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Involvement of leukotriene B_4 in itching in a mouse model of ocular allergy

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ABSTRACT

Itching of ocular allergy is alleviated but not completely relieved by H_1 histamine receptor antagonists, suggesting that histamine is not the sole itch mediator in ocular allergy. We investigated whether leukotriene B_4 (LTB_4), a mediator of cutaneous itch, is involved in the itch of ocular allergy in mice. Mice were immunized by the repeated subcutaneous injections of ragweed pollen and alum into the caudal back, and given a subconjunctival injection of ragweed pollen extract into the palpebra for allergic challenge. Challenge with ragweed pollen extract markedly elicited ocular scratching in sensitized mice. The scratching was almost abolished by mast cell deficiency. The H_1 antagonist terfenadine partially inhibited scratching at a dose that almost completely suppressed plasma extravasation. Scratching was inhibited by the glucocorticoid betamethasone and the 5-lipoxygenase inhibitor zileuton at doses that inhibited the challenge-induced production of LTB_4. A subconjunctival injection of LTB_4 at doses 1/10,000 or less than that required for histamine elicited ocular scratching in naïve mice. The LTB_4 receptor antagonist ONO-4057 inhibited the ragweed pollen challenge-induced ocular scratching at doses that suppressed LTB_4-induced ocular scratching. In addition to histamine, LTB_4 is involved in the ocular itching of pollen allergy. H_1 receptor antagonists with an inhibitory effect on the action and/or production of LTB_4 may have more potent anti-pruritic activity than selective H_1 antagonists.

1. Introduction

The common form of ocular allergy is seasonal allergic conjunctivitis, which is associated with exposure to airborne allergens such as ragweed and oak pollens. The main symptoms of allergic conjunctivitis are itching, lacrimation, and redness (Rosario and Bielory, 2011). Pollen allergy-induced itching of the conjunctiva is a severe clinical problem; frequent scratching or rubbing of itchy eyes may lead to cataracts (Nagaki et al., 1999). Histamine released from mast cells is an important itch mediator in pollen allergy, especially allergic conjunctivitis. Ocular instillation with an H_1 histamine receptor antagonist acutely reduced ocular itching compared to a placebo (Fujishima et al., 2006). However, H_1 histamine receptor antagonists with mast cell-stabilizing activity are both superior and used more commonly than selective H_1 histamine receptor antagonists are (Abelson et al., 2011; del Cuvillo et al., 2009). This suggests the possibility that histamine is not the sole itch mediator of ocular allergy.

Regarding to cutaneous itch, there are many endogenous itch mediators, including amines, peptides, proteases, cytokines, and eicosanoids (Paus et al., 2006). Among them, leukotriene B_4 (LTB_4), a 5-lipoxygenase metabolite of arachidonic acid, elicits an itch-related response (hind-paw scratching) at intradermal doses 1/10,000 or less than that required for histamine (Andoh and Kuraishi, 1998; Andoh et al., 2007); it is also involved in scratching induced by passive cutaneous anaphylaxis and chronic allergic dermatitis in mice (Andoh et al., 2011; Tsuji et al., 2010; Tsukumo et al., 2010). The inhibitory effects of some H_1 histamine receptor antagonists on cutaneous itching may be mediated by the inhibition of the action and/or production of LTB_4 (Andoh and Kuraishi, 2000, 2002, 2006). LTB_4 is elevated in guinea-pig conjunctiva after allergen challenge (Garceau et al., 1987) and in the tears of patients with giant papillary conjunctivitis (Akman et al., 1998). Thus, we investigated whether LTB_4 is responsible for the itching involved in ocular allergy.

Animal models of ocular allergy using various species such as mice, rats, guinea-pigs, rabbits, and dogs have already been reported (Bundoc and Keane-Myers, 2003; Groneberg et al., 2003). Although allergic inflammation has been extensively investigated using these animal models, there are only some reports of animal (i.e., guinea-pig and mouse) models of itch-related to ocular allergy (Fukushima et al., 2003; Kato et al., 2003; Sanchis-Merino et al., 2008; Shii et al., 2009; Woodward et al., 1996). Therefore, we

Abbreviations: Ig, immunoglobulin; LTB_4, leukotriene B_4; PBS, phosphate-buffered saline; RP, ragweed pollen.

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developed a mouse model of ocular itch of pollen allergy to investigate the role of LTB_4 in the itch of ocular allergy.

2. Materials and methods

2.1. Animals

Male ICR mice (4 weeks old at the start of experiment) were used in all experiments except for a series of experiments, in which male WBB6F1-W/W^v mice (mast cell-deficient, 9 weeks old) and the normal littermates (WBB6F1-+/+) were used. All mice were purchased from Japan SLC (Shizuoka, Japan). They were kept under controlled room temperature (ca. 22 °C), humidity (ca. 55%), and light (lit from 7:00 AM to 7:00 PM). Food and water were available *ad libitum*. The study protocol was approved by the Committee for Animal Experiments at the University of Toyama and was conducted in accordance with the guidelines of the Japanese Pharmacological Society.

2.2. Drugs

Zileuton, 5-[2-(2-carboxyethyl)-3-[6-(4-methoxyphenyl)-5E-hexenyl] oxyphenoxy] valeric acid (ONO-4057), betamethasone, and terfenadine were dissolved in 0.5% carboxymethyl cellulose (Wako Pure Chemical Ind., Osaka, Japan) and administered orally 1 h before allergen challenge. LTB_4 (Cayman Chemical, An Arbor, MI, USA) and histamine (Wako Pure Chemical Ind.) were dissolved in physiological saline containing 2% ethanol and physiological saline, respectively. These pruritogens were administered to the subconjunctiva in a volume of 2 μL.

