

Histamine Type 1 Receptor Deficiency Reduces Airway Inflammation in a Murine Asthma Model

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Key Words

Airway inflammation • Histamine • H1 receptor gene knockout mouse • Asthma, murine model • T helper type 2 response

Abstract

Background: Histamine plays an important role in immediate and late immune responses. The histamine type 1 (H1) receptor is expressed on several immune cell populations, but its role in a murine model of asthma remains unclear. The present study evaluated the role of histamine H1 receptors in airway allergic inflammation by comparing the development of bronchial asthma in histamine H1 receptor gene knockout (H1RKO) and wild-type mice. **Methods:** H1RKO and wild-type mice were sensitized by intraperitoneal injection of ovalbumin (OVA) or saline, and then challenged with aerosolized OVA or saline. Ventilatory timing in response to inhaled methacholine was measured, and samples of blood, bronchoalveolar lavage, and lung tissues were taken 24 h after the last OVA challenge. **Results:** OVA-treated wild-type mice showed significantly increased airway eosinophilic infiltration, and airway response to methacholine compared to OVA-treated H1RKO mice. The serum level of immunoglobulin

lin E and levels of interleukin (IL)-4, IL-5, IL-13, and TGF- β 1 in bronchoalveolar lavage fluid were lower in OVA-treated H1RKO mice than in OVA-treated wild-type mice, but there was no significant difference in interferon- γ expression. Overall, deletion of histamine H1 receptors reduced allergic responses in a murine model of bronchial asthma. **Conclusion:** Histamine plays an important role via H1 receptors in the development of T helper type 2 responses to enhance airway inflammation.

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Introduction

Histamine is an important proinflammatory mediator in bronchial asthma. It is produced by degranulation of mast cells, causing airway smooth muscle contraction, vasodilation, plasma exudation, and mucus hypersecretion. These actions are mediated by histamine type 1 (H1) receptors [1, 2]. In addition to these immediate immune responses, histamine also mediates late immune responses [3]. Frequent consequences of late immune responses are T cell-mediated airway inflammation and airway hyperresponsiveness (AHR), causing bronchial asthma.

Several immune cells including dendritic cells, T cells, B cells, monocytes, and macrophages are activated during allergic response in asthma, and predominant T helper type 2 (Th2) cell activation is a key event in allergic responses. Histamine influences T helper type 1 (Th1)/Th2 balance by modulating secretion of cytokines [3–5]. To date, four types of histamine receptors (H1, H2, H3, and H4) have been identified, and all of them are expressed on immune cells [3]. Two of the histamine receptors, H1 and H2, play a critical role in allergic inflammation associated with asthma. H1 receptors potentiate a proinflammatory activity leading to higher migration to the inflamed area, and increase effector functions. H2 receptors seem to be potent suppressors of inflammatory and effector functions [3].

The use of H1 receptor antagonists has not been recognized as a clinically effective therapy against asthma. However, second generation H1 receptor antagonists were shown to moderately improve airway obstruction and symptoms in patients with bronchial asthma treated with doses exceeding those recommended for first generation antihistamines [6]. In children with atopic dermatitis, specific H1 receptor antagonists such as ketotifen and cetirizine have been reported to delay or prevent development of asthma [7, 8]. In animal models of asthma, repetitive administration of H1 receptor antagonists during sensitization and/or challenge inhibited AHR, airway inflammation, and Th2 cytokine production [9, 10]. These observations suggested the potential usefulness of H1 receptor antagonists for prevention of bronchial asthma. However, evaluation of mechanisms using H1 receptor antagonists presents many limitations, as these receptor antagonists may also interact with monoamine receptors, which are not related to H1 receptors [11].

Genetically modified animals have been used for elucidating pathophysiological roles of histamine in asthma. For example, *L*-histidine decarboxylase gene knockout (HDCKO) mice show low accumulation of airway eosinophils, but increased AHR after allergic challenge [12]. In this study, all types of histamine receptors were affected because histamine levels were largely diminished in HDCKO mice. The use of a mouse model deficient in a specific type of histamine receptor, such as H1 receptor gene knockout (H1RKO) mice, would provide more straightforward information on the roles of histamine receptors in mechanisms of allergic inflammation and AHR. H1RKO mice grow normally, but present some behavioral alterations including abnormalities of locomotor activity, emotion, memory, learning, and nociception [13]. In vitro responses of T and B cells in H1RKO mice have been

reported, but the degree of bronchial asthma development in these mice is still unknown [4, 14]. In the present study, to clarify the roles of H1 receptor in allergic responses in vivo, the development of bronchial asthma was compared between H1RKO and wild-type (WT) mice.

