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Cutting Edge

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Cutting Edge: Histamine Is Required for IL-4–Driven Eosinophilic Allergic Responses

Julie A. Swartzendruber, Adam J. Byrne, and Paul J. Bryce

Histamine is an important allergic mediator, and studies have defined roles for both histamine 1 and 4 receptors in allergic airway inflammation. In this study, we show that histamine is necessary to generate IL-4–driven eosinophilic inflammation, as histamine-deficient mice cannot generate eosinophilic lung inflammation in response to intratracheal IL-4 and exogenous histamine restores responsiveness. This is histamine 2 receptor (H2R) dependent because H2R knockout mice fail to respond to IL-4, and a H2R agonist restores inflammation in histidine decarboxylase knockout. Furthermore, alveolar epithelial cells require H2R to produce CCL24, an eosinophil recruitment factor, whereas H2R blockade reduces CCL24 production from wild-type cells. In an allergic inflammation model, H2R knockout mice show significantly reduced eosinophilic inflammation and CCL24 expression. These data demonstrate a previously unidentified role for H2R in allergic inflammation and establishes a synergy between endogenous histamine and IL-4 that supports eosinophilic recruitment to the lung. ***The Journal of Immunology*****, 2012, 188: 000–000.**

Interleukin-4 is critically important for Th2-associated immunity, including allergic inflammation. Even in the absence of IL-5, IL-9, and IL-13, IL-4 is capable of eliciting the characteristic Th2 immune responses, including eosinophilia and IgE production, during helminth infection (1). Transgenic overexpression of IL-4 in the lung generates profound inflammatory responses without affecting airway reactivity (2), which is largely IL-13 dependent (3). Similarly, local delivery of rIL-4 leads to significant accumulation of inflammatory cells in the lung (4).

Although recruitment of eosinophils to the lungs of mice during allergic responses is regulated by both CCL11 and CCL24 (5), CCL11 expression has been shown to be mainly IL-13 driven (6) and lung eosinophilia in CCL11 knockout (KO) mice is only mildly affected (7). Conversely, CCL24 has a dominant role in promoting airway eosinophilia (7). Transgenic coexpression of CCL24 and IL-5 within the airway leads to chronic eosinophil-associated lung damage that mirrors severe asthma (8). During allergic airway responses or upon IL-4 overexpression, CCL24 is significantly upregulated (7, 9, 10), but the mechanisms controlling this local tissue response remain unclear.

During allergic responses, mast cells residing within tissues release a plethora of mediators capable of influencing inflammatory cell recruitment (11). One mediator is histamine, a highly diffusible bioactive molecule that exerts its biological functions via four receptors (histamine 1 receptor [H1R], histamine 2 receptor [H2R], histamine 3 receptor, and histamine 4 receptor [H4R]) (12). Whereas histamine is best known for its role in vasodilation and smooth muscle responses during immediate hypersensitivity, the histamine receptors also exert potent immunomodulatory influences. H4R has also been shown to regulate allergic sensitization, because H4R KO mice have defective dendritic cell priming of T cells and H4R blockade ameliorates allergic inflammation (13). We previously demonstrated that H1R on T cells is necessary for their recruitment to the lung and subsequent escalation of Th2-associated airway inflammation (14). In this H1R study, we demonstrated that exogenous delivery of IL-4 was sufficient to elicit inflammatory cell recruitment to the lungs of these mice because it bypassed the requirement for T cell migration.

In this study, we show that exogenous delivery of IL-4 cannot generate similar responses in mice specifically lacking histamine, suggesting that histamine is necessary for IL-4–driven lung eosinophilia. We demonstrate that this is mediated by H2R and, using both in vitro and in vivo approaches, that this receptor is critical for production of the eosinophilic chemokine CCL24. Consequently, our data suggest that histamine modulates the local lung responsiveness to IL-4 via H2R, permitting CCL24 production and subsequent eosinophil recruitment. We postulate that inhibition of this receptor may be useful in the therapeutic treatment of allergy and Th2-associated diseases.