2.3. Behavioral observation

The mice were placed in an acrylic cage consisting of four equal-sized cells (13 × 9 × 40 cm) for at least 1 h to allow them for acclimation. They were returned to the same cells immediately after injection of the allergen or pruritogen, and their behavior was videotaped for 2 h; all lab personnel were kept out of the observation room during this time. The number of times a mouse scratched its eye with its hind-paw was counted during video playback. A bout of scratching was defined as when a mouse stretched its hind-paw on the treated side toward its eye, leaned its head toward the paw, rapidly scratched its eye several times for approximately 1 s, and lowered its hind-paw. In a series of experiments, wiping of the treated eye with the ipsilateral forelimb was also observed; a single gentle stroke of the unilateral eye was counted as one wipe.

2.4. Sensitization and challenge

Ragweed pollen (RP) from *Ambrosia trifida* (Sigma Chem. Co., St. Louis, MO, USA) was suspended in a gel containing 40 mg/mL aluminum hydroxide (Imject® Alum, Pierce, Rockford, IL, USA), mixed for 30 min or more with a vortex mixer. The animals were immunized with subcutaneous injections of RP (100 μg) in the adjuvant alum gel (4 mg) into the caudal back in a volume of 100 μL twice a week for 3 weeks; in a series of experiments, mice were given repeated injections of RP suspended in phosphate-buffered saline (PBS; pH 7.4) or alum alone.

RP extract was used for allergen challenge. RP was suspended in PBS and left to stand overnight at 4 °C; it was then centrifuged at 4 °C at 2250 × *g* for 5 min. The supernatant was collected as RP allergen, and its protein concentration was determined using a protein assay kit (Bio-Rad Lab. Inc., Hercules, CA, USA). RP allergen was injected subconjunctivally into the unilateral palpebra for challenge at a dose of 2 μg/site in a volume of 2 μL.

2.5. Plasma extravasation

Mice were given an intravenous injection of 150 μL of 1% Evans blue dissolved in physiological saline and subconjunctival challenge with RP allergen 20 min later. Under deep pentobarbital (80 mg/kg, intraperitoneal) anesthesia, mice were euthanized by cervical dislocation 20 min after challenge, the ocular skin including the conjunctiva was immediately isolated. The bluish region of the sample was punched out using an 8 mm diameter punch and incubated in 1 mL of dimethyl sulfoxide overnight. The concentration of dye extracted was determined spectrophotometrically at 620 nm.

2.6. Determination of serum concentrations of total immunoglobulins

Blood was collected from the orbital sinus under sodium pentobarbital anesthesia (80 mg/kg, i.p.) and centrifuged at 4 °C at 2200 × *g* for 5 min. The serum was kept at −30 °C until assay. The concentrations of total IgE, IgG_1, IgG_{2a}, and IgG_{2b} in the serum were determined using a sandwich enzyme immunoassay (Ohtsuka et al., 2001). For each immunoglobulin determination, 96-well plates were coated with 100 μL/well of affinity-purified goat antibody against mouse IgE, IgG_1, IgG_{2a}, or IgG_{2b} (1/100; Bethyl Laboratories, Inc., Montgomery, TX, USA) at 4 °C overnight. The plates were washed with 200 μL/well of PBS containing 0.1% Tween 20 and blocked with PBS containing 1% bovine serum albumin (Sigma, USA) at room temperature for 1 h. After washing with PBS containing 0.1% Tween 20, 100 μL/well of diluted serum (1/50 for IgE, 1/2000 for IgG_1, IgG_{2a}, and IgG_{2b}) and standard (mouse IgE calibrator for IgE and mouse reference sera for IgG_1, IgG_{2a}, and IgG_{2b}; Bethyl Laboratories) were added to the coated plates at room temperature for 1 h. After washing with PBS containing 0.1% Tween 20, the plates were treated with horseradish peroxidase conjugated-goat antibodies against mouse (IgE (1/40,000), IgG_1 (1/80,000), IgG_{2a} (1/60,000), or IgG_{2b} (1/50,000), Bethyl Laboratories) at room temperature for 1 h. The plates were washed with PBS containing 0.1% Tween 20 and reacted with 100 μL/well of o-phenylene diamine solution (1 mg/mL, Wako Pure Chemical Ind., Osaka, Japan) at room temperature. After stopping the reaction with 2 N sulfuric acid, the absorbance was measured at 490 nm using a microplate reader (Immuno Mini NJ-2300, Thermo Fisher Scientific, Roskilde, Denmark).

2.7. LTB_4 determination

Under deep pentobarbital (80 mg/kg, intraperitoneal) anesthesia, mice were euthanized by cervical dislocation 5 min after challenge and the ocular skin, including the conjunctiva, was removed. The tissue sample was immediately weighed, shredded with scissors, and put into 2 mL of ice-chilled ethanol containing 10 μM indomethacin and 10 μM zileuton. Lipid extraction and the enzyme immunoassay for LTB_4 were performed as described previously (Andoh et al., 2009).

2.8. Statistical analysis

Data are presented as means ± SEM. Statistical comparisons between groups were performed using Dunnett's test, Tukey's test, or Bonferroni's method. Time course data were analyzed using repeated measures analysis of variance; $p < 0.05$ was considered significant.

3. Results

BALB/c mice are prototypical Th2-type mouse strain and useful for studies of allergic conjunctivitis. However, BALB/c mice are low responders to histamine; an intradermal injection of histamine does not elicit scratching behavior in BALB/c mice (Inagaki et al., 2001). In our preliminary experiments, instilling of histamine solution to the eye did not significantly increase ocular scratching in BALB/c mice; scratching bouts per hour were 7.5 ± 1.8, 10.0 ± 2.4, 14.3 ± 3.9, and 16.0 ± 3.1 ($n = 8$ each) after the instillation of PBS, 2, 5 and 10 μmol/site of histamine, respectively. In contrast, instilling of histamine at the same doses was reported to increase ocular scratching in ICR mice (Nakano et al., 2009). Therefore, we used ICR mice in this study to determine the role of histamine in itch of allergic conjunctivitis.

