



Establishment of an allergic rhinitis model in mice for the evaluation of nasal symptoms

Masako Tsunematsu*, Taketo Yamaji, Daisuke Kozutsumi, Rika Murakami, Shigeki Kimura, Kohsuke Kino

Research and Development Center, Division of Research and Development, Meiji Dairies Corporation, 540 Naruda, Odawara, Kanagawa 250-0862, Japan

Received 14 March 2006; accepted 21 December 2006

Abstract

The purpose of our study was to establish a new model of allergic rhinitis in mice, eliciting symptoms such as sneezing, infiltration of eosinophils into the nasal mucosa, and antigen-specific IgE production. One of the major human T-cell epitopes in Cry j 1, an allergen of Japanese cedar pollen, is also a major murine T-cell epitope in B10.S mice. Thus we tried to establish an allergic rhinitis model in B10.S mice with Cry j 1 as the antigen. We sensitized B10.S mice subcutaneously with Cry j 1/alum three times at intervals of 1 week. Five weeks after the final sensitization, we challenged the mice by instilling Cry j 1 intranasally from the day after intranasal histamine pretreatment. Soon after, we counted the number of sneezes. We then evaluated the infiltration of eosinophils into the nasal tissues and also measured the serum levels of antigen-specific IgE antibody. In addition, we confirmed the effects of ketotifen fumarate and dexamethasone hydrochloride on these animals. In Cry j 1-sensitized B10.S mice, sneezes, eosinophil peroxidase (EPO) activity in nasal tissues, and Cry j 1-specific IgE clearly increased after intranasal histamine pretreatment and 5 days of continuous intranasal Cry j 1 challenge. Both ketotifen and dexamethasone inhibited the increase in sneezing, and dexamethasone also inhibited EPO activity and Cry j 1-specific IgE. Thus we succeeded in establishing a new model of allergic rhinitis in Cry j 1-sensitized B10.S mice, which exhibited sneezing, eosinophil infiltration into the nasal mucosa, and Cry j 1-specific IgE production.

© 2007 Elsevier Inc. All rights reserved.

Keywords: Allergic rhinitis; Murine model; Cry j 1; Histamine

Introduction

The number of patients suffering from Japanese cedar (*Cryptomeria japonica*, or Cj) pollinosis has been increasing, and the prevalence has now reached 10 to 20% of the Japanese population (Savage and Roy, 2005; Okuda, 2003). Patients with Cj pollinosis have symptoms such as sneezing, watery rhinorrhea, and nasal blockage. Histological evaluation reveals a marked increase in eosinophils in the nasal submucosa and epithelium as well as chronic inflammation of the nasal mucosal membrane (Horak and Stubner, 1998; Igarashi et al., 1995). In addition to these allergen-specific symptoms, nasal hyperresponsiveness to nonspecific stimuli has been observed (Con-

nell, 1969). The majority of patients with allergic rhinitis are found to have high levels of allergen-specific IgE (Hashimoto et al., 1995). Many animal models for the study of allergic rhinitis have been reported (Nabe et al., 1998; Sato et al., 1999; Tamura et al., 1986; Kim et al., 2001; Murasugi et al., 2005). Murine models of allergic rhinitis are especially useful for investigating the immunological basis of this disease.

Two major allergens, Cry j 1 and Cry j 2, have been isolated from Cj pollen (Sone et al., 1994; Komiyama et al., 1994), and approximately 10 different T-cell epitopes in each allergen were found to be active in patients with Japanese cedar pollinosis (Sone et al., 1998). A major T-cell epitope has been identified in Cry j 1-sensitized B10.S mice (Kingsu and Saito, 1998; Kingsu et al., 2000), and this epitope sequence coincides with the most prevalent epitope in patients. Therefore B10.S mice should be good murine models for the study of patients with Cj pollinosis. However, to date, there has been no report of such a

* Corresponding author. Tel.: +81 465 37 3694; fax: +81 465 37 3624.

E-mail address: MASAKO.TSUNEMATSU@MEIJI-MILK.COM (M. Tsunematsu).

model. Therefore, in order to study the relevant immunological mechanisms of this disease and establish new therapeutic approaches, we developed a new murine model that mimics the major features of allergic rhinitis in humans.

On preliminary examination, continuous intranasal Cry j 1 challenges in Cry j 1-sensitized B10.S mice with alum adjuvant induced sneezing reflexes. Further, histamine is well-known as an important mediator in the initiation and the development of allergic reactions (Jutel et al., 2001). Therefore, because histamine in other studies was the most effective mediator for increasing nasal responses to other inflammatory mediators, we hypothesized that histamine pretreatment before continuous antigen challenges would increase the nasal responses.

In this study, we sought to establish a model of Cj pollinosis in B10.S mice with intranasal histamine and Cry j 1 challenges, hoping to parallel the rhinitis phenomenon in humans.

Materials and methods

Animals

Female B10.S mice were purchased from Japan SLC Inc. (Shizuoka, Japan). The animals were housed under controlled conditions of temperature ($21 \pm 2^\circ\text{C}$) and relative humidity ($55 \pm 15\%$), fed a standard diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan), and given water ad libitum. All experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals of Meiji Dairies Corporation.

