

RESEARCH ARTICLE

HDAC2 attenuates airway inflammation by suppressing IL-17A production in HDM-challenged mice

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¹Key Laboratory of Respiratory Disease of Zhejiang Province, Department of Respiratory and Critical Care Medicine, Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang, China; ²Department of Respiratory and Critical Care Medicine, Institute of Respiratory Diseases, Affiliated Hospital of Guangdong Medicine University, Zhanjiang, Guangdong, China; and ³State Key Laboratory for Respiratory Diseases, Guangzhou, Guangdong, China

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Lai T, Wu M, Zhang C, Che L, Xu F, Wang Y, Wu Y, Xuan N, Cao C, Du X, Wu B, Li W, Ying S, Shen H, Chen Z. HDAC2 attenuates airway inflammation by suppressing IL-17A production in HDM-challenged mice. *Am J Physiol Lung Cell Mol Physiol* 316: L269–L279, 2019. First published November 8, 2018; doi:10.1152/ajplung.00143.2018.—Histone deacetylase (HDAC)2 is expressed in airway epithelium and plays a pivotal role in inflammatory cells. However, the role of HDAC2 in allergic airway inflammation remains poorly understood. In the present study, we determined the role of HDAC2 in airway inflammation using *in vivo* models of house dust mite (HDM)-induced allergic inflammation and *in vitro* cultures of human bronchial epithelial (HBE) cells exposed to HDM, IL-17A, or both. We observed that HDM-challenged *Hdac2*^{+/-} mice exhibited substantially enhanced infiltration of inflammatory cells. Higher levels of T helper 2 cytokines and IL-17A expression were found in lung tissues of HDM-challenged *Hdac2*^{+/-} mice. Interestingly, IL-17A deletion or anti-IL-17A treatment reversed the enhanced airway inflammation induced by HDAC2 impairment. *In vitro*, HDM and IL-17A synergistically decreased HDAC2 expression in HBE cells. HDAC2 gene silencing further enhanced HDM- and/or IL-17A-induced inflammatory cytokines in HBE cells. HDAC2 overexpression or blocking IL-17A gene expression restored the enhanced inflammatory cytokines. Collectively, these results support a protective role of HDAC2 in HDM-induced airway inflammation by suppressing IL-17A production and might suggest that activation of HDAC2 and/or inhibition of IL-17A production could prevent the development of allergic airway inflammation.

airway inflammation; asthma; HDAC2

INTRODUCTION

Asthma is one of the most common chronic inflammatory airway diseases, and it affects an estimated 300 million individuals worldwide. Asthma remains poorly controlled in many patients, even in those under a specialist's care (15). There is

a critical need to identify the underlying basis of asthma and to develop an appropriate treatment strategy.

Chronic airway inflammation is a prominent pathological feature of asthma. Previous studies have shown that airway inflammation is mediated by the expression of inflammatory genes (4, 19). Certain histone deacetylases (HDACs) play an important role in suppression of inflammatory gene expression by reversing the hyperacetylation of core histones (14). HDAC2 is a class I histone deacetylase that regulates various cellular processes, such as proliferation, differentiation, cell cycle, apoptosis, senescence, development, and glucocorticoid function in inhibiting inflammatory response (14). HDAC2 activity has been shown to be reduced in alveolar macrophages, peripheral blood mononuclear cells, and bronchial biopsies in patients with asthma (4, 14, 15). However, the critical pathological function of HDAC2 in asthma has not been tested in murine models. The functional role of HDAC2 in airway epithelial cells, and its role in regulation of allergic airway inflammation remains unclear. Interleukin (IL)-17A has been described in multiple aspects of asthma pathogenesis, including airway inflammation, mucous cell metaplasia, and airway remodeling. Elevated levels of IL-17A mRNA and protein were found in asthmatic patients (2, 5, 6). Increased IL-17A levels are positively correlated with airway inflammation, airway hyperresponsiveness, and disease severity (1, 3, 22). However, whether HDAC2 can influence IL-17A-driven responses in asthma pathogenesis and exacerbation remains unknown.

In the present study, we sought to determine whether HDAC2 regulates allergic airway inflammation in a murine model of asthma and if so, to determine the mechanism by which this occurs. By use of an experimental model of allergic asthma, our results provide information on the possible effects of HDAC2 on allergic asthma and on the relationship between human bronchial epithelial (HBE) cells and IL-17A in airway inflammation.

METHODS

Animal studies. *Il17a*^{-/-} (C57BL/6 background) mice were purchased from the Center for Experimental Medicine and Systems Biology (Institute of Medical Science, University of Tokyo, Japan).

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Hdac2^{+/-} (C57BL/6 background) mice were kindly provided by Dr. J. A. Epstein (Univ. of Pennsylvania School of Medicine, Philadelphia, PA). *Hdac2*^{+/-} male mice were bred with *Il17a*^{-/-} female mice to create double-deficient (*Hdac2*^{+/-}*Il17a*^{-/-}) mice. Genotyping was performed with PCR assays using mouse tail genomic DNA. IL-17A wild-type (WT), and gene deletion were amplified using common primer 5'-ACTCTTCATCCACCTCACACGA-3', WT primer 5'-GTACACCAGCTATCCTCCAGATAG-3', and IL-17 knockout (KO) primer 5'-TCCTTGAAGTCGATGCCCTTCA-3'. The PCR product of the WT allele is ~750 bp, and gene deletion is ~500 bp, respectively. HDAC2 WT and gene deletion were amplified using HDAC2 WT forward (F) 5'-AGCATCAGACAAACG-GATAGCTTGCGATGA-3', HDAC2 WT reverse (R) 5'-AAGAGC ACTGAGTCTAAGTCCACCTAA-3'; HDAC2 KO F 5'-CTG-GAGAAGGCCCGACCATCC-3', and HDAC2 KO R 5'-CTG-GAGAAGGCCCGACCATCC-3', respectively. The PCR product of the WT allele is ~450 bp and gene deletion is ~250 bp, respectively. Representative genotyping of IL-17A and HDAC2 is shown in Fig. 1. All the animal experiments were strictly conducted in accordance with the protocols approved by the Ethics Committee for Animal Studies at Zhejiang University, China.

Induction of allergic airway inflammation. House dust mite (HDM) extract was purchased from Greer Laboratories (10.52 EU/mg endotoxin) as a lyophilized preparation and dissolved in endotoxin-free saline. Six- to eight-week-old mice were sensitized and challenged with vehicle (endotoxin-free saline) or HDM, and murine models of allergic asthma were established. Briefly, mice were sensitized on days 0, 1, and 2 by intratracheal instillation of 5 µg/50 µl HDM (or with vehicle as a control). On days 15, 16, and 17 after the initial sensitization, the mice were challenged with 5 µg/50 µl HDM (or with vehicle as a control) using intratracheal instillation. Mice were euthanized using a sodium pentobarbitone overdose intraperitoneally (ip) at 24 h after the final allergen challenge. For IL-17A neutralization, anti-IL-17A antibody (50 µg/mouse, R&D Systems) was administered ip 2 h before the challenge at day 15.

Collection of bronchoalveolar lavage fluid. Collection of bronchoalveolar lavage fluid (BALF) was performed as described in our previous study (21). Briefly, the lungs were administered lavage using a cannula inserted in the trachea, and then they were instilled with 0.8 ml of phosphate-buffered saline (PBS) after exsanguination. Cytospin slides were prepared using Wright-Giemsa staining. Total cell and eosinophil counts were determined under the microscope in a blinded manner.

Cell culture and treatment. HBE cells were purchased from American Type Culture Collection (ATCC, CRL-2741), and were maintained in RPMI 1640 with 10% FCS in a humidified incubator of 5% CO₂ atmosphere at 37°C. Cells were seeded in culture dishes and grown until 70% confluence. The medium was then replaced with a basal medium. The cells were stimulated with HDM and/or recombinant human (rh)IL-17A (R&D Systems) in a concentration-dependent manner for 24 h.

siRNA/plasmid studies. Small interfering (si)RNA for human HDAC2 and IL-17A were obtained from Santa Cruz Biotechnology. All the siRNAs were transfected into cells by GeneMute (SigmaGen). After 24 h of transfection, cells were exposed to HDM and/or rhIL-17A as described above. In plasmid studies, the cells were transfected with HDAC2 plasmid with Polyjet (SigmaGen), according to the manufacturer's protocols, when they reached 90% confluence. Twenty-four hours after plasmid transfection, the transfected cells were exposed to HDM and/or rhIL-17A.

