J Pharmacol Sci **106**, 354 – 360 (2008)

Journal of Pharmacological Sciences

Forum Minireview

Progress in Allergy Signal Research on Mast Cells: The Role of Histamine in Goblet Cell Hyperplasia in Allergic Airway Inflammation – a Study Using the Hdc Knockout Mouse

Kohei Yamauchi[1,*], Hong Mei Piao[1,5], Toshihide Nakadate[1], Toshiki Shikanai[1], Yutaka Nakamura[1], Miyuki Niisato[1], Harumasa Ito[1], Takashi Mouri[1], Hitoshi Kobayashi[1], Chihaya Maesawa[2], Takashi Sawai[3], Hiroshi Ohtsu[4], and Hiroshi Inoue[1]

[1]*Third Department of Internal Medicine,* [2]*Second Department of Pathology,* [3]*First Department of Pathology, Iwate Medical University School of Medicine, 19-1 Uchimaru, Morioka 020-8585, Japan*
[4]*Tohoku University Graduate School of Engineering, 6-6-4 Aramaki Aza Aoba, Aobaku, Sendai 980-8579, Japan*
[5]*Department of Respiratory Medicine, Affiliated Hospital of Yanbian University College of Medicine, Yanji, China*

Received December 5, 2007; Accepted December 19, 2007

Abstract. Although histamine is a central mediator in the immediate allergic reaction, its role in goblet cell hyperplasia in the airway of asthma is not completely understood. This study was designed to examine the role of histamine in goblet cell hyperplasia using histamine-deficient mice ($Hdc^{-/-}$ mice) with allergic airway inflammation. Wild-type and $Hdc^{-/-}$ C57BL/6 mice were sensitized with ovalbumin (OVA). After two-week exposure to OVA, goblet cell hyperplasia was evaluated. Cell differentials in BALF were analyzed. The mRNAs level of *MUC5AC* and *Gob-5* gene were quantitatively determined. The number of eosinophils in BALF increased in both the wild-type mice and $Hdc^{-/-}$ mice; however, their ratio in $Hdc^{-/-}$ mice was significantly lower than that in the wild-type mice. The mRNA levels of *Gob-5* and *MUC5AC* and the ratio of the goblet cells in the airway epithelium were significantly increased in $Hdc^{-/-}$ mice exposed to OVA compared to the wild-type mice under the same condition. These results suggested that histamine may play a regulatory role in goblet cell hyperplasia in allergic airway inflammation.

Keywords: airway remodeling, asthma, *Gob-5*, *MUC5AC*, allergy

Introduction

Goblet cell hyperplasia and mucus overproduction are important features of bronchial asthma (1, 2). Although goblet cell hyperplasia with mucus hypersecretion has been reported to be associated with the development of airway hyperresponsiveness and the increase of severity and mortality in bronchial asthma (1 – 4), the mechanisms responsible for goblet cell hyperplasia are not completely understood. To date, goblet cell hyperplasia has been thought to be induced after epithelial damage by numerous stimuli such as endogenous oxidants induced by neutrophil elastase (5), exogenous oxidants produced by cigarette smoke (6), and diesel engine emissions (7). On the other hand, it has been demonstrated that Th-2 lymphocyte–derived cytokines interleukin (IL)-4, IL-5, IL-9, and IL-13 induce goblet cell hyperplasia in animal models and in vitro studies (8 – 12).

According to previous reports, several genes are upregulated in association with goblet cell hyperplasia. Among them, *MUC* genes encode various mucin glycoproteins that are primary secretory proteins and consist of family genes (i.e., *MUC-1*, *MUC-2*, and *MUC-4*) (13, 14). *MUC5AC* is the predominant *MUC* gene expressed in the airway (14). To date, it has been demonstrated that the level of *MUC5AC* gene expression increased in animal models of asthma and tissue samples from asthmatic patients (15 – 17).

In addition, it has been reported that the overexpression of the *Gob-5* gene in airway epithelia exacerbates AHR, goblet cell hyperplasia, mucus overproduction, and eosinophil infiltration in a murine asthma model

*Corresponding author. kyamauch@iwate-med.ac.jp
Published online in J-STAGE
doi: 10.1254/jphs.FM0070262

(18). The *Gob-5* gene in the mouse corresponds to the *CLCA1* gene in humans, which regulates calcium-activated chloride conductance, has been shown to induce mucin gene expression (*MUC5AC*) in the human mucoepidermoid cell line NCI-H292 and its increased expression was demonstrated in asthmatic patients (18 – 20).

