# The H1 histamine receptor regulates allergic lung responses

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**Histamine, signaling via the type 1 receptor (H1R), has been shown to suppress Th2 cytokine production by in vitro cultured T cells. We examined the role of H1R in allergic inflammation in vivo using a murine asthma model. Allergen-stimulated splenic T cells from sensitized $H1R^{-/-}$ mice exhibited enhanced Th2 cytokine production. Despite this Th2 bias, allergen-challenged $H1R^{-/-}$ mice exhibited diminished lung Th2 cytokine mRNA levels, airway inflammation, goblet cell metaplasia, and airway hyperresponsiveness (AHR). Restoration of pulmonary Th2 cytokines in $H1R^{-/-}$ mice by intranasal IL-4 or IL-13 restored inflammatory lung responses and AHR. Further investigation revealed that histamine acts as a T cell chemotactic factor and defective T cell trafficking was responsible for the absence of lung inflammation. Cultured T cells migrated in response to histamine in vitro, but this was ablated by blockade of H1R but not H2R. In vivo, allergen-specific WT but not $H1R^{-/-}$ $CD4^+$ T cells were recruited to the lungs of naive recipients following inhaled allergen challenge. $H1R^{-/-}$ T cells failed to confer airway inflammation or AHR observed after transfer of WT T cells. Our data establish a role for histamine and H1R in promoting the migration of Th2 cells into sites of allergen exposure.**

## Introduction

Th2-type immune responses are central to the pathogenesis of atopic diseases. Elevated levels of $CD4^+$ Th2 cell–derived cytokines, including IL-4, IL-13, and IL-5, are strongly associated with asthma in humans (1–3). It is now known that these mediate several key characteristics of the allergic inflammatory process, including tissue eosinophilia, mucus production, and increased IgE levels (4–7).

At the onset of an allergic response, allergen interaction with specific Fcε receptor 1–bound IgE triggers the release of preformed histamine from cytoplasmic granules within mast cells and basophils. Histamine is responsible for many of the acute symptoms of allergic diseases, including vasodilation, enhanced smooth muscle contractility, neural reflexes, and vascular leak with resulting tissue edema (8). Histamine mediates its effects through 1 of 4 receptors, each characterized by a specific pattern of cellular expression and functions (9). Many of the allergic and inflammatory actions of histamine are mediated via the type 1 receptor (H1R). H1R is a Gαq/11 receptor that contains 7 membrane-spanning domains and an extracellular $NH_2$ terminal glycosylated domain. Activation of cellular process following histamine binding to H1R occurs via phospholipase C–mediated calcium mobilization (10), PKC activation, and NF-κB–mediated signaling pathways (11). The cellular expression of H1R includes airway smooth muscle, endothelial cells, neurons, dendritic cells, mast cells, monocytes/macrophages, and lymphocytes (reviewed in ref. 12). Interestingly, Jutel et al. showed that H1R is expressed at higher levels on Th1 T cells than on Th2 T cells (13).

Histamine can exert profound effects on the cytokine balance of immune responses in vivo and mediates its influence through complex and sometimes contrasting effects via H1R and H2R, as well as distinct influences on different immune effector cells. As a result, published data on histamine effects vary according to the specific cells or responses being analyzed and by whether the experimental approaches assess the global effects of exogenous histamine or the specific consequences of targeted deletion or blockade of 1 of its receptors. Histamine has been reported to inhibit IL-12, IL-2, and IFN-γ production by cultured cells (14), while, conversely, IL-4, IL-5, IL-10, and IL-13 are upregulated (15–18). Enhancement of Th2 responses by histamine in culture is likely the result of its effects on dendritic cells (19). Caron et al. (20) found that polarization of T cell responses by histamine could be accounted for by an H2R-driven differentiation of immature dendritic cells toward a Th2-generating DC2 dendritic cell phenotype.

Analysis of the role of H1R-mediated histamine signaling on T cell polarity has given somewhat contrasting results. Jutel et al. (13) clearly demonstrated suppression of IFN-γ production with concomitant enhancement of IL-4 secretion in anti-CD3–activated T cells from $H1R^{-/-}$ mice. $H1R^{-/-}$ mice also exhibited elevated antigen-specific serum IgE, $IgG_1$, $IgG_{2b}$, and $IgG_3$ levels. The investigators concluded that H1R is important for the generation of Th1 responses, while H2R regulates Th2, and that the enhanced Th2 profile in $H1R^{-/-}$ mice is due to reduction of Th1 responses.

Notwithstanding these findings, pharmacological inhibitors of H1R have been found to inhibit Th2-associated responses and are commonly prescribed in the treatment of allergic conditions. The clinical benefit of H1R blockade lies largely in inhibition of pruritus. However, broader antiinflammatory effects have been ascribed to H1R blockers, including inhibitory effects on adhesion molecule expression (21, 22) and inhibition of eosinophil chemotaxis (23). An H1R antagonist has also been shown to inhibit the generation of IL-4 and IL-13 by human basophils (24). We and others have recently shown that H1R antagonists prevent allergen-induced airway inflammation and hyperreactivity (25–27), but the cellular immunologic basis of this effect has not been clearly established.

**Nonstandard abbreviations used:** AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; H1R, histamine type 1 receptor; Penh, enhanced pause.

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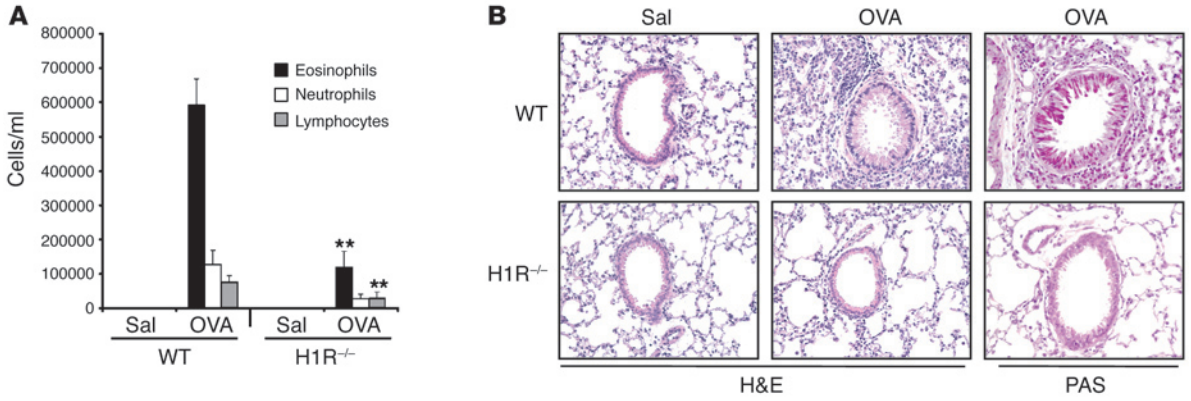


**Figure 1**
H1R$^{-/-}$ mice do not develop inflammatory airway responses to antigen. OVA-immunized (OVA) or sham-immunized (Sal) WT or H1R$^{-/-}$ mice were exposed to OVA by inhalation and studied for inflammatory airway changes. (**A**) The cellular composition of the BAL fluid was determined. Data represent mean ± SEM. $n$ = 9–10 individuals. **$P$ < 0.01 by Student's $t$ test. (**B**) Representative histological sections stained with H&E or PAS at ×200 magnification.