In out pilot experiments, instilling a drop of RP allergen (100 μg in a volume of 5 μL) to the eye did not increase ocular scratching in sensitized ICR mice; the number of scratching bouts per 2 h were 22 ± 5 and 25 ± 7 ($n = 8$ each) in mice given an eye drop of PBS and RP allergen, respectively. Therefore, in this study, RP allergen was injected subconjunctivally to the palpebra, as shown in Fig. 1.

3.1. RP allergy-induced ocular scratching

A subconjunctival injection of RP allergen significantly elicited hind-paw scratching of the treated eye in ICR mice immunized with RP together with alum (Fig. 2). Although there was a tendency toward increased ocular scratching bouts in mice immunized with RP alone, most mice (88%) did not exhibit increased ocular scratching (Fig. 2A). In mice immunized with RP together with alum, scratching began within several minutes after challenge, peaked during the first 10-min period, and decreased gradually until 1 h; however, scratching frequency appeared to be slightly higher 1–2 h after challenge (Fig. 2B).

Painful and itchy stimulation of the face elicits forelimb wiping and hind-paw scratching in mice, respectively (Shimada and LaMotte, 2008). Therefore, we examined whether a subconjunctival injection of allergen would elicit pain-related behaviors in addition to itch-related behaviors. In ICR mice immunized with RP together with alum, a subconjunctival injection of RP allergen markedly elicited hind-paw scratching of the treated eye; the numbers of scratching bouts per 2 h were 9.9 ± 1.5, 8.9 ± 3.1, and 86.6 ± 16.8 ($n = 8$ each) without injection, after vehicle injection, and after RP allergen injection, respectively. In contrast, no forelimb wiping behaviors were observed during the 2-h period in naïve mice and mice given subconjunctival injections of vehicle and RP allergen.

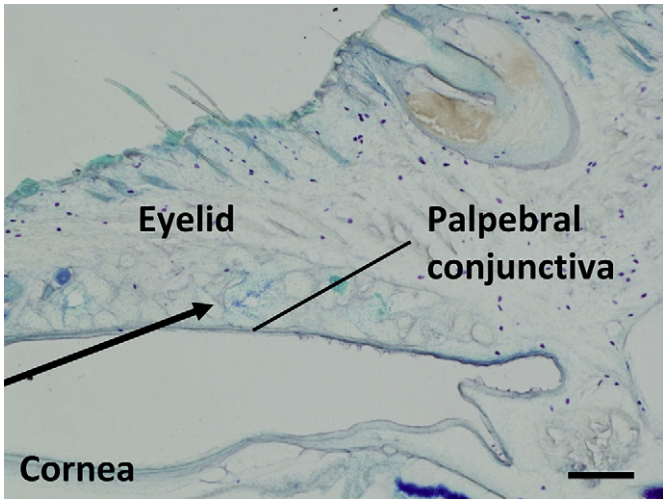

Fig. 1. Subconjunctival injection site in the *palpebra*. Toluidine blue staining of the murine eyelid. Arrow indicates the direction and site of allergen injection. Scale bar = 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

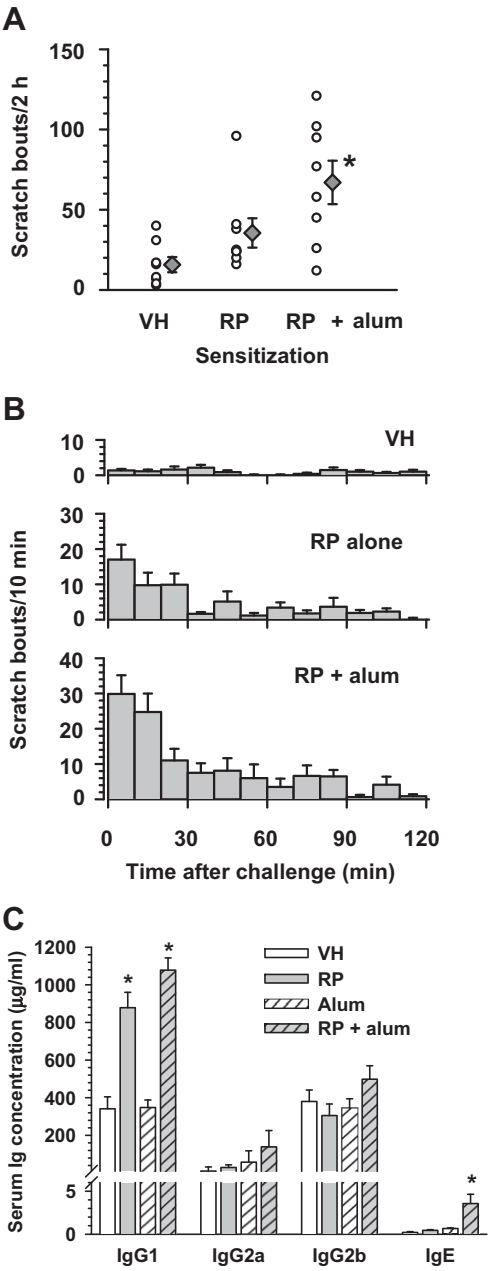

Fig. 2. Effects of ragweed pollen (RP) immunization on allergy-induced ocular scratching and serum immunoglobulin (Ig) concentration in ICR mice. Mice were immunized with RP alone or together with alum. (A) Cumulative scratch bouts after RP allergen challenge. Mice were challenged subcunjunctivally with RP allergen, and hind-paw scratching of the treated eye was quantified. Open circles, individual data for 24 mice; closed diamonds, mean of each group. *$p < 0.05$ vs. vehicle (VH) and RP (Bonferroni's method). Values represent the mean and SEM of eight animals. (B) Time course of scratching after RP allergen challenge. Values represent the mean and SEM of eight animals. Main effect of treatment, $F_{2231} = 13.53$, $p = 0.0002$; interaction between treatment and time, $F_{22,234} = 4.82$, $p < 0.0001$ (repeated measures of analysis of variance). (C) Serum concentrations of total IgG_1, IgG_{2a}, IgG_{2b}, and IgE. Immunoglobulin concentration was determined using a sandwich enzyme immunoassay (see text).

