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Oral administration of a T cell epitope inhibits symptoms and reactions of allergic rhinitis in Japanese cedar pollen allergen-sensitized mice

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Abstract

Although the concept of a T cell epitope in specific immunoprophylaxis was proposed more than a decade ago, it had not been well demonstrated since then that a T cell epitope inhibits symptoms and reactions of allergic disease in animal models. In this study, we have established a system to evaluate symptoms and reactions of allergic rhinitis in mice, and investigated whether oral administration of a T cell epitope relieves sensitized mice of allergic rhinitis. P2-246-259 (RAEVSYVHVNGAKF) is a BALB/c mouse T-cell epitope of Cry j 2, which is a major Japanese cedar (*Cryptomeria japonica*) pollen allergen. Mice were administered orally with 200 μg/animal of P2-246-259 four times within 2 weeks before sensitization, and sensitized intranasally with Cry j 2 twice. Of the cardinal symptoms of allergic rhinitis, we assessed sneezing and airway obstruction, but could not estimate rhinorrhea or pruritus. Sneezing frequency was significantly increased by challenge with Cry j 2. Concerning allergic reactions, vascular permeability of the nasal mucosa in the early phase and hyperreactivity to histamine in the late phase were also exacerbated by the challenge. These symptoms and reactions of allergic rhinitis were significantly inhibited by oral administration of P2-246-259. These results indicate utility of mice as models for allergic rhinitis; furthermore, the effects of P2-246-259 on allergic rhinitis imply that oral administration of a T cell epitope is a promising approach for specific immunoprophylaxis.

Keywords: Cry j 2; Pollinosis; Oral tolerance; Immunoprophylaxis; T cell epitope

1. Introduction

Although the strategy to cure allergic diseases is based on pharmacological therapy with the general principles of patient education and environmental control (WHO Position Paper, 2003), the only curative treatment in certain cases of allergic rhinitis and asthma would be specific immunoprophylaxis (Frew, 2003). Recently, molecular biological techniques have led us to develop immunoprophylaxis strategies to reduce side effects, especially using a T cell epitope. The potential success of intradermally administered T cell epitopes has also been reported in patients with allergic reactions to bee venom (Müller et al., 1998; Fellrath et al., 2003), cats (Pene et al., 1998; Maguire et al., 1999; Oldfield et al., 2002) and birch pollen (van Hage-Hamsten et al., 2002). Nevertheless, the route of sublingual administration of T cell epitopes has not been tested in clinical trials so far. In animals, oral administration of allergens or T cell epitopes is well known to induce "oral tolerance" (Garside and Mowat, 2001; Strobel, 2002). This phenomenon is considered as the basis of the above immunoprophylaxis.

In experiments on oral tolerance, researchers have highlighted unresponsiveness of T cells in sensitized mice. Consequently, allergenic symptoms and reactions seem to have been overlooked. Although the utility of an allergen

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itself (van Oosterhout et al., 1998) and a T cell epitope (Janssen et al., 2000a,b) has been shown in an allergic asthma mouse model, no demonstration in an allergic rhinitis mouse model has been reported yet. This would originate from the difficulties of evaluating the symptoms and reactions of allergic rhinitis in mice compared to other species such as guinea pigs (Szelenyi et al., 2000). However, considering the ease of handling of mice and accumulated immunological data, we set out to establish a system to evaluate allergic rhinitis in mice, and furthermore, to investigate the effect of oral tolerance on allergic rhinitis in mice.

