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Conditional Deletion of Histone Deacetylase 1 in T Cells Leads to Enhanced Airway Inflammation and Increased Th2 Cytokine Production

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Chromatin modifications, such as reversible histone acetylation, play a key role in the regulation of T cell development and function. However, the role of individual histone deacetylases (HDACs) in T cells is less well understood. In this article, we show by conditional gene targeting that T cell-specific loss of HDAC1 led to an increased inflammatory response in an in vivo allergic airway inflammation model. Mice with HDAC1-deficient T cells displayed an increase in all critical parameters in this Th2-type asthma model, such as eosinophil recruitment into the lung, mucus hypersecretion, parenchymal lung inflammation, and enhanced airway resistance. This correlated with enhanced Th2 cytokine production in HDAC1-deficient T cells isolated from diseased mice. In vitro-polarized HDAC1-deficient Th2 cells showed a similar enhancement of IL-4 expression, which was evident already at day 3 of Th2 differentiation cultures and restricted to T cell subsets that underwent several rounds of cell divisions. HDAC1 was recruited to the Il4 gene locus in ex vivo isolated nonstimulated CD4+ T cells, indicating a direct control of the Il4 gene locus. Our data provide genetic evidence that HDAC1 is an essential HDAC that controls the magnitude of an inflammatory response by modulating cytokine expression in effector T cells. The Journal of Immunology, 2010, 185: 3489–3497.

During T cell development and effector differentiation, cell fate decisions are made, and cell lineage-specific gene expression patterns are established and maintained. Epigenetic mechanisms, such as histone and DNA modifications, play a crucial role in this process. For instance, reversible changes in histone acetylation patterns accompany many important processes, ranging from VDJ recombination and CD4/CD8 cell fate decision during T cell development to the induction of cytokine expression during Th1/Th2 effector differentiation (1–5).

Modification of core histones by lysine acetylation is controlled by histone acetyltransferases and histone deacetylases (HDACs), which are considered transcriptional coactivators and corepressors, respectively. Eighteen HDACs subdivided into three classes have been identified in mammalian organisms (6, 7); however, dissecting individual roles for each member of the HDAC family in specific cell lineages and tissues remains a major scientific challenge. Several mammalian deacetylases, including HDAC1, HDAC2, HDAC3, HDAC7, and HDAC9, have been implicated in different T cell functions, and the application of HDAC inhibitors revealed important immunological processes that are dependent on the activity of HDACs (8, 9). Control of regulatory T cell development and function mediated by the transcriptional repressor FoxP3 involves HDAC7 and HDAC9, and interaction of HDAC9 and FoxP3 is antagonized by TCR stimulation (10, 11). The class I deacetylases HDAC1 and HDAC2 are highly expressed in thymus and spleen, and HDAC1-associated factors, such as Ikaros, Aiolos, and Sin3A, play important roles during T cell development (12, 13). These findings suggest a potential function of these epigenetic regulators in T cell-related processes, although the precise role of HDAC1 in T cell development and function has not been determined.

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Abbreviations used in this paper: AW, airways; BAL, bronchoalveolar lavage; bsl, baseline; BV, blood vessel; CA, central airways; CHB, chromatin immunoprecipitation; CL, central lung; con, control; DF, double-positive; E, eosinophil; HDAC, histone deacetylase; HS, hypersensitivity site; imm, immunized and diseased; L, lymphocyte; M, macrophage; MB2, methylCpG-binding domain protein-2; MHC, metacholine; MU, mucous; N, neutrophil; nd, not done; n.d., not detectable; n.s., not significant; PA, peripheral airways; PL, peripheral lung; SP, single-positive; TS, trichostatin A; wt, wild-type.

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We originally identified HDAC1, the founding member of the mammalian HDAC family, as an IL-2--induced gene in a cytolytic mouse T cell line (14). Loss of HDAC1 resulted in severe development problems and impaired proliferation in mouse embryos, causing embryonic lethality before embryonic day 10.5 (15, 16). In this study, we analyzed the role of HDAC1 on T cell development and function. Conditional deletion of HDAC1 in the T cell lineage using Cd4-Cre deleter strains revealed that HDAC1 is dispensable for late T cell development and the generation of the peripheral T cell pool. However, the T cell--specific loss of HDAC1 resulted in an increase in all critical parameters in a Th2--type allergic airway inflammation model, such as eosinophil recruitment into the lung, mucus hypersecretion, and parenchymal lung inflammation. HDAC1--deficient Th2 cells maintained their characteristic cytokine profile; however, they produced increased amounts of Th2--type cytokines, which led to enhanced allergic airway inflammation. The increase in IL--4 production in HDAC1--deficient CD4+ T cells was observed at day 3 of Th2--polarizing cultures and was restricted to CD4+ T cells that underwent several rounds of cell division. HDAC1 bound to the Il4 gene locus in nonstimulated CD4+ T cells, suggesting a direct regulation of the Il4 gene locus in nonactivated Th cells. Similarly to the enhanced Th2 cytokine expression in Th2 cells, HDAC1--deficient Th1 cells produced elevated levels of IFN-γ. Together, our data indicate that HDAC1 activity is essential for the regulation of the cytokine response in Th1 and Th2 effector cells and that HDAC1 modulates the severity of immune--mediated diseases.