Antigen

Cry j 1 was purified from Japanese cedar pollen by our group as described previously (Yasueda et al., 1983). The absence of Cry j 2 in purified Cry j 1 preparation was confirmed by Western blot analysis with two monoclonal antibodies (anti-Cry j 1 and anti-Cry j 2; Hayashibara Biochemical Laboratories Inc.). The purity of Cry j 1 determined by densitometric scanning after sodium dodecyl sulfate polyacrylamide gel electrophoresis and Coomassie brilliant blue staining was 88%.

Drugs

Histamine dihydrochloride was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Ketotifen fumarate (hereafter called ketotifen) and dexamethasone hydrochloride (dexamethasone) were purchased from Sigma-Aldrich (St Louis, MO).

Sensitization and nasal antigen challenge

Mice were sensitized with $10\text{ }\mu\text{g}$ of Cry j 1 mixed with 4 mg of aluminum hydroxide gel (Imject Alum, Pearce, Rockford, IL) by subcutaneous injection once a week for 3 consecutive weeks.

In order to investigate the effect of histamine pretreatment before continuous intranasal Cry j 1 challenges—5 weeks after final sensitization of Cry j 1 and prior to intranasal Cry j 1 challenge—histamine dihydrochloride [0.01 to $100\text{ }\mu\text{g/mL}$ in phosphate buffered saline (PBS)] or PBS only was dropped into

both nostrils ($10\text{ }\mu\text{L}$ per nostril) the day before the first intranasal antigen challenge. On the day after histamine pretreatment, $10\text{ }\mu\text{L}$ of Cry j 1 ($200\text{ }\mu\text{g/mL}$ in PBS) was dropped into each nostril, using a micropipette, for 5 consecutive days. Soon after the last intranasal antigen challenge, the number of sneezes was counted for 5 min.

To investigate the antigen specificity of the nasal response in our model, we composed the following three groups. Group 1: sensitized with Cry j 1+intranasally treated with histamine+intranasally challenged with PBS, Group 2: sensitized with Cry j 1+intranasally treated with histamine+intranasally challenged with Cry j 1. Group 3: no sensitization+intranasally treated with histamine+intranasally challenged with Cry j 1. In groups 1 and 2, five weeks after final sensitization of Cry j 1, mice were treated intranasally with histamine ($1\text{ }\mu\text{g/mL}$, $10\text{ }\mu\text{L}$ per nostril) and then intranasally challenged with PBS ($10\text{ }\mu\text{L}$ per nostril) in group 1 and Cry j 1 ($200\text{ }\mu\text{g/mL}$, $10\text{ }\mu\text{L}$ per nostril) in group 2, respectively, for 5 consecutive days. In group 3, nonsensitized mice were intranasally treated with histamine ($1\text{ }\mu\text{g/mL}$, $10\text{ }\mu\text{L}$ per nostril) and then intranasally challenged with Cry j 1 ($200\text{ }\mu\text{g/mL}$, $10\text{ }\mu\text{L}$ per nostril) for 5 consecutive days. Soon after the last intranasal challenge, the number of sneezes was counted for 5 min. Twenty-four hours after the last intranasal antigen challenge, the mice were killed and histologically examined; eosinophil peroxidase (EPO) activity in nasal tissues was also evaluated, and the serum level of Cry j 1-specific IgE was measured.

In addition, to determine the effectiveness of this system, the effects of ketotifen and dexamethasone on this model were evaluated. Five weeks after final sensitization with Cry j 1, mice were intranasally treated with histamine ($1\text{ }\mu\text{g/mL}$, $10\text{ }\mu\text{L}$ per nostril) and then intranasally challenged with Cry j 1 ($200\text{ }\mu\text{g/mL}$, $10\text{ }\mu\text{L}$ per nostril) for 5 consecutive days. Ketotifen (2 mg/kg , p.o.) and dexamethasone (1 mg/kg , p.o.) were administered orally 30 min and 1 h, respectively, prior to every intranasal antigen challenge. Soon after the last intranasal challenge, the number of sneezes were counted for 5 min. Twenty-four hours after the last intranasal antigen challenge, the mice were killed and both EPO activity in nasal tissues and the serum level of Cry j 1-specific IgE were evaluated.

Histological examination

For histological examination of the nasal mucosa, the head was fixed in 10% buffered formalin solution 24 h after the last intranasal antigen challenge. Then the head was severed between the upper and lower jaws and the facial skin stripped. The tip of the nose area was decalcified in Plank-Rychlo solution and embedded in paraffin. Frontal sections of the nasal tissues were stained with hematoxylin and eosin.

Measurement of eosinophil peroxidase (EPO) activity

Twenty-four hours after the last intranasal antigen challenge, the tip of the nose area was removed and homogenized with 5 mL of 0.5% hexadecyltrimethylammonium bromide (HTAB) in PBS. The homogenate was freeze-thawed three times and the