In order to more precisely resolve the roles of H1R in allergic lung responses, we studied the responses of H1R$^{-/-}$ mice, and specifically H1R$^{-/-}$ T cells, in a mouse model of asthma. Despite being able to mount significantly stronger systemic Th2 responses to antigen than their WT counterparts, OVA-sensitized H1R$^{-/-}$ mice failed to develop airway hyperresponsiveness (AHR) or airway inflammation. Th2 cytokine levels in the lungs of allergen-challenged H1R$^{-/-}$ mice were lower than those in WT lungs, and restoration of these cytokines by intranasal administration facilitated allergic inflammation. We observed that H1R regulates T cell migration and was essential for the recruitment of passively transferred antigen-specific CD4$^+$ and CD8$^+$ T cells into the site of antigen exposure and the subsequent generation of inflammatory responses. However, CD4$^+$ T cells alone were capable of generating the allergic lung responses. This finding establishes a critical role for histamine and H1R in orchestrating Th2 cell recruitment to sites of allergic inflammation.

## Results

*H1R$^{-/-}$ mice fail to develop lung inflammation following allergen challenge.* We analyzed OVA-immunized H1R$^{-/-}$ and WT mice following OVA challenge. AHR was assessed by methacholine challenge and whole-body plethysmography of unrestrained mice. Unimmunized WT and H1R$^{-/-}$ animals had similar baseline enhanced pause (Penh) values as well as methacholine responses. OVA-immunized WT animals developed significant AHR to methacholine ($P$ < 0.005), while no hyperresponsiveness was detected in H1R$^{-/-}$ mice (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI26150DS1). Analysis of the cellular composition of bronchoalveolar lavage (BAL) fluid revealed significantly fewer eosinophils ($P$ < 0.01) and lymphocytes ($P$ < 0.01) in OVA-immunized H1R$^{-/-}$ mice than in WT mice, which developed the eosinophil-dominated inflammatory infiltration with numerous lymphocytes, typical for this model (Figure 1A). The attenuation of inflammatory cell numbers in BAL fluid was mirrored by lung tissue histology. OVA-immunized WT mice developed a perivascular and peribronchial inflammation, as well as airway epithelial thickening and a strong increase in bronchial mucus production. In contrast, H1R$^{-/-}$ mice failed to develop these responses and had pulmonary histology remarkably similar to that of unimmunized mice (Figure 1B).

*Exaggerated systemic Th2 responses in H1R$^{-/-}$ mice.* In agreement with previous studies (13), antigen stimulation of splenocytes from H1R$^{-/-}$ mice induced an enhanced Th2 profile (Figure 2). Compared with WT splenocytes, H1R$^{-/-}$ cells produced significantly less IFN-γ ($P$ < 0.01) and significantly more IL-5 ($P$ < 0.01) and IL-13 ($P$ < 0.01). Levels of IL-4 in these cell culture supernatants were below the limits of detection (data not shown). These find-

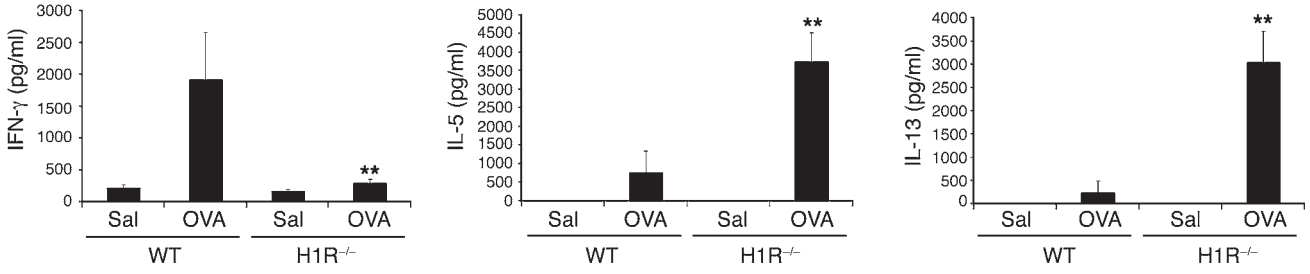


**Figure 2**
Hyperproduction of Th2 cytokines by splenocytes from H1R$^{-/-}$ mice. Splenocytes were stimulated for 72 hours in the presence of antigen (OVA). Cytokine secretion was determined by sandwich ELISA. Data represent mean ± SEM from 4 individuals per group. **$P$ < 0.01 by Student's $t$ test, compared with WT OVA values.

**Table 1**
Cytokine gene expression in lung tissue

| Cytokine | WT saline | WT OVA | H1R$^{-/-}$ saline | H1R$^{-/-}$ OVA |
|---|---|---|---|---|
| *IL-4* | 1.00 ± 0.12 | 5.50 ± 2.36 | 1.86 ± 0.44 | 3.21 ± 0.73 |
| *IL-5* | 1.00 ± 0.31 | 2.55 ± 0.18 | 0.86 ± 0.16 | 1.08 ± 0.28$^{A}$ |
| *IL-13* | 1.00 ± 0.27 | 105.7 ± 21.0 | 13.4 ± 5.23 | 34.1 ± 10.9$^{A}$ |
| *IL-10* | 1.00 ± 0.25 | 74.2 ± 25.7 | 4.07 ± 0.80 | 28.2 ± 9.76$^{B}$ |
| *IFN-γ* | 1.00 ± 0.19 | 5.38 ± 1.27 | 2.71 ± 0.64 | 5.12 ± 1.02 |

Real-time RT-PCR on mRNA from lung tissue from WT or H1R$^{-/-}$ mice was performed and the expression of cytokines determined. The level of each gene was calculated as a ratio of the expression of *β2-microglobulin* for each individual sample and expression normalized by comparing with the WT saline group. $n$ = 4–10 per group. $^{A}P < 0.005$, $^{B}P < 0.05$ compared with WT OVA by Student's $t$ test.

ings corroborate previous reports that the absence of H1R leads to a dominant systemic Th2 phenotype upon antigen or CD3 stimulation, but they contrast sharply with our observation of decreased allergen-driven pulmonary inflammation in H1R$^{-/-}$ mice.