Materials and Methods

Mice

H1RKO mice with a C57BL/6 background were kindly provided by Dr. Takeshi Watanabe from the Medical Institute of Bioregulation, Kyushu University, Japan. WT C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan). H1RKO mice were additionally backcrossed with C57BL/6 mice, and H1RKO and WT mice were maintained under specific pathogen-free conditions in our laboratory. Genotyping was confirmed as described previously [15]. Mice were maintained at 24°C, exposed to a daily 12-hour light-dark cycle under normoxic conditions, and were given food and water ad libitum. Male mice aged 7–8 weeks were used in all experiments. The study protocol was approved by the Showa University Animal Experiments Committee.

Sensitization and Allergen Challenge

Sensitization was performed by intraperitoneal injection of a solution (0.2 ml) containing 100 µg ovalbumin (OVA; grade VI; Sigma Chemical Co., St. Louis, Mo., USA) and 4 mg aluminum hydroxide on days 0, 7 and 14. Then, animals were challenged with aerosolized 1% OVA (grade III; Sigma Chemical Co.) dissolved in saline, using a compressor nebulizer (NE-C16; Omron, Tokyo, Japan; output <0.25 ml/min) for 30 min on days 15, 18, 17, 19, 20 and 21. Thus, OVA-sensitized and OVA-challenged WT mice were named WT/OVA, and OVA-sensitized and OVA-challenged H1RKO mice were named H1RKO/OVA. Control mice were sensitized and challenged with saline alone following the same protocols, and were named WT/saline and H1RKO/saline. Measurement of ventilatory timing and sampling of blood and bronchoalveolar lavage fluid (BALF) were assessed 24 h after the last challenge.

Response of Ventilatory Timing to Inhaled Methacholine

Ventilatory timing [16] was determined after each inhalation of incremental doses of methacholine. This was measured using a whole-body plethysmograph (Buxco Electronics, Sharon, Conn., USA). Each mouse was exposed to varying concentrations (0–48 mg/ml) of aerosolized methacholine (Sigma Chemical Co.) for 3 min. Mean enhanced pause [17] values representing ventilatory timings were assessed for 5 min after each inhalation.

Immunoglobulin E Level and Differential Cell Count in Blood

Blood was collected under anesthesia from the right ventricle on day 22. Immunoglobulin E (IgE) serum levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Yamasa, Chiba, Japan). Total cell number in blood was determined following standard hematological procedures. Blood smears were prepared, and stained using the Diff-Quick kit (Sysmex, Kobe, Japan) for differential cell count.

Histology

Animals were sacrificed by an overdose of anesthetics, and lung tissue samples were removed 24 h after the last challenge. Lung tissues were fixed in 10% buffered formalin, embedded in paraffin, cut into 4- μ m paraffin sections, and processed for hematoxylin and eosin staining.

Bronchoalveolar Lavage and Differential Cell Count

For sampling of BALF, the trachea was cannulated with a polyethylene tube, and the lungs were lavaged twice with phosphate-buffered saline. Total cell count was determined using a hemocytometer. For differential cell count, cells were centrifuged in a Cytospin (Thermo Shandon, Runcorn, UK), and stained with Diff-Quick.

Measurement of Cytokines

Interleukin (IL)-4, IL-5, IL-13, and TGF- β 1 levels in BALF were determined using commercially available ELISA kits (R&D Systems, Minneapolis, Minn., USA). Interferon (IFN)- γ levels in BALF were measured using a commercial ELISA kit from BioSource International (Camarillo, Calif., USA).

Statistical Analyses

Data were expressed as means $\pm$ SEM. Comparisons between groups were performed using a Mann-Whitney U test or a two-way analysis of variance (ANOVA). A p value <0.05 was considered statistically significant.

Results

Circulating Levels of Total IgE

Sensitization and challenge with OVA increased the total IgE level in serum in both genotype groups. The total IgE serum level was significantly increased in WT/OVA and H1RKO/OVA mice compared to WT/saline and H1RKO/saline mice. The IgE serum level was significantly higher in WT/OVA than in H1RKO/OVA mice (each group = 12 mice, fig. 1a).