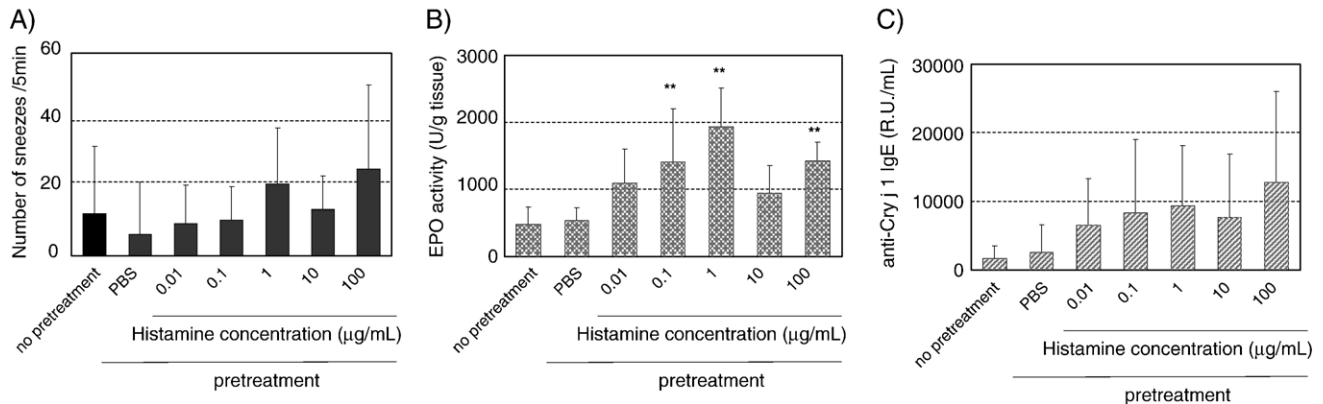


Fig. 1. Effects of histamine pretreatment on sneezing, EPO activity in nasal tissue, and antigen-specific IgE antibody induced by Cry j 1-administered for 5 consecutive days. Five weeks after the last subcutaneous Cry j 1 sensitization, 10 μ L of histamine (0.01–100 μ g/mL in PBS) was applied intranasally, and on the next day, the first nasal antigen challenge was given. A: the number of sneezes. B: EPO activities in nasal tissues. C: anti-Cry j 1 IgE antibody in serum measured by ELISA. Each value represents the mean $\pm$ S.D. of 8 mice. **: $p < 0.01$ (Dunnett's multiple comparison test), significantly different from PBS pretreatment group.

supernatant assayed for EPO after centrifugation. The supernatant (50 μ L) and substrate solution (16 mmol/L *o*-phenylenediamine and 0.01% H_2O_2 in 50 mmol/L Tris-HCL, pH 8.0) (100 μ L) were mixed and incubated for 15 min at room temperature. The reaction was stopped by adding 4 mol/L H_2SO_4 (50 μ L), and the optical density of the solution was read at 490 nm. Data were expressed in units of EPO activity, 1 unit being arbitrarily defined as the activity of 1×10^3 eosinophils isolated from guinea pigs.

Evaluation of serum level of Cry j 1-specific IgE

On the next day of the final intranasal antigen challenge, sera were obtained and the Cry j 1-specific IgE titer in serum was evaluated by enzyme-linked immunosorbent assay.

Briefly, 96-well plates (F16 Black Maxisorp, Nalge Nunc International) were coated with 100 μ L of purified rat antimouse IgE monoclonal antibody (50 μ g/mL, BD Bioscience, San Jose, CA) in bicarbonate buffer (0.1 mol/L, pH 8.2) 50 μ g/mL overnight at 4 $^{\circ}$ C. After removal of the coating solution, the plates were washed and blocked with PBS containing 1% bovine serum albumin for 30 min at room temperature (RT). After blocking, serum samples and serially diluted standard serum were added to the plate in duplicate. The standard serum was obtained by intraperitoneal immunization of a mouse with Cry j 1 and arbitrary assigned a value of 10,000 relative units (RU)/mL of Cry j 1-specific IgE. Plates were then washed and incubated with 100 μ L of 1/2000 diluted Cedar Pollen Allergen Cry j 1-Biotin (Hayashibara Biochemical Laboratories Inc.) for 1 h at