In this paper, to elucidate the roles of histamine in allergic airway inflammation, we produced an experimental asthma model with $Hdc^{-/-}$ mice. In particular, we analyzed effects of histamine on goblet cell hyperplasia.

Materials and Methods

Animals

Hdc knockout ($Hdc^{-/-}$) mice were generated by Ohtsu et al. (21). In the present study, we used 6- to 8-week-old female $Hdc^{-/-}$ mice backcrossed for six generations with C57BL/6 mice and used age/gender-matched C57BL/6N mice (Japan SLC, Shizuoka) as controls. Mice used for this study were generated by breeding of homozygous $Hdc^{-/-}$ mice in the facilities of Tohoku University (Sendai). Both $Hdc^{-/-}$ and wild-type mice were kept on a normal diet containing <0.3 mg of histamine/g of food. All experiments described in this study were performed according to the guidelines for the care and use of experimental animals as determined by the Japanese Association for Laboratory Animals Science in 1987.

Immunization and aerosolization protocol

The mice were sensitized according to the methods described in a previous paper (22). In brief, mice were sensitized at days 0 and 5 of the protocol by an intraperitoneal injection of 0.5 ml aluminum hydroxide–precipitated antigen containing 8 μg OVA (Sigma Chemical Co., St. Louis, MO, USA) adsorbed overnight at 4°C to 4 mg of aluminium hydroxide (Wako Chemical Co., Tokyo) in phosphate-buffered saline (PBS). Twelve days later, wild-type mice and $Hdc^{-/-}$ mice were divided into 3 groups, respectively, which consisted of 6 animals. One set of the wild-type mice group and the $Hdc^{-/-}$ mice group were killed for analysis, respectively, as a control without inhalation. To perform chronic exposure of OVA, the 2 groups of the wild-type mice and $Hdc^{-/-}$ mice, respectively, were placed in a plastic chamber (10 cm × 15 cm × 25 cm) and exposed to aerosolized OVA (5 mg/ml in 0.9% saline) for 1 h. The other 2 groups of the wild-type mice and $Hdc^{-/-}$ mice were each exposed to 0.9% saline only every other day for 14 days, also, as shown in Fig. 1. The aerosolized OVA was produced by Pulmo-Aide Compressor /Nebulizer (Devilbiss) (Sunrise Medical HHG, Inc., Somerset, PA, USA) at a flow rate of 5 – 7 liter/min.

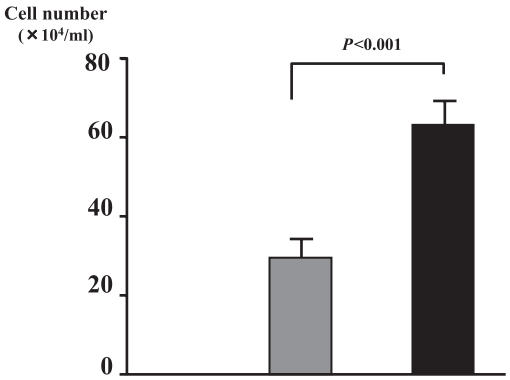

Fig. 1. Total cell numbers in BALF. Gray column: the wild-type mice, black column: $Hdc^{-/-}$ mice.

Collection and measurement of specimens

After being exposed to aerosolized saline or OVA every other day over 2 weeks, each group of mice was killed on the 14th day, 24 h after final inhalation, and bronchoalveolar lavage fluid (BALF) and lung tissue were collected as shown in Fig. 1. To collect BALF, the lungs were dissected and the trachea was cannulated with a polyethylene tube (Becton Dickinson, Sparks, MD, USA). The lungs were lavaged twice with 0.5 ml PBS, and ~0.8 ml of the instilled fluid was consistently recovered. The recovered fluid was centrifuged (300 × *g* for 6 min) and the cells were resuspended in 0.5 ml PBS. The total number of cells was counted using an improved Neubauer hemocytometer chamber. An air-dried slide preparation was made of each sample containing 10,000 cells by cytospin (Cytocentrifuge; Sakura Seiki, Tokyo) and stained with May-Grunwald-Giemsa stain. Differential counts of at least 500 cells were made according to standard morphologic criteria. The numbers of cells recovered per mouse were then expressed as the mean and S.E.M. for each treated group.