Materials and Methods

Mice

The generation of the conditional Hdac1 allele has been described (17). Cd4-Cre mice (18) were kindly provided by Dr. Chris Wilson (University of Washington, Seattle, WA). All mice were backcrossed onto C57BL/6 for at least five generations. Animal experiments were performed according to protocols approved by the Federal Austrian Ministry for Science and Research. The following primers were used for genotyping: A, Hdac1a-F, 5'-GGGA CTCT GCC CCA AAA AAC TAC TC-3'; B, Hdac1a-R, 5'-CAA AGA TGG GGC TGG AAG GCT AAG-3'; C, Hdac1Δ Δ, 5'-GGT ACG TCA ATG ACATCGTCCCT-3'; Dc4-Cre: CRE-FI 5'-TCT CTG TCG CTG CAA GCT TCT CCA-3'; and CRE-R1 5'-TCA AGG CCA GAC TAT GAG GCC TAT-3'.

Flow cytometric analysis and Abs

Cells were isolated and stained according to standard procedures. Cells were acquired on a FACS cautiously flow cytometer (BD Biosciences, San Jose, CA) and analyzed using Cell Quest Software. The following Abs were used: FITC--anti-B20 (clone RA3-6B2), allophycocyanin--anti-B20 (clone RA3-6B2), TC--anti-CD4 (clone CT-CD4), PE--anti-CD8 (clone CD8B9.11), PE--anti-CD80 (CT-CD8a), FITC--anti-CD3 (clone 500-A2), PE--anti-CD5 (clone 53-7.3), BIO--anti-CD80 (CT-CD8a), BIO--anti-CD11b, BIO--anti-CD11c, BIO--anti-CD45R, BIO--anti-Ly-6G (RB6-8C5), BIO--anti-Ter119, BIO--anti-NK, Alexa Fluor 647--anti-CD3 (clone 500A2), FITC--anti-CD3 (clone 145-2C11), PE--anti-CD44 (clone IM7), and Fc--block (all from BD Pharmingen, San Diego, CA), as well as PE--anti-CD25 (clone PC61.5), allophycocyanin--anti-CD62L (clone MEL-14), and Alexa Fluor 647--anti-Foxp3 (clone FJK-16a) (all from eBioscience, San Diego, CA).

T cell purification

CD4+ T cells were isolated from lymph nodes and spleens of Hdac1fl/fl and Hdac1a mice. Pooled cell suspensions were incubated with biotinylated anti-CD8a, anti-CD11b, anti-CD11c, anti-CD45R, anti-Ly-6G, anti-Ter119, and anti-NK Abs in PBS supplemented with 2% FBS. The CD4+ T cells were then purified by negative depletion using streptavidin beads (BD Pharmingen), according to the manufacturer’s instructions.

Th cell differentiation and analysis of cytokine production and cell division

Th2-polarization and cytokine measurements were previously described (19). For Th1 differentiation, 0.5 × 106 cells/well were activated with anti-CD3/CD28 and cultured in the presence of 20 U/ml IL-2, 5 ng/ml IL-12, and 1 µg/ml anti-IL-4. CD4+ T cell cultures were split 1:2 on day 3 after activation. After 6 d in culture, cells were purified over a Lymphoprep gradient and restimulated (5 × 105 cells/ml) with plate-bound anti-CD3e (0.1 µg/ml). Supernatant for cytokine quantification was collected 12 h later. IL-4, IFN-γ, IL-5, IL-10, IL-13, and IL-2 levels were determined using ELISA kits from BD Pharmingen, according to the manufacturer’s instructions.