Blood Eosinophils

Sensitization and challenge with OVA evoked systemic eosinophil increase in both genotype groups. Numbers of eosinophils in peripheral blood significantly increased in WT/OVA and H1RKO/OVA groups compared to WT/saline and H1RKO/saline groups (each group = 12 mice, fig. 1b). No significant difference in eosinophilia was found between WT/OVA and H1RKO/OVA mice.

Differential Cell Count in BALF

To clarify allergic inflammation in airways, a differential cell count in BALF was performed with 12 mice in each group. Numbers of eosinophils, lymphocytes, and neutrophils were significantly increased in BALF of WT/

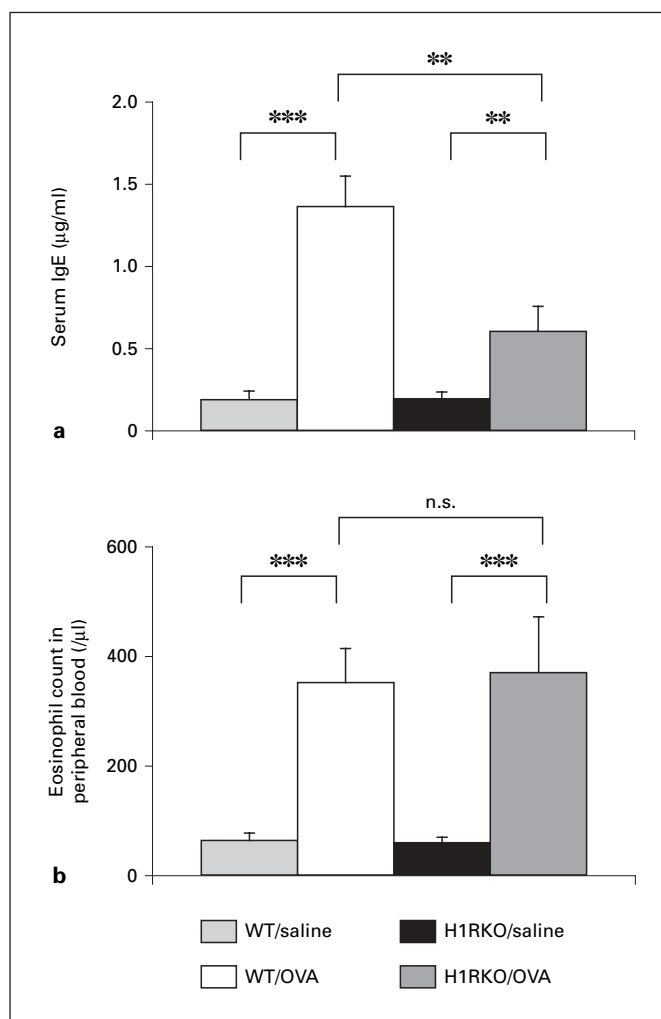


Fig. 1. Total serum IgE levels (a) and eosinophil counts in peripheral blood (b) in WT/OVA, WT/saline, H1RKO/OVA, and H1RKO/saline mice. Data are expressed as means $\pm$ SEM ($n = 12$). Statistical analysis was performed using a Mann-Whitney U test: ** $p < 0.01$; *** $p < 0.001$. n.s. = Not significant.

OVA and H1RKO/OVA compared to WT/saline and H1RKO/saline mice (fig. 2). Numbers of eosinophils, lymphocytes, and neutrophils were significantly enhanced in WT/OVA compared to H1RKO/OVA mice.

Lung Histological Findings

Representative lung sections are shown in figure 3. Four mice in each group were examined. There was a greater peribronchial leukocyte infiltration in WT/OVA mice compared to H1RKO/OVA mice. Airway goblet cell hyperplasia was more prominent in WT/OVA than in H1RKO/OVA mice.

Fig. 2. Differential cell counts in BALF of WT/OVA, WT/saline, H1RKO/OVA, and H1RKO/saline mice. Data are expressed as means $\pm$ SEM (n = 12). Statistical analysis was performed using a Mann-Whitney U test: * p < 0.05; ** p < 0.01; *** p < 0.001. n.s. = Not significant.