After harvesting BALF, lungs were fixed with formaldehyde and then embedded in paraffin. These 3-μm-thick sections were stained with hematoxylin eosin (HE), Elastica Masson's trichrome (EM), and periodic acid–Schiff (PAS). In addition, the other parts of left lungs were stored at −80°C for RNA extraction.

Histological measurement of goblet cell number

The 3-mm paraffin sections of the lungs described above were stained with PAS for evaluation of goblet cells. Goblet cell hyperplasia was determined by counting the number of PAS-positive cells in more than 5 large (diameter >200 μm) and more than 10 small bronchi (diameter <150 μm) per each lung under

microscopy. The results were expressed as % of PAS-positive cells per total epithelial cells. In addition, lung sections were stained with HE.

Isolation of total RNA and real-time quantitative PCR

Total RNA from tissues was obtained using ISOGEN (Wako Pure Chemicals, Osaka) and quantitated by spectrophotometry. Quality of the obtained RNA was examined by agarose gel electrophresis.

To quantify the expression of mRNA of *Gob-5*, *MUC5AC*, and glyceraldehyde-3-phosphatase dehydrogenase (*GAPDH*) in murine lungs, quantitative PCR was carried out using an ABI Prism 7700 Sequence Detector (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) as previously described (23).

Oligonucleotide PCR primer pairs and fluorogenic probes for murine *MUC5AC* and *Gob-5* were designed from the published sequences using Primer Express software (Perkin-Elmer): for *MUC5AC*, sense primer: 5'-ACTGTTACTATGCGATGTGTAGCCA-3', antisense primer: 5'-GAGGAAACACATTGCACCGA-3'; Taqman probe: 5'-[FAM]ACTGCCACCTGTCACTGGGCGG [TAMRA]-3' and for *Gob-5*, sense primer: 5'-AGGGCATCGTCATCGCC-3'; antisense primer: 5'-TCCTTTATGTGTTGAATGAGGGC-3'; Taqman probe: 5'-[FAM]AGACCACGACGTGCCGGAAGATG [TAMRA]-3' (24). Primers and labeled probe (VIC) for rodent *GAPDH* were purchased from Perkin-Elmer Applied Biosystems. Briefly, a 100-ng sample of RNA dissolved in 10 μl of water from each aliquot of murine lung tissue was denatured at 90°C for 90 s. Each RNA sample (100 ng/10 μl of water) was mixed in 40 μl of buffer containing the following reagents for the one-step RT-PCR reaction: 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01 mM EDTA, 60 nM Passive Reference 1 (Applied Biosystems), 5 mM $MgCl_2$, 100 nM sense primer, 100 nM antisense primer, 0.3 mM deoxynucleoside triphosphate (Boehringer), 0.4 U/μl RNase inhibitor (Promega), 0.4 U/μl Moloney murine leukemia virus RT (Perkin Elmer), 0.0025 U/μl *Taq* Gold Polymerase (Perkin Elmer), and 100 nM Taqman probe, as described above. The fragment of mRNA for *Gob-5*, *MUC5AC*, and *GAPDH* was reverse transcribed into cDNA (30 min at 48°C) and amplified by PCR for 40 cycles (15 s at 95°C and 1 min at 60°C). Whole reactions of the RT-PCR and detection of the fluorescence emission signal for every PCR cycle were performed at the same time in a single tube in a sequence detector (ABI 7700). The minimum PCR cycle to detect the fluorescent signal was defined as the cycle threshold (C), which is predictive of the quantity of an input target fragment (25). The standard curve was obtained between the fluorescence emission signals and C by means of duplicated serial dilutions of the total RNA from murine lung in medium alone. Expression of *MUC5AC* and *Gob-5* mRNA was normalized to a constitutive expression of *GAPDH* mRNA.

Statistical analyses

Data were each expressed as a mean ± S.E.M. Multiple comparisons of mean data among the groups were performed by the Mann-Whitney U test. Probability values of less than 0.05 were considered statistically significant.