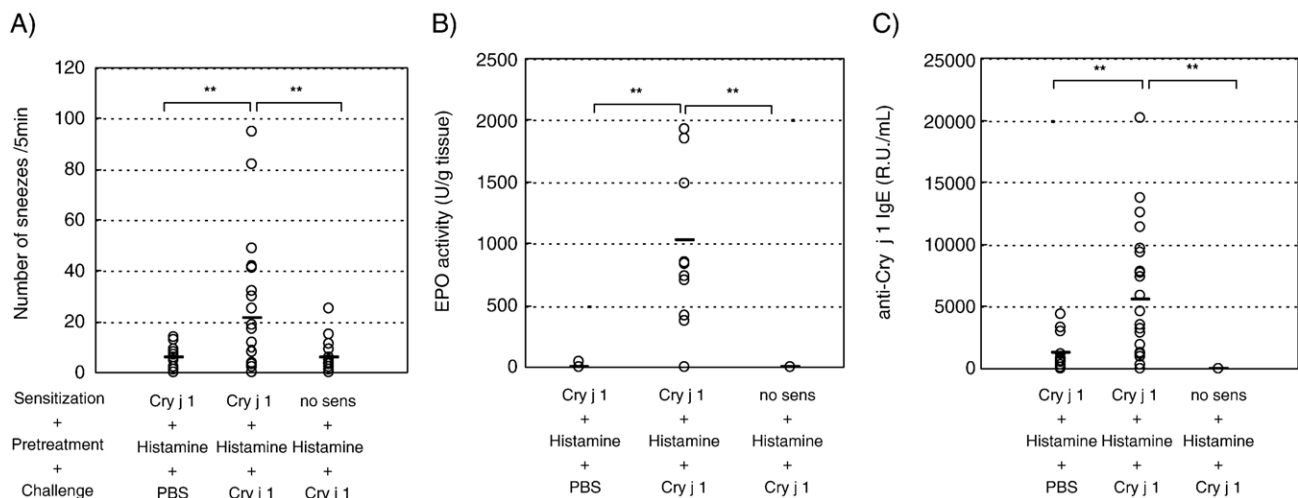


Fig. 2. The number of sneezes, EPO activity in nasal tissue and Cry j 1-specific IgE levels in three groups as follows. Group 1 ($n=15$): sensitized with Cry j 1 + intranasally treated with histamine (1 μ g/mL, 10 μ L/nostril)+intranasally challenged with PBS (10 μ L/nostril), Group 2 ($n=24$): sensitized with Cry j 1 + intranasally treated with histamine (1 μ g/mL, 10 μ L/nostril)+intranasally challenged with Cry j 1 (200 μ g/mL, 10 μ L/nostril), Group 3 ($n=15$): no sensitization+intranasally treated with histamine (1 μ g/mL, 10 μ L/nostril)+intranasally challenged with Cry j 1 (200 μ g/mL, 10 μ L/nostril). Each dot represents the individual value and “—” shows the mean value of each group. **: $p < 0.01$ (Aspin–Welch test), group 2 is significantly different from groups 1 and 3.

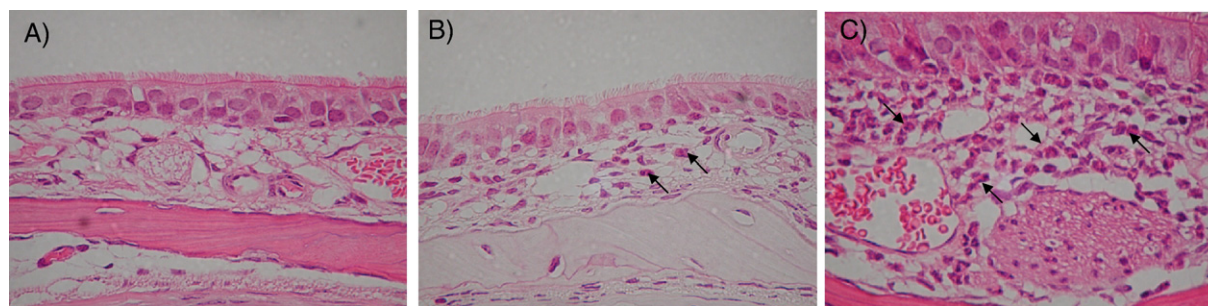


Fig. 3. Histopathological features of Cry j 1-induced allergic rhinitis in B10.S mice, 24 h after the last nasal antigen challenge. A: Control group; unsensitized mice intranasally challenged with PBS; B: no-pretreatment group— Cry j 1-sensitized mice that were challenged with Cry j 1 intranasally without histamine pretreatment; C: Histamine pretreatment group— Cry j 1-sensitized mice that were challenged with Cry j 1 intranasally after 1 µg/mL histamine pretreatment. Eosinophils are marked with the arrows. Stained with hematoxylin–eosin. Original magnification ×600.

RT. The plate was then washed and subsequently incubated with streptavidine- β -galactosidase conjugate (Molecular Probes, Eugene, OR) for 30 min at RT. After a final wash, 10 µL of substrate solution, consisting of Tris–HCl with 0.034 mg/mL of 4-methylumbelliferol- β -D-galactopyranoside, was added to the well. The reaction was stopped with 100 µL of glycine-NaOH, 0.2 mol/L, pH 10.3, and the fluorescence intensity measured by a microplate fluorescence reader (Fluoroskan Ascent, Thermo Electron Corp., Waltham, MA) at wavelengths of 355 nm (excitation) and 460 nm (emission).

Data and statistical analysis

For comparison between two groups, initially, the data were analyzed for homogeneity of variance by the *F* test. When the variances were homogeneous, Student's *t*-test was performed. When the variances were not homogeneous by the *F* test, the Aspin–Welch test was performed. For comparison among multiple groups, the data were analyzed for homogeneity of variance using Bartlett's test. When the variances were homogeneous, parametric Dunnett's multiple comparison test was performed. When the variances were not homogeneous by