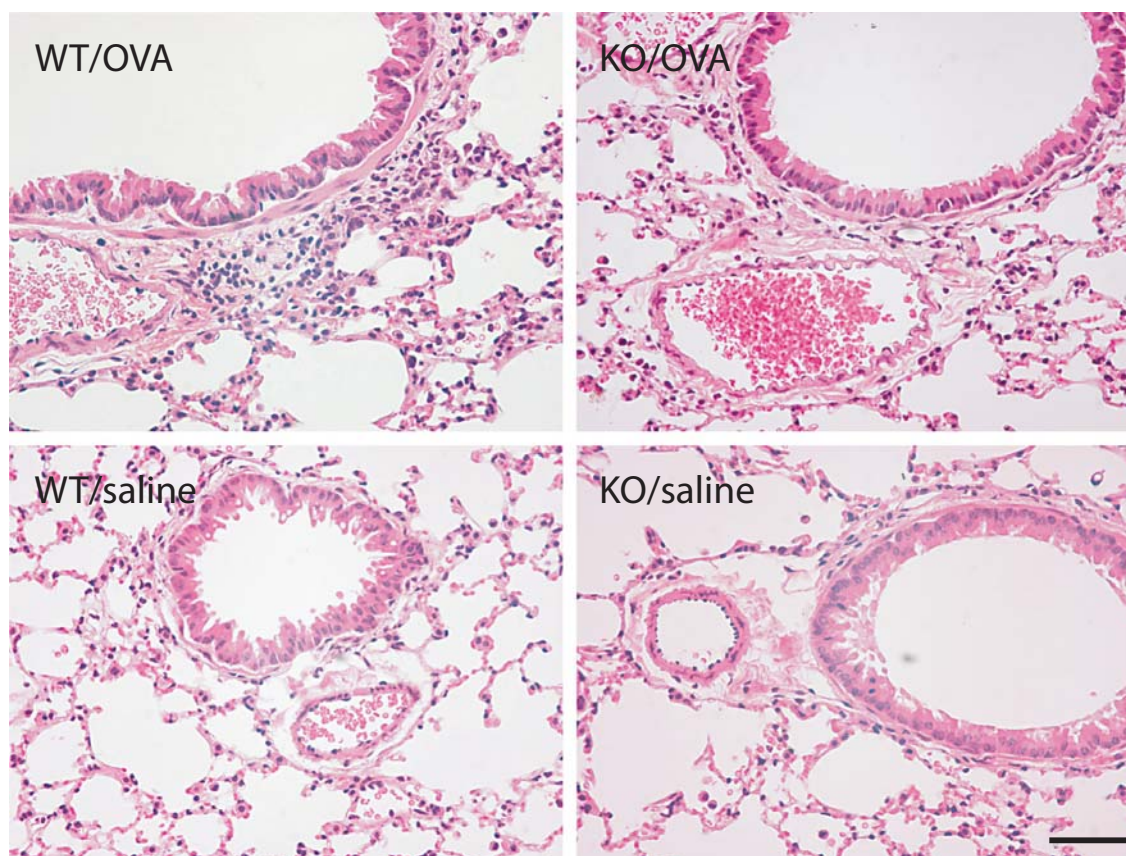
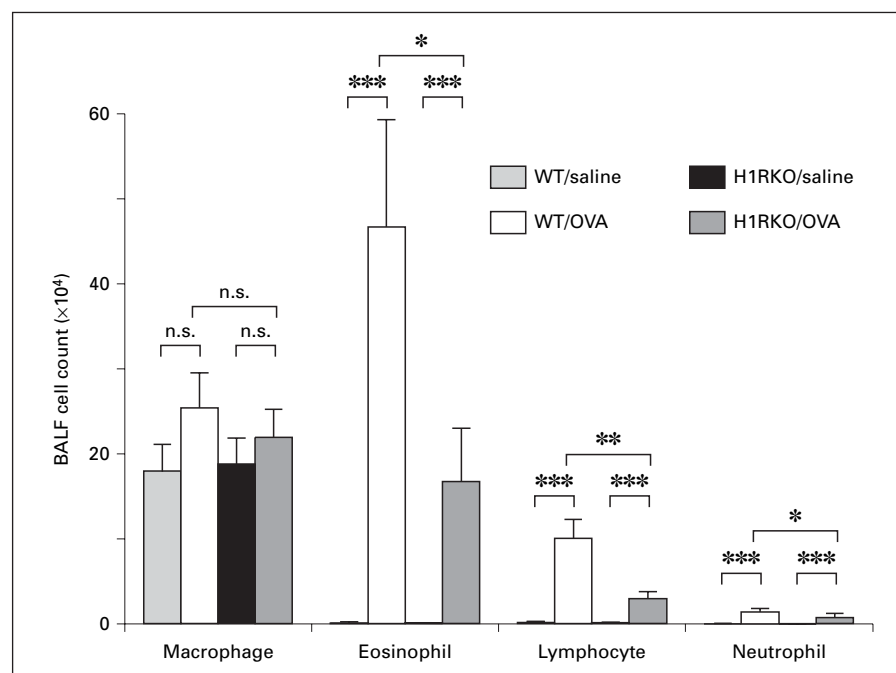