Simultaneous analysis of proliferation and cytokine production in differentiating Th cells was essentially performed as described (20). Briefly, purified CD4+ T cells were labeled with CFSE (Molecular Probes, Eugene, OR) by incubating 1 × 105 cells/ml in PBS with 10 nM CFSE for 8 min at room temperature. The CFSE-labelling reaction was stopped by adding 5 ml FCS, CD4+ T cells were washed twice with 10 ml cell--culture medium. For Th2 polarization, 0.1 µg/ml anti-CD3e, 0.5 µg/ml anti-CD28, 1 µg/ml anti-IL-12, 3.3 µg/ml anti-IFN-γ, 10 U/ml IL-2, and 5 U/ml IL-4 were added to the cell--culture medium. At day 3 of differentiation, cells were restimulated with PMA (50 ng/ml) and ionomycin (500 ng/ml) for 4 h in the presence of GolgiStop (BD). Cells were stained with TC--anti-CD4, fixed and permeabilized with Cytofix/Cytoflow solution (BD Pharmingen), and subsequently stained with FITC--anti-IFN-γ, PE--anti-IL-4, allophycocyanin--anti-IL-2, or with the respective isotype controls. Cells were acquired on a FACS Calibur (BD Biosciences), and data were analyzed with CellQuest Pro software (BD Biosciences). Some samples were analyzed on a FACSCanto (BD Biosciences), and data were evaluated with FACSDiva software (BD Biosciences).

Immunoblot analysis

Single-cell suspensions of Hdaclfl and Hdacla mice thymocytes and splenocytes were isolated and stained with appropriate Abs, according to standard procedures. Cells were sorted using a FACS Aria (BD Biosciences). Sorted cells were washed in PBS and resuspended in lysis buffer (20 mM Tris-HCl [pH 8], 138 mM NaCl, 10 mM EDTA, 100 mM NaF, 1% Nonidet-P40, and 10% glycerol) containing protease inhibitors (Roche, Basel, Switzerland). Lysates were snap-frozen in liquid nitrogen and stored at −80°C. PAGE and immunoblot analysis were performed according to standard protocols. The following Abs were used: anti-HDAC1 (60720), anti-HDAC2 (clone 3F3), anti-HDAC3 (7030), anti-HDAC6, anti-HDAC7 (4I3), anti-HDAC8 (18G7), and anti-HDAC9 (Abcam, Cambridge, U.K.), and anti-actin (AC-74; Sigma-Aldrich, St. Louis, MO).

OVA-induced allergic asthma and ex vivo stimulation of splenocytes

Mice were sensitized with 20 µg OVA (Sigma-Aldrich) i.p. emulsified in 2 mg aluminum hydroxide in a total volume of 200 µl on days 0 and 5. One week later, mice were aerosol challenged with OVA (1% in PBS) twice daily for 2 consecutive days. Two days after the last challenge (day 15), the lungs were lavaged with 1 ml sterile PBS, bronchoalveolar lavage fluid (BAL) cells were enumerated, and cell differentials were determined. Paraffin-embedded sections were stained with H&E, periodic-acid Schiff reagent, and Luna stains. OVA-specific IgG1 was measured by standard ELISA. Plates were first incubated with OVA (10 µg/ml) overnight at 4°C and blocked with PBS--BSA (2% w/v [pH 7.4]) for 2 h before adding sera. OD readings were taken at 450 nm following incubations with anti-mouse--IgG1--biotin (SouthernBioTech, Birmingham, AL), streptavidin--HRP, and tetramethylbenzidine substrate (Sigma-Aldrich).

For the analysis of splenic CD4+ T cell responses, spleens were isolated 2 d after the last challenge (day 15). Single-cell suspensions were made, and CD8+ T cells were depleted. Approximately 5 × 106 splenocytes (that contained 106 CD4+ T cells) were restimulated with 100 µg/ml OVA. Cytokines in the supernatant were determined after 72 h by ELISA.

Determination of airway resistance and airway compliance

Airway resistance and dynamic lung compliance were measured using a Finepoint Series RC site (Buxco Research Systems, Wilmington, NC), according to the manufacturer’s guidelines. In short, mice were anesthetized with an i.p. injection of ketamine/xylazine. The trachea was exposed and cannulated with a metal tube and connected to a ventilator; the esophagus was intubated with an ethanol (20%)-filled tube. Baseline signals for resistance and dynamic compliance were recorded, and mice were exposed to aerosolized 0.9% NaCl containing increasing doses of methacholine (0.5, 5, or 10 mg/ml). Results for airway resistance and compliance are expressed relative to baseline (baseline set to 1).