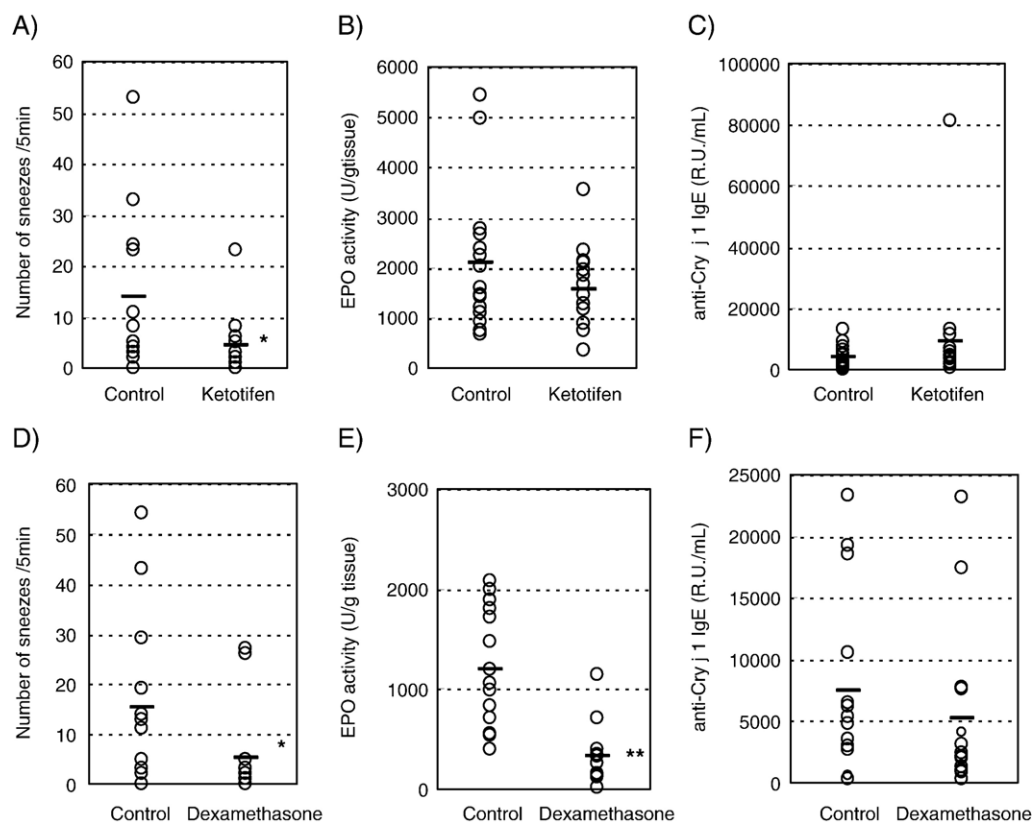


Fig. 4. Effects of ketotifen and dexamethasone on allergic rhinitis model in B10.S mice. The ketotifen treated group was treated orally 30 min prior to every nasal challenge (2 mg/kg) (16 mice/group). The dexamethasone treated group was treated orally 1 h prior to every nasal challenge (1 mg/kg) (15 mice/group). *: $p < 0.05$, **: $p < 0.01$ (Aspin–Welch test), significantly different from Control.

the Bartlett's test, nonparametric Dunnett's multiple comparison test was performed.

Results

Effects of histamine pretreatment on Cry j 1-induced sneezing, EPO activity, and Cry j 1-specific IgE

We constructed a dose-response curve of histamine pretreatment. The number of sneezes was increased in the histamine pretreatment group at a concentration of 1 µg/mL compared with the PBS pretreatment group and the no-pretreatment group, the value was not statistically significant (Fig. 1A). EPO activity was increased significantly at doses of 0.1, 1, and 100 µg/mL of histamine compared with the PBS pretreatment group (Fig. 1B). Cry j 1-specific IgE levels in serum were higher in histamine-pretreated groups than in the PBS-pretreated group and the no-pretreatment group, the value was not statistically significant (Fig. 1C).

To investigate the antigen specificity of the nasal response in our model, we examined the number of sneezes, EPO activity in nasal tissues, and Cry j 1-specific IgE levels in three groups as follows. Group 1: sensitized with Cry j 1 and intranasally treated with histamine and intranasally challenged with PBS. Group 2: sensitized with Cry j 1 and intranasally treated with histamine and intranasally challenged with Cry j 1. Group 3: no sensitization, intranasally treated with histamine, and intranasally challenged with Cry j 1. As shown in Fig. 2, the number of sneezes, EPO activity, and Cry j 1-specific IgE in group 2 were increased significantly compared with groups 1 and 3, respectively. There were no significant differences between groups 1 and 3 in the number of sneezes, EPO activity, and Cry j 1-specific IgE.

Histological analysis of nasal tissues

Unsensitized mice challenged intranasally with PBS showed no eosinophil infiltration (Fig. 3A). When Cry j 1-sensitized B10.S mice were challenged intranasally with Cry j 1 for 5 consecutive days without histamine pretreatment, a moderate infiltration of eosinophils occurred in the nasal mucosa (Fig. 3B). Furthermore, with pretreatment of histamine, eosinophils clearly infiltrated into the lamina propria more markedly than in the no-pretreatment group (Fig. 3C).

Effects of ketotifen and dexamethasone on the allergic rhinitis model

To measure the effects of the histamine H1-receptor antagonist, ketotifen (2 mg/kg) was administered orally 30 min before every intranasal antigen challenge. Ketotifen significantly inhibited the number of sneezes (Fig. 4A), but it did not inhibit EPO activity or lower than the anti-Cry j 1 IgE level (Fig. 4B,C).

To measure the effects of glucocorticoids, dexamethasone (1 mg/kg) was administered orally 1 h before every intranasal antigen challenge. Dexamethasone significantly inhibited

the number of sneezes and EPO activity (Fig. 4D,E). However, dexamethasone did not suppress the anti-Cry j 1 IgE level (Fig. 4F).