Fig. 3. Representative hematoxylin- and eosin-stained lung sections from WT/OVA, WT/saline, H1RKO/OVA, and H1RKO/saline mice. OVA sensitization and inhalation evoked marked leukocyte infiltration in airways of WT/OVA mice. In contrast, leukocyte infiltration was mild in H1 receptor knockout mice. Bar = 50 μ m.

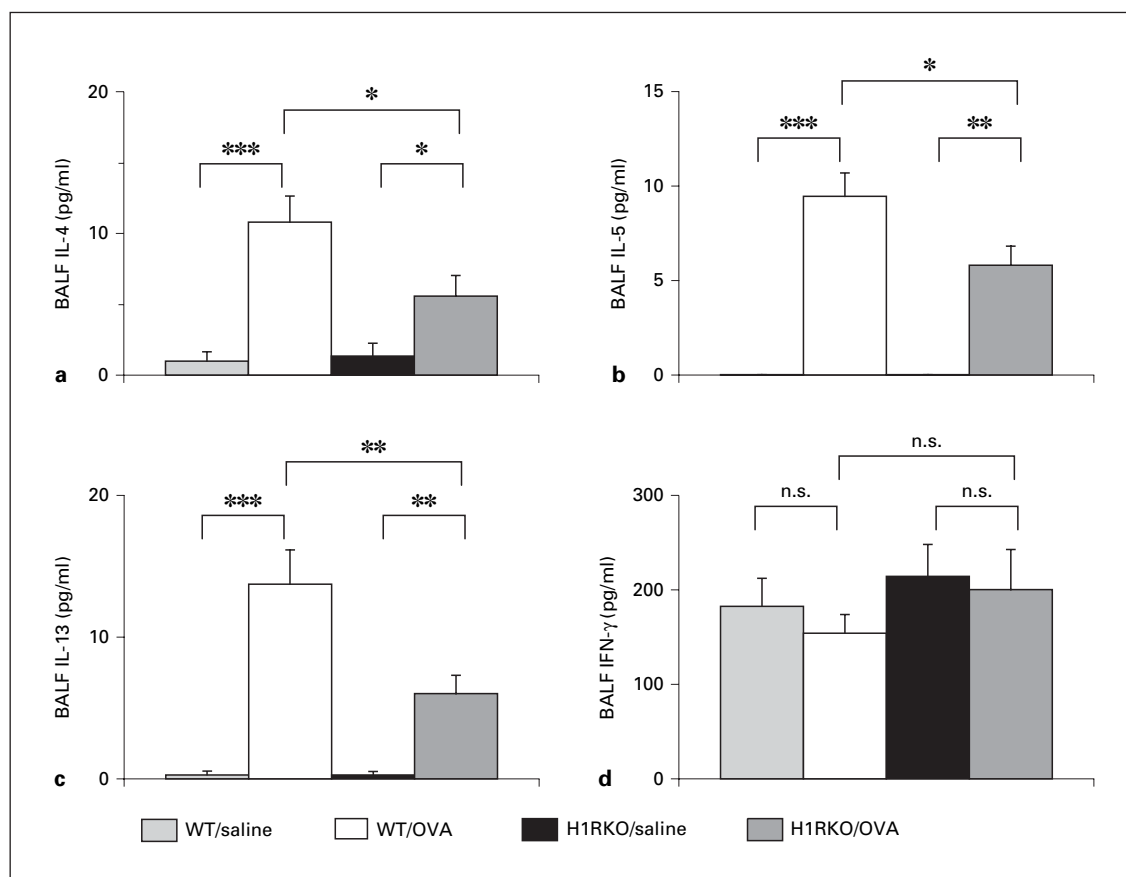


Fig. 4. Levels of IL-4 (a), IL-5 (b), IL-13 (c), and IFN-γ (d) in BALF of WT/OVA, WT/saline, H1RKO/OVA, and H1RKO/saline mice. Data are expressed as means $\pm$ SEM (n = 8). Statistical analysis was performed using a Mann-Whitney U test: * p < 0.05; ** p < 0.01; *** p < 0.001. n.s. = Not significant.

BALF Cytokine Levels

To confirm antigen-induced cytokine production, Th1 and Th2 cytokine levels were determined in BALF of 8 mice from each group. Levels of IL-4, IL-5, and IL-13 which are Th2 cytokines were significantly higher in WT/OVA and H1RKO/OVA mice than in WT/saline and H1RKO/saline mice (fig. 4a–c). WT/OVA mice showed significantly higher IL-4, IL-5, and IL-13 levels than H1RKO/OVA mice. Th1 cytokine IFN-γ levels in BALF