In Japan, there is a common disease which is known as Japanese cedar (*Cryptomeria japonica*) pollinosis. The morbidity of Japanese cedar pollinosis has gradually increased with more than 10% of the population suffering from this unpleasant disease (Hashimoto et al., 1995). Two major allergens were reported from Japanese cedar pollen, named Cry j 1 (Yasueda et al., 1983; Sone et al., 1994) and Cry j 2 (Sakaguchi et al., 1990; Komiyama et al., 1994; Namba et al., 1994). Patients with the pollinosis show high immunoglobulin E (IgE) titer (Hashimoto et al., 1995) and high T cell reactivity against both allergens (Sugimura et al., 1996). We have reported previously that, in BALB/c mice, immunogenicity of Cry j 2 is stronger than that of Cry j 1, and the peptide P2-246-259 corresponds to a dominant T cell epitope of Cry j 2 (Hirahara et al., 1998). Using this peptide, we have also demonstrated that oral administration of P2-246-259 inhibits T cell responses in Cry j 2-sensitized mice (Hirahara et al., 1998; Yoshitomi et al., 2002).

In this study, we first established a system to evaluate symptoms and reactions of allergic rhinitis in Cry j 2-sensitized BALB/c mice, mentioned above. Among the many responses, we focused on frequency of sneezing, airway obstruction, vascular permeability of nasal mucosa and hyperreactivity to histamine. To our knowledge, this is the first report to investigate vascular permeability of the nasal mucosa in mice. Finally, we investigated whether oral administration of a T cell epitope inhibits not only T cell responses but also allergic symptoms and reactions of allergic rhinitis in this model.

2. Materials and methods

2.1. Animals

Six-week-old female BALB/c mice were purchased from Charles River Japan, Inc. (Yokohama, Japan), and housed under conventional conditions. All experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals in Sankyo.

2.2. Reagents

Cry j 2 glycoprotein was isolated from Japanese cedar pollen by affinity chromatography with a monoclonal antibody specific for Cry j 2 (N26) (Kawashima et al., 1992). Synthetic peptide P2-246-259 (RAEVSYVHVNGAKF), based on the Cry j 2 sequence, was synthesized by Peptide Institute, Inc. (Osaka, Japan) using standard F-moc chemistry and purified by high-performance liquid chromatography. Cholera toxin and histamine were purchased from Sigma-Aldrich Co. (St. Louis, MO), cholera toxin B subunit was from Research Biochemicals Intl. (Natick, MA) and Evan's blue was from Merck (Darmstadt, Germany).

2.3. Induction of oral tolerance

Mice were dosed with 200 μg of P2-246-259 dissolved in 0.2 ml of phosphate-buffered saline (PBS) by gastric intubation with a plastic animal-feeding needle. This oral administration was repeated a total of 4 times, once each on Days 14, 11, 7 and 4 (Fig. 1) before sensitization. To investigate the effect of the peptide on allergic rhinitis, the mice were bilaterally intranasally sensitized with 1.75 μl immunizing solution per nostril on Days 0 and 14. The immunizing solution consisted of 1 μg of Cry j 2, 1 μg of cholera toxin B subunit and 25 ng of cholera toxin in 3.5 μl of PBS. After being housed for another 7 days, the sensitized mice were bilaterally intranasally challenged with 6.5 μl Cry j 2 solution per nostril on Day 22, and tested for allergic rhinitis. The solution contained 5 μg of Cry j 2 in 13 μl of PBS. In some groups, only PBS was administered to mice instead of oral administration of P2-246-259 and/or challenge with Cry j 2.

2.4. Sneezing frequency in early phase

In guinea pigs, sneezing is easily counted as sounds. Since sneezing in mice is inaudible, we counted each sneezing action accompanied with a tremble, which was similar to that of guinea pigs. Such actions seem to reflect sneezing, because anti-interleukin-5 antibody (Asakura et al., 1998) and histamine blockers (our unpublished data) inhibited the frequency. Immediately after intranasal challenge with Cry j 2 on Day 22, sneezing frequency was counted by sight for 5 min in an unrestrained whole body plethysmograph.

