ELSEVIER

European Journal of Pharmacology 440 (2002) 79–82

www.elsevier.com/locate/ejphar

# The role of histamine $H_1$ receptors in late-phase reaction of allergic conjunctivitis

Keiji Izushi [a], Hiroto Nakahara [a], Nobuyuki Tai [a], Mitsunobu Mio [a], Takeshi Watanabe [b], Chiaki Kamei [a,*]

[a] *Department of Pharmacology, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-naka 1-1-1, Okayama 700-8530, Japan*
[b] *Department of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan*

Received 13 September 2001; received in revised form 18 January 2002; accepted 25 January 2002

**Abstract**

The role of histamine $H_1$ receptors in the late-phase reaction of allergic conjunctivitis was studied using histamine $H_1$ receptor-deficient mice. To clarify the eosinophil infiltration, which is a reliable indicator of late-phase reaction, eosinophil peroxidase activity in the conjunctiva was measured. Mice were actively immunized with ovalbumin, and conjunctivitis was induced by topical instillation of ovalbumin. A significantly high eosinophil peroxidase level in the conjunctiva was observed in sensitized wild-type mice, whereas sensitized histamine $H_1$ receptor-deficient mice showed no significant increase in the conjunctival eosinophil peroxidase level. In addition, the elevation of eosinophil peroxidase level observed in sensitized wild-type mice was significantly antagonized by pretreatment with anti-P-selectin antibody. From these findings, it was concluded that eosinophil infiltration into the conjunctival tissue in late-phase reaction of allergic conjunctivitis is mediated by P-selectin stored in endothelial cells via histamine $H_1$ receptors. © 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Allergic conjunctivitis; Eosinophil peroxidase activity; Late-phase reaction; Histamine $H_1$ receptor; P-selectin

## 1. Introduction

Allergic conjunctivitis is an immediate type of hypersensitivity that develops minutes after exposure to antigen and lasts for a few hours. The symptoms of immediate hypersensitivity are itching, hyperemia, and edema accompanied by an increase in vascular permeability in the conjunctiva (Kamei et al., 1995; Calonge et al., 1990; Melamed et al., 2000). However, recent studies have indicated the participation of cellular late-phase reaction in animal models and in patients with allergic conjunctivitis (Trocmé et al., 1988). Leonardi et al. (1990) reported that the ocular late-phase reaction is a mast cell-dependent, delayed inflammatory reaction developing 4–12 h after the early phase reaction. The most prominent histological characteristic of the late-phase reaction is marked infiltration of granulocytes into the conjunctival substantia propria (Leonardi et al., 1990). In human skin, Solley et al. (1976) reported that histologically, the cutaneous late-phase reaction is characterized by a 10-fold increase in the number of infiltrating cells. Histamine is well known to play a dominant role in the process leading to the immediate phase in allergic conjunctivitis. We have shown that vascular permeability in the conjunctiva in allergic conjunctivitis associated with immediate hypersensitivity reaction (Woodward et al., 1986) is entirely regulated through histamine $H_1$ receptors (Nakahara et al., 2000). However, very little information is available about the participation of histamine in the late-phase reaction of allergic conjunctivitis.

The present study was performed to clarify the role of histamine $H_1$ receptors in late-phase reaction in allergic conjunctivitis 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 described (Inoue et al.,

* Corresponding author. Tel.: +81-86-251-7939; fax: +81-86-251-7939.
*E-mail address:* kamei@pheasant.pharm.okayama-u.ac.jp (C. Kamei).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)01304-3

1996). The mice were analyzed by the polymerase chain reaction of genomic DNAs from tail biopsies for the presence of the H1R mutant allele as previously described (Morimoto et al., 1999). Male wild-type mice (C57BL/6) were obtained from Japan SLC, Shizuoka, Japan. Male histamine $H_1$ receptor-deficient and wild-type mice weighing 20–25 g were used. Both strains were bred in our laboratory and housed in a temperature-controlled room at 24 ± 2 °C with 55 ± 15% humidity with food and water ad libitum.

