

The role of chemical mediators in eosinophil infiltration in allergic rhinitis in mice

Ryoji Kayasuga^a, Yoshinori Iba^a, Maria Alejandra Hossen^a,
Takeshi Watanabe^b, Chiaki Kamei^{a,*}

^aDepartment of Pharmacology, Faculty of Pharmaceutical Sciences, Okayama University, Okayama 700-8530, Japan

^bDepartment of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan

Received 4 November 2002; received in revised form 4 November 2002; accepted 4 November 2002

Abstract

The involvement of chemical mediators other than histamine in eosinophil infiltration in the nasal mucosa was studied using histamine H₁ receptor-deficient mice. Histamine H₁ receptor-deficient mice and wild-type controls were immunized with ovalbumin and consecutive topical antigen instillation was performed. Histological alterations and eosinophil infiltration into the nasal mucosa of mice were examined. Diffuse infiltration of inflammatory cells and edema after sensitization with antigen were observed in the nasal mucosa in both wild-type and histamine H₁ receptor-deficient mice. The number of eosinophils in the nasal mucosa in mice sensitized with antigen was significantly increased as compared with controls. The number of eosinophils in the nasal mucosa was significantly decreased by cetirizine and epinastine, ramatroban and zafirlukast in wild-type mice. Not only histamine but also thromboxane A₂ and leukotrienes play important roles in allergic rhinitis, especially in the late phase participating in nasal eosinophilia.

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Histamine H₁ receptor-deficient mice; Nasal eosinophilia; Histopathological changes; Thromboxane A₂; Leukotriene

1. Introduction

Allergic rhinitis is characterized by a marked increase in number of eosinophils in the nasal submucosa and epithelium [1], and eosinophils are well known to cause an increase in nasal cavity lavage fluid in patients with allergic rhinitis [2]. In the late phase of the allergic reaction, eosinophils, infiltrating into the

tissues from post-capillary venules, degranulate and release granule cytotoxic proteins such as eosinophil cationic protein and major basic protein, which are presumed to produce epithelial damage [3]. Lipid mediators such as leukotrienes and platelet-activating factor that cause edema of the nasal mucosa membrane [4,5], as well as cytokines that promote the chemotaxis, activation and degranulation of eosinophils are also generated by the activation of eosinophils [6]. It is widely accepted that chemical mediators such as histamine and leukotrienes facilitate leukocyte infiltration, followed by allergic inflammation and airway hyper-reactivity [7–9]. However, there are no interactions

* Corresponding author. Tel.: +81-86-251-7939; fax: +81-86-251-7939.

E-mail address: kamei@pheasant.pharm.okayama-u.ac.jp (C. Kamei).

between the increase in eosinophils and nasal symptoms, and the damage of the epithelium seen in the asthmatic airway was not observed in nasal allergy. Very little information is available about the participation of chemical mediators in nasal eosinophilia in the late phase of allergic rhinitis. Therefore, to clarify the roles of histamine H_1 receptors and other chemical mediators in animals, we performed histological analysis of the nasal mucosa of mice after active immunization using histamine H_1 receptor-deficient mice.

2. Materials and methods

2.1. Animals

Histamine H_1 receptor-deficient mice were generated by homologous recombination as previously

described by Inoue et al. [10]. Mutant mice have been back-crossed to C57BL/6 mice. Mutant and wild-type mice weighing 15–22 g were used for experiments. Both strains were housed in a temperature-controlled room at $24 \pm 2^\circ\text{C}$ with $55 \pm 15\%$ humidity and were given food and water ad libitum. All procedures involving animals were conducted in accordance with the guidelines of the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Okayama University.