The serum concentration of total immunoglobulin (Ig)G_1 was significantly increased by immunization with RP alone, but the serum concentrations of total IgG_{2a}, IgG_{2b}, and IgE were not increased in ICR mice (Fig. 2C). Immunization with RP together with alum significantly increased the serum concentration of total IgE without effects on the concentrations of total IgG_{2a} and IgG_{2b}, and IgG_1 concentration was similar to that of immunization with RP alone (Fig. 2C).

3.2. Roles of mast cells and histamine

In this series of experiments, we used mast cell-deficient WBB6F1-*W/W*^{v} mice and normal WBB6F1-+/+ mice to determine the role of mast cells in the RP allergy-induced ocular scratching. Subconjunctival injection of RP allergen markedly elicited ocular scratching in WBB6F1-+/+ mice immunized with RP and alum. Moreover, there was a tendency toward increased average ocular scratching in mice immunized with RP alone (Fig. 3A). However, the increase in ocular scratching after challenge was almost abolished in WBB6F1-*W/W*^{v} mice (Fig. 3A).

Pretreatment with terfenadine (30 mg/kg), a peripherally acting H_1 histamine receptor antagonist, partially but significantly inhibited ocular scratching induced by RP allergen in ICR mice immunized with RP and alum (Fig. 3B). The same dose of terfenadine almost abolished the plasma extravasation induced by challenge with RP allergen in the sensitized mice (Fig. 3C).

3.3. Effects of a glucocorticoid, 5-lipoxygenase inhibitor and LTB_4 receptor antagonist on RP allergy-induced ocular scratching

The glucocorticoid betamethasone (0.3 and 1 mg/kg), the 5-lipoxygenase inhibitor zileuton (30 and 100 mg/kg), and the LTB_4 receptor antagonist ONO-4057 (30 and 100 mg/kg) dose-dependently inhibited RP allergen-induced ocular scratching in ICR mice immunized with RP and alum (Fig. 4).

3.4. LTB_4- and histamine-induced ocular scratching

A subconjunctival injection of LTB_4 (1.2 pmol/site) markedly increased hind-paw scratching of the treated eye in naïve ICR mice; marked effect was observed for 30 min after injection (Fig. 5A).

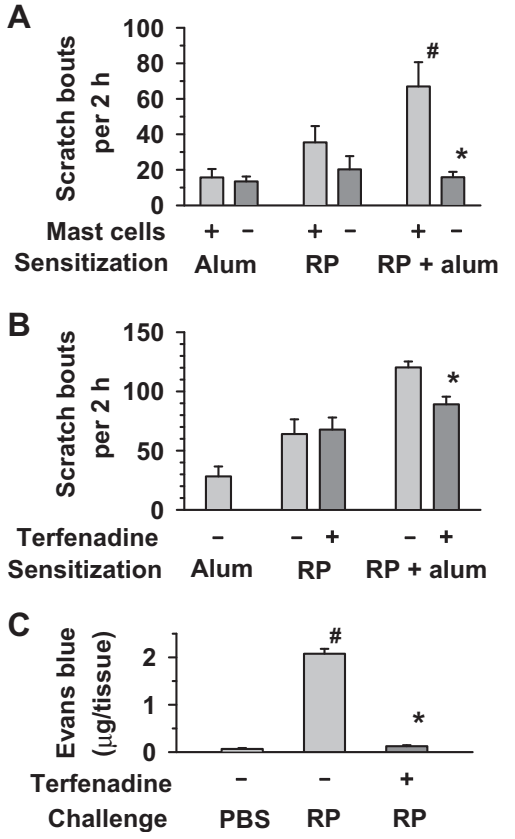

Fig. 3. Involvement of mast cells and histamine in RP allergy-induced ocular scratching. Mice were administered repeated subcutaneous injections of alum alone, RP alone or RP plus alum, and then RP allergen was injected subconjunctivally into the palpebra for challenge. (A) Effect of mast cell deficiency on the ocular scratching. −, mast cell-deficient WBB6F1-*W/W*^{v} mice; +, normal WBB6F1-+/+ littermates. $^*p < 0.05$ vs. mast cell+ and $^\#p < 0.05$ vs. alum alone and RP alone (Tukey's test). (B) Effects of terfenadine on the ocular scratching in ICR mice. Terfenadine (30 mg/kg) and vehicle (0.5% carboxymethyl cellulose) were administered orally 1 h before challenge. $^*p < 0.05$ vs. vehicle (Bonferroni's method). (C) Effects of terfenadine on plasma extravasation in ICR mice. Evans blue was injected intravenously into mice immunized with RP and alum, and 20 min later RP allergen or phosphate-buffered saline (PBS) was injected subconjunctivally; the amount in the treated eyelid was determined 20 min after challenge. Terfenadine (30 mg/kg) and the vehicle were administered orally 1 h before challenge. $^\#p < 0.05$ vs. PBS and $^*p < 0.05$ vs. RP without terfenadine (Tukey's test). The number of animals per group was 8 (A), 8–12 (B), or 6 (C).