Isolation and restimulation of lung T cells from diseased mice

Lungs were removed following perfusion with 15 ml ice-cold PBS via the left ventricle of the heart, and single-cell suspensions were prepared by colla-
genase (Invitrogen, Carlsbad, CA) and DNase I (Sigma-Aldrich) treatment (150 U/ml collagenase D and 50 U/ml DNase I). Digested lung tissue was gently dispensed by passage through a 40-μm pore nylon tissue strainer (BD Biosciences); the resultant single-cell suspension was treated with Pharm Lyse buffer (BD Biosciences) to remove any residual RBCs, washed twice, and dead cells were removed by a Lympholyte gradient (Cedarlane Laboratories, Hornby, ON, Canada). Lung cells (1.5 × 10⁶) were resuspended in PM1 (75 ng/ml; Sigma-Aldrich) and dithiothreitol (150 ng/ml; Sigma-Aldrich) in the presence of GolgiStop (BD Biosciences) for 5 h at 37°C. Alternatively, splenocytes from CD45.1 mice were homogenized, treated with Pharm Lyse buffer (BD Biosciences), γ-irradiated with 3000 rad, and incubated overnight at 37°C with 200 μg/ml OVA at 1.5 × 10⁶ cells/ml. Subsequently, 1.5 × 10⁶ OVA-loaded splenocytes were added to 1.5 × 10⁶ lung cells, and the mixed-cell populations were incubated for 8 h at 37°C. GolgiStop was added during the last 5 h of incubation. Lung cells stimulated with PM/α/ι/ι/ι/ι in OVA-loaded splenocytes were stained with FITC-anti–CD45.2, PE-Cy7-anti–CD44, PerCP-Cy5.5-anti–CD4, and V450-anti–CD8α (all from BD Biosciences). The cells were then fixed with 2% paraformaldehyde and permeabilized using Perm Wash buffer (BD Biosciences), according to the manufacturer’s protocol. Cells were washed with 2% FCS/PBS, and intracellular cytokine expression was detected using allophycocyanin–anti–IL-4 (BD Biosciences) or PE–anti–IL-5 and A647–anti–IL-13 (eBioscience). Data were acquired on an LSR II flow cytometer and analyzed using FlowJo software (Tree Star, Ashland, OR).

Chromatin immunoprecipitation assays

Chromatin immunoprecipitation (ChIP) was performed as previously described (21), with some modifications. Purified splenic and lymph node CD4⁺ T cells (5 × 10⁶ for a single precipitation) were resuspended in PBS and cross-linked in 1% formaldehyde for 10 min at 37°C. Cross-linking was stopped by the addition of glycine to final concentration of 125 mM; cells were washed and lysed, and chromatin was sonicated, as described (21). Chromatin was diluted 1:10 in dilution buffer, precleared, and precipitated with magnetic Dynabeads Protein A (Invitrogen) blocked with 1 mg/ml BSA in slurry, 100 μg/ml salmon sperm DNA, 500 μg/ml BSA) or with 50 μg/ml Dynabeads Protein A (Invitrogen) blocked with 1 mg/ml BSA in dilution buffer while rocking at 4°C for 2 h. The beads were washed, and chromatin-Ab complexes were eluted from the protein A-Sepharose beads (50% Triton X-100, 5 mM EDTA, 1 mM NaDodSO₄, 0.1 M NaHCO₃, and 10 mM DTT). After de-cross-linking, the beads were washed and lysed, and chromatin was sonicated, as described (21). Chromatin was diluted 1:10 in dilution buffer, preclreated, and precipitated overnight with anti-HDAC1 Ab. The next day, chromatin-Ab complexes were harvested by incubation with 30 μl protein A-Sepharose beads (50% slurry, 100 μg/ml salmon sperm DNA, 500 μg/ml BSA) or with 50 μg/ml Dynabeads Protein A (Invitrogen) blocked with 1 mg/ml BSA in dilution buffer while rocking at 4°C for 2 h. The beads were washed, and chromatin-Ab complexes were eluted from the protein A-Sepharose beads using 2% NaDodSO₄, 0.1 M NaHCO₃, and 10 mM DTT. After de-cross-linking and proteinase K digestion, DNA was extracted with phenol-chloroform, precipitated with ethanol, and dissolved in water. The abundance of distinct DNA fragments was quantified by semiquantitative PCR, and PCR products were resolved by 2% agarose gel electrophoresis. Alternatively to semiquantitative PCR, the analysis of precipitated material was done by quantitative real-time PCR using the iCycler iQ system from Bio-Rad (Hercules, CA) and 2× SensiMix SYBR and fluorescein from Peqlab (Erlangen, Germany). Primer pairs used for ChIP assays were β-actin, 5'-TTG AAA CCA GGA CTA CGG TTC 3'; 5'-CAC CGG CCT CAC CCC ATT TG-3'; amplicon 1, 5'-GCC ACA TCA TGG AC-3'; amplicon 2, 5'-ATC AGA ACA TCA CGT CHT C-3'; 5'-ATG CCA ACC TGA AGA ACT AAC-3'; amplicon 3, 5'-CTC TCC TCT TGC TTA TAA TTC-3'; 5'-CTG ATC AGG ATT CTT CGT GCA G-3'; amplicon 4, 5'-CCC CAC AAC AAT TAG TTT G-3'; 5'-CCC CAC TTG GTT ATT TAT GAC-3'; amplicon 5, 7'-GGG TGT GAA TAA GCC ATA G-3'; amplicon 6, 5'-GCC TGT GAA TAA GCC ATA G-3'; amplicon 7, 5'-GCC TGT GAA TAA GCC ATA G-3'; amplicon 8, 5'-GCC TGT GAA TAA GCC ATA G-3'; amplicon 9, 5'-GCC TGT GAA TAA GCC ATA G-3'; amplicon 10, 5'-GCC TGT GAA TAA GCC ATA G-3'; and amplicon 11, 5'-GCC TGT GAA TAA GCC ATA G-3'; amplicon 12, 5'-GCC TGT GAA TAA GCC ATA G-3'; and amplicon 13, 5'-GCC TGT GAA TAA GCC ATA G-3'; and amplicon 14, 5'-GCC TGT GAA TAA GCC ATA G-3'; and amplicon 15, 5'-GCC TGT GAA TAA GCC ATA G-3'; and amplicon 16, 5'-GCC TGT GAA TAA GCC ATA G-3'.

