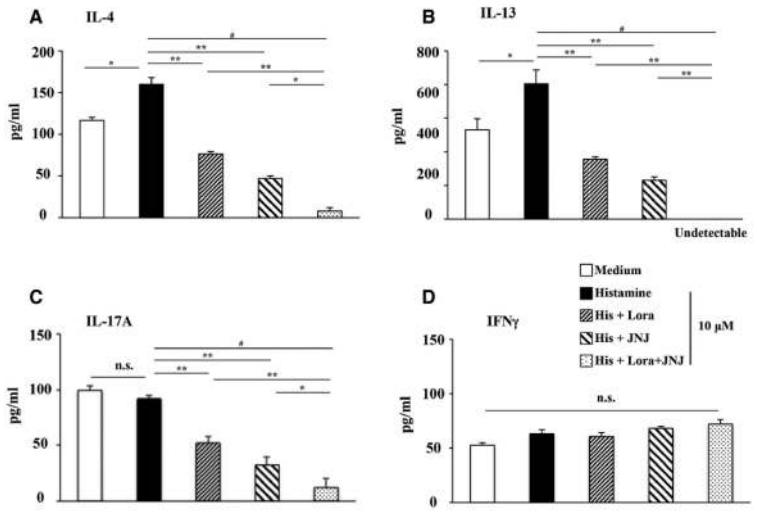

Figure 5.

Effects of H1R and H4R antagonism on DC antigen presenting cell function. $CD4^+CD45RB^+$ T cells from OT-II mice were co-cultured with OVA-loaded and loratadine- or JNJ7777120-treated DCs for 4 days. After coculture, the $CD4^+CD45RB^+$ T cells were restimulated for 24 hrs, and the levels of cytokines secreted by $CD4^+$ T cells were determined. Data represent means±SEM from 3 independent experiments. *P<0.05, **P<0.01, and #P<0.001. n.s., not significant.

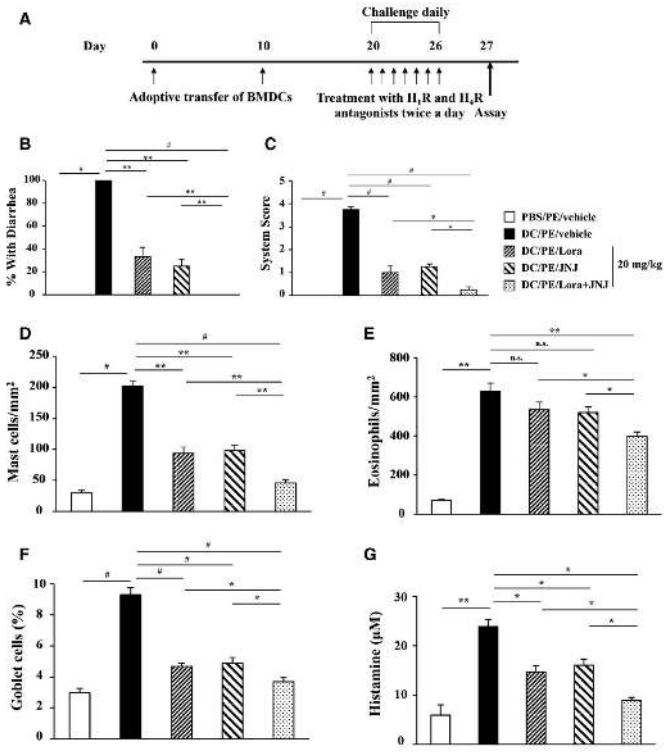

Figure 6.

Effects of H1 and H4 receptor antagonism on intestinal responses following DC transfer and allergen challenge. (A) Protocol for dendritic cell induction of peanut allergy and treatment with H1R or H4R antagonists. Adoptive transfer of BMDCs was performed on days 1 and 10. Challenge and treatment with H1R and H4R antagonists were performed from day 20 to day 26. (B) Development of diarrhea after treatment with JNJ7777120 and/or loratadine. (C) Scores based on the severity of clinical symptoms were assessed 30 minutes after oral challenge. (D, E, F) Quantitation of mucosal mast cell, eosinophil, and goblet cell numbers in jejunum. (G) Plasma histamine levels were assessed within 30 minutes of the last oral challenge. Results are from 2 independent experiments, and each experiment included 3-4 mice per group. *P<0.05, **P<0.01, and #P<0.001. n.s., not significant. PBS/PE/vehicle, sham sensitized and peanut challenged and sham treatment; DC/PE/vehicle, DC sensitized and PE challenged and sham treatment; DC/PE/Lora, DC sensitized and PE challenged and treated with loratadine; DC/PE/JNJ, DC sensitized and PE challenged and treated with JNJ7777120; DC/PE/Lora+JNJ, DC sensitized and PE challenged and treated with loratadine and JNJ7777120.

Table 1

Decreased numbers of $CD11c^+CD11b^+$ DCs in the MLN and lamina propria (LP) of the small intestines of PE sensitized and challenged mice treated with HIR and/or H4R antagonists. Results are from 3 independent experiments; mean values±SEM are given.

	$CD11b^+CD11c^+$(in MLN)	$CD11b^+CD11c^+$ (in LP)
PBS/PE/vehicle	0.24 ± 0.04	0.12 ±0.02
PE/PE/vehicle	0.64 ± 0.06[a]	2.23 ± 0.34[a]
PE/PE/Lora	0.45 ± 0.03[b]	0.21 ±0.03[b]
PE/PE/JNJ	0.46 ± 0.02[b]	0.25 ± 0.04[b]
PE/PE/Lora+JNJ	0.34 ± 0.02[b]	0.17 ±0.04[b]

[a] $P<0.01$ comparing PBS/PE/vehicle treatment vs. PE/PE/Lora; PE/PE/JNJ; and PE/PE/Lora+JNJ treatment.

[b] $P<0.01$ comparing PE/PE/vehicle treatment vs. PE/PE/Lora; PE/PE/JNJ; and PE/PE/Lora+JNJ treatment.

Table 2

Decreased numbers of MHCII$^+$CD11c$^+$ and CD80$^+$CD11c$^+$ DCs in the MLN of PE sensitized and challenged mic treated with HIR and/or H4R antagonists. The percentages of MHCII$^+$CD11c$^+$, DC80$^+$CD11c, and CD86$^+$CD11c$^+$ DCs in the MLN were determined by flow cytometry. Results are from 3 independent experiments; mean values±SEM are given.

	MHCII$^+$CD11c$^+$	CD80$^+$CD11c$^+$	CD86$^+$CD11c$^+$
PBS/PE/vehicle	0.42 ± 0.05	0.43 ± 0.04	0.41 ±0.03
PE/PE/vehicle	1.33 ± 0.14[a]	1.32 ±0.09[a]	0.6± 0.06
PE/PE/Lora	0.62 ± 0.04[c]	0.95 ± 0.08[c]	0.52 ±0.04
PE/PE/JNJ	0.54 ± 0.03[c]	0.67 ± 0.06[b]	0.45 ± 0.03
PE/PE/Lora+JNJ	0.47 ± 0.04[c]	0.57 ± 0.04[b]	0.43 ± 0.02

[a] P<0.01 comparing PBS/PE/vehicle treatment vs. PE/PE/vehicle treatment.

[b] P<0.01 comparing PE/PE/vehicle treatment vs. PE/PE/Lora; PE/PE/JNJ; and PE/PE/Lora+JNJ treatment.

[c] P<0.05 comparing PE/PE/vehicle treatment vs. PE/PE/Lora, PE/PE/JNJ; and PE/PE/Lora+JNJ treatment.