Materials and Methods

Animals

Female C57BL/6 (4–8 wk old; Taconic Farms, Hudson, NY), histidine decarboxylase (HDC) KO [from H. Ohtsu, Tohoku University, Japan (15)], and H2R KO [from T. Watanabe, Kyoto University, Japan (16)] mice were housed under specific pathogen-free conditions and maintained on an OVA-

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Abbreviations used in this article: ATII, alveolar type II; BAL, bronchoalveolar lavage; HDC, histidine decarboxylase; H1R, histamine 1 receptor; H2R, histamine 2 receptor; H4R, histamine 4 receptor; KO, knockout; WT, wild-type.

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free diet. All experiments were approved by the Northwestern University Animal Care and Use Committee.

Intratracheal IL-4–driven airway inflammation

A total of 5 μg BSA, carrier-free recombinant murine IL-4 (eBioscience, Franklin Lakes, NJ), histamine (Calbiochem, Rockland, MA), or dimaprit dihydrochloride (Tocris Bioscience, Ellisville, MO) was administered intratracheally for three consecutive days. Mice were studied on day 4.

Primary alveolar type II cell isolation

Alveolar type II (ATII) epithelial cells were isolated by Pulmonary Core B (Northwestern University, Chicago, IL), as previously described (17). Cells were cultured in DMEM with 10% FCS, 100 U/ml penicillin, and 100 g/ml streptomycin and treated with 10 ng/ml recombinant murine IL-4 (PeproTech) and 50 μM hydrochloride ranitidine (Sigma-Aldrich, St. Louis, MO).

CCL24 ELISA

CCL24 was measured by ELISA (R&D Systems, Minneapolis, MN), according to the manufacturer's instruction.

Allergic airway inflammation model

Mice received i.p. injection of 10 μg OVA (grade VI; Sigma-Aldrich) in alum (3 mg) or alum alone at days 0 and 14, followed by 20 min of aerosolized 1% OVA on days 21–23, and studied on day 24.

Lung inflammation

Bronchoalveolar lavage (BAL) was collected by flushing lungs with 0.8 ml BAL fluid (10% FCS, 1 mM EDTA, 1× PBS). BAL fluid was counted and cytospun onto slides, and differential cell counts were performed after staining with DiffQuik (Dade Behring, Newark, DE). H&E and periodic acid-Schiff histology was performed by Histo-Scientific Research Laboratories (Mount Jackson, VA).

Real-time RT-PCR

Total RNA was isolated from 50–100 mg lung tissue using a RNeasy kit (Qiagen, Valencia, CA). cDNA was prepared using qScript cDNA Super Mix (Quanta, Gaithersburg, MD). Gene expression was determined, as previously described (14, 18).

Statistics

Data are represented as mean ± SEM. Statistical significance was determined using Student *t* test. All analysis was done using GraphPad Prism Software (La Jolla, CA).

Results and Discussion

Histamine is necessary for IL-4–induced eosinophil recruitment and CCL24 production

Because we previously showed that intranasal IL-4 could restore eosinophilic inflammatory responses in H1R KO mice (14), we wished to examine responses in the absence of histamine itself. In contrast to H1R KO mice, HDC KO mice failed to mount eosinophilic inflammation after IL-4 delivery (Fig. 1*A*), and this could be restored with addition of exogenous histamine at the time of IL-4 administration. Mechanistically, histamine can directly promote eosinophil migration (19), whereas work using allergen challenge in patients also showed that histamine affects eosinophil migration indirectly by promoting local eotaxin expression (20, 21). Within the time frame we examined, histamine alone failed to induce any inflammatory infiltration (Fig. 1*A*). Conversely, whereas wild-type (WT) mice displayed a robust expression of the eosinophilic chemokine CCL24, this was ablated in the HDC KO mice and restored by exogenous histamine (Fig. 1*B*). We were unable to detect significant upregulation of the other major eosinophilic chemokine CCL11 in any group (data not shown), concurrent with its proposed IL-13 dependence (6). Consequently,