2.5. Airway obstruction in early phase

Responsiveness to Cry j 2 in airway obstruction was determined in conscious and unrestrained mice using barometric plethysmography to measure enhanced pause (Penh). This parameter is a unitless measure that has been shown to correlate with the changes in airway resistance

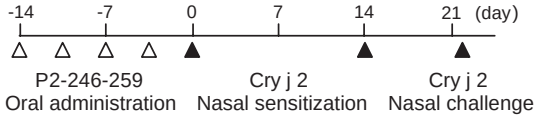

Fig. 1. Experimental protocols.

(Hamelmann et al., 1997). On Day 21, Penh before challenge was measured in an unrestrained whole body plethysmograph for 10 min. The plethysmography system consisted of an amplifier, a flow regulator, a chamber and software (Buxco Electronics, Inc., Wilmington, NC). On Day 22, 13 min after challenge, Penh was measured for 10 min. The averaged Penh data after challenge was normalized using the value before challenge.

2.6. Vascular permeability of nasal mucosa in early phase

We measured the amount of extravasated Evan's blue to evaluate vascular permeability of the nasal mucosa according to the method in guinea pigs (Mizutani et al., 1999) with some modifications. In only this experiment, the sensitized mice were intravenously injected with 2 mg/ml of the dye immediately before challenge on Day 22. The injected volume was 1 ml/25 g of body weight, and the volume induced no side effects. Thirty minutes after the challenge, the mice were sacrificed, and perfused with about 10 ml of saline via the heart. The removed nasal cavity without skin and muscle was incubated in 0.15% Na_2SO_4 containing 70% (w/w) of acetone for 2 days. The extract solution was filtered through cotton, and the absorbance at 620 nm was measured with a spectrometer.

2.7. Airway obstruction and hyperreactivity to histamine in late phase

On Day 23, about 18 h after challenge, Penh was again measured in the late phase for 10 min to check for airway obstruction. After this, 50 mM of histamine was intranasally administered to the challenged mice, and Penh was measured for another 10 min. After that, 500 mM of histamine was sequentially administered, and Penh was estimated again in the same way. Histamine solution was administered bilaterally by 5 μl per nostril (total of 0.5 and 5 μmol, respectively).

2.8. Statistical analysis

All data in this study are expressed as means±S.E.M. Significant differences of data were calculated by Tukey's multiple range test after analysis of variance or *t*-test. Probability values of <0.05 were considered to represent significant differences.

3. Results

3.1. Sneezing frequency in early phase

Sneezing, itching and rhinorrea are characteristic symptoms during early phase allergic rhinitis, and some degree of nasal congestion is also observed (Skoner, 2001). Therefore, we first investigated the former three symptoms in Cry j 2-sensitized mice. Sneezing frequency was counted for 5 min immediately after challenge with Cry j 2. The challenge significantly increased sneezing frequency from 2.3±0.7 (intranasally treated with only PBS) to 13.8±3.6 times/5 min (Fig. 2). Oral administration of P2-246-259 itself did not affect the frequency; however, it almost completely reduced the sneezing frequency to the basal level in Cry j 2-challenged mice. While counting sneezing frequency, we also checked whether mice showed itching behavior such as rubbing the nose. Although grooming behavior was observed in all groups, no mice particularly rubbed their nose (data not shown). Concerning rhinorrhea, in preliminary experiments using guinea pigs, it was possible to directly evaluate the amount of rhinorrhea by weighing a paper string inserted into the nostril. Due to the small amount of rhinorrhea, however, this method is impractical in mice (data not shown).

3.2. Airway obstruction in early phase

A parameter of airway obstruction, Penh, was measured in an unrestrained conscious state 13 min after challenge with Cry j 2. The challenge showed an increase in Penh (to 140.3±14.7% of control), but did not reach statistical significance (Fig. 3). In addition, other parameters such as tidal volume and breathing frequency did not change (data not shown). Penh in a P2-246-259-administered group was 102.0±8.5%.