Discussion

There have been many reports on models of experimental rhinitis (Nabe et al., 1998; Sato et al., 1999; Tamura et al., 1986; Kim et al., 2001; Murasugi et al., 2005). Nabe et al. (1998) reported on an experimental allergic rhinitis model in the guinea pig, with development of nasal blockage and sneezing during repeated inhalation challenges with Cj pollen. Sato et al. (1999) reported on the study of a mucosal immune response in an ovalbumin-prensensitized BALB/c mouse model of allergic rhinitis. Tamura et al. (1986) reported on the investigation of IgE antibody responses against Cj pollen in DBA/2, BDF1, C57BL/6, C57BL/10, and BALB/c mice and suggested that DBA/2, BDF1, and BALB/c mice were responders to Cj pollen antigen whereas C57BL/6 and C57BL/10 mice were nonresponders. In our preliminary examination, we confirmed that B10.S mice were responder to Cry j 1 (data not shown). It has been reported that a mouse model of oral tolerance to Cj pollen as antigen was developed in C3H/HeN mice (Kim et al., 2001). C3H/HeN mice were immunized with intraperitoneal injection of Cj pollen in Freund's complete adjuvant, and serum levels of IgE, IgG, IgG1, and IgG2a were measured. Murasugi et al. (2005) reported that they had succeeded in establishing a system to evaluate symptoms and reactions of allergic rhinitis in Cry j 2-sensitized BALB/c mice. In their model, sneezing frequency, vascular permeability of the nasal mucosa, and hyperreactivity to histamine were increased by intranasal challenge with Cry j 2 solution. Murasugi et al. (2005) used cholera toxin for sensitization as adjuvant; thus an inflammatory response might be caused in nasal mucosa by the cholera toxin, and eosinophil infiltration could not be evaluated. To our knowledge, there have been no reports that the infiltration of eosinophils into nasal tissues was evaluated by EPO activity in mice. In our model, eosinophil infiltration could be evaluated by measuring the EPO activity in nasal tissue in Cry j 1-sensitized B10.S mice. Eosinophil infiltration into the nasal mucosa is a major symptom of allergic rhinitis; therefore our model promises to be very useful.

One of the major human T-cell epitopes in Cry j 1 is also a major T-cell epitope in B10.S mice (Kingsu and Saito, 1998; Kingsu et al., 2000), but there has been no report of an allergic rhinitis model in B10.S mice whose symptoms are sneezing, eosinophil infiltration into the nasal mucosa, and antigen-specific IgE production elicited by histamine pretreatment and continuous intranasal antigen challenges for 5 days. In the present study, we sought to develop such a model of allergic rhinitis in B10.S mice with Cry j 1 as antigen.

In preliminary examination, when Cry j 1-sensitized mice were challenged by intranasal instillation of Cry j 1 for 5 consecutive days, sneezing reflexes were observed only after the last challenge of Cry j 1 by intranasal instillation. Histamine is a well-known chemical mediator of inflammation and allergic

response, directly causing an increase in vascular permeability, smooth-muscle contraction, mucus hypersecretion, and edema formation (Jutel et al., 2005). In addition, high levels of histamine have been identified in the nasal secretions of patients with allergic rhinitis after antigen challenge (Miadonna et al., 1999). Therefore we hypothesized that histamine pretreatment before continuous Cry j 1 challenge would enhance nasal response. In the present study, stimulation of nasal mucosa with histamine in the histamine pretreatment group enhanced the sneezing reflex, EPO activity, and antigen-specific IgE production compared with the no-pretreatment group and the PBS-pretreatment group (Fig. 1). These results suggest that histamine pretreatment enhances the local sensitivity to allergen at the nasal mucosa and also the systemic reactions to allergen, such as allergen-specific IgE production. Pretreatment with histamine may cause the opening of the intraepithelial tight junctions, allowing the penetration of allergen into the epithelium and lamina propria, where allergen further interacts with sensitized mast cells (Kawaguchi et al., 1995). One of the reasons for the enhancement of sensitivity at the nasal mucosa might be that histamine increases nasal epithelial permeability because it reduces ZO-1 protein expression (Takeuchi et al., 2001). ZO-1 is one of the tight junction proteins and thereby participates in the regulation of paracellular permeability.

Chronic allergic diseases such as asthma, rhinitis, and atopic dermatitis are characterized by the preferential infiltration of eosinophils and/or diffuse deposition of eosinophil granule protein (Donald and Leung, 1999; Bochner and Busse, 2005). Eosinophils increase in number in both nasal secretions and the nasal mucosa after allergen challenge or during natural allergen exposure (Terada et al., 1994; Ciprandi et al., 2004). Eosinophils are thought to be responsible for some of the histopathologic and physiologic changes observed in these diseases. In histopathological studies, intranasal stimulation of Cry j 1 without histamine pretreatment induced slight eosinophil infiltration. On the other hand, considerable numbers of eosinophils clearly infiltrated into the nasal mucosa after the intranasal stimulation of histamine pretreatment and repeated antigen challenge. Eosinophils are known to contain relatively large amounts of peroxidase, so EPO has been used as a marker enzyme for tissue eosinophil content. In our model, we succeeded in the quantitative measurement of EPO activity in mouse nasal tissue and confirmed that EPO activity is proportional to eosinophil infiltration in nasal tissues. Thus our model suggests that mice can be useful for evaluating the infiltration of eosinophils into nasal tissues in allergic rhinitis. This study shows that pretreatment of histamine increases eosinophil infiltration into nasal mucosa. Histamine might facilitate the recruitment of airway eosinophils by stimulating eosinophil chemotaxis.