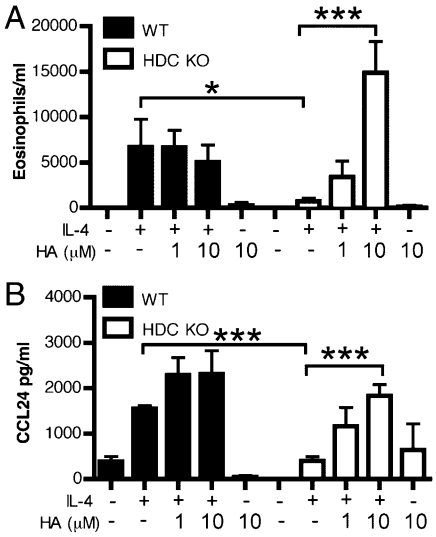

FIGURE 1. Endogenous histamine is necessary for IL-4–induced eosinophil recruitment and CCL24 production. A total of 5 μg IL-4 or BSA, with or without histamine (HA), was administered intratracheally to WT or HDC KO mice for two consecutive days, and inflammation was determined on day 3. Eosinophils in the BAL fluid were identified using DiffQuik staining of cytospun BAL fluid cells (*A*). CCL24 levels were measured in BAL fluid by ELISA (*B*). Data represent mean ± SEM from three independent experiments (n = 3–7). $^{*}p < 0.05$, $^{***}p < 0.005$ by Student *t* test.

rather than directly promoting eosinophil migration, histamine serves to regulate the expression of the eosinophil chemoattractant signals upon IL-4 exposure in the lung tissue. Importantly, these findings suggest that there is a homeostatic role for histamine in facilitating responsiveness to IL-4 in WT animals, and this basal tone is lost in HDC KO mice. Because our previous work established that these effects of histamine were H1R independent and H4R-mediated eosinophil migration has been shown to be mediated via CCL16 (22), we next examined the role of H2R using H2R KO mice.

IL-4–mediated eosinophil recruitment to the lung requires H2R

Similar to HDC KO mice, IL-4 was unable to induce eosinophilic inflammation in H2R KO mice (Fig. 2*B*). CCL24 production was also impaired in H2R KO mice (Fig. 2*C*). Furthermore, coadministration of IL-4 with a H2R agonist, dimaprit, was sufficient to increase both the eosinophil numbers (Fig. 2*E*) and CCL24 production (Fig. 2*F*) in the HDC KO. Whereas the eosinophil numbers failed to reach statistical significance due to some variability (although all dimaprit-treated mice had eosinophils in the BAL fluid versus none of the PBS treated), the CCL24 response was highly significant, supporting H2R being the critical histamine receptor in mediating the effects of histamine on eosinophilic lung responses. Although the established role for H2R is in acid secretion in the stomach and the H2R KO mice have abnormal gastric responses (16), H2R has a wide expression profile that includes both nonhematopoietic and hematopoietic cells (23). Because intratracheal administration of IL-4 acts locally to drive inflammation, we concluded that H2R was mostly likely necessary at the level of a tissue resident cell.

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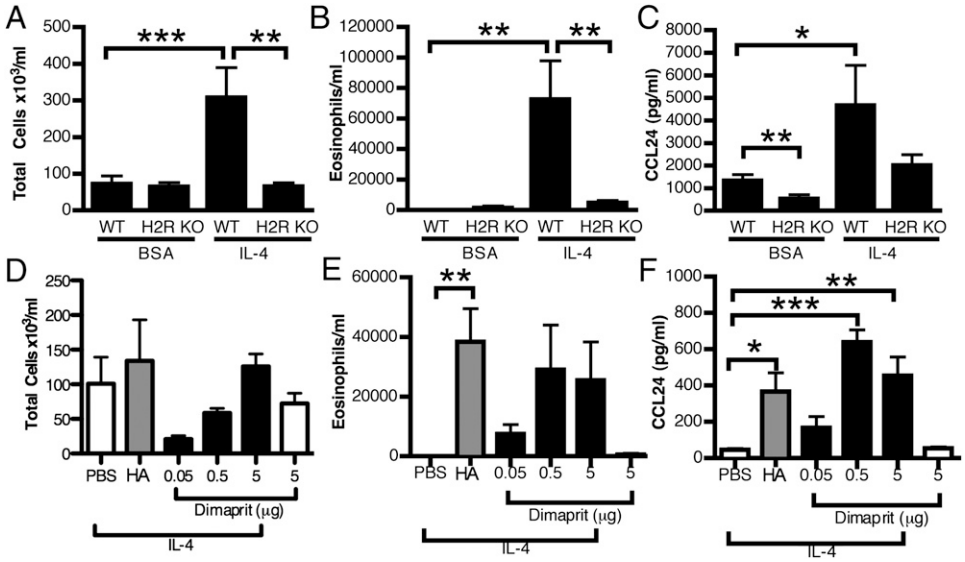