3.3. Vascular permeability of the nasal mucosa in early phase

Challenge with an allergen triggers release of chemical mediators such as histamine, leukotriene and serotonin from

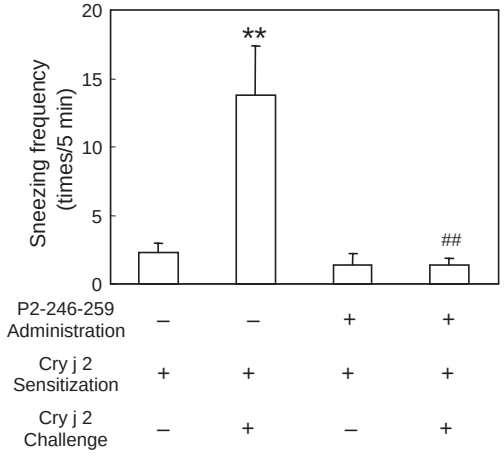

Fig. 2. Oral administration of P2-246-259 inhibits increase in sneezing frequency induced by challenge with Cry j 2. Each column from the left corresponds to the following groups: group A, PBS-administered, Cry j 2-sensitized and PBS-challenged mice; group B, PBS-administered, Cry j 2-sensitized and Cry j 2-challenged mice; group C, P2-246-259-administered, Cry j 2-sensitized and PBS-challenged mice; and group D, P2-246-259-administered, Cry j 2-sensitized and Cry j 2-challenged mice. $^{**}P<0.01$ vs. group A, $^{\#\#}P<0.01$ vs. group B, n=5–10.

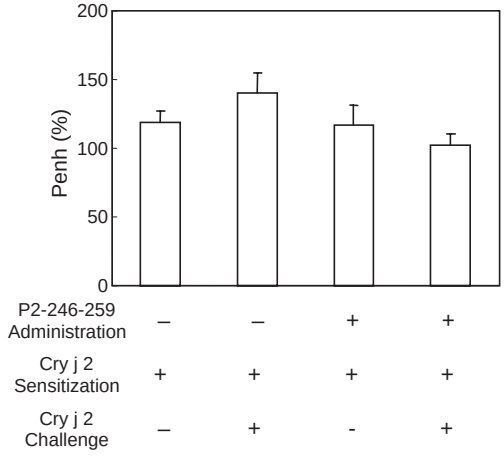

Fig. 3. Oral administration of P2-246-259 shows a tendency to inhibit increase in Penh induced by challenge with Cry j 2. n=5. Other legends are as in Fig. 2.

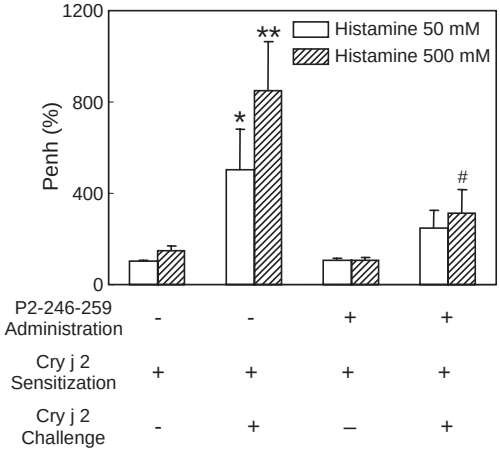

Fig. 5. Oral administration of P2-246-259 inhibits increase in Penh induced by challenge with histamine. Open and hatched columns indicate Penh challenged with 50 and 500 mM histamine, respectively. *P<0.05, **P<0.01 vs. group A, $^{\#}P$<0.05 vs. group B, n=5. Other legends are as in Fig. 2.

mast cells. Among them, histamine is reported to induce nasal obstruction by allergic reactions such as increased vascular permeability and vasodilation (Togias, 2003). Because airway obstruction increased after challenge with Cry j 2, we investigated whether vascular permeability of the nasal mucosa was increased after challenge. The permeability was measured 30 min after challenge using Evan's dye extravasation as a parameter. Challenge with Cry j 2 caused significant increase in dye extravasation in Cry j 2-challenged mice (14.0±1.0 μg/site) compared to PBS-challenged mice (4.3±0.4 μg/site) (Fig. 4). In the same way as for sneezing frequency, oral administration of P2-246-259 significantly inhibited the increase induced by challenge with Cry j 2.