To investigate the antigen specificity of the nasal response in our model, we examined the number of sneezes, EPO activity in nasal tissue, and Cry j 1-specific IgE levels in three groups. In Cry j 1-sensitized mice, intranasal challenge of Cry j 1 significantly increased the number of sneezes, EPO activity, and Cry j 1-specific IgE compared with intranasal challenge of PBS. In Cry j 1 intranasally challenged mice, sensitizations of Cry j 1 significantly increased the number of sneezes, EPO

activity, and Cry j 1-specific IgE compared with no sensitization. It is therefore suggested that the response in our model is antigen-specific.

It is necessary to establish an animal model showing similar nasal allergic symptoms to that of humans in order to evaluate the effectiveness of antiallergy drugs. Ketotifen, an antiallergy drug having H1-receptor antagonist activity, is widely used for allergic disease and known to be effective in reducing sneezing (Grant et al., 1990). Glucocorticoid is also widely used in the treatment of inflammatory disease, including allergic inflammation (Caramori and Adcock, 2005). Thus we evaluated the effects of ketotifen and dexamethasone on our model of allergic rhinitis in B10.S mice. Ketotifen decreased sneezing but had no effect on EPO activity or Cry j 1 IgE level. Numerous previous studies have shown that dexamethasone significantly relieves all symptoms of rhinitis. In our model, ketotifen and dexamethasone showed the same effects on allergic rhinitis as other groups have reported previously (Fukuda et al., 2003; Sugimoto et al., 2000; Yamasaki et al., 2001). Therefore these results suggest that our murine model will prove to be useful in the investigation of allergic rhinitis.

Conclusion

We have herewith reported the development of a new model of allergic rhinitis in Cry j 1-sensitized B10.S mice, producing sneezing, eosinophil infiltration into the nasal mucosa, and Cry j 1-specific IgE production. In addition, we succeeded in the quantitative evaluation of eosinophil infiltration into the nasal mucosa of mice by measuring the EPO activity in nasal tissue. The symptoms in mice—such as sneezing, eosinophil infiltration into the nasal mucosa, and allergen-specific IgE production—were similar to those observed in human cedar pollinosis. Therefore this new model of allergic rhinitis in Cry j 1-sensitized mice should prove to be useful in studying human cedar pollinosis.

References

- Bochner, B.S., Busse, W.W., 2005. Allergy and asthma. *Journal of Allergy and Clinical Immunology* 115 (5), 953–959.
- Caramori, G., Adcock, I., 2005. Anti-inflammatory mechanisms of glucocorticoids targeting granulocytes. *Current Drug Targets. Inflammation and Allergy* 4 (4), 455–463.
- Ciprandi, G., Cirillo, I., Vizzaccaro, A., Milanese, M., Tosca, M.A., 2004. Nasal obstruction in patients with seasonal allergic rhinitis: relationships between allergic inflammation and nasal airflow. *International Archives of Allergy and Immunology* 134 (1), 34–40.
- Connell, J.T., 1969. Quantitative intranasal pollen challenges. 3. The priming effect in allergic rhinitis. *The Journal of Allergy* 43 (1), 33–44.
- Donald, Y.M., Leung, M.D., 1999. Pathogenesis of atopic dermatitis. *Journal of Allergy and Clinical Immunology* 104, S99–S108.
- Fukuda, S., Midoro, K., Gyoten, M., Kawano, Y., Ashida, Y., Nabe, T., Kohno, S., Nagaya, H., 2003. Effects of TAK-427 on acute nasal symptoms and nasal obstruction in guinea pig model of experimental allergic rhinitis. *European Journal of Pharmacology* 476 (3), 239–247.
- Grant, S.M., Goa, K.L., Fitton, A., Sorkin, E.M., 1990. Ketotifen. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in asthma and allergic disorders. *Drugs* 40 (3), 412–448.
- Hashimoto, M., Nigi, H., Sakaguchi, M., Inouye, S., Imaoka, K., Miyazawa, H., Taniguchi, Y., Kurimoto, M., Yasueda, H., Ogawa, T., 1995. Sensitivity to two major allergens (Cry j I and Cry j II) in patients with Japanese cedar