FIGURE 2. H2R is necessary for IL-4–induced eosinophil recruitment and CCL24 production. A total of 5 μg IL-4 or BSA was administered intratracheally for three consecutive days to WT or H2R KO mice. On day 4, total cells (*A*), eosinophils (*B*), and CCL24 levels (*C*) in BAL fluid were determined. Data represent mean ± SEM from three independent experiments (n = 7–9). A total of 5 μg IL-4 or BSA was administered intratracheally for three consecutive days to HDC KO mice with or without histamine (10 μM) or dimaprit, and total cells (*D*), eosinophils (*E*), and CCL24 levels (*F*) in BAL fluid were measured on day 4. Data represent mean ± SEM from three independent experiments (n = 4–6). $^{*}p < 0.05$, $^{**}p < 0.05$, $^{***}p < 0.005$ by Student t test.

H2R is critical for IL-4–induced CCL24 production by ATII cells

Airway smooth muscle cells express IL-4R, but it has recently been shown that they do not contribute significantly to IL-4–induced inflammation (24). Therefore, we focused our interest on the lung epithelium. ATII cells have previously been shown to produce CCL24 in response to IL-4 (25). We initially determined that these cells also express high levels of H2R and that both WT and H2R KO cells express similar levels of IL-4R (data not shown). In response to IL-4, H2R KO ATII cells produced significantly less CCL24 than WT cells (Fig. 3*A*). We further interrogated the role of H2R in CCL24 production using pharmacological blockade of H2R. WT ATII cells pretreated with ranitidine 30 min prior to IL-4 treatment demonstrated a dose-dependent significant reduction in CCL24 production (Fig. 3*B*). Therefore, we propose that IL-4–driven eosinophilic inflammation is most likely facilitated by endogenous histamine, acting via H2R on ATII cells within the lung to promote the generation of a CCL24 gradient. Interestingly, mast cells are commonly located in or around the airway epithelium, and the frequency of intraepithelial mast cells is increased in asthmatics with a dominant Th2 phenotype (26). Therefore, this increased mast cell presence may serve to enhance the local tissue responsiveness to IL-4 by increasing local histamine levels.

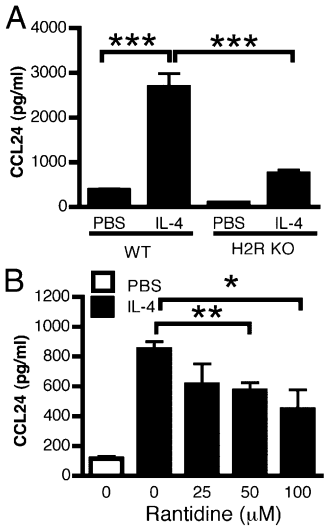

FIGURE 3. IL-4–induced CCL24 production by ATII cells is H2R dependent. Primary mouse ATII cells were isolated and stimulated with 10 ng of murine rIL-4 for 48 h. CCL24 levels were measured in supernatant from WT and H2R KO ATII cells (*A*) or from WT ATII cells treated with ranitidine (*B*). Data represent mean ± SEM from three independent experiments (n = 3–6). $^{*}p < 0.5$, $^{**}p < 0.05$, $^{***}p < 0.005$ by Student t test.

Paradoxically, our in vitro culture experiments did not receive exogenous histamine, and yet, H2R still regulated IL-4–driven CCL24 production. Histamine receptors are known to possess constitutive basal activity (27). This includes H2R, which ranitidine inhibits via inverse agonist activity (28). It has also been previously shown that histamine is present in cell culture media and required for optimal T cell activation in vitro (29). We determined that our FCS did contain histamine such that our cultures were receiving $\sim 10^{-7}$ M histamine (data not shown), but we were unable to eliminate this because the ATII cells failed to grow well in serum-free media. However, because our in vivo data using the HDC KO mice demonstrate that histamine is required for IL-4–driven eosinophila and CCL24 expression, we conclude that histamine is most likely acting upon its receptor.