2.2. Reagents

Ovalbumin (Sigma, St. Louis, MO, USA), pertussis toxin (Sigma) and aluminum hydroxide hydrate gel suspension (Cosmo Bio, LSL, Tokyo, Japan) were used. These agents were dissolved in saline. Epinastine hydrochloride (Böehringer Ingelheim, Ingelheim, Ger-

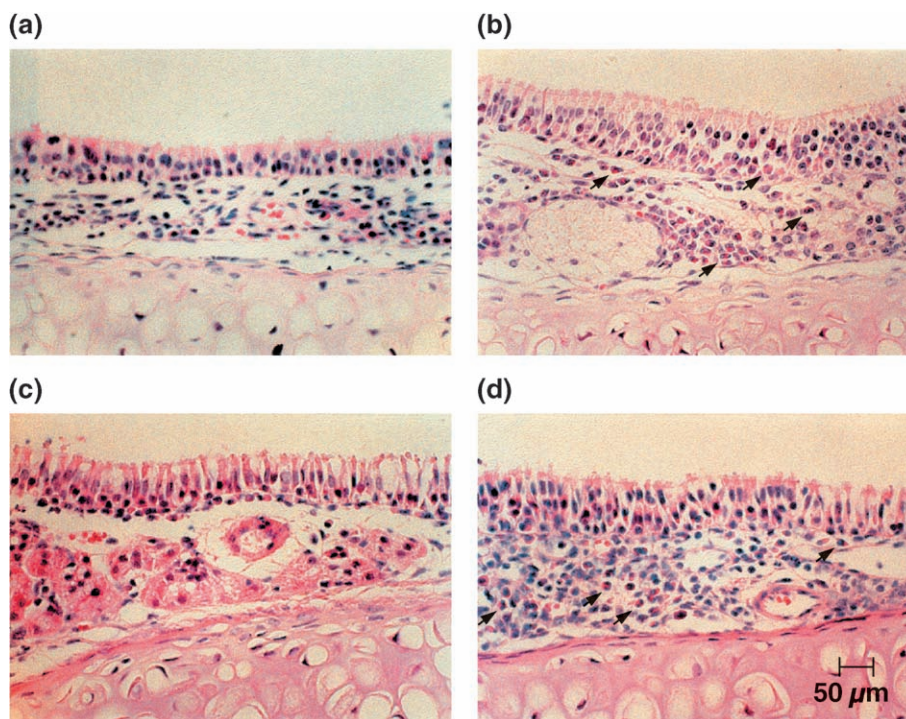


Fig. 1. Histopathological changes of nasal septal mucosa from mice sensitized with ovalbumin or saline. Wild-type and histamine H_1 receptor-deficient mice were immunized followed by repeated instillation into the bilateral nostrils of saline (a, c) or ovalbumin (b, d). Twenty-four hours after final antigen challenge, mice were killed and decapitated. Nasal specimens were fixed, decalcified and stained with hematoxylin and eosin. Many eosinophils were present in the nasal mucosa of sensitized mice (arrows). (a) Wild-type mouse challenged with saline. (b) Wild-type mouse challenged with ovalbumin. (c) Histamine H_1 receptor-deficient mouse challenged with saline. (d) Histamine H_1 receptor-deficient mouse challenged with ovalbumin.

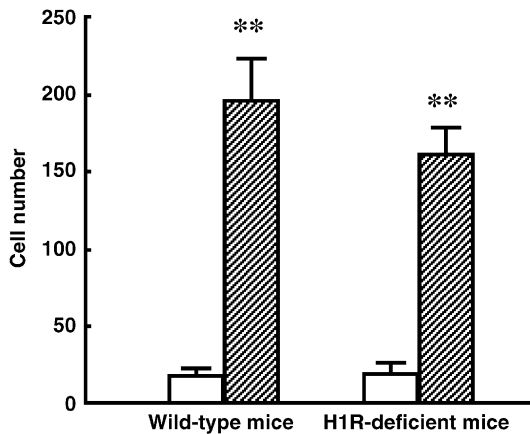


Fig. 2. The numbers of eosinophils in the nasal mucosa of mice sensitized with ovalbumin. Eosinophils were counted in the mucosa on both sides of the nasal septum of wild-type and histamine H_1 receptor-deficient mice immunized with saline (open columns) or ovalbumin (hatched columns) 24 h after final antigen challenge. Each column and vertical bar shows the mean $\pm$ S.E.M. of five experiments. **Significantly different from saline-treated control group at $P < 0.01$.

many), cetirizine hydrochloride (UCB, Brussels, Belgium), ramatroban (Bayer Yakuhin, Osaka, Japan) and zafirlukast (Astra Zeneca, Osaka, Japan) were also used. These agents were suspended in 5% gum arabic and administered orally 1 h before antigen instillation.