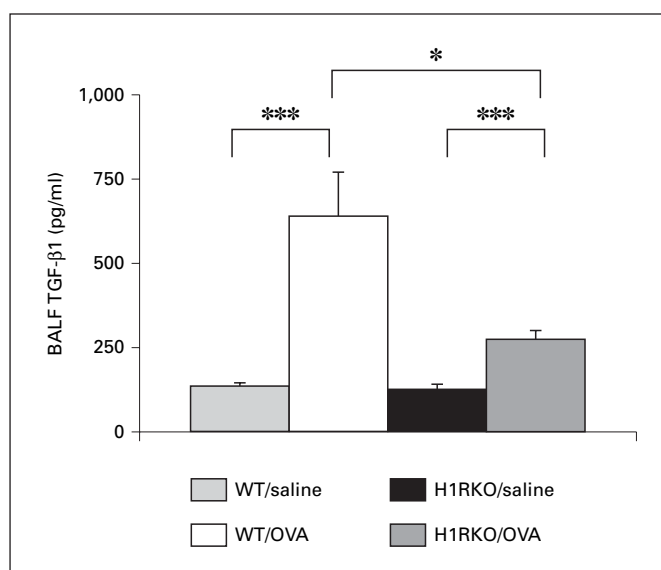


Fig. 5. TGF-β1 levels in BALF of WT/OVA, WT/saline, H1RKO/OVA, and H1RKO/saline mice. Data are expressed as means $\pm$ SEM (n = 8). Statistical analysis was performed using a Mann-Whitney U test: * p < 0.05, *** p < 0.001.

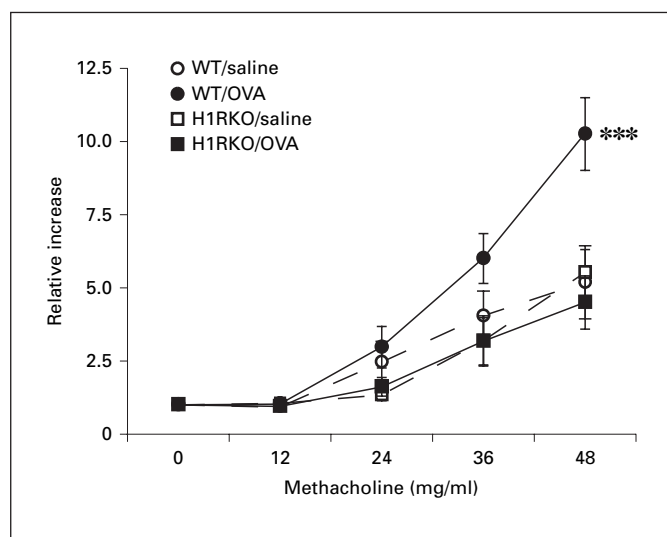


Fig. 6. Ventilatory timing in response to inhalation of aerosolized methacholine in WT/OVA, WT/saline, H1RKO/OVA, and H1RKO/saline mice. Data are expressed as means $\pm$ SEM ($n = 8$). Values represent relative changes in ventilatory timing at each methacholine concentration compared with baseline values. There was a significant change (***) as analyzed by a two-way ANOVA.

were not significantly different between the genotype groups (fig. 4d). TGF- β 1 is a cytokine involved in airway remodeling, whose level was significantly higher in mice treated with aerosolized OVA than with saline in both WT and H1RKO mice (fig. 5). WT/OVA mice showed higher levels of TGF- β 1 than H1RKO/OVA mice.