### 2.2. Reagents

The following reagents were obtained from the sources shown in parentheses: aluminum hydroxide hydrate gel suspension (ALUM, LSL, Tokyo, Japan), ovalbumin (Sigma, St. Louis, MO, USA), pertussis toxin (Research Biochemicals International, Natrick, MA, USA), *o*-phenylenediamine dihydrochloride (Wako, Osaka, Japan), purified-antimouse P-selectin (BD PharMingen, San Diego, CA, USA), purified immunoglobulin reagent grade rat IgG (Sigma), Triton X-100 (Sigma). All other chemicals used in the study were of the highest quality and commercially available.

### 2.3. Antigen and immunization

The histamine $H_1$ receptor-deficient and wild-type mice were given an intraperitoneal injection of ovalbumin (100 μg), ALUM (1 mg), and pertussis toxin (300 μg). Five days later, they received a booster injection of 50 μg of ovalbumin alone subcutaneously at the back (Oettgen et al., 1994). Eighteen days after the first immunization, the mice were used as actively sensitized animals.

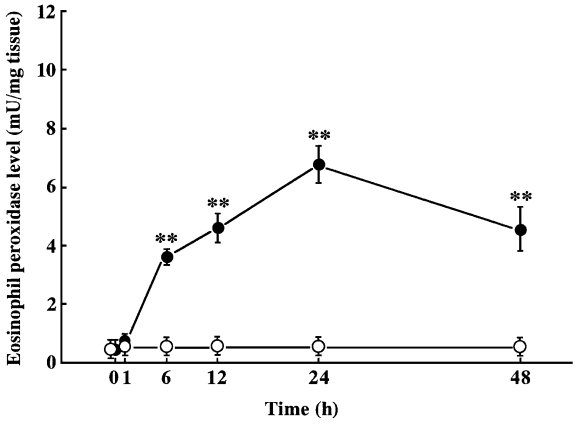


Fig. 1. Time course of changes in conjunctival eosinophil peroxidase level in sensitized wild-type mice. Mice were actively immunized with ovalbumin, and conjunctivitis was induced by topical instillation of ovalbumin. Nonsensitized wild-type mice (open circle), sensitized wild-type mice (solid circle). Each value represents the mean ± S.E.M. ($n = 6$). **: Significantly different from control at $P < 0.01$.

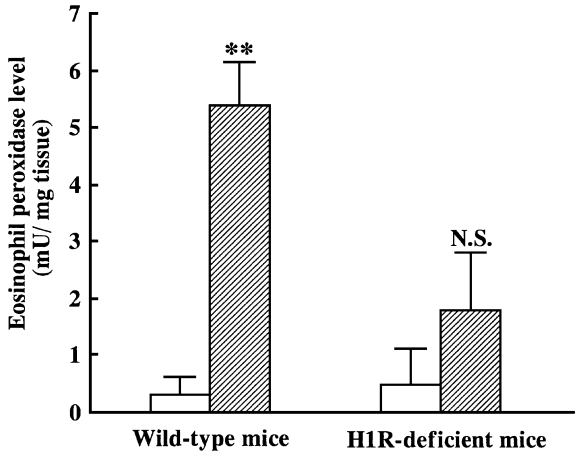


Fig. 2. Changes in conjunctival eosinophil peroxidase level in sensitized wild-type and histamine $H_1$ receptor-deficient mice. Conjunctival eosinophil peroxidase levels were measured 24 h after antigen challenge in sensitized wild-type and histamine $H_1$ receptor-deficient mice. H1R-deficient mice: histamine $H_1$ receptor-deficient mice. Control (open columns), ovalbumin (750 μg/site) (hatched columns). Each value represents the mean ± S.E.M. ($n = 8$). **: Significantly different from control at $P < 0.01$.