Results

Normal T cell development in the absence of HDAC1

To test the role of HDAC1 in T cell development and function, we took advantage of a conditional HDac1 allele that we previously published (17). In this allele (Hdac1F/F), the targeting strategy for the generation of a conditional Hdac1 allele. A, Schematic map of the endogenous Hdac1 locus (upper part, wt allele, “floxed” allele, Hdac1F/F), and the Hdac1 locus after Cre recombinase-mediated deletion of the neo cassette (lower middle part, “floxed” allele, Hdac1F/F), and the Hdac1 locus after Cre-mediated deletion of exon 6 (lower part, wt allele, “floxed” allele, Hdac1F/F), as previously shown (17). The filled arrowheads indicate loxP sites. B, PCR analysis of DNA isolated from Hdac1F/F (wt), Hdac1F/F (F), or Hdac1D/Δ (Δ) CD4⁺ T cells. The location of the genotyping primers A, B, and C is shown at the left. All three primers were used in one PCR reaction to detect the various alleles. wt, 487 bp; F, 567 bp; Δ, 535 bp. C, PCR analysis of DNA isolated from Hdac1F/F (F) and Hdac1D/Δ (Δ) double-negative, DP, and CD4 SP thymocytes.

Cre deleter mice, which initiate deletion from the double-negative 3 stage on (Fig. 1) (22). There were no apparent T cell developmental defects in the Hdac1F/F × Cd4-Cre mice (for simplicity, we refer to them as Hdac1F/F) (Fig. 2A). All of the major thymocyte subsets were present in normal numbers (Fig. 2B) and CD3, CD5, and CD69 surface marker expression on single-positive (SP) thymocytes was similar in Hdac1F/F and Hdac1D/Δ SP cells (data not shown). Immunoblot analysis revealed wild-type (wt) HDAC1 protein levels in Hdac1D/Δ double-positive (DP) cells but diminished levels in CD4SP thymocytes (Fig. 2C), suggesting that HDAC1 remains stable in DP cells and is progressively reduced during positive selection upon Cd4+Cre-mediated deletion. In peripheral wt (Hdac1D/Δ) CD4⁺ and CD8⁺ T cells, as well as Hdac1D/Δ B220⁻ B cells, HDAC1 expression is detectable, whereas Hdac1D/Δ CD4⁺ and CD8⁺ T cells did not show detectable levels of HDAC1. Furthermore, we observed upregulation of HDAC2 but not of HDAC3, HDAC6, HDAC7, or HDAC9 in Hdac1D/Δ mice (Fig. 2C). Loss of HDAC1 expression during late stages of thymocyte differentiation and in peripheral T cells did not lead to alterations in peripheral CD4⁺ and CD8⁺ T cell distribution (Fig. 2D, 2E) and numbers (Fig. 2F). In addition, there was no difference in the distribution of naive (CD4⁺CD62L⁺) and effector/memory (CD4⁺CD69⁺ CD44⁺ CD62L⁻) CD4⁺ T cell subsets (Fig. 2G), and there were normal numbers of FoxP3⁺CD4⁺CD25⁺ regulatory T cells (Fig. 2H).