Ventilatory Timing in Response to Inhalation of Methacholine

Exposure to aerosolized methacholine dose-dependently increased ventilatory timing in all four groups including 8 mice each. The response was more significant in WT/OVA than in WT/saline mice. WT/OVA mice apparently showed an increased response, while the response in H1RKO/OVA mice was not obvious. The response in H1RKO/OVA mice was not different from those in WT/saline and H1RKO/saline mice (fig. 6).

Discussion

In our study, H1 receptor deficiency attenuated airway allergic inflammation, which was associated with decreased levels of BALF Th2 cytokine and circulating IgE.

Our results suggested that histamine enhanced Th2 response via H1 receptors, leading to enhanced airway allergic inflammation.

Extensive infiltrations by eosinophils and lymphocytes were detected in the airways in our bronchial asthma mouse model, indicating the occurrence of an allergic inflammation. Th2 cytokines including IL-4, IL-5, and IL-13 have been demonstrated to cause allergic inflammation [1, 18]. In addition, Th2 cytokines may also stimulate production of IgE from B cells, activation of eosinophils, and their accumulation in airways [18]. In the present asthma model, we observed increased levels of IL-4, IL-5, and IL-13 in BALF, and of IgE in serum of WT/OVA mice, indicating the induction of an enhanced Th2 response. The Th2 response was mild in H1RKO/OVA mice compared to WT/OVA mice. Overall, these observations suggested that H1 receptor-mediated effects affected Th2 responses.

Both WT and H1RKO mice showed eosinophilia in peripheral blood after OVA challenge. However, eosinophilia occurred in peripheral blood despite reduced numbers of BALF eosinophils in H1RKO/OVA mice. This finding was probably due to the inability of histamine to stimulate migration, adhesion, infiltration and/or survival of eosinophils into the airways. It is known that histamine is capable of upregulating expression of adhesion molecules such as P-selectin, E-selectin, ICAM-1, and VCAM-1 via H1 receptors. These adhesion molecules are crucial for rolling and migration of eosinophils through the vascular endothelium [19, 20]. In addition, histamine also stimulates secretion of CC chemokines including eotaxin, macrophage chemoattractant proteins, and thymus- and activation-regulated chemokines that induce chemotaxis of leukocytes via H1 receptor [21]. Low accumulation of eosinophils in airways of H1RKO mice after OVA challenge compared to their WT counterparts may be due to inactivity of these H1 receptor-mediated pathways. In agreement with this, it has been reported that induction of P-selectin and recruitment of eosinophils in lungs were significantly reduced in HDCKO mice after OVA challenge [12]. Another important factor that causes eosinophilia is IL-5, which stimulates migration, and prolongs survival of eosinophils [18]. In the present study, the decreased number of eosinophils in BALF of H1RKO/OVA mice compared to their WT counterparts could also be explained by low levels of IL-5 in H1RKO mice.

In the present study, no difference was observed in the Th1 cytokine IFN- γ levels between H1RKO and WT mice. In agreement with our findings, a murine model of

allergic inflammation involving daily administration of an H1 receptor antagonist throughout sensitization and challenge did not affect Th1 responses [10]. However, Jutel et al. [4] showed suppression of IFN- γ with increases in IL-4 and IL-13 in H1RKO mice *in vitro*. A possible explanation for this discrepancy is that H1 receptors are more sensitive to dendritic cells compared with Th1 and Th2 cells in the process of allergic response *in vivo*. In fact, histamine affects dendritic cell polarization by stimulating the expression of CD86 and production of several chemokines, and causes inhibition of IL-12 production from matured dendritic cells; consequently histamine initiates predominant Th2 responses via H1 and H2 receptors [3, 22–24]. Th1 cells highly express H1 receptors, therefore, IFN- γ might be significantly decreased once the removed Th1 cells are stimulated by an antibody in H1RKO mice. In contrast, in an antagonist study and in our H1RKO mice study *in vivo*, the deficiency of H1 receptors did not affect the Th1 cytokine level and attenuated the Th2 cytokine response. These results suggested that effects of H1 receptor on dendritic cells would be dominant to increase Th2 response *in vivo*. On the other hand, H2 receptor expression on Th2 cells is known to attenuate Th2 cytokine levels and proliferation, which relates to peripheral tolerance [3]. H2 receptors may be involved in reduced Th2 responses in H1RKO mice.