Results

Cell differentials in BALF of $Hdc^{-/-}$ mice

Repetitive exposure of OVA induced a marked increase of total cell numbers in BALF from both wild and $Hdc^{-/-}$ mice sensitized with OVA, compared with those from the wild-type mice group and mice sensitized with OVA exposed to saline (Fig. 1). Total cell number in BALF from the $Hdc^{-/-}$ mice sensitized with OVA after exposure to OVA increased significantly more than that from the wild-type mice in the same condition. Eosinophils were predominant cells in BALF from the wild-type mice exposed to OVA. In contrast, both eosinophils and lymphocytes increased in BALF from the $Hdc^{-/-}$ mice exposed with OVA, and the ratio of eosinophils in BALF was significantly lower in the $Hdc^{-/-}$ mice (Fig. 2).

Gob-5 gene expression in the lung of $Hdc^{-/-}$ mice

Levels of *Gob-5* mRNA in lung tissue of both wild-type and $Hdc^{-/-}$ mice sensitized with OVA increased markedly compared with those from wild-type and $Hdc^{-/-}$ mice sensitized with OVA exposed to saline.

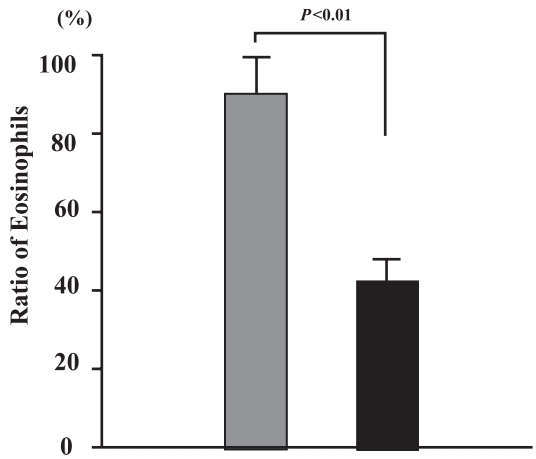

Fig. 2. The ratio of eosinophils in BALF. Gray column: the wild-type mice, black column: $Hdc^{-/-}$ mice.

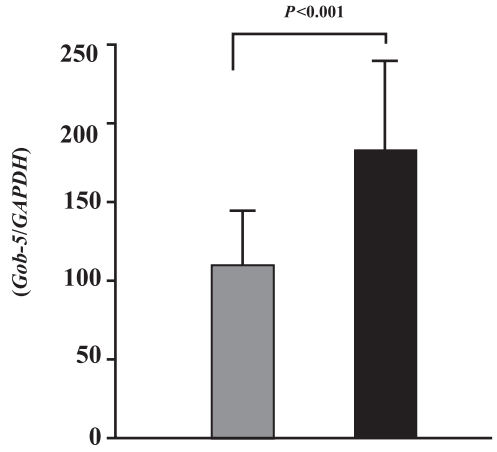

Fig. 3. *Gob-5* gene expression in the lung. Gray column: the wild-type mice, black column: *Hdc*$^{-/-}$ mice.

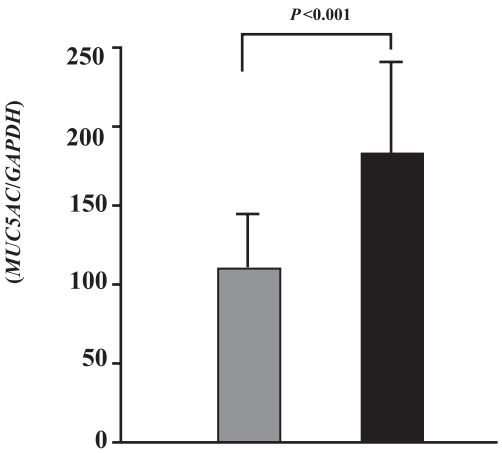

Fig. 4. *MUC5AC* gene expression in the lung. Gray column: the wild-type mice, black column: *Hdc*$^{-/-}$ mice.

The level of *Gob-5* mRNA in the *Hdc*$^{-/-}$ mice exposed to OVA was significantly higher than that in the wild-type mice in the same condition (Fig. 3).

MUC5AC gene expression in the lung of Hdc$^{-/-}$ *mice*

Levels of *MUC5AC* mRNA in lung tissue of both wild-type and *Hdc*$^{-/-}$ mice sensitized with OVA and exposed to OVA increased markedly compared with those from wild-type and *Hdc*$^{-/-}$ mice sensitized with OVA exposed to saline. The level of *MUC5AC* mRNA in the *Hdc*$^{-/-}$ mice exposed to OVA was significantly higher than that in the wild-type mice under the same condition (Fig. 4).