RT-PCR analysis. cDNA was prepared by reverse transcription of RNA using random hexamer primers and Superscript II (Invitrogen). Real-time PCR was performed for 50 cycles on a Step One Real-time PCR System (Applied Biosystems). The sequence of primers is listed in Table 1.

Western blot. HBE cells were lysed in RIPA buffer. Proteins in lysates of cells were separated by SDS-PAGE and immunoblotted using antibodies against the following proteins: IL-17A, HDAC2, and β-actin (Santa Cruz Biotechnology).

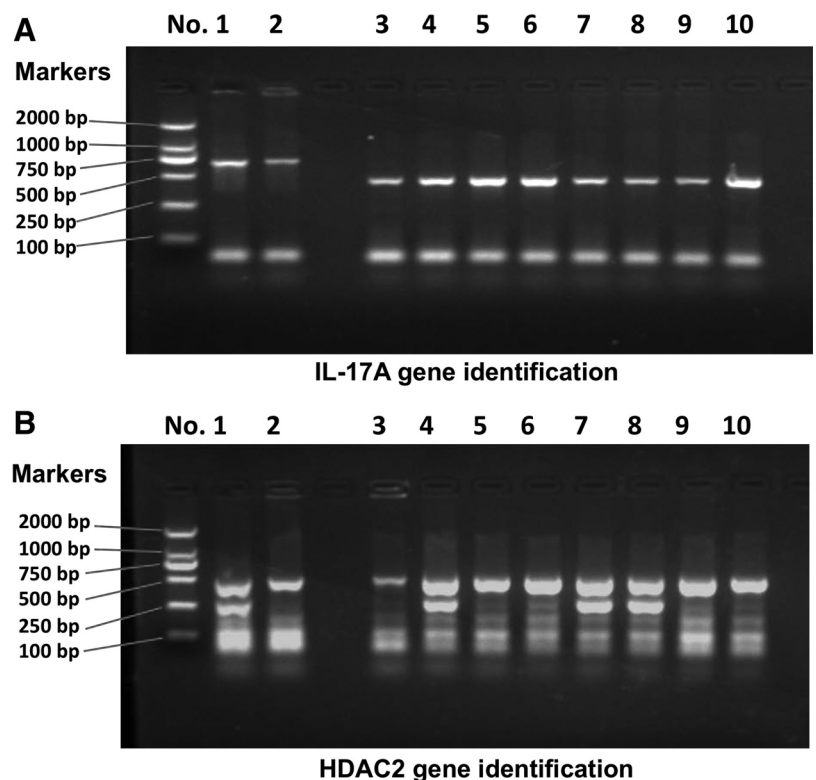


Fig. 1. Representative genotyping of IL-17A and histone deacetylase-2 (HDAC2). Genotyping performed with PCR assays using mouse tail genomic DNA. A: PCR product of the wild-type (WT) allele is ~750 bp and IL-17A gene deletion is ~500 bp, respectively; IL-17A homozygote: nos. 3, 4, 5, 6, 7, 8, 9, 10; WT: nos. 1, 2. B: PCR product of the wild-type allele is ~450 bp and gene deletion is ~250 bp, respectively. HDAC2 heterozygote: nos. 1, 4, 7, 8; WT: nos. 2, 3, 5, 6, 9, 10. HDAC2 and IL-17A double-deficient mice (*Hdac2*^{+/-}*Il17a*^{-/-}): 4, 7, 8.

Table 1. Sequence of primers for real-time PCR

Gene	Sequence (5'–3')	Direction
Human <i>IL6</i>	TGATGGATGCTACCAAACTGG	Sense
	TTCATGTACTCCAGGTAGCTATGG	Antisense
Human <i>IL8</i>	ACTGAGAGTGATTGAGAGTGGAC	Sense
	AACCTCTGCACCCAGTTTTC	Antisense
Human <i>Eotaxin</i>	CCCCTTCAGCGACTAGAGAG	Sense
	TCTTGGGGTCCGCACAGAT	Antisense
Human <i>IL17A</i>	CGGCTGGAGAAGATACTGGT	Sense
	TTAGTCCGAAATGAGGCTGTC	Antisense
Human <i>GAPDH</i>	TGTTGCCATCAATGACCCCTT	Sense
	CTCCACGAGTACTACGG	Antisense

Mediator assays. The levels of mouse IL-17A were measured with ELISA kits (R&D Systems). Mouse IL-5, IL-6, IL-13, and C-X-C motif chemokine ligand 1 (CXCL1) in lung homogenate were measured using an ELISA kit (eBioscience).

Flow cytometry and antibodies. Mouse-specific antibodies Pacific APC-Cy7-CD3, fluorescein isothiocyanate (FITC)-CD4, and PE-Cy7-CD8 were purchased from eBioscience. APC- γ TCR, PE-IL-17A, and Percp-CD45 were purchased from BD Pharmingen. HDAC2 was purchased from Abcam. Flow cytometry was performed with Fortessa (BD Biosciences), and data were analyzed with FlowJo (TreeStar).

Immunohistochemistry. Hematoxylin-eosin (H&E) staining sections were semiquantitated (score: 0–4) as previously described for the inflammatory situation. Mucus production of goblet cells were

assessed by Periodic Acid-Schiff (PAS) staining (8). The area of peribronchial Masson trichrome staining (blue) was visualized and quantified by the software as a percentage of the total band area. The slides were incubated with either a rabbit polyclonal antibody against IL-17A or HDAC2 (Santa Cruz Biotechnology). Quantitative measurements of IL-17A- or HDAC2-positive cells in the lung tissue were performed according to previously described methods (18). Briefly, HDAC2- or IL-17A-positive and -negative cells were counted in each specimen. The lung cells positive for the HDAC2 or IL-17A antibody staining were expressed as a percentage of total cells. Sections were examined using a light microscope (BX51; Olympus, Japan) and quantified by Image Pro 6.1 software (Media Cybernetics). At least 10 bronchioles were counted on each slide, and the data were used for statistical analysis. Intra-observer error was assessed by performing three independent counts on the same section on separate occasions.

Immunofluorescence. HBE cells were plated on sterilized coverslips. After treatment, the cells were washed with PBS three times, fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.1% Triton X-100 (Biosharp) for 20 min, blocked with 3% bovine serum albumin (BSA) for 1 h, and then incubated overnight with primary antibody (HDAC2, IL-17A, CC10). Subsequently, the cells were stained with FITC-conjugated secondary antibody (Alexis fluor). DAPI was used for nucleus staining.

Chromatin immunoprecipitation assays. Chromatin immunoprecipitation (ChIP) assays were performed according to the manufacturer's protocol. Immunoprecipitation was performed using an anti-retinoid-related orphan nuclear receptor- γ t (ROR γ t) antibody (Santa