*Local Th2 responses are diminished in H1R$^{-/-}$ lung.* Our findings suggested that H1R$^{-/-}$ mice have a dominant systemic Th2 response and yet fail to develop lung inflammation. In order to assess whether the generation of Th2 cytokines was impaired or intact at the site of allergen challenge in the lungs, we studied pulmonary expression of cytokine genes. Levels of transcripts encoding IL-4, IL-5, IL-13, IL-10, and IFN-γ in lung tissue were determined by quantitative real-time PCR (Table 1). Following allergen inhalation, OVA-immune WT mice exhibited markedly increased lung expression of each gene (normalized to the levels of gene expression in unimmunized WT tissues), consistent with the inflammatory responses shown in Figure 1B. In contrast, OVA-exposed H1R$^{-/-}$ tissues consistently displayed only modest increases in gene expression with statistically significant reductions in transcripts for IL-5, IL-13, and IL-10 compared with WT levels ($P < 0.005$, $P < 0.005$, and $P < 0.05$ respectively). The level of IL-5 protein in the BAL fluid was also significantly reduced in H1R$^{-/-}$ mice (1,058 ± 128.8 pg/ml in WT mice and 234.9 ± 72.2 pg/ml in H1R$^{-/-}$ mice). Interestingly, unimmunized H1R$^{-/-}$ mice possessed higher basal expression of IL-10, IL-13, and IFN-γ, perhaps indicating a higher constitutive activation of resident T cells in the airways of H1R$^{-/-}$ mice. However, this basal activation did not involve Th2, as we observed in response to antigen (Figure 2), and clearly did not confer airway inflammation or AHR (Figure 1 and Supplemental Figure 1). We considered the possibility that these altered cytokine profiles might be secondary to alterations in H2R expression in H1R$^{-/-}$ mice, since H2R has also been shown to influence T cell cytokine production (13). However, the levels of H2R mRNA in the lungs were found to be similar between groups (fold inductions of 1.00 ± 0.63 in WT mice and 0.92 ± 0.47 in H1R$^{-/-}$ mice; $P = 0.70$). In addition, the expression of H3R and H4R was not significantly different between WT and H1R$^{-/-}$ mice (H3R: 1.00 ± 0.11 vs. 1.19 ± 0.80, respectively, $P = 0.85$; H4R: 1.00 ± 0.38 vs. 0.78 ± 0.48, respectively, $P = 0.74$).

*H1R$^{-/-}$ mice produce robust B cell immune responses.* Our observation (Figure 2) that antigen-stimulated T cells from H1R$^{-/-}$ mice displayed robust cytokine responses indicates that systemic immune sensitization in these mutants is intact at the level of T cell–derived cytokines. In order to examine whether H1R deficiency might specifically affect B cell sensitization and antibody production, we measured OVA-specific antibodies in WT and H1R$^{-/-}$ mice. H1R$^{-/-}$ mice produced antigen-specific IgM, IgE, $IgG_1$, $IgG_3$, and $IgG_{2b}$ of amounts equal to or greater than those in WT mice (Supplemental Figure 2). The levels of total serum

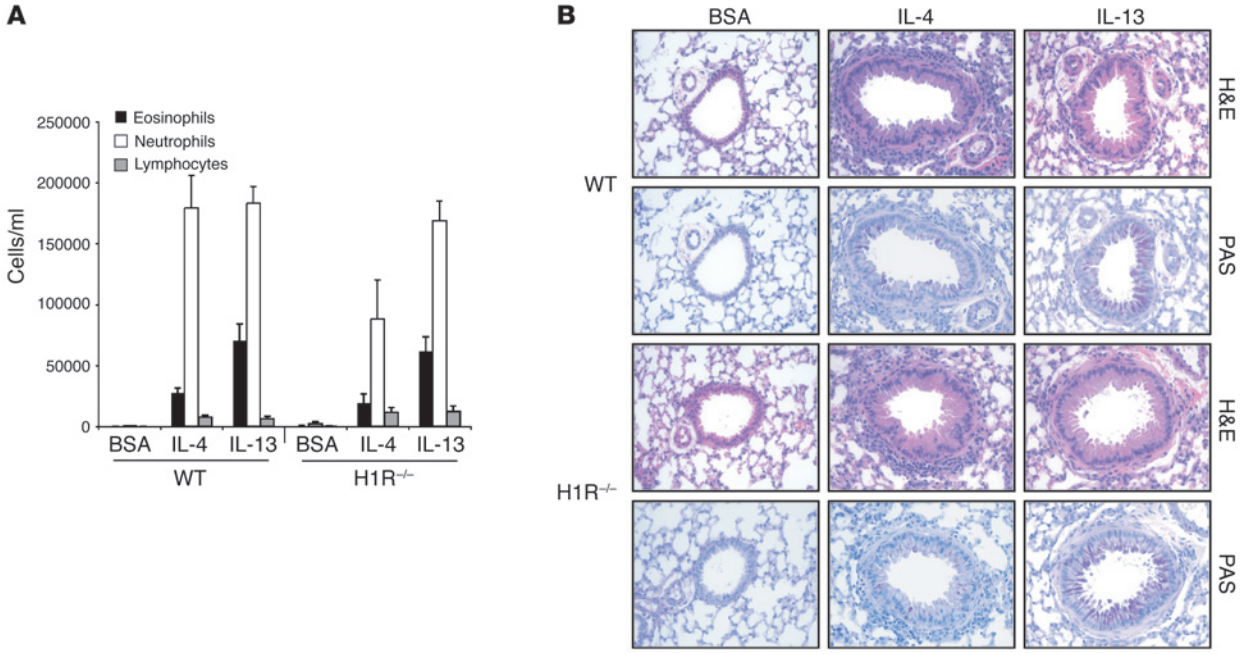


**Figure 3**
IL-4 and IL-13 induce airway inflammation in H1R$^{-/-}$ mice. Five micrograms of recombinant mouse IL-4 or IL-13 or 5 μg BSA was administered intranasally for 3 days, and inflammation was determined. Each group consisted of 5 mice. (**A**) The numbers of eosinophils, neutrophils, and lymphocytes in the BAL fluid. (**B**) Representative airway histology in WT and H1R$^{-/-}$ lung tissue. Sections were examined at ×200.