Histology of large and small airways

PAS staining demonstrated an increase of goblet cells in the epithelium in large and small airways of both wild and *Hdc*$^{-/-}$ mice sensitized with OVA (Fig. 5: A and B and Fig. 6: A and B). Goblet cell hyperplasia was more prominent in the *Hdc*$^{-/-}$ mice exposed to OVA than that in the wild-type mice under the same condition (Fig. 5: A and B and Fig. 6: A and B). Repetitive saline inhalation induced a mild increase of goblet cells in large and small airways of both wild-type and *Hdc*$^{-/-}$ mice (Fig. 5: A and B and Fig. 6: A and B).

Goblet cell hyperplasia in large and small airways

The ratios of goblet cells in the epithelium in large and small airways of both wild-type and *Hdc*$^{-/-}$ mice sensitized with OVA and exposed to OVA increased markedly compared with those from wild-type and *Hdc*$^{-/-}$ mice sensitized with OVA exposed to saline. The ratios of goblet cells in the epithelium in large and small airways of the *Hdc*$^{-/-}$ mice exposed to OVA was significantly higher than those in the wild-type mice under the same condition (Fig. 7).

Discussion

We demonstrated in the present paper that goblet cell hyperplasia was augmented in the *Hdc*$^{-/-}$ mice sensitized with OVA after repetitive OVA exposure over 14 days. We also demonstrated that higher levels of Gob-5 and MUC5AC mRNA expressed in the *Hdc*$^{-/-}$ mice after repetitive OVA exposure compared with the wild-type mice.

Histamine has been recognized as a chemical mediator playing a central role in allergic reactions including mucosal edema, mucous gland secretion, smooth muscle contraction, and so on. Recent studies have demonstrated that histamine plays critical roles in immunomodulation by acting on different types of histamine receptors such as H1R, H2R, and H4R on the surface of immune cells and inflammatory cells (26 – 31). Using mutant mice lacking H1R and H2R, Jutel et al. demonstrated that histamine enhanced TH1-type responses by triggering H1R, whereas both TH1- and TH2-type responses were negatively regulated by H2R. Actually, deletion of H1R resulted in suppression of IFN-γ, IL-4, and IL-13; and deletion of H2R showed upregulation of both TH1 and TH2 cytokines (IL-4 and IL-13) (27). Mazzoni et al. demonstrated that histamine inhibited INF-α and TNF-α release from activated plasmacytoid dendritic cells, through H2R (32). Histamine also inhibited lipopolysaccharide-induced TNF-α production by down-regulating intercellular adhesion molecules (ICAM)-1 in human monocytes through H2R (33). We speculate that the changes of cytokine production in the *Hdc*$^{-/-}$ mice might influence on the goblet cell hyperplasia.

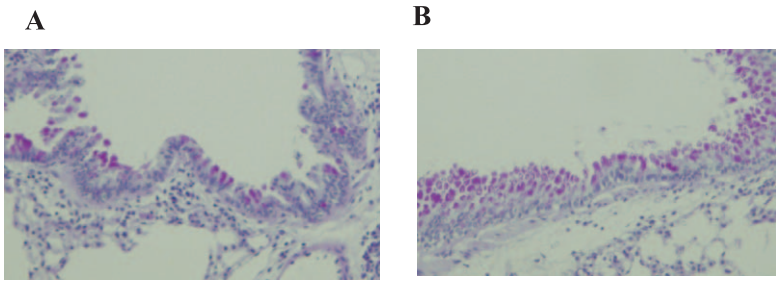

Fig. 5. Goblet cells in the large airways. The goblet cells in the epithelium were stained with PAS. Panel A: the wild-type mice, panel B: *Hdc*$^{-/-}$ mice.