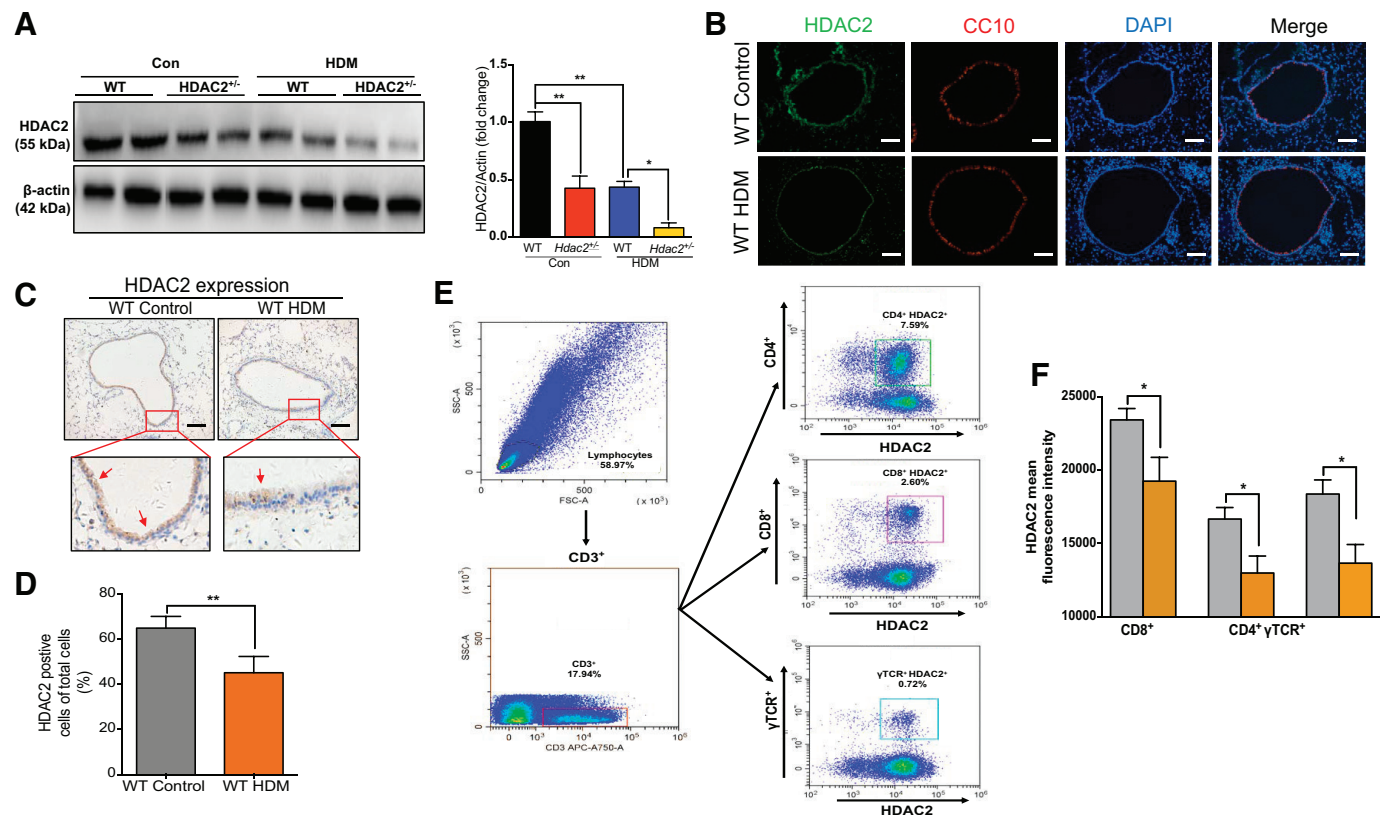


Fig. 2. Histone deacetylase-2 (HDAC2) reduced in epithelial cells and T helper 17 (Th17) cells in house dust mite (HDM)-induced mice. **A:** HDAC2 expression in lung homogenate was analyzed by Western blot and quantified using ImageJ software. **B:** representative HDAC2 and CC10 (Clara cell-specific protein in airway epithelial cells) are shown in green or red, as depicted, and DAPI staining in blue (Scale bar, 100 μ m). **C** and **D:** expression of HDAC2 in the lung of mice was measured by immunohistochemistry (IHC) (scale bar, 100 μ m). Semiquantification of IHC in lungs was done using Image Pro 6.1 software; **E** and **F:** flow cytometry was performed with Fortessa (BD Biosciences). The cell population shown in the FACS plots gated out from whole lung cells; the entire gating strategy is shown. Quantification of HDAC2 fluorescence intensity was analyzed with FlowJo (TreeStar). Data are representative of 2 independent experiments ($n = 5-6$ mice per group per study). Results are expressed as means $\pm$ SE. Student's *t*-test was employed for comparison for 2 groups. One-way ANOVA with post hoc Tukey's corrections for comparison of more than 2 groups: * $P < 0.05$, ** $P < 0.01$.

Cruz Biotechnology) with rotation overnight at 4°C. Protein/DNA complexes were captured in elution buffer, and cross-links were reversed to free DNA. The PCR was addressed for the IL-17A promoter region -52 to -246 and was performed using the following primers: 5'-GCA GCTCTGCTCAGCTTCTA-3' and 5'-GGG-CTTTTCTCCTTCTGTGG-3' (11). The primers for GAPDH, 5'-TACTAGCGGTTTACGGGCG-3'; 5'-TCGAACAGGAGGAGCA-GAGAGCGA-3', were used as a control. After DNA purification, quantitative PCR was performed.

Statistical analysis. Data are presented as means $\pm$ SE unless otherwise stated. Normality of distribution was checked with a Kolmogorov-Smirnov test. Statistical analyses were performed using the parametric two-tailed Student's *t*-test or nonparametric Mann-Whitney *U*-test for the comparison of two groups, and ANOVA with post hoc Tukey's corrections for the comparison of more than two groups.

Spearman's test was used for correlation analysis between the two data. A *P* value < 0.05 (two-sided) was considered significant. Statistical analysis was performed using GraphPad Prism 7.0 software (San Diego, CA).

RESULTS

Impairment of HDAC2 enhances Th2 cytokines and IL-17A expression. To determine whether HDAC2 is functionally linked to the pathogenesis of airway inflammation, WT and *Hdac2*^{+/-} mice were exposed to HDM. We observed that HDAC2 expression was significantly decreased in HDM-exposed WT mice compared with the saline control, and it was further suppressed by HDM in *Hdac2*^{+/-} mice relative to the

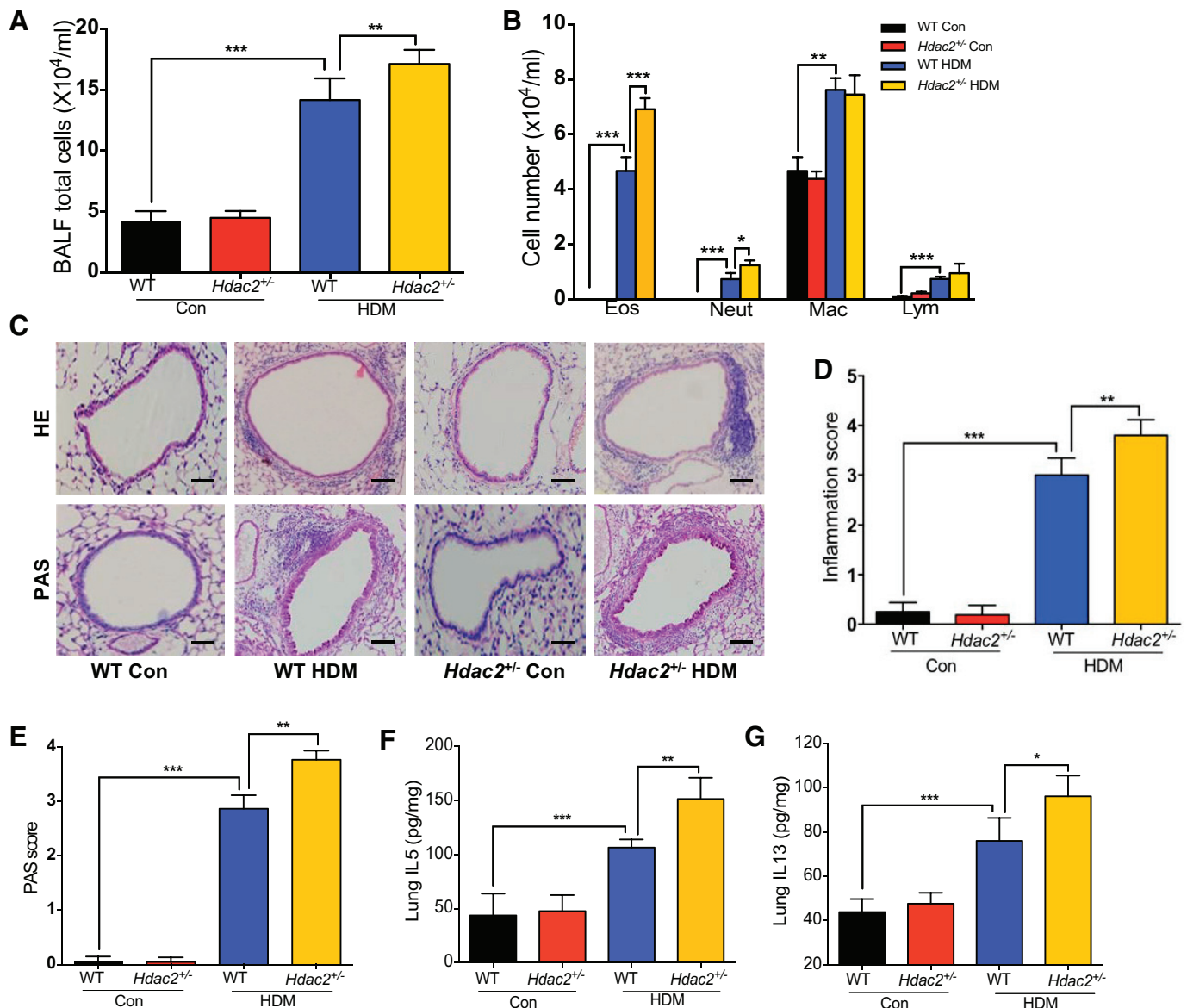


Fig. 3. Impairing of histone deacetylase-2 (HDAC2) exacerbates house dust mite (HDM)-induced airway inflammation. *A* and *B*: total cells and differential cell counts in bronchoalveolar lavage fluid (BALF). *C*: representative photomicrographs of lung inflammation expression (hematoxylin-eosin; H&E) and mucus production (periodic acid-Schiff; PAS) are shown (Scale bar, 100 μ m). *D* and *E*: semiquantification of H&E and PAS in the lungs were done using Image Pro 6.1 software. *F* and *G*: protein levels of inflammatory cytokines in lung homogenate from *Hdac2*^{+/-} and wild-type (WT) mice were determined by ELISA. Data are representative of 3 independent experiments (*n* = 5–6 mice per group per study). Results are expressed as means $\pm$ SE. One-way ANOVA with post hoc Tukey's corrections for comparison of more than 2 groups: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