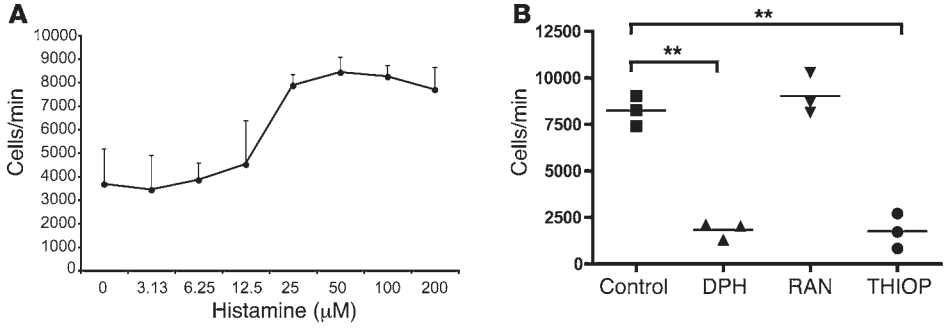


**Figure 4**
OVA-specific T cells migrate toward histamine via an H1R-dependent process. Chemotaxis to histamine was investigated using the OVA-specific DO11.10 T cell hybridoma. (**A**) DO11.10 cells migrate in response to histamine. Cells were added to the upper chamber of Transwell chambers, and histamine was added to the lower chamber. After 4 hours, the number of cells in the lower chamber was determined by FACS. (**B**) Diphenhydramine (DPH), an H1R antagonist; ranitidine (RAN), an H2R antagonist; and thioperamide (THIOP), an H3R/H4R antagonist, were added to assess their effects on T cell chemotaxis induced by histamine (100 μM). Data represent the number of cells moving through Transwell chambers (mean cells ± SEM) from 3 separate experiments. $^{**}P < 0.01$ by Student's *t* test.

immunoglobulins were also increased following immunization and similar to those in WT mice (data not shown). These findings are similar to those observed in previous studies (13, 28). Therefore, H1R$^{-/-}$ mice display both strong systemic T cell and efficient B cell responses to antigen.

*Inhalation of either IL-4 or IL-13 induces lung inflammation in H1R$^{-/-}$ mice.* Taken together, our findings of attenuated allergic airway inflammation and decreased pulmonary Th2 cytokine levels in allergen-challenged H1R$^{-/-}$ mice (Figure 1 and Table 1) led us to hypothesize that the absence of AHR and inflammatory lung changes might be due to an inability to mount efficient local cytokine responses. If so, restoration of local cytokine levels should elicit inflammation, even on an H1R$^{-/-}$ background. Alternatively, if attenuated inflammation in the lungs of H1R$^{-/-}$ mice results from a requirement for H1R in lung tissue to mediate Th2-driven recruitment of effector cells, then restoration of cytokines per se should not correct the defect in H1R$^{-/-}$ animals. Direct administration of IL-4 or IL-13 to the lung has been reported to result in the development of AHR and airway inflammation (29–31). We gave IL-4 or IL-13 (5 μg) intranasally daily for 3 days to WT and H1R$^{-/-}$ mice. IL-4 and IL-13 induced increases in airway responsiveness to methacholine in both WT and H1R$^{-/-}$ mice (Supplemental Figure 3). The magnitude of response was greater in response to IL-13 than to IL-4, but both cytokines induced statistically increased hyperresponsiveness compared with a control protein, BSA. Additionally, intranasal IL-4 and IL-13 caused an increase in the numbers of inflammatory cells present in the BAL fluid of both WT and H1R$^{-/-}$ mice (Figure 3A). Histological investigation showed that IL-4 and IL-13 led to distinct changes in the lung tissue morphology (Figure 3B). IL-4 elicited peribronchial inflammation in both WT and H1R$^{-/-}$ mice, with a modest increase in PAS$^{+}$ (mucus-secreting) cells. In contrast, IL-13 administration led to a more modest inflammatory response but evoked an intense goblet cell metaplasia with mucus secretion, again with similar responses in both WT and H1R$^{-/-}$ recipients. Together, these data clearly show that H1R$^{-/-}$ mice are fully capable of mounting inflammatory lung responses to Th2 cytokines and indicate that the attenuation of allergic airway inflammation in H1R$^{-/-}$ animals is the consequence of inadequate levels of inflammatory cytokines in the lung after antigen exposure (Table 1), rather than a direct effect of absent H1R expression in cells of the lung tissue.

*H1R mediates T cell chemotaxis to histamine.* The absence of Th2 cytokines in the lungs of allergen-challenged H1R$^{-/-}$ mice suggested a local paucity of Th2 cells. We hypothesized that H1R-deficient

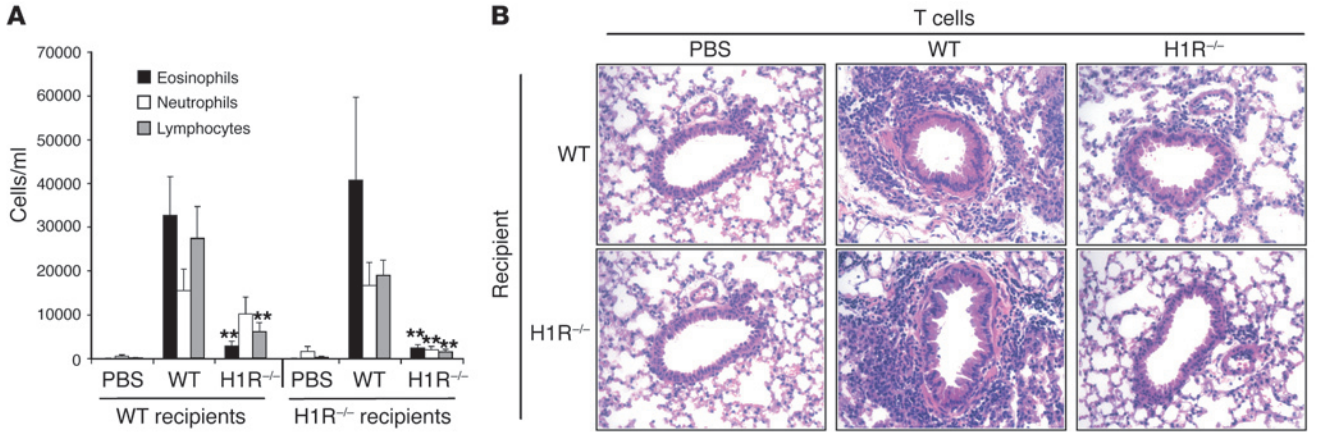


**Figure 5**
Inflammatory airway responses to antigen can be induced by WT T cells but not by H1R$^{-/-}$ T cells. Antigen-expanded CD4$^{+}$ T cells from WT or H1R$^{-/-}$ mice were adoptively transferred into naive WT or H1R$^{-/-}$ recipients, and the development of airway inflammation was studied after inhaled OVA challenge. (**A**) The numbers of eosinophils, neutrophils, and lymphocytes in BAL fluid were determined ($n$ = 9/group). $^{**}P < 0.01$, Student's *t* test. (**B**) Representative lung histology at ×200 magnification. Sections were stained with H&E.