Here, we presented overall suppression of Th2 responses using a murine asthma model for H1RKO mice, but further studies are needed to clarify how histamine receptors affect the immune cells in allergic responses *in vivo* using techniques such as the adopted T cell transfer assay and H2 receptor knockout mice.

AHR is believed to result from airway inflammation, and to correlate with different grades of bronchial infiltration by inflammatory cells such as eosinophils, lymphocytes, and mast cells in animal models of bronchial asthma [22, 25, 26]. In the present study, increased lung infiltration by eosinophils as demonstrated by large numbers of eosinophils in BALF and in the peribronchial area might account for increased ventilatory timing in response to inhaled methacholine in WT/OVA mice, and this was associated with increased BALF levels of IL-4, IL-5, and IL-13. These Th2 cytokines have been shown to directly induce AHR even in the absence of inflammatory cell recruitment or mucus production [27]. In contrast, the response of ventilatory timing was mild, and was associated with decreased airway inflammation, and Th2 response in H1RKO/OVA mice. It remains controversial whether increased ventilatory timing or mean enhanced pause correspond to increased AHR [16]. However, over-

all results including airway inflammation suggested that H1 receptors might play significant roles in AHR in bronchial asthma. Some studies reported reduction of AHR using treatments with H1 receptor antagonists, and by neutralization of histamine in murine models [9, 10, 28]. In addition, OVA-induced AHR is not inhibited by H2 antagonist [28]. These findings showed that H1 receptors contributed to AHR in murine models.

Airway remodeling is another important contributing factor in mechanisms of AHR. It was shown to be associated with progressive deterioration of lung function in patients with bronchial asthma. TGF- β 1 is a growth factor promoting airway remodeling by stimulating secretion of extracellular matrix proteins such as collagen and proteoglycans and it is mainly produced by eosinophils. Recently, it has been reported that IL-5 as well as eosinophils were involved in allergen-induced subepithelial and peribronchial fibrosis by producing TGF- β 1 [29]. Indeed, in the present study, concentrations of TGF- β 1 in four groups were compatible with numbers of eosinophils in BALF and with concentrations of IL-5 in BALF. A decreased concentration of TGF- β 1 in BALF of H1RKO/OVA mice may result from reduced airway infiltration by eosinophils and from reduced production of IL-5, attenuating AHR probably due to reduced remodeling.

Attenuation in the Th2 response was partial in H1RKO mice, which might be related to other genetic differences. In H1RKO mice, turnover rate of 5-hydroxytryptamine (5-HT) significantly increased in the brain [13]. It is unknown whether 5-HT in the peripheral circulation increases in H1RKO mice. However, De Bie et al. [30] demonstrated that 5-HT and histamine were involved in allergic responses in a mouse model, using antagonists. Murine mast cells are known to release both histamine and 5-HT upon stimulation *in vivo* [31]. 5-HT concentration secondarily increased by deficiency of H1 receptors might enhance allergic responses, and attenuation of Th2 response might be partial in H1RKO mice.

In brief, the present study showed that histamine enhanced Th2 responses via H1 receptor, leading to enhanced airway allergic inflammation. Our data were consistent with previous findings showing that repetitive administration of H1 receptor antagonists inhibited allergic responses in murine models of asthma, and that there was a delay or prevention in the development of asthma in children with atopic dermatitis [7–10]. Although H1 receptor antagonists are believed to result in a very limited efficacy in human asthma, our results suggested that blockade of H1 receptors by administration of specific,

long-acting H1 antagonists might reduce airway inflammation, and would improve symptoms and severity of bronchial asthma. Overall, our observations suggested that administration of H1 receptor antagonists might be a promising strategy for the prevention and treatment of asthmatic inflammation.

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