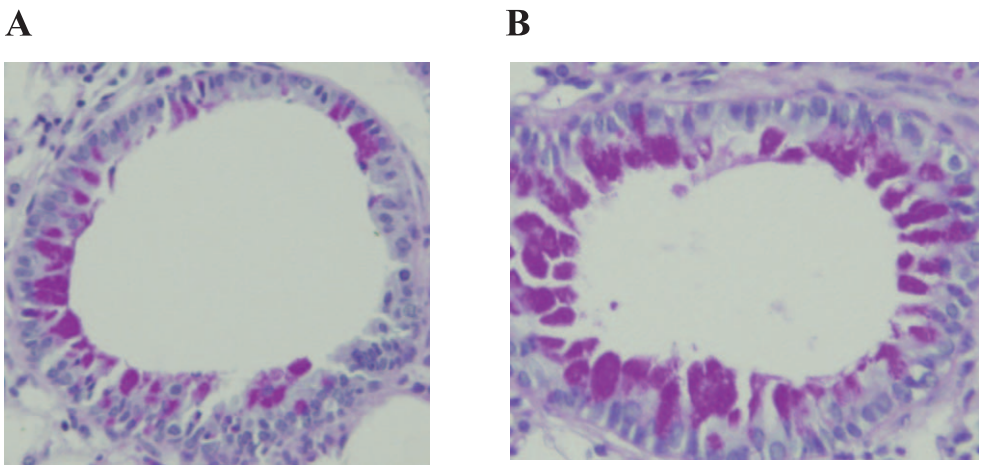

Fig. 6. Goblet cells in the small airways. The goblet cells in the epithelium were stained with PAS. Panel A: the wild-type mice, panel B: *Hdc*$^{-/-}$ mice.

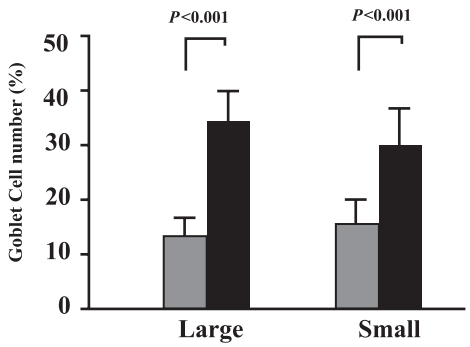

Fig. 7. Goblet cell ratio in the large and small airways. Gray column: the wild-type mice, black column: *Hdc*$^{-/-}$ mice.

We demonstrated the increase of goblet cells in the epithelium of both large and small airways in the *Hdc*$^{-/-}$ mice exposed to OVA compared to the wild-type mice under the same condition. Previous reports have revealed that Th2 cytokines such as IL-4, IL-5, IL-9, and IL-13 were involved in goblet cell hyperplasia in allergic airway inflammation (8 – 11). Recently, Busse et al. demonstrated that chronic exposure of TNF-α is related to airway-induced goblet cell hyperplasia (34). We need to measure these cytokines to elucidate the mechanism of goblet cell hyperplasia in the *Hdc*$^{-/-}$ mice.

In association with the increase of goblet cells, the higher levels of Gob-5 and *MUC5AC* mRNA were evaluated in the *Hdc*$^{-/-}$ mice exposed to OVA. Previous reports suggested that the *Gob-5* gene, which corresponds to *hCLCA1* in human beings, was thought to be one of first steps in mucus cell metaplasia and hyperplasia inducing mucin gene expression (18). The *MUC5AC* gene, a member of the mucin gene family, has been evaluated to be exclusively expressed in the airway and associated with hyperplasia of goblet cells. In addition to IL-4, IL-5, IL-9, and IL-13 (35 – 38), TNF-α was reported to increase the expressions of the *Gob-5* and *MUC5AC* genes. These evidences suggested that the changes in the production of these cytokines in the *Hdc*$^{-/-}$ mice might cause the higher mRNA levels of the *Gob-5* and *MUC5AC* genes, leading to the increased number of goblet cells in the airway.

The cell differentials in the BALF of the sensitized the

Hdc$^{-/-}$ mice exposed to OVA were different from those of the wild-type mice under the same condition. The ratio of eosinophils in the BALF of the *Hdc*$^{-/-}$ mice was lower compared to that of the wild-type mice. Koarai et al. demonstrated the lower expression of P-selectin in the lung of the *Hdc*$^{-/-}$ mice compared to the wild-type mice, and they suggested that the low level of P-selectin expression might cause a low influx of eosinophils from blood into the alveolar space in the *Hdc*$^{-/-}$ (39). A recent study has demonstrated that the H4R mediates eosinophil chemotaxis, cell shape change, and upregulation of adhesion molecules (30). In our experiment, we could speculate that H4R-dependent eosinophil chemotaxis could not be fully activated in allergic airway inflammation in the *Hdc*$^{-/-}$ mice; and as a result, eosinophil influx into alveolar space might be suppressed after allergen challenge.