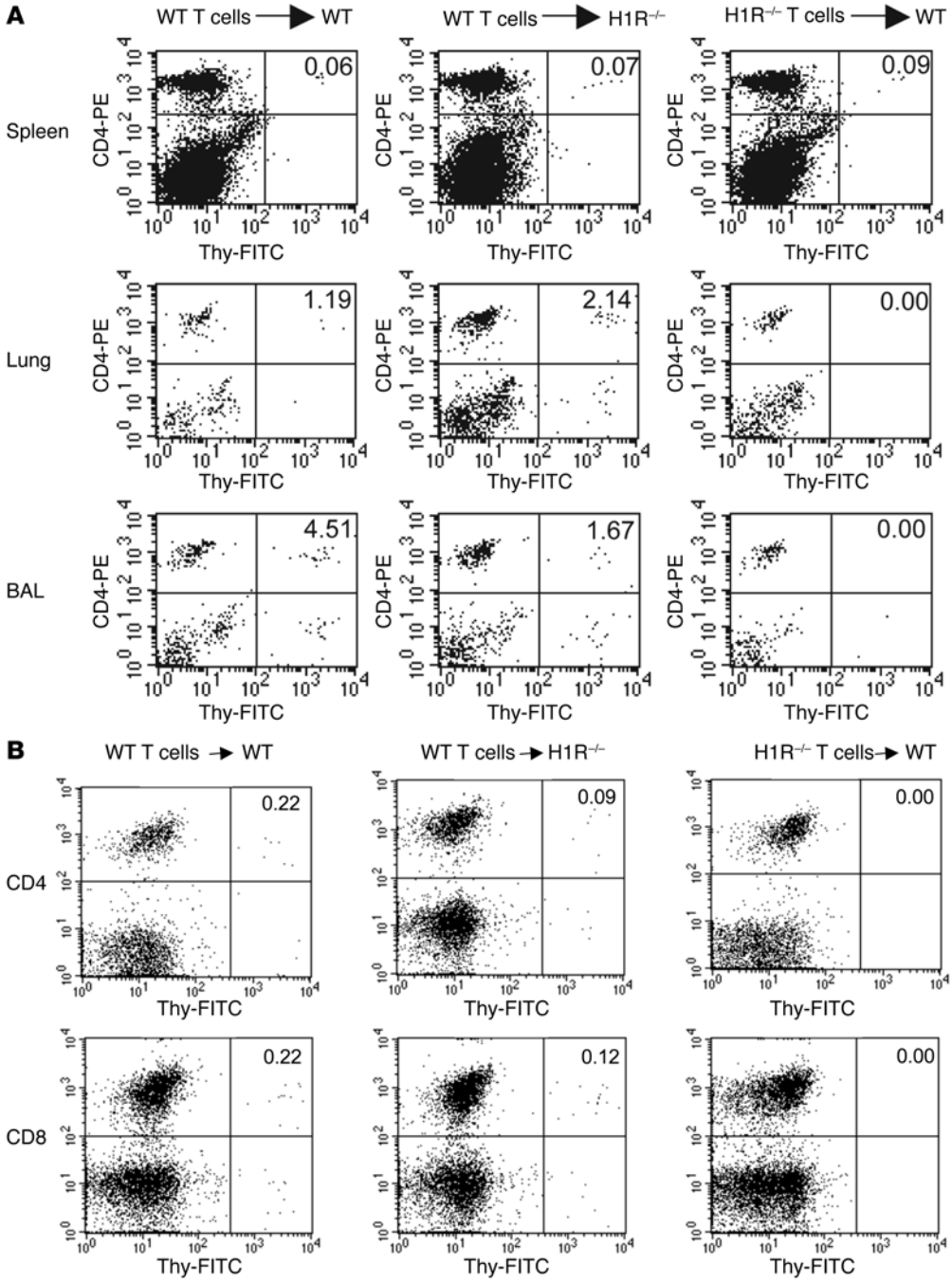


**Figure 6**
H1R$^{-/-}$ T cells do not migrate into the lung following antigen challenge. Thy1 congenic antigen-expanded CD4$^+$ T cells (**A**) or CD3$^+$ T cells (**B**) from WT or H1R$^{-/-}$ mice were adoptively transferred into naive WT or H1R$^{-/-}$ recipients. Cells were adoptively transferred into naive recipients and their movement into spleen, whole lung tissue, and BAL fluid studied. Single-cell suspensions were stained using FITC-Thy1.1 or FITC-Thy1.2 (see Methods) and PE-CD4 or PE-CD8 to determine the presence of the transferred cells. The plots shown were obtained by analysis of the pooled cells of 5 animals of each group and represent 3 individual experiments.

antigen-specific T cells might not be receiving sufficient signals to move into the lung and that the effects of histamine could be important in this process. In order to determine the role of histamine receptors in chemotactic responses of T cells, we employed an in vitro Transwell method. By using the OVA-specific DO11.10 T cell hybridoma for these experiments, we were able to assess responses in a homogenous T cell population in the absence of any other histamine receptor–expressing cells, such as antigen-presenting cells. We found that histamine stimulated the migration of cells into the lower chamber in a dose-responsive manner and within a 12.5–100 μM range (Figure 4A). Coincubation of cells with diphenhydramine hydrochloride, a specific antagonist of H1R; ranitidine hydrochloride, a specific antagonist of H2R; or thioperamide, an inhibitor of H3R and H4R, revealed that H1R blockade inhibited the migration of cells, while H2R blockade did not (Figure 4B). Intriguingly, thioperamide also inhibited the T cell migration. Since H3R is predominately expressed in neuronal cells and not found on T cells, these data indicate that histamine directly influences the chemotactic movement of T cells and that this effect is mediated via H1R and H4R.