HDM-exposed WT mice (Fig. 2A). We next assessed which cell types accounted for the decrease in whole lung HDAC2 signal in response to HDM. Previous studies have shown that HDAC2 is expressed by airway epithelial cells (7, 9). We also observed that HDM exposure decreased HDAC2 expression in mouse airway epithelium (Fig. 2, B–D). To identify whether HDAC2 was modulated in T cells, lung CD4⁺, CD8⁺, and $\gamma\delta$ T cells were analyzed. The entire gating strategy of the CD3⁺, CD4⁺, CD8⁺, and $\gamma\delta$ -T cells is shown in Fig. 2E. It revealed that HDAC2 was significantly decreased in the CD4⁺, CD8⁺, and $\gamma\delta$ -T cells from HDM-exposed mice compared with those from controls (Fig. 2F). Collectively, the airway epithelium cells and T cells at least partly accounted for HDAC2 reduction in HDM-treated mice.

We next assessed the ability of *Hdac2*^{+/-} mice to develop airway inflammation after exposure to HDM. We found that the total inflammatory cells and eosinophils were significantly increased in BALF from HDM-exposed *Hdac2*^{+/-} mice compared with HDM-exposed WT mice (Fig. 3, A and B). H&E-stained lung sections showed an increase in peribronchial infiltrates of inflammatory cells in *Hdac2*^{+/-} mice in response

to HDM exposure (Fig. 3, C and D). Moreover, mucus production was also significantly increased in HDM-exposed *Hdac2*^{+/-} mice relative to WT controls (Fig. 3, C and E). The inflammatory T helper 2 (Th2) cytokines such as IL-5 and IL-13 were increased in lung homogenates of HDM-exposed *Hdac2*^{+/-} mice (Fig. 3, F and G).

In addition to Th2 cytokines, IL-17A is closely involved in the pathogenesis of asthma (1, 3, 22). We next sought to determine whether HDAC2 protects against allergic airway inflammation via an IL-17A-dependent mechanism. We observed that immunolocalization of IL-17A in the lung tissues was significantly increased in HDM-exposed *Hdac2*^{+/-} mice (Fig. 4A). The IL-17A expression was further confirmed by using ELISA analysis (Fig. 4B). To identify the cellular sources of IL-17A production in this context, lung T cells were analyzed using flow cytometry. It revealed that IL-17A was predominantly expressed by CD4⁺ and $\gamma\delta$ -TCR⁺ cells in HDM-exposed *Hdac2*^{+/-} mice (Fig. 4, C–F).

It has been reported that Th17 cell differentiation and IL-17A expression are controlled transcriptionally by the retinoid-related orphan nuclear receptor- γ t (ROR γ t) (11). Ichiyama et

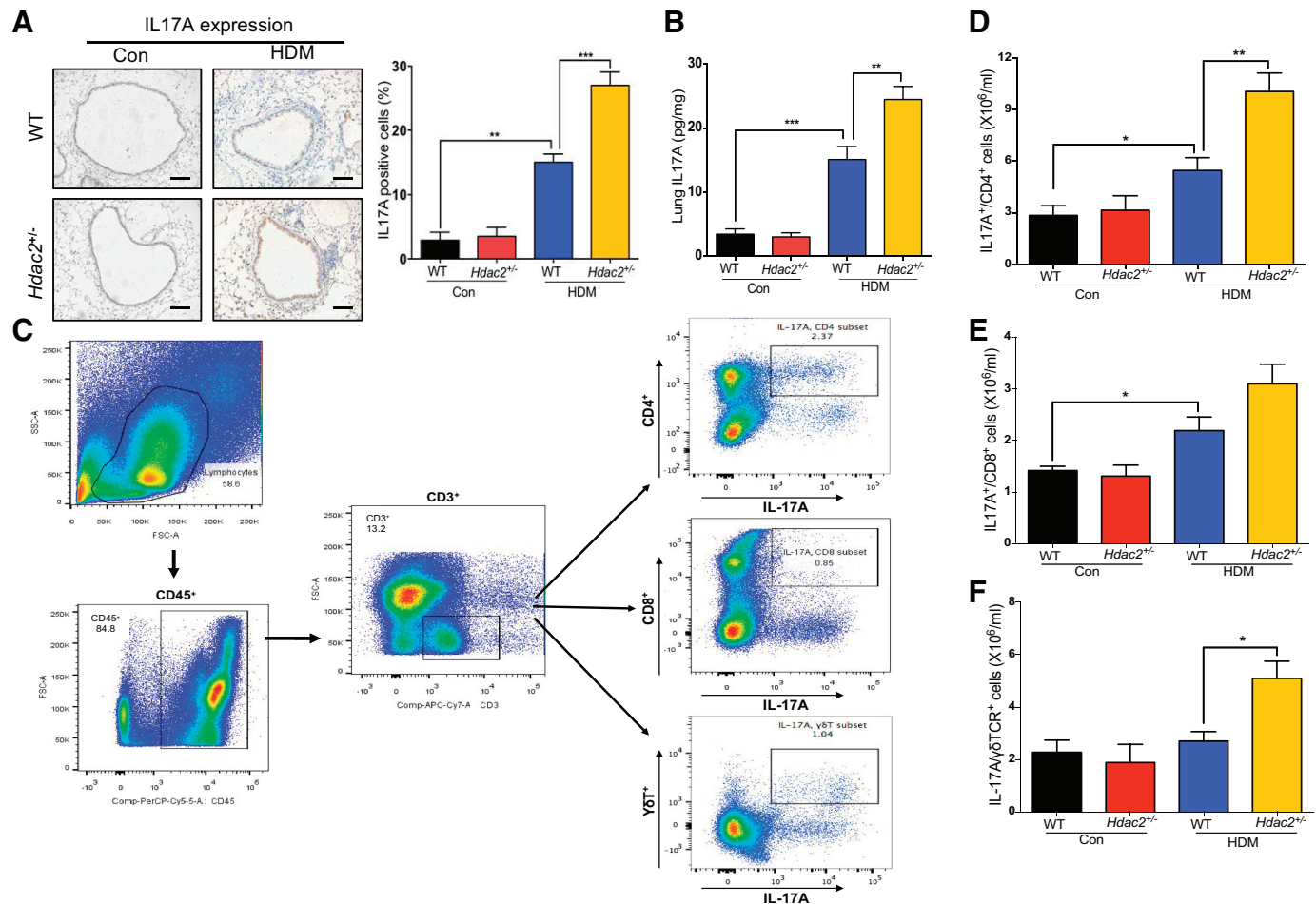


Fig. 4. Impairing of histone deacetylase-2 (HDAC2) enhanced IL-17A expression in allergic mice. A: expression of IL-17A in lung homogenate of mice was measured by immunohistochemistry (IHC) (scale bar, 100 μ m). Semiquantification of IHC in lungs was done using Image Pro 6.1 software. B: protein levels of IL-17A in lung homogenate from *Hdac2*^{+/-} and wild-type (WT) mice was determined by ELISA. C: flow cytometry was performed with Fortessa (BD Biosciences). Representative flow plots are shown for total CD3⁺ T cell subsets isolated from lungs of control (Con-) and house dust mite (HDM)-exposed mice; the entire gating strategy is shown. D–F: quantification of IL-17A⁺ cells was analyzed with FlowJo (TreeStar). Data are representative of 2 independent studies ($n = 4$ –6 mice per group per study). Results are expressed as means $\pm$ SE. One-way ANOVA with post hoc Tukey's corrections for the comparison of more than 2 groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

al. (13) have shown that ROR γ t-mediated IL-17A mRNA transcription through direct interaction with ROR γ t. We observed that ROR γ t was increased in HBE cells treated with HDAC2 siRNA (Fig. 5A). To further elucidate the specific association of HDAC2 and ROR γ t with the IL-17A promoter in airway epithelial cells, a ChIP assay was performed with an anti-ROR γ t antibody in the HDAC2 siRNA-treated cells. The data revealed that HDM caused ROR γ t to accumulate on the IL-17A promoter (Fig. 5B), and in HDAC2-siRNA treated cells, the interaction of ROR γ t with the IL-17A promoter was further increased (Fig. 5C). On the other hand, the interaction of ROR γ t with the IL-17A promoter was decreased in HDAC2-overexpressed cells (Fig. 5D).