3.4. Airway obstruction and hyperreactivity to histamine in late phase

During late phase allergic rhinitis, chemoattractant cytokines play a key role, and airway obstruction becomes a main symptom. Therefore, we measured airway obstruction again about 18 h after challenge with Cry j 2, but no significant increase in Penh in Cry j 2-challenged mice was observed (109.1±10.2%). In an attempt to study allergic reactions in the late phase of this model, we finally investigated the mucosal hyperreactivity. Histamine was sequentially administered to nostrils at the concentrations of 50 and 500 mM, and Penh was measured for 10 min immediately after each challenge of histamine. We found that sensitivity to histamine was significantly increased by nasal challenge with Cry j 2 (Fig. 5). At the challenge concentration of 500 mM, oral administration of P2-246-259 significantly inhibited the hyperreactivity.

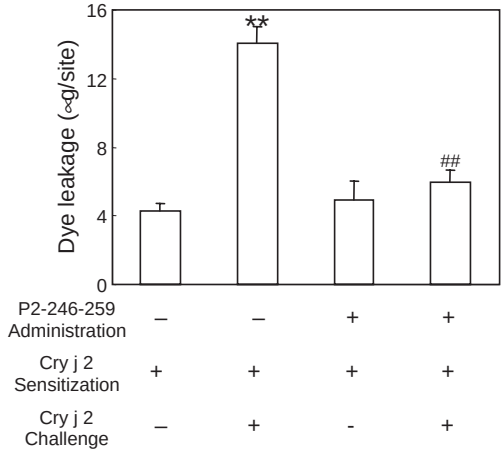

Fig. 4. Oral administration of P2-246-259 inhibits extravasation of dye in nasal mucosa induced by challenge with Cry j 2. n=7. Other legends are as in Fig. 2.

4. Discussion

In this study, we have succeeded in establishing a system to evaluate symptoms and reactions of allergic rhinitis in Cry j 2-sensitized BALB/c mice. Among the tested responses, sneezing frequency, vascular permeability of the nasal mucosa and hyperreactivity to histamine were significantly increased by challenge with Cry j 2. Furthermore, oral administration of a T cell epitope of Cry j 2 inhibited the symptom of sneezing and the reactions of vascular permeability and hyperreactivity in Cry j 2-sensitized mice. Previously, we demonstrated that oral administration of a T cell epitope inhibits proliferative responses of T cells, anti-Cry j 2 IgE antibody and cytokine production in a Cry j 2-sensitized mouse model (Hirahara et al., 1998). Furthermore, the administration after sensitization of Cry j 2 was effective in inhibition proliferative responses of T cells. These series of ex vivo and in vivo data suggest the utility of a T cell epitope in immunoprophylaxis of allergic rhinitis.