*H1R$^{+/+}$ T cells confer inflammatory lung responses to H1R$^{-/-}$ mice.* To determine whether the absence of inflammatory lung responses in OVA-sensitized H1R$^{-/-}$ mice (Figure 1 and Supplemental Figure 1) was due to an inability of OVA-specific T cells to migrate into the

lung upon antigen exposure, we transferred WT or H1R$^{-/-}$ CD4$^+$ T cells from OVA-sensitized donors into naive WT or H1R$^{-/-}$ recipients. The lungs of the recipients were antigen challenged by exposure to nebulized 1% OVA for 20 minutes for 6 days and then studied for the development of allergic inflammation and AHR. WT recipients of CD4$^+$ T cells from WT donors developed an AHR to methacholine, compared with allergen-challenged mice receiving no cells (Supplemental Figure 4). In contrast, WT recipients that received H1R$^{-/-}$ CD4$^+$ T cells developed significantly less AHR ($P < 0.05$), indicating that, even in an H1R$^{+/+}$ endothelium and lung environment, H1R$^{-/-}$ CD4$^+$ T cells were unable to promote AHR. Furthermore, the transfer of WT CD4$^+$ T cells into H1R$^{-/-}$ mice conferred airway responses to methacholine, while, as expected, transferring H1R$^{-/-}$ CD4$^+$ T cells did not. This result indicated that H1R expression by CD4$^+$ effector T cells is sufficient for the induction of allergic lung inflammation and that the expression of H1R in host tissues is dispensable.

Both the capacity of WT CD4$^+$ T cells to restore airway responses in H1R$^{-/-}$ recipients and the failure of H1R$^{-/-}$ T cells to transfer AHR were corroborated by analyses of BAL fluid and tissue sections. Transfer of WT CD4$^+$ T cells into either WT or H1R$^{-/-}$ recipients led to increases in the numbers of eosinophils, neutrophils, and lymphocytes (Figure 5A). Conversely, H1R$^{-/-}$ CD4$^+$ T cells induced markedly less cellular accumulation in the BAL, although, interestingly, the numbers of cells were slightly greater than those found in mice that did not receive cells, indicating a minimal residual proinflammatory capacity of H1R$^{-/-}$ T cells. Peribronchial inflammation and increased epithelial thickness were clearly evident in both WT and H1R$^{-/-}$ recipients of WT CD4$^+$ T cells, whereas these features were absent in mice receiving H1R$^{-/-}$ CD4$^+$ T cells (Figure 5B). Potentially consistent with the presence of inflammatory cells in the BAL fluid of H1R$^{-/-}$ T cell recipients was some mild epithelial thickening in these animals as well.

*H1R$^{-/-}$ T cells fail to migrate into the site of antigen exposure*. The failure of H1R$^{-/-}$ T cells to confer AHR and inflammation to naive recipients along with the absence of Th2 cytokines in the lungs of allergen-challenged H1R$^{-/-}$ mice strongly suggested a trafficking defect at the level of the Th2 cell. The use of Thy1 congenic donor/recipient pairs for transfer of WT or H1R$^{-/-}$ T cells allowed us to track the movement of transferred cells to tissues of allergen-challenged recipients. This analysis revealed that WT CD4$^+$ T cells transferred into WT recipients were present in the spleen but, importantly, were also detectable in the lung tissue and the BAL fluid following allergen challenge (Figure 6A). Similarly, WT cells were also detectable in the tissues of H1R$^{-/-}$ recipients. In contrast, H1R$^{-/-}$ CD4$^+$ T cells were found in the spleen of WT recipients, but there were no detectable H1R$^{-/-}$ CD4$^+$ T cells in lung or BAL fluid of WT animals. Analysis of H1R$^{-/-}$ CD4$^+$ T cells transferred into H1R$^{-/-}$ recipients was not possible, since Thy1 congenic H1R$^{-/-}$ strains are not yet available. Recent studies have demonstrated an involvement of CD8$^+$ T cells in the generation of allergic lung inflammation (32). In order to determine the requirements for H1R on antigen-driven CD8$^+$ T cell movement into the lung, we adoptively transferred CD3$^+$ T cells and studied the migration of both CD4$^+$ and CD8$^+$ T cells into the lung. H1R$^{-/-}$ CD8$^+$ T cells also failed to migrate toward the lung (Figure 6B), while WT cells did, indicating a role for H1R in also regulating CD8$^+$ T cell trafficking.

Taken together, these data show that H1R is required for CD4$^+$ and CD8$^+$ T cell migration to the lung upon antigen inhalation and for the development of AHR and airway inflammation.

## Discussion

The local release of histamine during allergic reactions has long been recognized as an important step in immediate hypersensitivity reactions to antigen. Vasodilation, smooth muscle contraction, tissue edema, as well as inflammatory changes, such as increased expression of E-selectin, have all been attributed to the effects of histamine and its receptors. Pharmacological antagonists of the histamine receptors, especially H1R blockers, are commonly used for the treatment of acute allergic reactions. The role of H1R in chronic allergic conditions, including asthma, in humans is less clear, although 1 study has suggested that H1R blockade might impede the "allergic march," preventing the common clinical progression from atopic dermatitis to allergic asthma in susceptible children (33).

Analyses of the role of H1R using mice with a targeted mutation of the receptor gave some initially unexpected results. Jutel et al. (13) showed a dominant Th2 cytokine response in H1R$^{-/-}$ animals. Th2 cytokines are enhanced in allergic disease and are important regulators of allergic lung responses. Several studies have demonstrated enhanced allergic responses in animals with Th2-dominated phenotypes (34–37). Thus, the reported Th2 bias of H1R$^{-/-}$ mice might have predicted an exaggerated allergic inflammatory response. In contrast with this expectation, however, we and several others have reported that H1R antagonists inhibit the generation of allergic lung inflammation in mouse models of asthma (25–27). This contradiction prompted us to independently assess the cytokine polarity and the inflammatory effector capacity of H1R$^{-/-}$ Th cells using an in vivo model of airway allergy. We observe an absence of inflammatory lung responses in H1R$^{-/-}$ mice, and our findings resolve the paradox of impaired allergic inflammation in the setting of Th2 hyperresponsiveness by revealing that a critical defect of H1R$^{-/-}$ T cells resides in their inability to home to sites of allergic inflammation. We and others have recently observed similar suppression of the airway response to allergen in mice subjected to pharmacologic H1R blockade. Like H1R$^{-/-}$ mice, WT animals receiving either fexofenadine or desloratadine exhibited marked attenuations in BAL cellularity, peribronchial inflammation, and bronchial responsiveness to methacholine (25–27). The effect of histamine on T cell trafficking was not assessed. IgE responses to allergen were intact in drug-treated animals, suggesting that Th2 function was present. However, in contrast to Jutel et al.'s published results and our own data using H1R$^{-/-}$ mice, which exhibited high-intensity systemic Th2 responses, T cells from mice treated with H1R inhibitors displayed decreased production of Th2 cytokines. We speculate that pharmacologic H1R blockade may not ablate H1R signaling as completely as targeted deletion of the receptor. Additionally, perhaps greater sensitivity of certain immune cells (i.e., Th2-polarizing dendritic cells) compared with effector Th cells might result in different global effects on polarity. It is also possible that pharmacologic H1R blockade has nonspecific effects, including anticholinergic influences, that could also modulate cytokine production independently of H1R.