2.3. Immunization

Mice were given an intraperitoneal injection of ovalbumin (100 μ g), aluminum hydroxide gel (1 mg) and pertussis toxin (300 ng) by the method of Kayasuga et al. [11]. Five days later, they received a booster injection of 50 μ g of ovalbumin alone subcutaneously in the back. From 18 days after the first immunization, daily intranasal sensitization with ovalbumin (50 μ g) was performed for 1 week.

2.4. Histological examination

Twenty-four hours after the challenge on day 25, mice were anesthetized with diethylether. The animals were killed by exsanguination, then their heads were removed. They were fixed with 10% neutral buffered formalin for several days, and decalcified with 10% EDTA solution (pH 7.4) for 1 week. Samples were embedded in paraffin, and frontal sections of the nose

4- μ m thick were stained with hematoxylin and eosin to evaluate the number of eosinophils. All eosinophils that had infiltrated the mucosa at both sides of the nasal septum were determined microscopically [12].

2.5. Effects of drugs on antigen-induced nasal eosinophilia

Test drugs were administered orally 1 h before nasal antigen challenge, and the number of eosinophils was counted under a light microscope.

2.6. Statistical analysis

Data are presented as means $\pm$ S.E.M. Statistical analysis was performed by one-way analysis of variance with Dunnett's test. Probability values of less than 0.05 were considered significant.

3. Results

3.1. Histopathological changes

Typical microscopic findings in the nasal mucosa are shown in Fig. 1. Photomicrographs indicated infiltration of inflammatory cells comprised of eosinophils in both wild-type and histamine H_1 receptor-deficient mice after sensitization with ovalbumin (b,

Table 1

Effects of certain drugs on nasal eosinophilia after antigen challenge in sensitized wild-type mice

Drugs	Dose (mg/kg, p.o.)	Eosinophil influx
Vehicle		239.0 $\pm$ 31.3
Cetirizine	3	146.5 $\pm$ 34.9
	10	103.8 $\pm$ 31.2*
Epinephrine	3	176.3 $\pm$ 14.8
	10	145.6 $\pm$ 22.6*
Ramatroban	30	180.8 $\pm$ 39.3
	100	110.3 $\pm$ 19.2*
Zafirlukast	30	205.3 $\pm$ 31.4
	100	127.8 $\pm$ 14.5*

Mice were immunized with ovalbumin, and 24 h after final ovalbumin challenge, the number of eosinophils was counted on both sides of the nasal septum. The drugs were given orally 1 h before antigen instillation. Each value shows the mean $\pm$ S.E.M. of four to six experiments.

*Significantly different from vehicle group at $P < 0.05$.

d). On the other hand, inflammatory cells and edema of the nasal mucosa were rarely seen in the nasal mucosa in either strain treated with saline (a, c).

3.2. Assessment of nasal eosinophilia

The numbers of eosinophils in the nasal mucosa in wild-type and histamine H₁ receptor-deficient mice sensitized with ovalbumin are shown in Fig. 2. In both strains, a significant increase in eosinophils number was observed in the antigen-treated group as compared with the saline-treated group.

3.3. Effects of drugs on antigen-induced nasal eosinophilia

Table 1 shows the effects of chemical mediator antagonists on nasal eosinophilia induced by antigen in wild-type mice. The histamine H₁ receptor antagonists cetirizine and epinastine significantly decreased the number of eosinophils induced by antigen at a dose of 10 mg/kg. The thromboxane A₂ receptor antagonist ramatroban and cys leukotriene 1 receptor antagonist zafirlukast also inhibited nasal eosinophilia significantly at a dose of 100 mg/kg.