### 2.4. Eosinophil peroxidase assay

Conjunctivitis was induced by topical instillation of ovalbumin (750 μg/site, 5 μl). Twenty-four hours after antigen application, the mice were sacrificed under ether anesthesia, the conjunctiva was carefully excised, weighed, and washed twice with ice-cold phosphate buffered saline. The tissues were homogenized with 1 ml of 50 mM Tris–HCl buffer (pH 8.0) with a Polytron® (Kinematica, Lucerne, Switzerland) on ice. After addition of 350 μl of 50 mM Tris–HCl buffer and 150 μl of 0.1% Triton X-150, the homogenates were placed in ice bath for 1 h. Substrate solution (400 μl of 50 mM Tris–HCl containing 0.1% Triton X-100, 1 mM *o*-phenylenediamine, and 0.5 mM hydrogen peroxide) was added to the sample (200 μl), and incubated at 37 °C for 10 min before addition of 200 μl of 2 N sulfuric acid. The absorbance at 490 nm was then determined using a spectrophotometer (Hitachi, U-2000, Tokyo, Japan). Eosinophil peroxidase activity was measured according to the method of Strath et al. (1985) which is based on the oxidation of *o*-phenylenediamine by eosinophil peroxidase in the presence of hydrogen peroxide and 1 unit means 1 mmol of hydrogen peroxidase decomposed for 10 min.

### 2.5. Data analysis

Data are expressed as means ± S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison was used for evaluation of differences between the groups. Student's *t*-test was used for comparisons between two groups. Probability values less than 0.05 were considered significant.

## 3. Results

### 3.1. Time course of changes in conjunctival eosinophil peroxidase level in sensitized wild-type mice

As shown in Fig. 1, eosinophil peroxidase level in the conjunctivitis was increased significantly 6 h after application of antigen and lasted for 48 h. The peak effect was seen 24 h after application of antigen.

### 3.2. Changes in conjunctival eosinophil peroxidase level in sensitized wild-type and histamine $H_1$ receptor-deficient mice

Conjunctival eosinophil peroxidase levels were measured 24 h after antigen (ovalbumin) challenge in sensitized wild-type and histamine $H_1$ receptor-deficient mice. As shown in Fig. 2, a significantly high eosinophil peroxidase level was observed in sensitized wild-type mice following antigen instillation, whereas sensitized histamine $H_1$ receptor-deficient mice showed no significant increase in the conjunctival eosinophil peroxidase level.

### 3.3. Effects of anti-P-selectin antibody on conjunctival eosinophil peroxidase level in sensitized wild-type and histamine $H_1$ receptor-deficient mice

Results were shown in Fig. 3. Anti-P-selectin antibody was injected intravenously into sensitized wild-type and histamine $H_1$ receptor-deficient mice. Purified immunoglobulin reagent grade rat IgG was used as a control antibody. Thirty minutes after injection of anti-P-selectin antibody, antigen (ovalbumin) was instilled, and conjunctival eosinophil peroxidase levels were measured 24 h later. The elevation of eosinophil peroxidase level observed after antigen challenge in sensitized wild-type mice was significantly antagonized by pretreatment with anti-P-selectin antibody. On the other hand, no significant antagonism was observed in sensitized histamine $H_1$ receptor-deficient mice by administration of anti-P-selectin antibody.

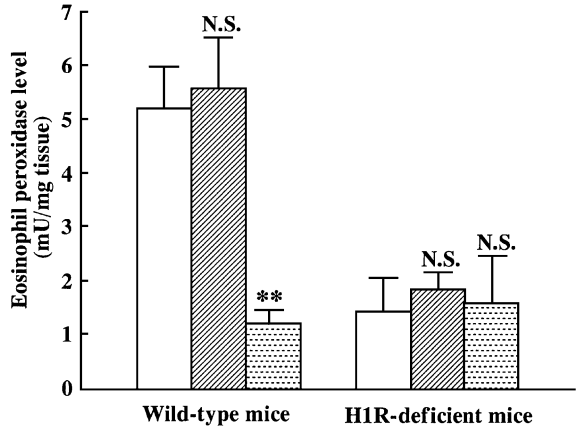


Fig. 3. Effects of anti-P-selectin antibody on conjunctival eosinophil peroxidase level in sensitized wild-type and histamine $H_1$ receptor-deficient mice. H1R-deficient mice: histamine $H_1$ receptor-deficient mice. Anti-P-selectin antibody was injected intravenously 30 min before topical instillation of ovalbumin. Purified immunoglobulin reagent grade rat IgG was used as a control antibody. Ovalbumin (750 μg/nite) (open columns), control antibody (20 μg) (hatched column), anti-P-seletin antibody (20 μg) (stripped columns). Each value represents the mean ± S.E.M. ($n = 8$). **: Significantly different from control at $P < 0.01$.