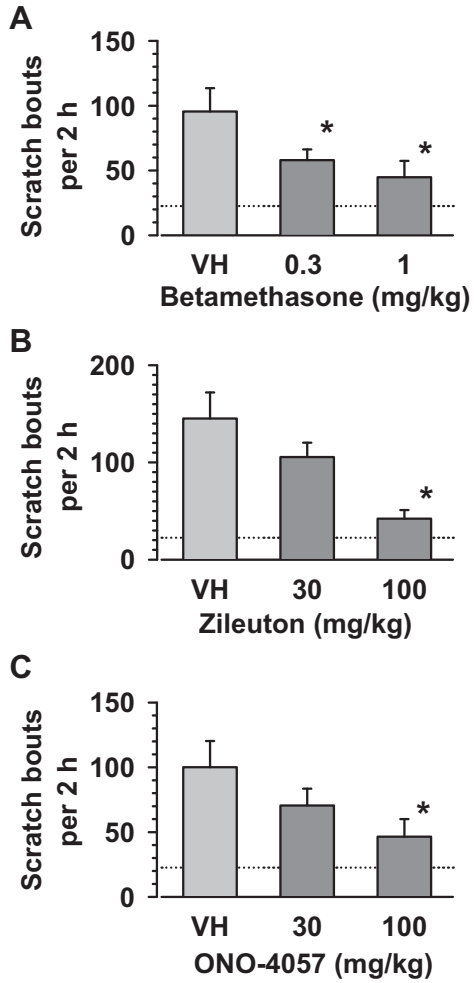

Fig. 4. Effects of a glucocorticoid, 5-lipoxygenase inhibitor, and LTB_4 receptor antagonist on RP allergen-induced ocular scratching in sensitized ICR mice. Betamethasone (A), zileuton (B), ONO-4057 (C), and vehicle (VH, 0.5% carboxymethyl cellulose) were administered orally 1 h before the subconjunctival injection of RP allergen in mice sensitized with RP and alum. Dotted lines denote the number of scratching bouts after allergen injection in naïve ICR mice. Values represent the mean and SEM of eight animals. $^*p < 0.05$ compared with VH (Dunnett's test).

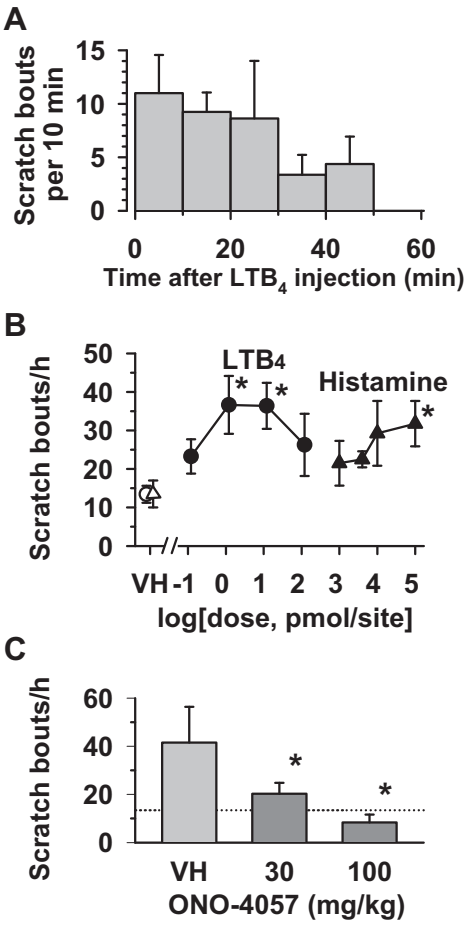

Fig. 5. Ocular scratching after subconjunctival injections of LTB_4 and histamine in naïve ICR mice. (A) Time course of scratching after the injection of LTB_4 (1.2 pmol/site). (B) Dose-response curves for LTB_4 and histamine. LTB_4 (closed circles), histamine (closed triangles), vehicle (VH) for LTB_4 (2% ethanol in saline, open circle), and VH for histamine (saline, open triangle) were injected subconjunctivally. Values represent the mean and SEM of seven to eight animals. $^*p < 0.05$ compared with the corresponding VH (Dunnett's test). (C) The effects of the LTB_4 receptor antagonist ONO-4057. ONO-4057 was administered orally 1 h before the injection of LTB_4 (1.2 pmol/site). A dotted line denotes the number of scratching bouts after subconjunctival VH injection. Values represent the mean and SEM of eight animals. $^*p < 0.05$ compared with VH (Dunnett's test).

There was a tendency toward increased ocular scratching at 0.12 pmol/site, and significant increases at 1.2 and 12 pmol/site; however, the effect of a 120 pmol/site dose was less than that of 12 pmol/site (Fig. 5B). The effective doses of LTB_4 were 1/10,000 or less than that (100 nmol/site) required for histamine (Fig. 5B). Oral pretreatment with ONO-4057 (30 and 100 mg/kg) significantly and dose-dependently inhibited ocular scratching induced by LTB_4 at 1.2 pmol/site; near complete inhibition was observed at 100 mg/kg (Fig. 5C).

3.5. LTB_4 production

A subconjunctival injection of RP allergen markedly increased LTB_4 content in the conjunctiva and subconjunctiva of ICR mice immunized with RP and alum, although it had no effects in non-sensitized mice (Fig. 6). LTB_4 production induced by RP allergen challenge was significantly and almost completely inhibited by oral pretreatment with betamethasone (1 mg/kg) and zileuton (100 mg/kg) (Fig. 6).

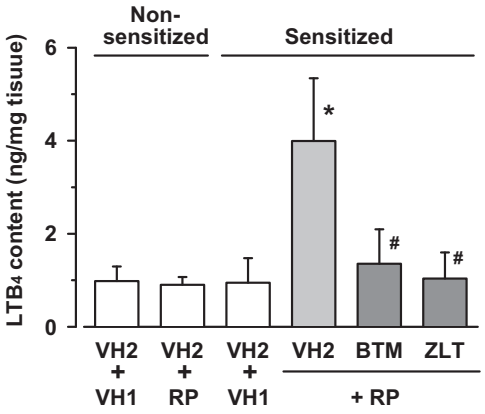

Fig. 6. The production of LTB_4 after RP allergen challenge in the ocular skin including conjunctiva in ICR mice. RP allergen or vehicle (VH1, phosphate-buffered saline) was administered to the subconjunctiva in sensitized and naïve (non-sensitized) mice; the ocular skin including conjunctiva were removed 5 min later to determine the LTB_4 contents. Betamethasone (BTM, 1 mg/kg), zileuton (ZLT, 100 mg/kg), and vehicle (VH2, 0.5% carboxymethyl cellulose) were administered orally 1 h before allergen challenge. The content of LTB_4 was determined using an enzyme immunoassay (see text). Values represent the mean and SEM of four to six animals. $^*p < 0.05$ compared with VH1 + VH2 in the sensitized group (Dunnett's test).