IL-17A deficiency restores HDM-induced HDAC2 reduction and attenuates airway inflammation. We next questioned whether HDAC2 reduction in response to HDM is IL-17A dependent. WT and *Il17a*^{-/-} mice were treated with HDM, as described in METHODS. Interestingly, we observed that, although there was a significant decrease in HDAC2 expression in the lungs of HDM-exposed WT mice, HDAC2 reduction was not observed in HDM-exposed *Il17a*^{-/-} mice (Fig. 6A). Furthermore, we found that total and differential cell counts in BALF were significantly decreased in HDM-exposed *Il17a*^{-/-} mice compared with HDM-exposed WT mice (Fig. 6, B and C). HDM-exposed *Il17a*^{-/-} mice also showed reduced inflamma-

tory cell infiltration, mucus production, and inflammatory cytokine expression vs. HDM-exposed WT mice (Fig. 6, D–H).

Blocking IL-17A reverses the enhanced airway inflammation caused by HDAC2 impairment. We next sought to determine whether IL-17A mediates the enhanced airway inflammation caused by defective HDAC2. To address this, the *Hdac2*^{+/-}/*Il17a*^{-/-} mice were generated. We found that IL-17A deletion greatly diminished airway inflammation and cytokine production in HDM-exposed *Hdac2*^{+/-} mice (Fig. 6, B–H). Similar results were observed in *Hdac2*^{+/-} mice treated with anti-IL-17A antibody. We found that the administration of anti-IL-17A antibody to HDM-exposed *Hdac2*^{+/-} mice also significantly decreased the airway inflammation compared with those mice receiving isotype control antibody (Fig. 7).

Interplay between HDAC2 and IL-17A orchestrates the inflammatory response in HBE cells. Airway epithelial cells represent the first line of defense against allergens, viruses, and environmental pollutants that contribute to asthma pathogenesis. Previous studies have shown that IL-17A is expressed by airway epithelial cells (17, 18). We next investigated the underlying molecular mechanism in human bronchial epithelial (HBE) cells. Consistent with animal data, HDM-stimulated HBE cells showed a significant decrease in HDAC2 but a notable increase in IL-17A (Fig. 8, A and B). Costimulation with rhIL-17A and HDM further decreased HDAC2 expression

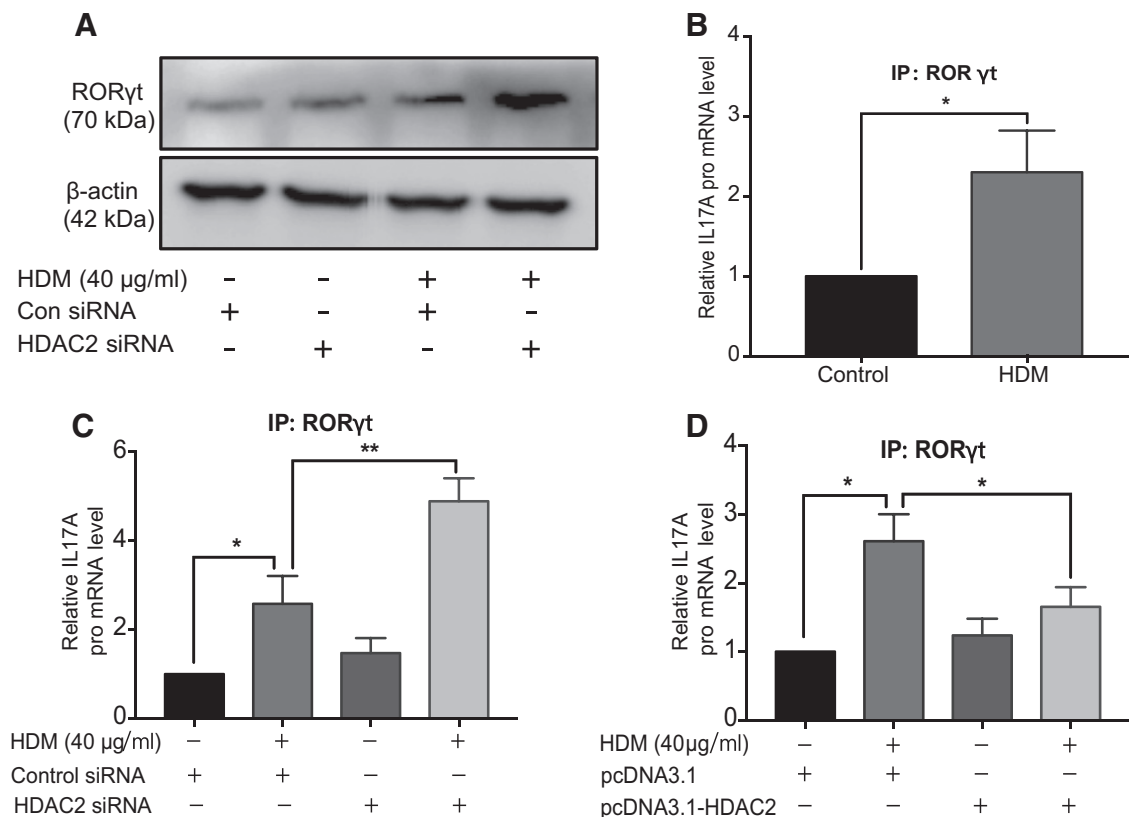


Fig. 5. Anti-retinoid-related orphan nuclear receptor- γ t (ROR γ t) is increased after histone deacetylase-2 (HDAC2) knockdown in epithelial cells. A: human bronchial epithelial (HBE) cells were transfected with HDAC2 siRNA for 24 h and then treated with house dust mites (HDM) for another 24 h. Expression of ROR γ t induced by HDM was studied using Western blot. B–D: chromatin fragments were incubated with anti-ROR γ t antibody. Eluted chromatin-ROR γ t fragments underwent qPCR detection covering the IL-17A promoter. Data are representative of 3 independent studies. Results are expressed as means $\pm$ SE. Student's *t*-test was employed for comparison for 2 groups; 1-way ANOVA with post hoc Tukey's corrections for comparison of more than 2 groups: **P* < 0.05, ***P* < 0.01.