H2R is necessary for lung eosinophilia during allergic airway inflammation

Although we demonstrated a role for H1R in allergic inflammation (14), H2R has not been investigated. Using the same model of allergic airway inflammation we used in the H1R study (14), H2R KO mice showed reduced cellular infiltration in BAL fluid compared with WT (Fig. 4*A*). This was not due to altered H1R expression because the relative expression of all histamine receptors in lung tissue from the H2R KO was similar to WT levels (data not shown). As predicted from our in vitro studies, this reduced cellular infiltrate was due largely to a significant reduction in eosinophils in the lungs of the H2R KO mice, with a concurrent

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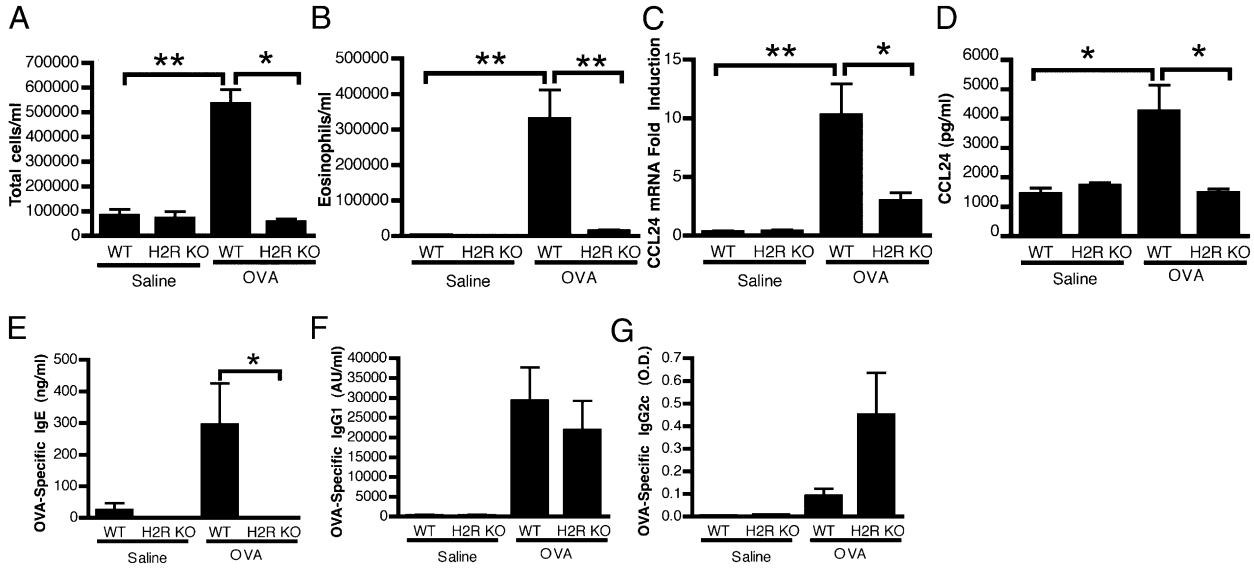

FIGURE 4. H2R is necessary for eosinophilic airway inflammation to Ag. Sham (saline)- or OVA/alum-sensitized WT and H2R KO mice were challenged with aerosolized OVA and studied for inflammatory responses. Total cells (*A*) and eosinophil (*B*) counts in BAL fluid were determined. CCL24 protein levels in BAL fluid (*C*) and mRNA levels in lung tissue (*D*) were also measured. OVA-specific Ab levels in serum were measured by ELISA (*E–G*). Data represent mean ± SEM from two independent experiments (n = 5–6). $^{*}p < 0.05$, $^{**}p < 0.05$ by Student t test.