In summary, we demonstrated the augmented goblet cell hyperplasia in the airway of OVA-sensitized histamine-deficient mice exposed to OVA during two weeks compared with the wild-type mice under the same condition.

Acknowledgment

This study was supported by the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

References

1 Laitinen A, Laitinen LA. Pathology of asthma. Allergy Proc. 1994;15:323–328.
2 Shimura S, Andoh Y, Haraguchi M, Shirato K. Continuity of airway goblet cells and intraluminal mucus in the airways of patients with bronchial asthma. Eur Respir J. 1996;9:1395–1401.
3 Carroll N, Carello S, Cooke C, James A. Airway structure and inflammatory cells in fatal attacks of asthma. Eur Respir J. 1996;9:709–715.
4 Aikawa T, Shimura S, Sasaki H, Ebina M, Takishima T. Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack. Chest. 1992;101:916–921.
5 Voynow JA, Fischer BM, Malarkey DE, Burch LH, Wong T, Longphre M, et al. Neutrophil elastase induces mucus cell metaplasia in mouse lung. Am J Physiol Lung Cell Mol Physiol. 2004;287:L1293–L1302.
6 Harkema JR, Hotchkiss JA. Ozone- and endotoxin-induced mucous cell metaplasias in rat airway epithelium: novel animal models to study toxicant-induced epithelial transformation in airways. Toxicol Lett. 1993;68:251–263.
7 Ichinose T, Takano H, Miyabara Y, Sadakaneo K, Sagai M, Shibamoto T. Enhancement of antigen-induced eosinophilic inflammation in the airways of mast-cell deficient mice by diesel exhaust particles. Toxicology. 2002;180:293–301.
8 Dabbagh K, Takeyama K, Lee HM, Ueki IF, Lausier JA, Nadel JA. IL-4 induces mucin gene expression and goblet cell metaplasia *in vitro* and *in vivo*. J Immunol. 1999;162:6233–6237.
9 Longphre M, Li D, Gallup M, Drori E, Ordonez CL, Redman T, et al. Allergen-induced IL-9 directly stimulates mucin transcription in respiratory epithelial cells. J Clin Invest. 1999;104:1375–1382.
10 Temann UA, Prasad B, Gallup MW, Basbaum C, Ho SB, Flavell RA, et al. A novel role for murine IL-4 in vivo: induction of MUC5AC gene expression and mucin hypersecretion. Am J Respir Cell Mol Biol. 1997;16:471–478.
11 Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J Clin Invest. 1999;103:779–788.
12 Trifilieff A, Fujitani Y, Coyle AJ, Kopf M, Bertrand C. IL-5 deficiency abolishes aspects of airway remodelling in a murine model of lung inflammation. Clin Exp Allergy. 2001;31:934–942.
13 Rose MC, Voynow JA. Respiratory tract mucin genes and mucin glycoproteins in health and disease. Physiol Rev. 2006;86:245–278.
14 Fahy JV. Goblet cell and mucin gene abnormalities in asthma. Chest. 2002;122:320S–326S.
15 Groneberg DA, Eynott PR, Lim S, Oates T, Wu R, Carlstedt I, et al. Expression of respiratory mucins in fatal status asthmaticus and mild asthma. Histopathology. 2002;40:367–373.
16 Ordonez CL, Khashayar R, Wong HH, Ferrando R, Wu R, Hyde DM, et al. Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. Am J Respir Crit Care Med. 2001;163:517–523.
17 Zuhdi Alimam M, Piazza FM, Selby DM, Letwin N, Huang L, Rose MC. Muc-5/5ac mucin messenger RNA and protein expression is a marker of goblet cell metaplasia in murine airways. Am J Respir Cell Mol Biol. 2000;22:253–260.
18 Nakanishi A, Morita S, Iwashita H, Sagiya Y, Ashida Y, Shirafuji H, et al. Role of gob-5 in mucus overproduction and airway hyperresponsiveness in asthma. Proc Natl Acad Sci U S A. 2001;98:5175–5180.
19 O'Neill L. Gob genes, mucus and asthma. Trends Immunol. 2001;22:353.
20 Zhou Y, Shapiro M, Dong Q, Louahed J, Weiss C, Wan S, et al. A calcium-activated chloride channel blocker inhibits goblet cell metaplasia and mucus overproduction. Novartis Found Symp. 2002;248:150–165.
21 Ohtsu H, Tanaka S, Terui T, Hori Y, Makabe-Kobayashi Y, Pejler G, et al. Mice lacking histidine decarboxylase exhibit abnormal mast cells. FEBS Lett. 2001;502:53–56.
22 Ohkawara Y, Lei XF, Stampfli MR, Marshall JS, Xing Z, Jordana M. Cytokine and eosinophil responses in the lung, peripheral blood and bone marrow compartments in a murine model of allergen-induced airways inflammation. Am J Respir Cell Mol Biol. 1997;16:510–520.
23 Martell M, Gomez J, Esteban JI, Sauleda S, Quer J, Cabot B, et al. High-throughput real-time reverse transcription-PCR quantitation of hepatitis C virus RNA. J Clin Microbiol. 1999;37:327–332.
24 Ryseck RP, Macdonald-Bravo H, Mattei MG, Bravo R. Structure, mapping, and expression of fisp-12, a growth factor-inducible gene encoding a secreted cysteine-rich protein. J Cell