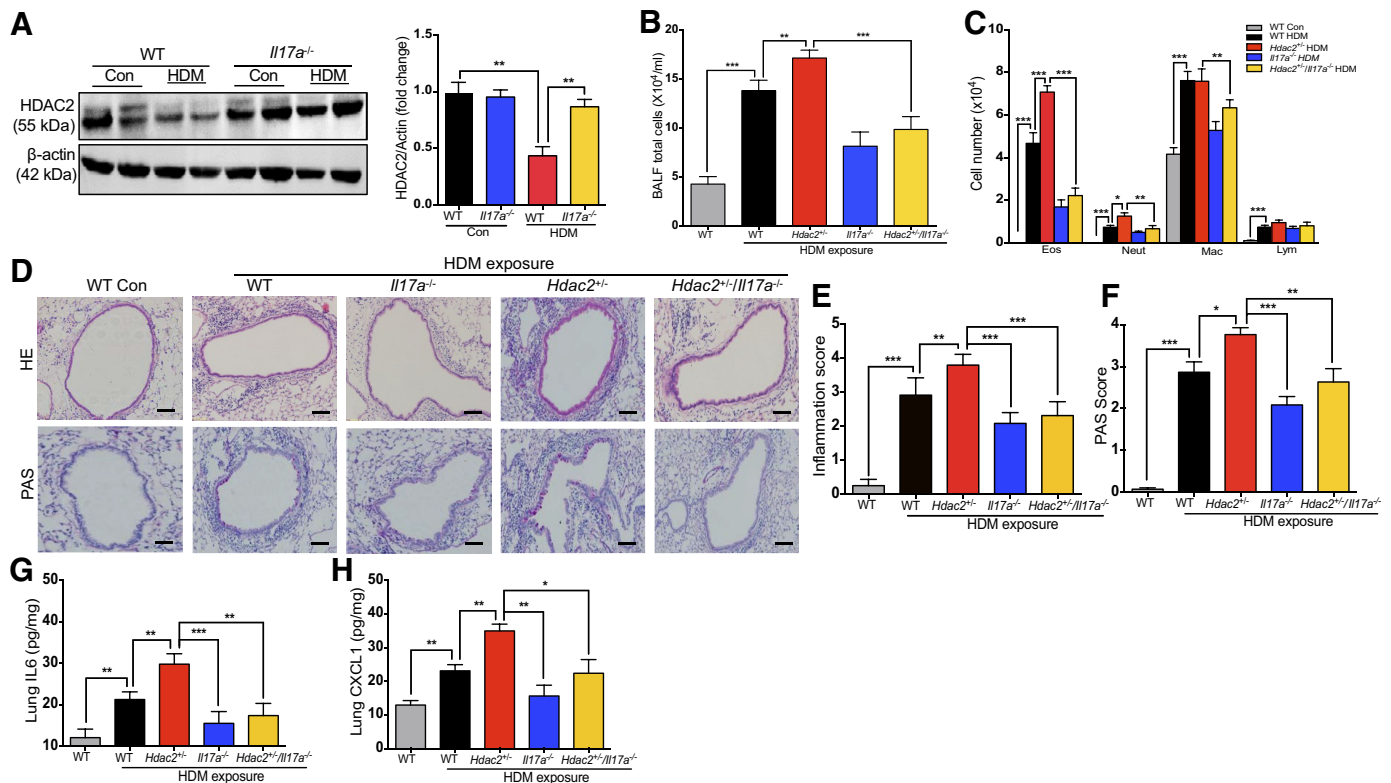


Fig. 6. *Il17a* deficiency attenuates house dust mite (HDM)-induced histone deacetylase-2 (HDAC2) reduction and airway inflammation. **A:** HDAC2 expression in lung homogenate of mice was measured by Western blot, and quantified using ImageJ software. **B** and **C:** total cells and differential cell counts in bronchoalveolar lavage fluid (BALF) from wild-type (WT), *Hdac2*^{+/-}, *Il17a*^{-/-}, and *Hdac2*^{+/-}/*Il17a*^{-/-} mice after HDM administration. **D:** representative photomicrographs of lung inflammation expression (hematoxylin-eosin; H&E) and mucus production (periodic acid-Schiff; PAS) are shown (scale bar, 100 μ m). **E** and **F:** semiquantification of H&E and PAS in lungs was done using Image Pro 6.1 software. **G** and **H:** protein levels of inflammatory cytokines in lung homogenate were measured by ELISA. Data are representative of 3 independent studies ($n = 5$ –6 mice per group per study). Results are expressed as means $\pm$ SE. One-way ANOVA with post hoc Tukey's corrections for comparison of more than 2 groups: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

(Fig. 8, C and D). Moreover, IL-17A gene silencing attenuated the HDM-induced HDAC2 reduction in HBE cells (Fig. 8E).

On the other hand, HDAC2 siRNA further increased HDM-induced IL-17A expression compared with the control cells that received scrambled siRNA (Fig. 9A). HDAC2 knockdown also significantly exacerbated HDM-induced expression of other cytokines. Moreover, rhIL-17A in conjunction with HDM resulted in a further increase in the expression of these cytokines compared with the culture without rhIL-17A (Fig. 9, B–D). However, IL-17A knockdown reduced the expression of these cytokines compared with the control cells receiving scrambled siRNA (Fig. 9, E and F). To clarify the dependence of inflammation on derepression of inflammatory pathways by the decrease in HDAC2, HDAC2 expression vector was transfected into HBE cells. We found that HDAC2 overexpression significantly reduced HDM-induced IL-17A expression and other inflammatory cytokines compared with the control cells (Fig. 10).

Collectively, these data indicate that an interplay between HDAC2 and IL-17A exists in HDM-stimulated airway epithelial cells, thereby playing a pivotal role in amplification of allergic airway inflammation.

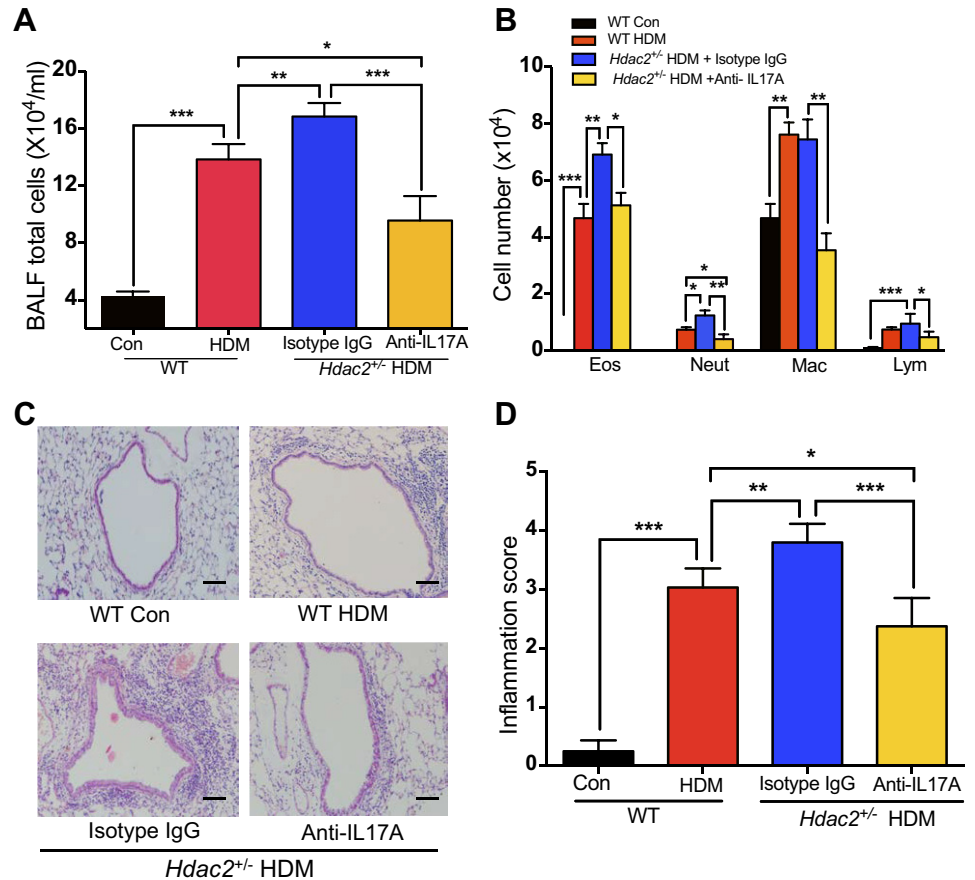
DISCUSSION

In the present study, using an experimental mouse model of asthma, *Hdac2*-impaired mice were used for the first time to

demonstrate its suppressive effect on HDM-induced airway inflammation. IL-17A deletion or anti-IL-17A antibody attenuated the exacerbated airway inflammation in HDM-exposed *Hdac2*^{+/-} mice. IL-17A expression mediated the HDAC2 reduction and served as an important mediator in the protective beneficial effect of HDAC2 on airway inflammation.