4. Discussion

The results of the present study show that subconjunctival challenge with RP allergen elicited ocular scratching with the hind-paw in sensitized mice. The challenge did not elicit ocular wiping with the forelimb. Intradermal injections of pruritogen (histamine) and algogen (capsaicin) into the murine cheek elicit hind-paw scratching (i.e., an itch-related response) and forelimb wiping (i.e., a pain-related response) at the injection site, respectively (Shimada and LaMotte, 2008). Therefore, the present results suggest that subconjunctival allergen injection induces itch but not pain in sensitized mice.

The present study demonstrated that ocular challenge with RP allergen elicited hind-paw scratching in mice immunized with allergen and the adjuvant alum, but not in mice immunized with allergen alone. Ocular scratching peaked within the first 10-min period and subsided by 1 h, and it was almost abolished by mast cell deficiency; these results suggest that it is due to immediate ocular allergy. When RP adheres to the conjunctiva, antigenic components may be eluted in tears, permeating the conjunctiva and causing allergic reactions in sensitized individuals. In this study, topical instillation of RP allergen to the eye did not increase scratching. This result is concordant with a previous report, in which topical instillation of 40 μg ragweed to the eye did not elicit scratching in sensitized mice although it caused mast cell degranulation (Shii et al., 2009). It was also reported that topical instillation of ovalbumin elicits scratching in ovalbumin-sensitized mice (Shii et al., 2009). In RP-sensitized mice, topical instillation with RP allergen causes moderate eyelid edema (Magone et al., 1998) and increases plasma extravasation by approximately 2.5 times (Shii et al., 2009), while subconjunctival challenge produced an approximately 30-fold increase in the present experiment. Tight junctions connect conjunctival epithelial cells and function as a barrier to allergen penetration (Irkec and Bozkurt, 2003). Thus, the conjunctiva may act as a mechanical barrier to RP allergen, and the amount of RP allergen permeating the conjunctiva might not be sufficient to cause itching in sensitized mice.

Although the ocular scratching induced by RP allergen injection was almost abolished by mast cell deficiency, it was only partially inhibited by the H_1 histamine receptor antagonist terfenadine at

a dose that almost completely inhibited the challenge-induced plasma extravasation; this raises the possibility that mast cell mediator(s) other than histamine are also involved in the itching of ocular allergy. The LTB_4 receptor antagonist ONO-4057 inhibited ocular scratching induced by RP allergen challenge at doses similar to those that suppressed LTB_4-induced ocular scratching; this suggests that LTB_4 plays a role in itching in our model of ocular allergy. Zileuton and betamethasone also inhibited RP allergen challenge-induced ocular scratching at doses that suppressed the LTB_4 production induced by the challenge; this also suggests that LTB_4 is involved in itching in our model of ocular allergy.

A subconjunctival injection of LTB_4 significantly increased ocular scratching at 1.2 and 12 pmol/site in naïve mice. These doses were roughly similar to doses at which intradermal injection of LTB_4 elicits cutaneous scratching in naïve mice (Andoh and Kuraishi, 1998), and 1/10,000 or less than that of subconjunctival injection of histamine. Thus, LTB_4 is a potent itch mediator in the conjunctiva as well as the skin.

The present results did not provide information concerning the primary site of pruritogenic action of LTB_4. There are two LTB_4 receptor subtypes: BLT1 and BLT2; they are G-protein-coupled receptors and have high and low binding affinities for LTB_4, respectively (Yokomizo et al., 1997, 2000). BLT1 mRNA is predominantly expressed in leukocytes in humans and mice (Tager and Luster, 2003). However, the distribution of BLT receptors in the eye and trigeminal ganglion neurons is unknown. BLT1, but not BLT2, mRNA is expressed in the dorsal root ganglia and skin in mice (Andoh and Kuraishi, 2005). LTB_4 increases intracellular Ca^{2+} concentration in cultured murine dorsal root ganglion neurons, approximately half of which also responds to capsaicin, suggesting the expression of BLT1 receptor in TRPV1 channel-expressing sensory neurons (Andoh and Kuraishi, 2005). Therefore, the trigeminal sensory neurons are a possible site of LTB_4 action.

Subconjunctival challenge with RP allergen increased the LTB_4 concentration in the ocular skin and conjunctiva in sensitized mice, which was inhibited by zileuton. These results are concordant with those of a previous report in which ovalbumin challenge increased LTB_4 concentration in guinea-pig conjunctiva (Garceau et al., 1987). LTB_4 is derived from arachidonic acid via the catalytic actions of 5-lipoxygenase and LTA_4 hydrolase (Murphy and Gijón, 2007). The distributions of these enzymes in the eye are unknown. However, the fact that the ocular scratching induced by RP allergen challenge was almost abolished by mast cell deficiency in the present study permits us to speculate that these cells play a key role in the production of pruritogenic LTB_4 in the allergic eye. In this context, stimulation of mast cells, including the IgE-mediated activation, causes the production of LTB_4 (Miyahara et al., 2009; Yamashita et al., 2000). Mast cells express H_4 histamine receptors (Lippert et al., 2004), the stimulation of which results in Ca^{2+} mobilization from intracellular Ca^{2+} stores (Hofstra et al., 2003) and the production of LTB_4 (Takeshita et al., 2003). Thus, histamine released from mast cells may act in an autocrine/paracrine manner to produce LTB_4, thus enhancing itching.