Among histamine receptors, H1R is not unique in modulating T cell responses. Others have previously shown that H2R can also influence several of the disease parameters we were investigating. Histamine can stimulate IL-5 production from CD3-stimulated T cells (38) and increase airway mucus secretion (39) via H2R. Additionally, H2R has been shown to promote Th2 responses by influencing dendritic cell activity (19) and regulating T cell proliferative responses (40). Increased expression of H2R has been found in the nasal mucosa of patients with allergic rhinitis (41). We postulated

that an absence of H1R could result in an increased expression of H2R and thereby influence airway inflammation. However, the expression of H2R in $H1R^{-/-}$ lungs was similar to that in WT mice, indicating that the changes in lung responses we observed were not due to increased H2R. Our results indicate that H2R alone is not sufficient to support the induction of Th2-driven allergic airway responses and that H1R must be functional. This observation is consistent with previous work using $H1R^{-/-}$ mice that showed H1R but not H2R regulates allergic nasal responses in animals (42).

Studies using histidine decarboxylase–deficient ($HDC^{-/-}$) mice, which lack the ability to produce histamine, have highlighted the importance of histamine in allergic lung responses. Koarai et al., using antigen-challenged 129Sv $HDC^{-/-}$ mice, showed a significant reduction in airway eosinophilia and a reduction in P-selectin expression but normal AHR (43), while Kozma et al., using $HDC^{-/-}$ mice on the BALB/c background, showed reduced eosinophilia, AHR, and antigen-specific IgE (44). Additionally, rEV131, a histamine-binding protein produced by the *Rhipicephalus appendiculatus* tick, when given intranasally in a mouse model of asthma, prevents AHR, airway inflammation, eosinophilia, and mucus production (45). Interestingly, we found that the levels of P-selectin were statistically lower in the lungs of $H1R^{-/-}$ immunized mice (data not shown), suggesting that this feature of the responses in $HDC^{-/-}$ mice is due to an absence of H1R-mediated histamine action. Our findings reveal many of the defective responses of $HDC^{-/-}$ mice are likely secondary to an absence of H1R-mediated T cell migration into the lung.

Chemoattractant properties of histamine might be predicted from the 7-transmembrane G protein–coupled structure of its receptors, and indeed such effects of histamine have been reported in several systems. Histamine has recently been shown to drive the migration of mast cells (46) and eosinophils via H4R-mediated effects (47). A chemotactic effect of histamine on whole-blood mononuclear cells has been proposed to function via an enhanced production of lymphocyte chemoattractant factor (LCF) (48), now referred to as IL-16. Histamine-driven IL-16 would likely influence T cell migration. However, this effect, on partially purified cells, was shown to be via H2R and at concentrations of histamine more than 100-fold higher than those in our study, and more recent work has demonstrated that the increased production of IL-16 by histamine functions via H2R and H4R (49). In addition, we did not observe any significant differences in IL-16 expression between WT and $H1R^{-/-}$ lungs (data not shown). H1R antagonists have been reported to reduce the migration of activated human T cells (50, 51), although doubts have been raised as to whether these effects were via H1R or via additional properties. We studied the chemoattractant properties of histamine in vitro using the OVA-specific DO11.10 T cell line. Histamine caused a dose-dependent increase in T cell migration within an effective range of 12.5–100 μM that was similar to that described for the chemotactic properties of histamine on mast cells and eosinophils (46, 47). These concentrations of histamine are also within the range of histamine levels found in the tissues of asthma patients with active disease symptoms (52), although somewhat higher than those described when patients who were currently free from symptoms were included (53). In our hands, the H1R antagonist diphenhydramine hydrochloride completely blocked the effects of histamine on T cell migration, while ranitidine hydrochloride had no effect and the dual H3R/H4R antagonist also inhibited migration. Since H3R is predominantly expressed in the central nervous system, this is likely to be an effect via the H4R receptor, which has been previously described as mediating chemotactic responses in immune cells (46, 47). Our data show that histamine is chemotactic for T cells and that this response is mediated via H1R, as well as H4R. We also demonstrate that H1R is required for antigen-specific $CD4^+$ T cells to migrate to the site of antigen exposure. Despite recent evidence that highlights an involvement of $CD8^+$ T cells in the generation of allergic lung inflammation (32) and our finding that $CD8^+$ T cell migration is also regulated by H1R, we have been able to restore the inflammatory responses in $H1R^{-/-}$ mice using purified $CD4^+$ T cells alone, implying that the H1R-mediated $CD4^+$ T cell migration into the lung is a critical component of responses in our model.

H1R has been recently identified as encoded by *Bphs*, a gene linked to susceptibility to several autoimmune diseases (54). In this study, $H1R^{-/-}$ mice were shown to exhibit a diminished clinical disease onset and severity in models of experimental allergic encephalomyelitis and autoimmune orchitis. These diseases are typically associated with a Th1-dominanted T cell response, and the authors concluded that the skewing of $H1R^{-/-}$ T cell responses toward a Th2 profile was responsible for the less-severe disease phenotype. Our findings indicate that the progression of disease in the absence of H1R is also determined by influences on the ability of T cells to migrate toward antigen. Indeed, our findings show that the dominant Th2 cytokine profile in $H1R^{-/-}$ mice does not augment the responses in our Th2-driven allergic airway responses; the inability of the T cells to migrate determines the disease outcome. It might, therefore, be postulated that the lack of disease onset seen in models of autoimmunity are, in fact, due to an absence of T cell migration.

In conclusion, we have demonstrated that H1R is an essential receptor in the efficient generation of allergic lung responses. Despite the induction of enhanced Th2 responses to antigen in the absence of H1R, T cells require H1R for migration toward histamine in vitro and for recruitment to sites of allergen challenge in vivo. We speculate that H1R blockade might be useful in blocking T cell trafficking and allergic inflammation in clinical settings. Despite antihistamines not being currently utilized in the treatment of asthma, the advancement of our understanding of the roles of histamine in allergic disease indicate that blockade of the H1R with currently available drugs might have potential benefit for patients.

## Methods

$H1R^{-/-}$ mice have been previously described (55). Mutant mice had been backcrossed 8 times onto the C57BL/6 strain. WT C57BL/6 mice were purchased from Jackson Laboratory. Mice were maintained in specific pathogen–free conditions at Children's Hospital Animal Resource Facility and under the guidelines of the National Research Council's *Guide for the Care and Use of Laboratory Animals*. All experiments were carried out under experimental protocols that had been approved by the Children's Hospital Animal Care and Use Committee. Animals were fed an OVA-free diet.