## 4. Discussion

Not only the early-phase reaction but also the late-phase reaction are generated in allergic diseases such as asthma, skin reaction, and rhinitis. In allergic conjunctivitis, the early-phase reaction is characterized by extensive degranulation of mast cells, whereas marked neutrophil, eosinophil, and basophil accumulation are seen in late-phase reaction (Leonardi et al., 1990). Among these leukocytes, activated eosinophils liberate cytotoxic proteins such as major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil peroxidase, and these proteins may cause damage to other cells, aggravating allergic inflammation (Martin et al., 1996). In ocular late-phase reaction, Leonardi et al. (1990) found that eosinophil accumulation was most marked in guinea pigs passively sensitized with IgG1 antibodies. Trocmé et al. (1988) also reported that greater participation of eosinophil in conjunctival anaphylaxis was observed than has been reported in cutaneous anaphylaxis in rats. Therefore, we measured eosinophil peroxidase level in the conjunctiva as an index of eosinophil infiltration. Eosinophil peroxidase level was increased 6 h after antigen challenge and lasted for 48 h with the peak effect at 24 h. This finding suggested that an increase in eosinophil peroxidase level in the conjunctiva is a good index of the late-phase reaction of allergic conjunctivitis.

As described in the text, a significant increase in eosinophil peroxidase level was observed in sensitized wild-type mice, whereas no increase was found in sensitized histamine $H_1$ receptor-deficient mice. These results clearly indicated that the increase in eosinophil peroxidase level of the conjunctiva in allergic conjunctivitis is completely regulated by histamine release from activated mast cells through histamine $H_1$ receptors. Similar findings were demonstrated by Santing et al. (1994) that blockade of histamine $H_1$ receptors in sensitized guinea pigs significantly reduced not only the early but also the late allergic reaction, as well as infiltration of inflammatory cells in the airway. The elevation of eosinophil peroxidase level observed after antigen challenge in sensitized wild-type mice was antagonized by pretreatment with anti-P-selectin antibody. The movement of leukocytes from the bloodstream to the afflicted tissue is a key feature of inflammation, and this process consists of two events: (1) the initial contact between the leukocytes and endothelium described as leukocyte rolling, and (2) firm or stationary adhesion (Atherton and Born, 1973; Subramaniam et al., 1995).

Kubes and Kanwar (1994) reported that P-selectin is a likely candidate as a mediator of the initial phase of leukocyte rolling and that histamine induces leukocyte rolling via a P-selectin-dependent mechanism in vivo. Mayadas et al. (1993) reported that P-selectin-deficient mice, generated by gene targeting of embryonic stem cells exhibit a number of defects in leukocyte behavior, including elevated numbers of circulating neutrophils, virtually total absence of leukocyte rolling in mesenteric venules. Therefore, it seems likely that inhibition of eosinophil peroxidase infiltration into the conjunctiva in histamine $H_1$ receptor-deficient mice is due to inhibition of the expression of P-selectin.

From these findings, we concluded that eosinophil infiltration in the late-phase reaction of allergic conjunctivitis is mediated by P-selectin through histamine $H_1$ receptors.

## References


Atherton, A., Born, G.V.R., 1973. Relationship between the velocity of rolling granulocytes and that of the blood flow in venules. J. Physiol. 233, 157–165.

Calonge, M.C., Pastor, J.C., Herreras, J.M., González, J.L., 1990. Pharmacologic modulation of vascular permeability in ocular allergy in the rat. Invest. Ophthalmol. Visual Sci. 31, 176–180.