Repeated immunization with RP alone significantly increased serum concentration of total IgG_1 but not IgE. There was a tendency toward increased ocular scratching after RP allergen challenge in ICR and WBB6F1-++ mice immunized with RP alone and the tendency was almost abolished in WBB6F1-W^v mice. In our preliminary experiments, intradermal injection of RP allergen markedly increased scratching in ICR and WBB6F1-++ mice immunized with RP alone and the increase was partially but significantly inhibited by mast cell deficiency. Although the first mosquito bites do not increase scratching, repeated bites increase scratching in ICR mice, in which serum concentration of total IgG_1 increases and there is a tendency toward increased concentration of total IgE (Ohtsuka et al., 2001). Scratching of mosquito-bitten skin is not inhibited by terfenadine at a dose that almost abolishes mosquito bite-induced increase in plasma extravasation (Ohtsuka et al., 2001). Thus, mechanisms of allergic itch and the role of mast cell mediators appear to be different between the conjunctiva and skin. Although the precise causes of the difference are unknown, it is possible that IgG_1 is involved in itching of cutaneous but not ocular allergy. In this context, the high-affinity IgG receptor FcγRI is present in the primary afferent fibers in the skin, and RP allergen binds to cutaneous nerve fiber in mice immunized with RP alone (Andoh and Kuraishi, 2004).

In summary, we developed a mouse model of ocular itching of immediate pollen allergy that critically depends on mast cells. We confirmed the role of histamine, a mast cell mediator, and demonstrated the involvement of LTB_4, another mast cell mediator, in the ocular itching due to pollen allergy. H_1 antagonists with inhibitory effect on the action and/or production of LTB_4 may have more potent anti-pruritic activity than selective H_1 antagonists. The present results provide basic evidence for the idea that H_1 antagonists with mast cell-stabilizing action are superior to specific H_1 antagonists for the treatment of pruritic ocular allergy (Abelson et al., 2011).

References

Abelson, M.B., McLaughlin, J.T., Gomes, P.J., 2011. Antihistamines in ocular allergy: are they all created equal? Curr. Allergy. Asthma. Rep. 11, 205–211.

Akman, A., Irkec, M., Orhan, M., Erdener, U., 1998. Effect of lodoxamide on tear leukotriene levels in giant papillary conjunctivitis associated with ocular prosthesis. Ocul. Immunol. Inflamm. 6, 179–184.

Andoh, T., Kuraishi, Y., 1998. Intradermal leukotriene B_4, but not prostaglandin E_2, induces itch-associated responses in mice. Eur. J. Pharmacol. 353, 93–96.

Andoh, T., Kuraishi, Y., 2000. Involvement of blockade of leukotriene B_4 action in anti-pruritic effects of emedastine in mice. Eur. J. Pharmacol. 406, 149–152.

Andoh, T., Kuraishi, Y., 2002. Inhibitory effects of azelastine on substance P-induced itch-associated response in mice. Eur. J. Pharmacol. 436, 235–239.

Andoh, T., Kuraishi, Y., 2004. Direct action of immunoglobulin G on primary sensory neurons through Fc gamma receptor I. FASEB. J. 18, 182–184.

Andoh, T., Kuraishi, Y., 2005. Expression of BLT1 leukotriene B_4 receptor on the dorsal root ganglion neurons in mice. Brain. Res. Mol. Brain. Res. 137, 263–266.

Andoh, T., Kuraishi, Y., 2006. Suppression by bepotastine besilate of substance P-induced itch-associated responses through the inhibition of the leukotriene B_4 action in mice. Eur. J. Pharmacol. 547, 59–64.

Andoh, T., Nishikawa, Y., Yamaguchi-Miyamoto, T., Nojima, H., Narumiya, S., Kuraishi, Y., 2007. Thromboxane A_2 induces itch-associated responses through TP receptors in the skin in mice. J. Invest. Dermatol. 127, 2042–2047.

Andoh, T., Saito, A., Kuraishi, Y., 2009. Leukotriene B_4 mediates sphingosylphosphorylcholine-induced itch-associated responses in mouse skin. J. Invest. Dermatol. 129, 2854–2860.

Andoh, T., Haza, S., Saito, A., Kuraishi, Y., 2011. Involvement of leukotriene B_4 in spontaneous itch-related behaviour in NC mice with atopic dermatitis-like skin lesions. Exp. Dermatol. 20, 894–898.

Bundoc, V.G., Keane-Myers, A., 2003. Animal models of ocular allergy. Curr. Opin. Allergy. Clin. Immunol. 3, 375–379.

del Cuvillo, A., Sastre, J., Montoro, J., Jáuregui, I., Dávila, I., Ferrer, M., Bartra, J., Mullol, J., Valero, A., 2009. Allergic conjunctivitis and H_1 antihistamines. J. Investig. Allergol. Clin. Immunol. 19, 11–18.

Fujishima, H., Fukagawa, K., Takano, Y., Okamoto, S., Nakagawa, Y., Uchio, E., Yokoi, N., Fukushima, A., Takamura, E., 2006. The early efficacy of topical levocabastine in patients with allergic conjunctivitis. Allergol. Int. 55, 301–303.

Fukushima, Y., Nabe, T., Mizutani, N., Nakata, K., Kohno, S., 2003. Multiple cedar pollen challenge diminishes involvement of histamine in allergic conjunctivitis of guinea pigs. Biol. Pharm. Bull. 26, 1696–1700.

Garceau, D., Ford-Hutchinson, A.W., Charleson, S., 1987. 5-Lipoxygenase inhibitors and allergic conjunctivitis reactions in the guinea-pig. Eur. J. Pharmacol. 143, 1–7.

Groneberg, D.A., Bielory, L., Fischer, A., Bonini, S., Wahn, U., 2003. Animal models of allergic and inflammatory conjunctivitis. Allergy 58, 1101–1113.