- (*Cryptomeria japonica*) pollinosis. *European Journal of Pharmacology* 25 (9), 848–852.
- Horak, F., Stubner, P., 1998. New trends in allergic rhinitis. *Allergy and Clinical Immunology International* 10 (3), 81–87.
- Igarashi, Y., Goldrich, M.S., Kaliner, M.A., Irani, A.M., Schwartz, L.B., White, M.V., 1995. Quantitation of inflammatory cells in the nasal mucosa of patients with allergic rhinitis and normal subjects. *Journal of Allergy and Clinical Immunology* 95, 716–725.
- Jutel, M., Watanabe, T., Klunker, S., Akdis, M., Thomet, O.A., Malolepszy, J., Zak-Nejmark, T., Koga, R., Kobayashi, T., Blaser, K., Akdis, C.A., 2001. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature* 413 (6854), 420–425.
- Jutel, M., Blaser, K., Akdis, C.A., 2005. Histamine in allergic inflammation and immune modulation. *International Archives of Allergy and Immunology* 137 (1), 82–92.
- Kawaguchi, S., Ukai, K., Jin, C.S., Sakakura, Y., 1995. Effect of histamine on nasal epithelial permeability to horseradish peroxidase in allergic guinea pigs. *Annals of Otolaryngology & Laryngology* 104 (5), 394–398.
- Kim, J.H., Mun, Y.J., Ahn, S.H., Park, J.S., Woo, W.H., 2001. Induction of oral tolerance to Japanese cedar pollen. *Archives of Pharmacological Research* 24 (6), 557–563.
- Kingetsu, I., Saito, S., 1998. Identical recognition of T-cell epitopes by Th1 and Th2 subsets in mice immunized with Japanese cedar pollen antigens. *Jikeikai Medical Journal* 45, 95–105.
- Kingetsu, I., Ohno, N., Hayashi, N., Sakaguchi, M., Inouye, Saito, S., 2000. Common antigenicity between Japanese cedar (*Cryptomeria japonica*) pollen and Japanese cypress (*Chamaecyparis obtusa*) pollen. I. H-2 complex affects cross responsiveness to Cry j 1 and Cha o 1 at the T- and B-cell level in mice. *Immunology* 99 (4), 625–629.
- Komiyama, N., Sone, T., Shimizu, K., Morikubo, K., Kino, K., 1994. cDNA cloning and expression of Cry j II the second major allergen of Japanese cedar pollen. *Biochemical and Biophysical Research Communications* 201 (2), 1021–1028.
- Miadonna, A., Milazzo, N., Gibelli, S., Salmaso, C., Lorini, M., Tedeschi, A., 1999. Nasal response to a single antigen challenge in patients with allergic rhinitis— inflammatory cell recruitment persists up to 48 hours. *European Journal of Pharmacology* 29 (7), 941–949.
- Murasugi, T., Nakagami, Y., Yoshitomi, T., Hirahara, K., Yamashita, M., Taniguchi, Y., Sakaguchi, M., Ito, K., 2005. Oral administration of a T cell epitope inhibits symptoms and reactions of allergic rhinitis in Japanese cedar pollen allergen-sensitized mice. *European Journal of Pharmacology* 510, 143–148.
- Nabe, T., Mizutani, N., Shimizu, K., Takenaka, H., Kohno, S., 1998. Development of pollen-induced allergic rhinitis with early and late phase nasal blockage in guinea pigs. *Inflammation Research* 47 (9), 369–374.
- Okuda, M., 2003. Epidemiology of Japanese cedar pollinosis throughout Japan. *Annals of Allergy, Asthma and Immunology* 91 (3), 288–296.
- Sato, J., Asakura, K., Murakami, M., Uede, T., Kataura, A., 1999. Topical CTLA4-Ig suppresses ongoing mucosal immune response in presensitized murine model of allergic rhinitis. *International Archives of Allergy and Immunology* 119 (3), 197–204.
- Savage, J., Roy, D., 2005. Allergic rhinitis: an update. *Journal of the Royal Society of Health* 125 (4), 172–175.
- Sone, T., Komiyama, N., Shimizu, K., Kusakabe, T., Morikubo, K., Kino, K., 1994. Cloning and sequencing of cDNA coding for Cry j I, a major allergen of Japanese cedar pollen. *Biochemical and Biophysical Research Communications* 199 (2), 619–625.
- Sone, T., Morikubo, K., Miyahara, M., Komiyama, N., Shimizu, K., Tsunoo, H., Kino, K., 1998. T cell epitopes in Japanese cedar (*Cryptomeria japonica*) pollen allergens: choice of major T cell epitopes in Cry j 1 and Cry j 2 toward design of the peptide-based immunotherapeutics for the management of Japanese cedar pollinosis. *Journal of Immunology* 161 (1), 448–457.
- Sugimoto, Y., Kawamoto, E., Chen, Z., Kamei, C., 2000. A new model of allergic rhinitis in rats by topical sensitization and evaluation of H(1)-receptor antagonists. *Immunopharmacology* 48 (1), 1–7.
- Takeuchi, K., Kishioka, C., Ishinaga, H., Sakakura, Y., Majima, Y., 2001. Histamine alters gene expression in cultured human nasal epithelial cells. *Journal of Allergy and Clinical Immunology* 107 (2), 310–314.
- Tamura, S., Kobayashi, T., Kikuta, K., Nakagawa, M., Sakaguchi, M., Inouye, S., 1986. IgE antibody responses against Japanese cedar pollen in the mouse. *Microbiology and Immunology* 30 (9), 883–891.
- Terada, N., Konno, A., Togawa, K., 1994. Biochemical properties of eosinophils and their preferential accumulation mechanism in nasal allergy. *Journal of Allergy and Clinical Immunology* 94, 629–642.
- Yamasaki, M., Mizutani, N., Sasaki, K., Nabe, T., Matsumoto, T., Ashida, Y., Kohno, S., 2001. Involvement of thromboxane A2 and peptide leukotrienes in early and late phase nasal blockage in a guinea pig model of allergic rhinitis. *Inflammation Research* 50 (9), 466–473.
- Yasueda, H., Yui, Y., Shimizu, T., Shida, T., 1983. Isolation and partial characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. *Journal of Allergy and Clinical Immunology* 71, 77–86.