*Immunization to OVA*. Four- to 8-week-old mice were sensitized to OVA by intraperitoneal injection of 10 μg OVA (Grade VI; Sigma-Aldrich) in alum (3 mg) or alum alone at days 0 and 14. These mice were then challenged for 20 minutes to aerosolized 1% OVA by ultrasonic nebulization on days 28, 29, and 30 and were studied on day 31.

*Airway inflammation*. Analysis of AHR to methacholine and of BAL fluid as well as lung histology were performed as previously described (25). Briefly, whole-body plethysmography on conscious, unrestrained mice was performed under continuous airflow conditions. Penh values were

calculated from the flow-derived flow whole-body plethysmography measurements, avoiding the influence of conditioning on airway functions. Penh is a unitless indicator of changes in airway resistance and correlates well with specific airway resistance. Methacholine was nebulized into the airflow for 2-minute periods and the average Penh value calculated for this and the subsequent 8 minutes. After airway function analysis, mice were killed and the lungs immediately flushed with 0.8 ml BAL fluid (10% FCS, 1 mM EDTA, ×1 PBS) via the trachea. The recovered fluid was cytospun onto slides and differential cell counts performed after staining with DiffQuik (Baxter). Tissue sections and histological staining were performed by Histo-Scientific Research Laboratories.

*Real-time RT-PCR*. Total RNA was isolated from 50–100 mg lung tissue using the QIAGEN RNeasy Fibrous Tissue Mini Kit. cDNA was prepared using a SuperScript II RNase H-Reverse Transcriptase kit (Invitrogen Corp.) and analyzed by PCR on an ABI 7300 Thermal Cycler (Applied Biosystems). Taqman probes and Taqman Universal Master Mix were used as directed (Applied Biosystems) for each gene of interest.

*In vitro cytokine production*. Single-cell suspensions of splenocytes were prepared from individual mice, as previously described (25), and cultured in the presence of OVA (50 μg/ml). Supernatants were collected after 72 hours of culture and cytokine levels determined by sandwich ELISA using capture and biotinylated detection antibodies (BD Biosciences — Pharmingen).

*Serum antibody levels*. Serum from immunized mice was collected at day 31 and specific antibody levels determined by sandwich ELISA. OVA-specific IgM, $IgG_1$, $IgG_3$, and $IgG_{2b}$ levels were calculated by coating plates with OVA (10 μg/ml) and isotype-specific secondary antibodies (SouthernBiotech). Individual levels of OVA-specific antibodies were calculated as arbitrary units by comparison with a pooled serum standard from 8 OVA-immunized mice. OVA-specific IgE was determined as previously described (25).

*IL-4– and IL-13–induced airway responses*. As in previous studies (29–31), AHR and airway inflammation were induced by administration of recombinant IL-4 or IL-13 to the airways. Five micrograms of recombinant mouse IL-4 (PeproTech) or recombinant mouse IL-13 (a gift from Talal Chatila, UCLA, Los Angeles, California, USA) or 5 μg BSA was given intranasally for 3 consecutive days and the mice studied 24 hours later.

*In vitro T cell migration*. The in vitro chemotaxis of OVA-specific DO11.10 T cells, a previously described T cell hybridoma (56), to histamine was assessed using 24-well Transwell chambers with polycarbonate membranes (6.5 mm diameter, 5.0 μm pore size) (Corning Inc.). Briefly, histamine in complete RPMI 1640 (Sigma-Aldrich) with 10% fetal calf serum, 1 mmol/l sodium pyruvate, 2 mmol/L-glutamine, 0.05 mmol/l 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin was placed in the lower chamber and $1 \times 10^6$ DO11.10 T cells added to the upper chambers. The chambers were then incubated at 37°C in 5% $CO_2$ for 4 hours and then the numbers of migrated cells in the lower chambers counted, as previously described (46). In separate experiments, diphenhydramine hydrochloride, ranitidine hydrochloride, or thioperamide (obtained from Sigma-Aldrich) were added to the upper and lower chambers and migrated cell numbers determined in response to histamine in the lower chamber.

*T cell transfer*. OVA-sensitized $CD4^+$ T cells were prepared as previously described (57), with minor modifications. Briefly, mice were immunized with OVA (10 μg) in alum and single-cell suspensions of splenocytes ($5 \times 10^6$ cells/ml) prepared at day 7. Cells were cultured in serum-free HL-1 media (Cambrex Corp.) in the presence of 200 μg/ml OVA. After 4 days, $CD4^+$ cells were negatively selected using a MACS $CD4^+$ T cell isolation kit (Miltenyi Biotec), as per the manufacturer's instructions. The purity of the purified $CD4^+$ cell population was determined by FACS analysis and was 85–98%. $2 \times 10^6$ $CD4^+$ cells were intravenously injected into naive recipients. These mice were then exposed to OVA via the airways by ultrasonic nebulization of 1% OVA for 20 minutes every day for 6 days.

The migration of $CD4^+$ T cells was investigated by utilizing Thy1 congenic mice on the C57BL/6 background. B6.PL-*Thy1*$^a$/Cy mice (Jackson Laboratory) carry the *Thy1*$^a$ (Thy1.1) allele on T cells, rather than the *Thy1*$^b$ (Thy1.2) allele normally present in the C57BL/6 strain. OVA-immunized $CD4^+$ T cells from these mice were injected into wild-type C57BL/6 mice or $H1R^{-/-}$ mice, while cells from $H1R^{-/-}$ animals were injected into B6.PL-*Thy1*$^a$/*Cy* or $H1R^{-/-}$ recipients. The recipient mice were studied for development of AHR to methacholine, cellular infiltration in BAL fluid, and tissue inflammation by histology, while the movement of the transferred $CD4^+$ cells into lung tissue, BAL fluid, or spleen was determined by FACS analysis using PE-labeled anti-CD4 antibody (BD Biosciences — Pharmingen) and FITC-labeled anti-Thy1.1 or anti-Thy1.2 (BD Biosciences — Pharmingen).

In a similar manner, OVA-specific $CD3^+$ T cells were also negatively selected using a Pan T Cell Isolation Kit (Miltenyi Biotec) to evaluate the ability of both $CD4^+$ and $CD8^+$ T cells to migrate to the lung. Isolated $CD3^+$ T cells were 86–95% pure, and $5.2 \times 10^6$ cells were intravenously injected into naive recipients, which were then treated as outlined above. PE-labeled anti-CD8 antibody (BD Biosciences — Pharmingen) was used to detect $CD8^+$ cells in the lungs and BAL fluid.

*Statistics*. Statistical analysis was carried out using Microsoft Excel data analysis for 2-tailed Student's *t* test and GraphPad Prism 4 for ANOVA. *P* values less than 0.05 were considered statistically significant.

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