4. Discussion

It has been suggested that eosinophils could be responsible for the organ damage in hypersensitivity reactions. We developed a mouse model of allergic rhinitis with topical application of antigen, and histological changes and infiltration of eosinophils in the nasal mucosa were also studied. The numbers of eosinophils in the nasal mucosa of sensitized wild-type and histamine H₁ receptor-deficient mice were significantly increased as compared with the control group. Therefore, this allergic model appears to be useful for studies of the involvement of chemical mediators in the late phase of allergic rhinitis. However, as described in the Results section, no marked histological alterations and no significant differences in eosinophil infiltration were observed between wild-type and histamine H₁ receptor-deficient mice treated with antigen in the membrane of the nasal mucosa. These findings were essentially inconsistent with the reports that histamine increases the infiltration of

leukocytes through histamine H₁ receptors in the late phase of allergic responses [7,8]. On the other hand, Takeda et al. [13] reported that there was no significant difference in the number of eosinophils in bronchoalveolar lavage fluid between mast cell-deficient mice (W/W^v) and their congenic littermates. Almost the same findings were demonstrated by de Van Rijn et al. [14], who reported that the intensity of eosinophil infiltration into the nasal mucosa of immunoglobulin E-dependent mice was not different from that of wild-type controls. These findings suggested that there is no intimate relationship between infiltration of eosinophils and histamine/histamine H₁ receptor, similarly to the findings of the present study.

Eosinophil infiltration into the nasal mucosa after antigen challenge was significantly inhibited by both epinastine and cetirizine in sensitized wild-type mice. Epinastine shows selective and potent histamine H₁ receptor antagonism. Cetirizine has been reported to significantly block the accumulation of eosinophils at skin sites after antigen challenge in atopic subjects [15], and the drug caused a significant reduction of ICAM-1/CD54 expression after conjunctival allergen challenge [16]. Therefore, cetirizine probably reduces adhesion molecule expression in addition to its histamine H₁ receptor antagonistic activity resulting in inhibition of eosinophil infiltration into the nasal mucosa. The experiment using histamine H₁ receptor antagonists suggested that histamine H₁ receptors are also responsible for eosinophil infiltration to some extent.

To investigate the participation of chemical mediators other than histamine on nasal eosinophilia, the effects of some chemical mediators were studied. Both ramatroban and zafirlukast significantly inhibited the infiltration of eosinophils. Narita et al. [12] reported that ramatroban significantly inhibited antigen-induced nasal eosinophilia in a guinea pig allergic rhinitis model. In addition, Shi et al. [17] reported that a thromboxane A₂ synthase inhibitor and a thromboxane prostanoid receptor antagonist significantly reduced the increase in number of eosinophils in bronchoalveolar lavage fluid in sensitized mice. Zafirlukast caused a significant inhibition of eosinophil migration from the lamina propria to the lumen induced by chemotaxin formyl-met-leu-phe [18]. Underwood et al. [19] and Krell et al. [20] found that pranlukast and zafirlukast significantly inhibited eosi-

nophilia in bronchoalveolar lavage fluid after leukotriene D₄ challenge, suggesting the involvement of leukotriene in nasal eosinophilia. Taken together, these observations suggested that histamine H₁ receptors, thromboxane A₂ receptors and cys leukotriene 1 receptors are involved in the eosinophil influx into the nasal mucosa in actively sensitized wild-type mice.