reduction in CCL24 mRNA and protein production (Fig. 4*B–D*). Additionally, we measured several other chemokine and cytokine levels, including eosinophil-associated IL-16, IL-5, and CCL5 (RANTES) (Supplemental Fig. 1*A–G*). Rather than a general defect in expression of all responses, H2R KO mice displayed an apparent disregulation, with some mediators upregulated, similar to WT in response to challenge, whereas others were unchanged. Histology revealed substantial inflammation and mucus production in WT lung tissue that were significantly reduced in the H2R KO mice, which exhibited only minor perivascular inflammation (Supplemental Fig. 1*H*). Quantification of periodic acid-Schiff^{+} cells in the airway showed significantly fewer mucus-expressing cells in H2R KO mice versus WT (data not shown). The presence of some inflammation and mucus production in the H2R KO mice does indicate some inflammatory responses occurred in the H2R KO mice, albeit significantly reduced. Furthermore, analysis of the OVA-specific Igs generated during sensitization showed an absence of specific IgE in H2R KO mice (Fig. 4*E*), but competent production of IgG1 (Fig. 4*F*) and IgG2c (Fig. 4*G*), a similar pattern to that reported by Jutel et al. (30). They also demonstrated enhanced Th1- and Th2-associated OVA-specific recall responses from H2R KO splenocytes (30), a phenotype we have also observed (data not shown). Consequently, H2R KO mice do exhibit Ag sensitization, and so the lack of airway eosinophilia and IgE is not likely due to defects in Ag processing and presentation. In agreement, we determined that CD11c^{+} dendritic cells from H2R KO mice pulsed with OVA$^{323–329}$ peptide induced similar proliferation of OTII CD4^{+} T cells as those from WT mice (data not shown). Jutel et al. (30) proposed that the lack of IgE was due to the heightened levels of IFN-γ. However, it has since been shown that IL-21, and not IFN-γ, is the critical suppressive cytokine of IgE generation during allergic sensitization to OVA (31). IL-21 expression levels were similar between WT and H2R KO mice (data not shown), suggesting this was not responsible for the differences seen in IgE. Instead, we hypothesized that H2R may be also directly impacting B cell responses, because class switching to IgE is critically dependent upon IL-4 signaling (32, 33). Using B cells cultured in vitro with LPS plus increasing concentrations of IL-4, we determined that H2R KO cells were significantly impaired in their ability to generate IgE (Supplemental Fig. 2), despite similar levels of IL-4R expression (data not shown). Consequently, we conclude that H2R is directly regulating the production of IgE by influencing the ability of B cells to respond to IL-4. It is possible that the lack of responsiveness to IL-4 by cells other than the airway epithelium underlies the relatively suppressed inflammatory response seen in the OVA model. However, further investigation is required to dissect the significance of H2R expression on other cell types and how their cumulative response to IL-4 shapes allergic airway inflammation. Overall, these results suggest that H2R facilitates IL-4–driven eosinophil recruitment and IgE production in vivo.

In conclusion, our data define a requirement for histamine in facilitating responsiveness to IL-4. We are proposing that the endogenous low levels of histamine present during homeostasis are sufficient for this effect. Whereas histamine can be sequestered from diet or produced by bacteria, the HDC KO mice used in our study were not maintained on a histamine-deficient diet, and so this basal histamine is most likely derived from host immune cells, which could include stored histamine from mast cells or basophils, but also inducible producers such as dendritic cells (13). Although we previously demonstrated that H1R is critical for regulating the migration of Ag-specific T cells to the lung (14), this regulation of tissue responsiveness to IL-4 functions via H2R. Therefore, we propose that histamine exerts its varied influences on both systemic and local inflammatory responses via different receptors that can be targeted for the therapeutic treatment of allergy and Th2-associated diseases.

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Disclosures

The authors have no financial conflicts of interest.