Hofstra, C.L., Desai, P.J., Thurmond, R.L., Fung-Leung, W.P., 2003. Histamine H_4 receptor mediates chemotaxis and calcium mobilization of mast cells. J. Pharmacol. Exp. Ther. 305, 1212–1221.

Inagaki, N., Nagao, M., Igeta, K., Kawasaki, H., Kim, J.F., Nagai, H., 2001. Scratching behavior in various strains of mice. Skin. Pharmacol. Appl. Skin. Physiol. 14, 87–96.

Irkec, M., Bozkurt, B., 2003. Epithelial cells in ocular allergy. Curr. Allergy. Asthma. Rep. 3, 352–357.
Kato, M., Kurose, T., Oda, T., Miyaji, S., 2003. The role of platelet activating factor and the efficacy of apafant ophthalmic solution in experimental allergic conjunctivitis. J. Ocul. Pharmacol. Ther. 19, 315–324.
Lippert, U., Artuc, M., Grutzkau, A., Babina, M., Guhl, S., Haase, I., Blaschke, V., Zachmann, K., Knosalla, M., Middel, P., Krüger-Krasagakis, S., Henz, B.M., 2004. Human skin mast cells express H_2 and H_4, but not H_3 receptors. J. Invest. Dermatol. 123, 116–123.
Magone, M.T., Chan, C.C., Rizzo, L.V., Kozhich, A.T., Whitcup, S.M., 1998. A novel murine model of allergic conjunctivitis. Clin. Immunol. Immunopathol. 87, 75–84.
Miyahara, N., Ohnishi, H., Miyahara, S., Takeda, K., Matsubara, S., Matsuda, H., Okamoto, M., Loader, J.E., Joetham, A., Tanimoto, M., Dakhama, A., Gelfand, E.W., 2009. Leukotriene B_4 release from mast cells in IgE-mediated airway hyperresponsiveness and inflammation. Am. J. Respir. Cell. Mol. Biol. 40, 672–682.
Murphy, R.C., Gijón, M.A., 2007. Biosynthesis and metabolism of leukotrienes. Biochem. J. 405, 379–395.
Nagaki, Y., Hayasaka, S., Kadoi, C., 1999. Cataract progression in patients with atopic dermatitis. J. Cataract. Refract. Surg. 25, 96–99.
Nakano, Y., Takahashi, Y., Ono, R., Kurata, Y., Kagawa, Y., Kamei, C., 2009. Role of histamine H_4 receptor in allergic conjunctivitis in mice. Eur. J. Pharmacol. 608, 71–75.
Ohtsuka, E., Kawai, S., Ichikawa, T., Nojima, H., Kitagawa, K., Shirai, Y., Kamimura, K., Kuraishi, Y., 2001. Roles of mast cells and histamine in mosquito bite-induced allergic itch-associated responses in mice. Jpn. J. Pharmacol. 86, 97–105.
Paus, R., Schmelz, M., Biro, T., Steinhoff, M., 2006. Frontiers in pruritus research: scratching the brain for more effective itch therapy. J. Clin. Invest. 116, 1174–1186.
Rosario, N., Bielory, L., 2011. Epidemiology of allergic conjunctivitis. Curr. Opin. Allergy. Clin. Immunol. 11, 471–476.
Sanchis-Merino, M.E., Montero, J.A., Ruiz-Moreno, J.M., Rodriguez, A.E., Pastor, S., 2008. Comparative efficacy of topical antihistamines in an animal model of early phase allergic conjunctivitis. Exp. Eye. Res. 86, 791–797.
Shii, D., Oda, T., Shinomiya, K., Katsuta, O., Nakamura, M., 2009. Cyclosporine A eye drops inhibit the early-phase reaction in a type-I allergic conjunctivitis model in mice. J. Ocul. Pharmacol. Ther. 25, 321–328.
Shimada, S.G., LaMotte, R.H., 2008. Behavioral differentiation between itch and pain in mouse. Pain 139, 681–687.
Tager, A.M., Luster, A.D., 2003. BLT1 and BLT2: the leukotriene B_4 receptors. Prostaglandins. Leukot. Essent. Fatty. Acids. 69, 123–134.
Takeshita, K., Sakai, K., Bacon, K.B., 2003. Critical role of histamine H_4 receptor in leukotriene B_4 production and mast cell-dependent neutrophil recruitment induced by zymosan in vivo. J. Pharmacol. Exp. Ther. 307, 1072–1078.
Tsuji, F., Aono, H., Tsuboi, T., Murakami, T., Enomoto, H., Mizutani, K., Inagaki, N., 2010. Role of leukotriene B_4 in 5-lipoxygenase metabolite- and allergy-induced itch-associated responses in mice. Biol. Pharm. Bull. 33, 1050–1053.
Tsukumo, Y., Harada, D., Manabe, H., 2010. Pharmacological characterization of itch-associated response induced by repeated application of oxazolone in mice. J. Pharm. Sci. 113, 255–262.
Woodward, D.F., Nieves, A.L., Friedlaender, M.H., 1996. Characterization of receptor subtypes involved in prostanoid-induced conjunctival pruritus and their role in mediating allergic conjunctival itching. J. Pharmacol. Exp. Ther. 279, 137–142.
Yamashita, M., Kushihara, M., Hirasawa, N., Takasaki, W., Takahagi, H., Takayanagi, M., Ohuchi, K., 2000. Inhibition by troglitazone of the antigen-induced production of leukotrienes in immunoglobulin E-sensitized RBL-2H3 cells. Br. J. Pharmacol. 129, 367–373.
Yokomizo, T., Izumi, T., Chang, K., Takuwa, Y., Shimizu, T., 1997. A G-protein-coupled receptor for leukotriene B_4 that mediates chemotaxis. Nature 387, 620–624.
Yokomizo, T., Kato, K., Terawaki, K., Izumi, T., Shimizu, T., 2000. A second leukotriene B_4 receptor, BLT2. A new therapeutic target in inflammation and immunological disorders. J. Exp. Med. 192, 421–432.