Allergic rhinitis is clinically characterized by symptoms such as sneezing, rhinorrhea, pruritus of the nose and eyes, and nasal obstruction (Naclerio, 1991). Sneezing was observed immediately after challenge with Cry j 2 in our model, and this immediate appearance correlates with data from patients with allergic rhinitis in a nasal allergen challenge test (Wang et al., 1995). We counted sneezing frequency for 5 min immediately after challenge, because the released amount of histamine reaches a plateau 5 min after allergen challenge in mast cells of human nasal mucosa (Otsuka et al., 1995). Furthermore, only two-time nasal sensitization was enough to trigger significant increase in sneezing frequency, therefore, our shortened protocol would allow prompt evaluation of drugs for allergic rhinitis. Concerning rhinorrea, as mentioned in the Results, it was impossible to measure the small amount in mice. The other parameter of early phase symptoms, itching behavior, also was not observed under our experimental conditions. However, Asakura et al. (1998) reported that, in addition to sneezing, nasal scratching movements immediately after challenge is a good parameter in intraperitoneally ovalbumin-sensitized BALB/c mice. Because pruritus immediately after challenge is mainly induced by histamine release in the same way as sneezing (White, 1990), this raises the possibility that itching behavior could also become a good parameter to add to our model. Early and late phase airway obstruction is mainly mediated by chemical mediators and inflammatory factors, respectively. Although no significant increase in Penh was observed in either phase, a subtle increase in the early phase should be further assessed to establish a more complete mouse model to show most of the symptoms of allergic rhinitis. In addition, the component of nasal obstruction would much contribute to the airway obstruction, because the nasally administered Evan's blue solution of 5 μl did not reach the larynx (data not shown). In terms of reactions of allergic rhinitis, we evaluated early phase vascular permeability of the nasal mucosa and late phase hyperreactivity to histamine. Evan's dye extravasation approximately tripled by challenge with Cry j 2. This increase would be sufficient to serve as a parameter, since it has been reported that challenge with histamine induced about 1.5 times as much extravasation as saline-challenged guinea pigs (Mizutani et al., 1999), which are the most commonly used species to evaluate allergic rhinitis. Concerning hyperreactivity, the applied concentrations of histamine were high compared to the common threshold in guinea pigs, but hyperreactivity to histamine would be also useful for assessing reactions of allergic rhinitis in mice.

In this study, we have demonstrated that oral administration of a T cell epitope significantly inhibits sneezing frequency and vascular permeability of the nasal mucosa in Cry j 2-sensitized mice, possibly by the mechanisms of oral tolerance. Our previous report showed that repeated sensitization with Cry j 2 for 6 weeks increases Cry j 2-specific IgE titer (Hirahara et al., 1998), but the increase was not significant under the present conditions of 3 weeks after the first sensitization (data not shown). One explanation is that the increased level of the antibody was too small to be detected in our ELISA system. Although involvement of antigen-specific IgE triggering the above symptoms and reactions of allergic rhinitis is unclear, it is likely that hyperreactivity to chemical mediators released after allergic challenge contributes to the allergic rhinitis in this model. Indeed, hyperreactivity to histamine was also inhibited by oral administration of P2-246-259. This change in hyperreactivity would be elaborately regulated by cytokines released from subsets of T cells. We have reported previously that oral administration of a T cell epitope decreases levels of interferon-γ, interleukin-2 and interleukin-4 (Hirahara et al., 1998), and many reports also elucidate the involvement of cytokines in the development of allergic rhinitis (Asakura et al., 1998; Okano et al., 2000; Iwasaki et al., 2003). The procedures of presenting orally-administered allergen to T cells in Peyer's patches, modifying the balance of cytokines, and inducing tolerance in specific T cells, seem a most plausible explanation for oral tolerance (Garside and Mowat, 2001; Strobel, 2002), but its precise mechanisms such as clonal deletion (Chen et al., 1995), clonal anergy (Friedman and Weiner, 1994) and active suppression (Chen et al., 1994) still remain to be elucidated.

Our established mouse model would help in the research on allergic rhinitis immunologically from the point of view of symptoms and reactions, and furthermore, implies that not only Brown Norway rats and guinea pigs but also mice are available for evaluating allergic rhinitis (Szelenyi et al., 2000). Although the data concerning oral administration of a T cell epitope before sensitization is reported in this paper, we also confirmed that the oral administration after sensitization also inhibits symptoms of allergic rhinitis in a similar protocol (our unpublished data), supporting the concept that an orally administered T cell epitope is also a promising strategy for immunotherapy. The utility of immunoprophylaxis has been based on many clinical trials, but scientific evidence to support immunoprophylaxis has not been sufficient so far. Therefore, further studies linking clinical trials to animal experiments are certainly needed to achieve higher success in clinical trials.

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