Growth Differ. 1991;2:225–233.
25 Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res. 1996;6:986–994.
26 Siegel JN, Schwartz A, Askenase PW, Gershon RK. T-cell suppression and contrasuppression induced by histamine H2 and H1 receptor agonists, respectively. Proc Natl Acad Sci U S A. 1982;79:5052–5056.
27 Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OA, Malolepszy J, et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature. 2001;413:420–425.
28 Oda T, Matsumoto S, Masuho Y, Takasaki J, Matsumoto M, Kamohara M, et al. cDNA cloning and characterization of porcine histamine H4 receptor. Biochim Biophys Acta. 2002; 1575:135–138.
29 Nakayama T, Kato Y, Hieshima K, Nagakubo D, Kunori Y, Fujisawa T, et al. Liver-expressed chemokine/CC chemokine ligand 16 attracts eosinophils by interacting with histamine H4 receptor. J Immunol. 2004;173:2078–2083.
30 Ling P, Ngo K, Nguyen S, Thurmond RL, Edwards JP, Karlsson L, et al. Histamine H4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. Br J Pharmacol. 2004;142:161–171.
31 Hofstra CL, Desai PJ, Thurmond RL, Fung-Leung WP. Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. J Pharmacol Exp Ther. 2003;305:1212–1221.
32 Mazzoni A, Leifer CA, Mullen GE, Kennedy MN, Klinman DM, Segal DM. Cutting edge: histamine inhibits IFN-alpha release from plasmacytoid dendritic cells. J Immunol. 2003;170:2269–2273.
33 Morichika T, Takahashi HK, Iwagaki H, Yoshino T, Tamura R, Yokoyama M, et al. Histamine inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production in an intercellular adhesion molecule-1- and B7.1-dependent manner. J Pharmacol Exp Ther. 2003;304:624–633.
34 Busse PJ, Zhang TF, Srivastava K, Lin BP, Schofield B, Sealfon SC, et al. Chronic exposure to TNF-alpha increases airway mucus gene expression *in vivo*. J Allergy Clin Immunol. 2005;116:1256–1263.
35 Temann UA, Prasad B, Gallup MW, Basbaum C, Ho SB, Flavell RA, et al. A novel role for murine IL-4 *in vivo*: induction of MUC5AC gene expression and mucin hypersecretion. Am J Respir Cell Mol Biol. 1997;16:471–478.
36 Lee JJ, McGarry MP, Farmer SC, Denzler KL, Larson KA, Carrigan PE, et al. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. J Exp Med. 1997;185:2143–2156.
37 Longphre M, Li D, Gallup M, Drori E, Ordonez CL, Redman T, et al. Allergen-induced IL-9 directly stimulates mucin transcription in respiratory epithelial cells. J Clin Invest. 1999;104:1375–1382.
38 Atherton HC, Jones G, Danahay H. IL-13-induced changes in the goblet cell density of human bronchial epithelial cell cultures: MAP kinase and phosphatidylinositol 3-kinase regulation. Am J Physiol Lung Cell Mol Physiol. 2003;285:L730–L739.
39 Koarai A, Ichinose M, Ishigaki-Suzuki S, Yamagata S, Sugiura H, Sakurai E, et al. Disruption of L-histidine decarboxylase reduces airway eosinophilia but not hyperresponsiveness. Am J Respir Crit Care Med. 2003;167:758–763.