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Supplemental Figures

Histamine is required for IL-4-driven eosinophilic allergic responses

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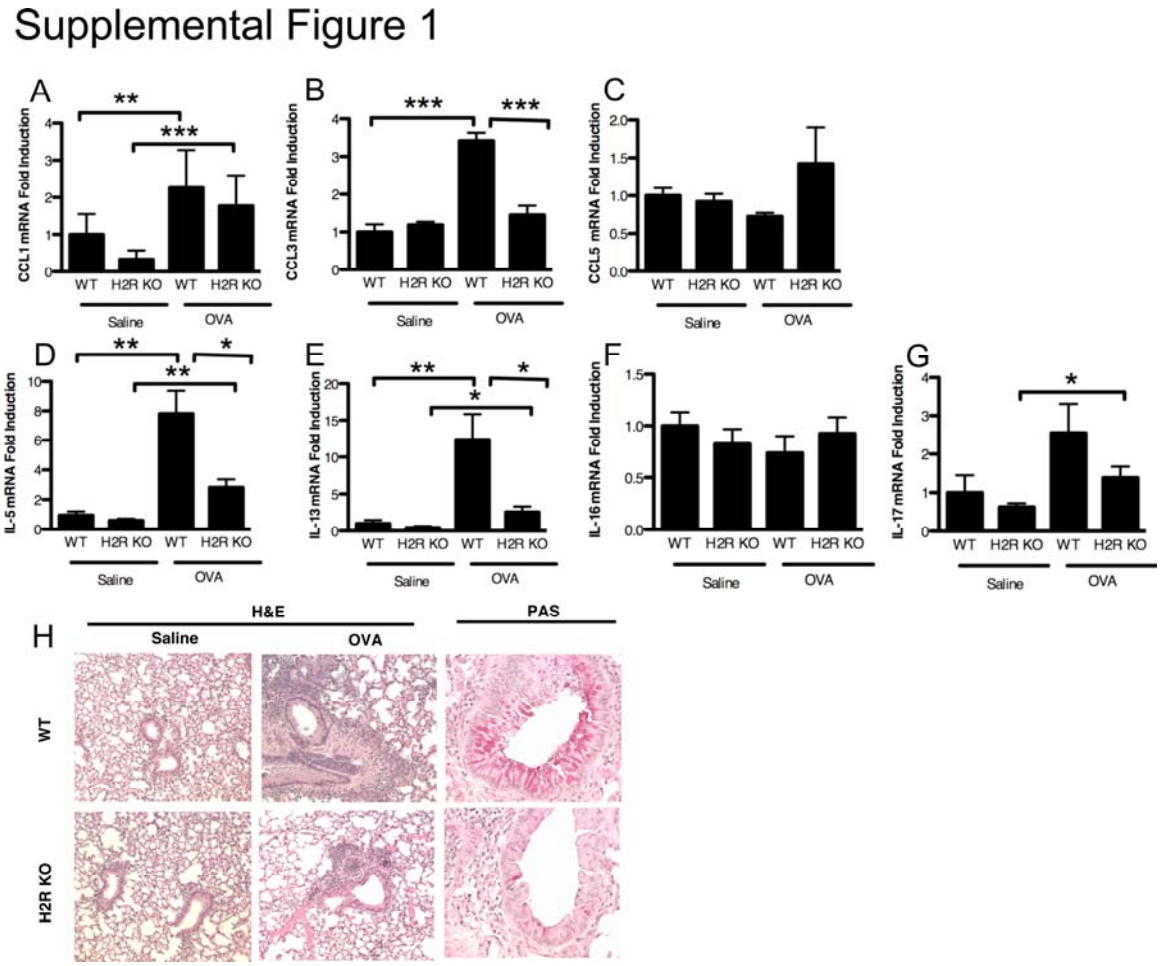
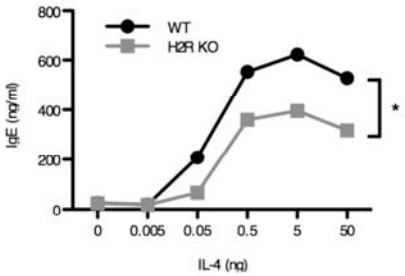


Supplemental Figure 1: Lung responses are disregulated in the absence of H2R. Sham (saline) or OVA/alum sensitized WT and H2R KO mice were challenged with aerosolized OVA and chemokine and cytokine mRNA levels in lung tissue were measured. Data represent mean ± SEM from 2 independent experiments (n = 4-6). *$p < 0.05$; ** $p < 0.05$, ***$p < 0.005$ by Students t-test. Lung tissue was also studied for inflammation (by H&E staining) or mucus (by Periodic Acid Schiff (PAS) staining). Sections are representative of 5-6 mice.

Supplemental Figure 2



Supplemental Figure 2: H2R deficient B cells have impaired IgE production in response to IL-4. B220^{+} B cells were isolated from spleens of WT and H2R KO mice using magnetic beads. The cells were cultured for 6 days in the presence of LPS and increasing concentrations of IL-4. Antibody production was measured by ELISA on day 6 from cell culture supernatant. Data are representative of 3 independent experiments with similar results. *$p < 0.05$ by 2-tailed ANOVA.