Increased allergic airway inflammation and enhanced Th2 cytokine production in the absence of HDAC1

HDACs have been implicated in asthma and allergic diseases (23, 24). The HDAC inhibitor trichostatin A (TSA) and the class III HDAC inhibitor sirtinol attenuate airway inflammation in a murine model of allergic asthma (25, 26) which displays several features of a Th2-type reaction and characteristic IL-5–dependent infiltration of eosinophils into the lung (27). To analyze whether HDAC1
The numbers indicate the percentage of cells in the indicated regions.

(BAL) were increased in and mucus production. Total inflammatory cells in the airways were 13 (28). Two days later, mice were analyzed for lung inflammation response.

whether loss of HDAC1 influences the extent of the inflammatory activity in T cells regulates allergic airway inflammation, we tested whether loss of HDAC1 influences the extent of the inflammatory response. 

Two days later, mice were analyzed for lung inflammation and mucus production. Total inflammatory cells in the airways (BAL) were increased in 

mucus hypersecretion (Fig. 3C, 3D) and parenchymal lung inflammatory infiltrates containing eosinophils (Fig. 3E).

Moreover, the analysis of serum OVA-specific IgG1 levels revealed Ag specificity (Fig. 3F). Moreover, 

FIGURE 2. Conditional deletion of HDAC1 using transgenic Cd4-Cre does not affect thymocyte development or the generation of peripheral T cells. A, CD4/CD8 expression on thymocytes from 

Hdac1F mice. Whole-cell lysates from 3 mice were used for each population. 

Data are representative of three mice analyzed. 

FIGURE 3. Enhanced allergic airway disease in HDAC1-deficient mice. 

Hdac1F and 

Hdac1F mice were primed i.p. twice with OVA, followed by OVA aerosol challenges (OVA; n = 5). Untreated 

Hdac1F and 

Hdac1F mice were used as control (con; n = 3). A, BAL differential cell count. Mean ± SEM are shown. B, Eosinophil numbers at specified areas of lung parenchyma. Luna-stained lung tissue sections were counted with a 100× oil-immersion objective. C, Representative photomicrographs of lung sections. Arrows indicate fuchsia-stained mucus in epithelial goblet cells. Scale bar, 50 μm; original magnification ×400. D, Grading of mucus production. The distribution of goblet cells in the central and peripheral airways was scored on periodic-acid Schiff reagent-stained sections. Grade 0, no goblet cells; grade 1, >20%; grade 2, 20–40%; grade 3, 41–60%; grade 4, 61–80%; and grade 5; >80% goblet cells. E, Representative photomicrographs of H&E-stained lung tissue sections. Areas of highest-density inflammation are indicated. Scale bar, 200 μm; original magnification ×400. F, Representative photomicrographs of 

Hdac1F mice showed enhanced
airway resistance (Fig. 3G) and reduced airway compliance (Fig. 3H) compared with Hdac1F mice. Together, these data suggest a negative regulatory role for HDAC1 in the generation of airway inflammation.

To characterize the CD4+ T cell response of diseased mice, lung T cells were isolated 48 h after the last OVA aerosol challenge. The T cells were restimulated with OVA-pulsed irradiated splenic APCs or with PMA/ionomycin. Under both conditions, enhanced Th2 cytokine production of lung CD4+ T cells in the absence of HDAC1 was observed (Fig. 4A–C), although we were able to detect IL-4 expression only upon PMA/ionomycin restimulation (Fig. 4C). Enhanced IL-4 and IL-5 cytokine production was also detected when CD8+ T cell-depleted splenocytes isolated from diseased Hdac1F and Hdac1Δ mice were restimulated with OVA (Fig. 4D), whereas IL-13 levels were similar (Fig. 4D). No IFN-γ production above control levels was detected in splenocytes or Ag-restimulated lung CD4+ T cells of diseased Hdac1F and Hdac1Δ mice (data not shown). Because Th2 cytokines are clearly linked with the induction and maintenance of airway inflammation (29), these data suggest that enhanced Th2 cytokine production is responsible for enhanced airway inflammation in Hdac1Δ mice.