Chronic inflammation in asthma is associated with increased expression of multiple inflammatory proteins. Increased expression of these inflammatory proteins occurs mainly at the level of gene expression. HDAC2 play a critical role in regulating inflammatory genes by suppressing gene expression via reversing core histone hyperacetylation (4). However, to date, no murine models have been used to investigate the functions of HDAC2 in asthma. For the first time, we used *Hdac2*^{+/-} mice exposed to HDM to further confirm the pivotal role of HDAC2 in airway inflammation. We found that impairing *Hdac2* in mice further amplified airway inflammation and mucus production during experimental asthma and that the suppressive role of HDAC2 was most likely related to its regulation of inflammatory molecule production, including IL-17A. IL-17A plays an important role in the pathogenesis of asthma (5). ROR γ t is the critical transcription factor for orchestrating the expression of IL-17A (11, 13). We found that the expression of ROR γ t was increased in HDM-exposed HBE cells with knockdown of *Hdac2* by siRNA. HDM caused ROR γ t to accumulate on the IL-17A promoter, and in

Fig. 7. Anti-IL-17A antibody attenuates enhanced airway inflammation in histone deacetylase-2-deficient (*Hdac2*^{+/-}) mice. **A** and **B**: total bronchoalveolar lavage fluid (BALF) cells and differential cell counts were assessed. **C**: representative photomicrographs of lung inflammation expression (hematoxylin-eosin; H&E) are shown (scale bar, 100 μ m). **D**: semiquantification of H&E in lungs using Image Pro 6.1 software. Data are representative of 2 independent studies (*n* = 4–5 mice per group per study). Results are expressed as means $\pm$ SE. One-way ANOVA with post hoc Tukey's corrections for comparison of more than 2 groups: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.



HDAC2-siRNA treated cells the interaction of ROR γ t with the IL-17A promoter was further increased. These data indicate that the binding of ROR γ t to the IL-17A promoter is promoted in case of HDAC2 reduction. We further found that additional IL-17A deletion greatly improved airway inflammation, mucus production, and cytokine production in *Hdac2*^{+/-} mice when using *Hdac2*^{+/-}/*Il17a*^{-/-} mice. Administration of anti-IL-17A antibody to HDM-exposed *Hdac2*^{+/-} mice also attenuated the airway inflammation. Collectively, these data provide experimental evidence that elevated IL-17A mediates the effect of HDAC2 reduction in asthma pathogenesis.

Although previous studies demonstrated that HDAC2 expression was decreased in asthma and related to disease severity, there is a poor understanding of disease-related endogenous mediators that result in HDAC2 reduction (4, 16, 19). In the present study, we observed that HDM exposure significantly reduced HDAC2 expression in HBE cells, and costimulation with IL-17A further decreased HDAC2 expression. HDAC2 reduction was not observed in HDM-exposed *Il17a*^{-/-} mice, and pretreatment with anti-IL-17A antibody or IL-17A siRNA attenuated the HDM-induced HDAC2 reduction in HBE cells. In contrast, HDAC2 overexpression attenuated the production of cytokines. Collectively, these data suggest that IL-17A serves as a critical modulator of HDAC2 in airway epithelium in asthma pathogenesis.

Both HDAC2 and IL-17A have been implicated in steroid resistance in asthma. HDAC2 activity is reduced in patients with asthma, and the reduction in HDAC2 activity is associated

with corticosteroid resistance. Hew et al. (10) showed that HDAC activity is also reduced in peripheral blood mononuclear cells from severe asthmatic patients, and this is correlated with resistance to the anti-inflammatory effects of corticosteroids in vitro. Steroid treatment caused a significant reduction in IL-17A-induced IL-6 expression in epithelial cells obtained from normal controls but not in those from asthmatic subjects (10). Zijlstra et al. (22) showed that IL-17A induces glucocorticoid insensitivity in the airway epithelium, which is probably mediated by PI 3-kinase activation and subsequent reduction of HDAC2 activity, suggesting a possible molecular mechanism for IL-17A-induced steroid insensitivity. Evidence of increased glucocorticoid receptor (GR)- β expression in epithelial cells following IL-17A stimulation suggests a possible role for Th17-associated cytokines in the mechanism of steroid hyporesponsiveness in asthmatic subjects (1). Our current study demonstrates the paradigm of an interplay between HDAC2 and IL-17A, further suggesting that these two factors may cooperatively mediate steroid resistance in chronic airway inflammation.

In summary, our experiments in mice and human epithelial cells demonstrate that HDAC2 has a protective function in airway inflammation involving inhibition of IL-17A production. Our findings support the importance of IL-17A as a mediator in the protective effect of HDAC2 on airway inflammation in asthma and may suggest that activation of HDAC2 and/or inhibition of IL-17A production could prevent the development of allergic airway inflammation.

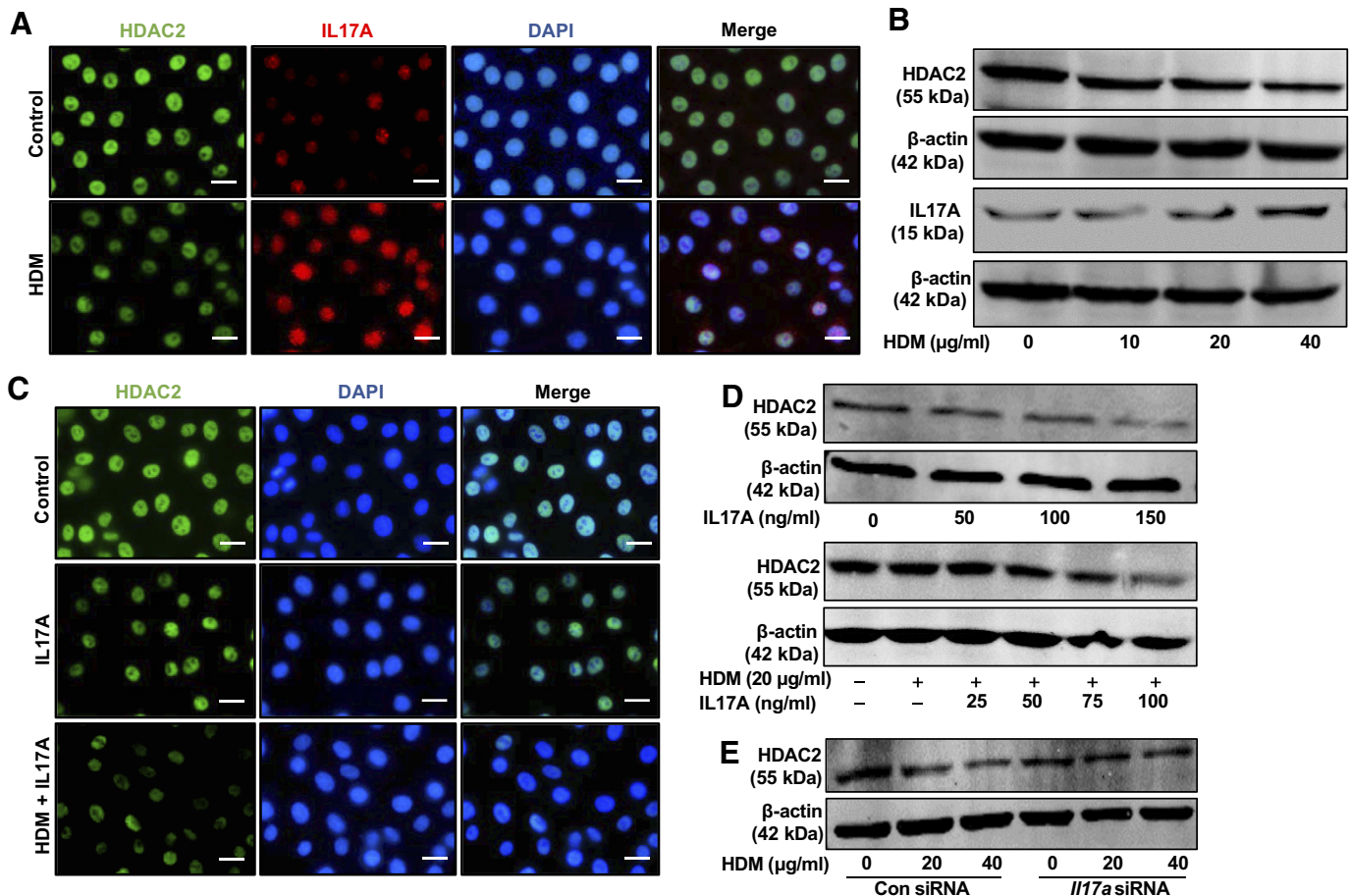


Fig. 8. IL-17A mediates house dust mite (HDM)-induced histone deacetylase-2 (HDAC2) reduction in human bronchial epithelial (HBE) cells. **A:** HDAC2 and IL-17A expressions in HDM-treated HBE cells were determined by immunofluorescence staining (scale bar, 100 μ m). **B:** HDAC2 and IL-17A expressions in HDM-treated HBE cells were determined by Western blot analysis. **C:** HDAC2 expression in HBE cells stimulated with IL-17A and HDM was determined by immunofluorescence staining (scale bar, 100 μ m). **D:** HDAC2 expression in HBE cells stimulated with IL-17A and HDM was determined by Western blot. **E:** HDAC2 expression in HDM-treated HBE cells with IL-17A siRNA was determined by Western blot. Results are expressed as means $\pm$ SE of 3 independent experiments.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

T.L., M.W., C.Z., L.C., F.X., Y. Wang, Y. Wu, N.X., and X.D. performed experiments; T.L., M.W., C.Z., L.C., F.X., Y. Wang, Y. Wu, N.X., and C.C. analyzed data; T.L., M.W., C.C., B.W., W.L., S.Y., H.S., and Z.C. interpreted results of experiments; T.L. and M.W. prepared figures; T.L., M.W., L.C., and C.C. drafted manuscript; T.L., M.W., Y. Wu, and Z.C. edited and revised manuscript; T.L., M.W., C.Z., L.C., F.X., Y. Wang, Y. Wu, N.X., C.C., X.D., B.W., W.L., S.Y., H.S., and Z.C. approved final version of manuscript; H.S. and Z.C. conceived and designed research.