References

- [1] Jankowski R, Persoons M, Foliguet B, Coffinet L, Thomas C, et al. Eosinophil count in nasal secretions of subjects with and without nasal symptoms. *Rhinology* 2000;38:23–32.
- [2] Wang D-Y, Clement P. Pathogenic mechanisms underlying the clinical symptoms of allergic rhinitis. *Am J Rhinol* 2000;14:325–33.
- [3] Gleich GJ, Loefering DA. Immunobiology of eosinophils. *Annu Rev Immunol* 1984;2:429–59.
- [4] Mizutani N, Nabe T, Imai A, Sakurai H, Takenaka H, et al. Markedly increased nasal blockage by intranasal leukotriene D₄ in an experimental allergic rhinitis model: contribution of dilated mucosal blood vessels. *Jpn J Pharmacol* 2001;86:170–82.
- [5] Misawa M, Iwamura S. Platelet-activating factor (PAF)-induced rhinitis and involvement of PAF in allergic rhinitis in guinea pigs. *Jpn J Pharmacol* 1990;54:217–26.
- [6] Martin LB, Kita H, Leiferman KM, Gleich GJ. Eosinophils in allergy: role in disease, degranulation, and cytokines. *Int Arch Allergy Immunol* 1996;109:207–15.
- [7] Asako H, Kurose I, Wolf R, DeFrees S, Zheng Z-L, et al. Role of H₁ receptors and P-selectin in histamine-induced leukocyte rolling and adhesion in postcapillary venules. *J Clin Invest* 1994;93:1508–15.
- [8] Kubes P, Kanwar S. Histamine induces leukocyte rolling in post-capillary venules. *J Immunol* 1994;152:3570–7.
- [9] Spada CS, Nieves AL, Krauss AH-P, Woodward DF. Comparison of leukotriene B₄ and D₄ effects on human eosinophil and neutrophil motility in vitro. *J Leukoc Biol* 1994;55:183–91.
- [10] Inoue I, Yanai K, Kitamura D, Taniuchi I, Kobayashi T, et al. Impaired locomotor activity and exploratory behavior in mice lacking histamine H₁ receptors. *Proc Natl Acad Sci U S A* 1996;93:13316–20.
- [11] Kayasuga R, Sugimoto Y, Watanabe T, Kamei C. Participation of chemical mediators other than histamine in nasal allergy signs; a study using mice lacking histamine H₁ receptors. *Eur J Pharmacol* 2002;449:287–91.
- [12] Narita S, Asakura K, Kataura A. Effects of thromboxane A₂ receptor antagonist (Bay u 3405) on nasal symptoms after antigen challenge in sensitized guinea pigs. *Int Arch Allergy Immunol* 1996;109:161–6.
- [13] Takeda K, Hamelmann E, Joetham A, Shultz LD, Larsen GL, et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J Exp Med* 1997;186:449–54.
- [14] Van de Rijn M, Mehlhop PD, Judkins A, Rothenberg ME, Luster AD, et al. A murine model of allergic rhinitis: studies on the role of IgE in pathogenesis and analysis of the eosinophil influx elicited by allergen and eotaxin. *J Allergy Clin Immunol* 1998;102:65–74.
- [15] Fadel R, Herpin-Richard N, Rihoux JP, Henocq E. Inhibitory effect of cetirizine 2HCL on eosinophil migration in vivo. *Clin Allergy* 1987;17:373–9.
- [16] Ciprandi G, Buscaglia S, Pesce G, Passalacqua G, Rihoux JP, et al. Cetirizine reduces inflammatory cell recruitment and ICAM-1 (or CD54) expression on conjunctival epithelium in both early- and late-phase reactions after allergen-specific challenge. *J Allergy Clin Immunol* 1995;95:612–21.
- [17] Shi H, Yokoyama A, Kohno N, Hirasawa Y, Kondo K, et al. Effect of thromboxane A₂ inhibitors on allergic pulmonary inflammation in mice. *Eur Respir J* 1998;11:624–9.
- [18] Muñoz NM, Douglas I, Mayer D, Herrnreiter A, Zhu X, et al. Eosinophil chemotaxis inhibited by 5-lipoxygenase blockade and leukotriene receptor antagonism. *Am J Respir Crit Care Med* 1997;155:1398–403.
- [19] Underwood DC, Osborn RR, Newsholme SJ, Torphy TJ, Hay DWP. Persistent airway eosinophilia after leukotriene (LT) D₄ administration in the guinea pig: modulation by the LTD₄ receptor antagonist, pranlukast, or an interleukin-5 monoclonal antibody. *Am J Respir Crit Care Med* 1996;154:850–7.
- [20] Krell RD, Dehaas CJ, Lengel DJ, Kusner EJ, Williams JC, et al. Preclinical exploration of the potential antiinflammatory properties of the peptide leukotriene antagonist ICI 204,219 (Accolate). *Ann N Y Acad Sci* 1994;744:289–98.