HDAC1-deficient in vitro-polarized Th1 and Th2 T cells produce increased amounts of cytokines

The establishment and maintenance of the cytokine-expression pattern during Th effector differentiation is regulated at the epigenetic level and is accompanied by an increase in a Th1 and Th2 lineage-specific histone acetylation pattern at the Ifng and Il4 gene loci, respectively (30, 31). To investigate whether enhanced cytokine levels can also be detected upon in vitro activation of HDAC1-deficient T cells, peripheral CD4+ T cells were isolated from Hdac1F and Hdac1Δ mice and polarized into Th2 lineages. The analysis of the cytokine-expression profile revealed that Hdac1Δ Th2 cells still expressed IL-4 but not IFN-γ (Fig. 5A), thus indicating that the helper lineage-specific cytokine-expression pattern is maintained in the absence of HDAC1. However, the levels of IL-4, IL-5, IL-10, and IL-13 in Th2 cultures were higher in Hdac1Δ Th2 cells compared with Hdac1F cells (Fig. 5A), in agreement with the enhanced Th2 cytokine levels observed in diseased Hdac1Δ mice (Fig. 4). Similarly, Hdac1Δ Th1 cultures produced enhanced levels of IFN-γ and maintained a Th1-type cytokine profile (Fig. 5B). This indicates an essential role for HDAC1 in controlling expression levels of Th lineage-specific cytokines.

![Figure 4](http://www.jimmunol.org/)

**FIGURE 4.** Enhanced Th2 cytokine production in diseased Hdac1Δ mice. A, Lung cells from imm or nonimmunized con Hdac1F and Hdac1Δ mice (CD45.2+) were isolated and stimulated for 5 h with PMA/ionomycin as described in **Materials and Methods.** Dot plots show representative CD44 versus intracellular IL-5 (**upper panel**) or IL-13 (**lower panel**) expression on gated CD45.2+ cells. Numbers in the quadrants indicate the percentage of cells in the respective quadrant. B, Summary of all individual CD4+CD45.2+ lung cells analyzed as described in A. Experiments were performed in two independent batches with at least three mice per experiment. Each dot represents a mouse. The p values were calculated using an unpaired Student t test. C, Lung cells from imm or nonimmunized Hdac1F and Hdac1Δ mice were isolated and stimulated for 5 h with PMA/ionomycin as described in **Materials and Methods.** Dot plots show CD44 versus intracellular IL-4 (**upper panel**), IL-5 (**middle panel**), or IL-13 (**lower panel**) expression on CD4+ CD45.2+ cells. Each dot plot shows the pool of lung cells isolated from three mice (lung cells from mice were pooled prior to activation and staining). One representative experiment of two is shown. D, IL-4, IL-5, and IL-13 cytokine production of OVA-restimulated CD8+ T cell-depleted splenocytes from imm or nonimmunized con Hdac1F and Hdac1Δ mice. Cytokine levels in the supernatant were determined 72 h after restimulation. Mean ± SEM are shown. Data are the summary of three (for IL-4 and IL-5) or two (for IL-13) independent experiments with at least four mice per experiment for diseased mice (n = 13 for IL-4 and IL-5; n = 9 for IL-13) and at least two mice per experiment for control mice (n = 7 for IL-4 and IL-5; n = 5 for IL-13). *p* < 0.05, unpaired Student t test. Δ, Hdac1Δ; con, control; F, Hdac1F; imm, immunized and diseased; n.s., not significant.
To determine the kinetics of cytokine production in the absence of HDAC1, CD4+ T cells were isolated from Hdac1F and Hdac1D mice, labeled with CFSE, and stimulated with anti-CD3 plus anti-CD28 under Th2-polarizing conditions for 3 d. Under these conditions, only a small subset of wt (Hdac1F) CD4+ T cells started to produce IL-4, whereas there was a 3–4-fold increase in the percentage of IL-4–producing cells within the Hdac1D CD4+ T cell population (Fig. 6A, upper panels). In contrast, IL-2 levels were similar between Hdac1F and Hdac1D T cells (Fig. 6A, 6B, middle panels). Thus, our data indicate that HDAC1 might be the major HDAC regulating the epigenetic status of the activated IL-4 locus to ensure appropriate cytokine levels. In line with previous data (20, 32) we also observed that IL-4 production was restricted to cells that underwent several rounds of cell divisions (Fig. 6A, 6B, upper panels). This may imply that cell proliferation is required to remove other repressive epigenetic marks, such as DNA methylation, from...
the Th2 cytokine loci (5). In addition to the enhanced IL-4 production, Hdac1ΔT cells activated under Th2-polarizing conditions displayed increased proliferation, because the percentage of Hdac1ΔT CD4+ T cells that underwent more than four divisions was greater compared with Hdac1WT CD4+ T cells (Fig. 6A, 6B, lower panels). Similar to enhanced IL-4 expression in Hdac1ΔT Th2-polarizing cultures, we observed enhanced IFN-γ expression in Hdac1ΔT Th1-polarizing cultures (data not shown).