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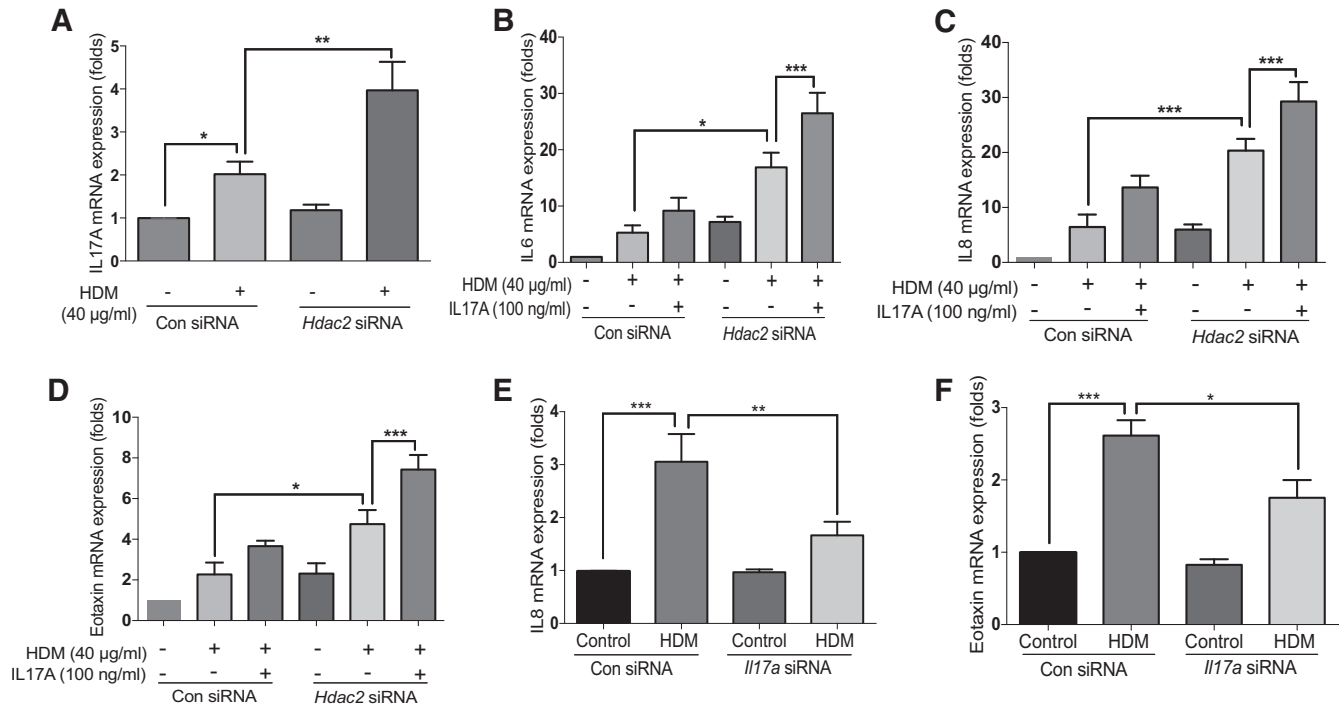


Fig. 9. Histone deacetylase-2 (*Hdac2*) deficiency exacerbates cytokine production in house dust mite (HDM)- and/or recombinant human (rh)IL-17A-treated human bronchial epithelial (HBE) cells. A–F: HBE cells were transfected with HDAC2 siRNA for 24 h and then treated with HDM and/or rhIL-17A for another 24 h. Inflammatory cytokine expression was determined by RT-PCR. Data are representative of 3 independent experiments. Results are expressed as means $\pm$ SE. One-way ANOVA with post hoc Tukey's corrections for comparison of more than 2 groups: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

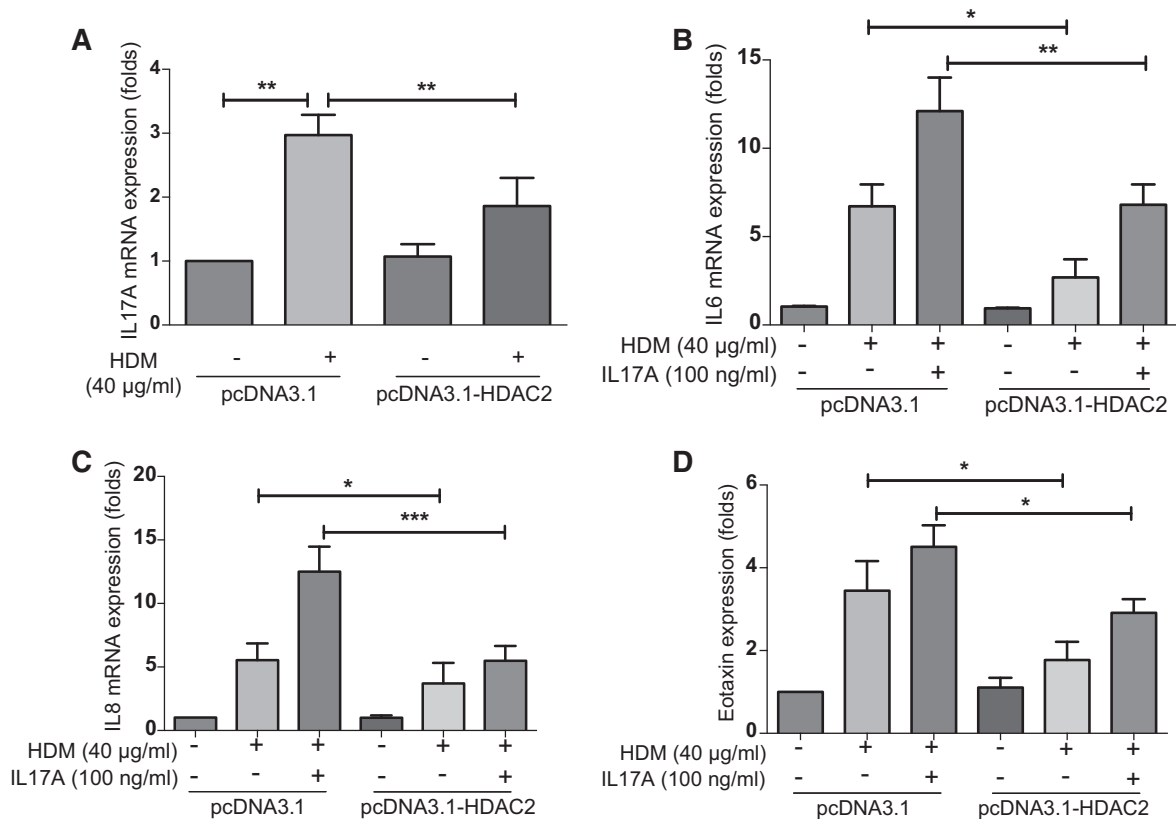


Fig. 10. Effects of histone deacetylase-2 (*Hdac2*) gene overexpression on house dust mite (HDM)-induced inflammatory cytokines. Cells were transfected with Control or HDAC2 plasmid and stimulated with HDM and/or recombinant human (rh)IL-17A. A–D: relative levels of inflammatory cytokines were assessed by qPCR. Data are representative of 3 independent experiments. Results are expressed as means $\pm$ SE. One-way ANOVA with post hoc Tukey's corrections for comparison of more than 2 groups: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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