Inoue, I., Yanai, K., Kitamura, D., Taniuchi, I., Kobayashi, T., Niimura, K., Watanabe, T., Watanabe, T., 1996. Impaired locomotor activity and exploratory behavior in mice lacking histamine $H_1$ receptors. Proc. Natl. Acad. Sci. U. S. A. 93, 13316–13320.

Kamei, C., Izushi, K., Nakamura, S., 1995. Effects of certain antiallergic drugs on experimental conjunctivitis in guinea pigs. Biol. Pharm. Bull. 18, 1518–1521.

Kubes, P., Kanwar, S., 1994. Histamine induces leukocyte rolling in post-capillary venules. A P-selectin-mediated event. J. Immunol. 152, 3570–3577.

Leonardi, A., Bloch, K.J., Briggs, R., Allansmith, M.R., 1990. Histology of ocular late-phase reaction in guinea pigs passively sensitized with IgG1 antibodies. Ophthalmic Res. 22, 209–219.

Martin, L.B., Kita, H., Leifeman, K.M., Gleich, G.J., 1996. Eosinophils in allergy: role in disease, degranulation, and cytokines. Int. Arch. Allergy Immunol. 109, 207–215.

Mayadas, T.N., Johnson, R.C., Rayburn, H., Hynes, R.O., Wagner, D.D., 1993. Leukocyte rolling and extravasation are severely compromised in *P* selectin-deficient mice. Cell 74, 541–554.

Melamed, J., Schwartz, R.H., Blumenthal, M.N., Zeitz, H.J., 2000. Efficacy and safety of nedocromil sodium 2% ophthalmic solution b.i.d. in the treatment of ragweed seasonal allergic conjunctivitis. Allergy Asthma Proc. 21, 235–239.

Morimoto, T., Yamamoto, Y., Mobarakeh, J.I., Yanai, K., Watanabe, T., Watanabe, T., Yamatodani, A., 1999. Involvement of the histaminergic system in leptin-induced suppression of food intake. Physiol. Behav. 67, 679–683.

Nakahara, H., Izushi, K., Sugimoto, Y., Watanabe, T., Kamei, C., 2000. Vascular permeability in allergic conjunctivitis in mice lacking histamine $H_1$ receptors. Eur. J. Pharmacol. 409, 313–317.

Oettgen, H.C., Martin, T.R., Wynshaw-Boris, A., Deng, C., Drazen, J.M., Leder, P., 1994. Active anaphylaxis in IgE-deficient mice. Nature 370, 367–370.

Santing, R.E., Schraa, E.O., Wachters, A., Olymulder, C.G., Zaagsma, J., Meurs, H., 1994. Role of histamine in allergen-induced asthmatic reactions, bronchial hyperreactivity and inflammation in unrestrained guinea pigs. Eur. J. Pharmacol. 254, 49–57.

Solley, G.O., Gleich, G.J., Jordon, R.E., Schroeter, A.L., 1976. The late phase of the immediate wheal and flare skin reaction. Its dependence upon IgE antibodies. J. Clin. Invest. 58, 408–420.

Strath, M., Warren, D.J., Sanderson, C.J., 1985. Detection of eosinophils using an eosinophil peroxidase assay. Its use as an assay for eosinophil differentiation factors. J. Immunol. Methods 83, 209–215.

Subramaniam, M., Saffaripour, S., Watson, S.R., Mayadas, T.N., Hynes, R.O., Wagner, D.D., 1995. Reduced recruitment of inflammatory cells in a contact hypersensitivity response in P-selectin-deficient mice. J. Exp. Med. 181, 2277–2282.

Trocmé, S.D., Bonini, S., Barney, N.P., Bloch, K.J., Allansmith, M.R., 1988. Late-phase reaction in topically induced ocular anaphylaxis in the rat. Curr. Eye Res. 7, 437–443.

Woodward, D.F., Ledgard, S.E., Nieves, A.L., 1986. Conjunctival immediate hypersensitivity: re-evaluation of histamine involvement in the vasopermeability response. Invest. Ophthalmol. Visual Sci. 27, 57–63.