**HDAC1 is recruited to the Th2 cytokine locus**

As described above, conditional deletion of HDAC1 in the T cell lineage resulted in increased levels of Th1- and Th2-type cytokines in differentiating and in polarized effector T cells. This suggests that HDAC1 limits the extent of cytokine production upon activation of T cells. HDAC1 is part of multiprotein repressive chromatin remodeling complexes that are recruited to target genes (6). Thus, the lack of HDAC1 activity might impair the repressive activity of such complexes. To determine whether HDAC1-containing remodeling complexes bind to the Il4 gene locus, ChIP assays with non-stimulated wt (Hdac1WT) CD4+ T cells and as a control with Hdac1ΔT CD4+ T cells were performed. Several known DNase I hypersensitivity sites (HSs) at the Il4 gene locus (33, 34) were tested as potential candidate target regions. This led to the identification of multiple HDAC1 recruitment sites at the Il4 gene locus and the surrounding cytokine genes in Hdac1ΔT CD4+ T cells (Fig. 7), suggesting a direct control of the Il4 gene locus by HDAC1.

**Discussion**

Reversible changes in histone acetylation patterns accompany many important processes in T cells. Eighteen HDACs have been identified in mammalian organisms (6, 7), and it remains a major scientific challenge to dissect the individual roles for each member of the HDAC family in specific cell lineages and tissues. In this study, we used conditional gene targeting to investigate the role of HDAC1 in T cells. We observed that HDAC1 deletion in T cells enhances allergic airway inflammation by modulating cytokine production in Th1/Th2 effector T cells. Thus, our study indicates a crucial negative regulatory role for HDAC1 in T cells.

One important finding of our study was that T cell-specific loss of HDAC1 leads to enhanced allergic airway inflammation. HDACs were implicated previously in lung inflammation (23). Patients suffering from chronic obstructive pulmonary disease display reduced HDAC activity and show decreased HDAC2 expression levels in peripheral lung and alveolar macrophages (35). Reduced HDAC activities and HDAC2 expression were also observed in bronchial biopsies of asthmatic patients (36). Anti-inflammatory drugs, such as glucocorticoids, reduce inflammation via glucocorticoid receptor-mediated recruitment of HDAC2 and the downregulation of target genes (37). Thus, reduced expression of HDAC2 in certain lung diseases may increase inflammatory gene expression. In contrast, treatment of patients with the anti-inflammatory drug theophylline increases HDAC activity and, therefore, decreases the inflammatory response in asthma (38). Our data provide genetic evidence that HDAC1 regulates the extent of the inflammatory response by modulating T cell function. Thus, HDAC1 and HDAC2 may negatively regulate lung disease, although they may act in different cell subsets. Given that HDAC2 is upregulated in HDAC1-deficient T cells, it is possible that the entire impact of HDAC1 loss on airway inflammation is partially masked by increased HDAC2 levels. A partial redundancy of HDAC1 and HDAC2 functions was observed for the control of cardiac morphogenesis (16), adipogenesis (39), neuronal development (40), cell proliferation (17), and transcription (41). Whether HDAC2 activity is also important in T cells in controlling the inflammatory response will be an important aspect of future studies. However, the HDAC inhibitor TSA attenuates allergic airway inflammation in mice by reducing T cell infiltration and Th2 cytokine production (25). In addition, inhibition of the class III HDAC sirtuin 1 leads to attenuated allergic airway inflammation (26). Thus, other TSA-sensitive and -insensitive members of the HDAC family that may be expressed in T cells, as well as in other cell types (e.g., alveolar macrophages), are involved in the pathophysiology of allergic airway inflammation.

**FIGURE 7.** HDAC1 binds to several cis-regulatory sites at the Th2 gene complex in nonstimulated CD4+ T cells. A. **Upper panel,** Map of the Il4 and Il13 gene loci indicating the location of exons, the identified DNase I HSs, and the neighboring Kif3a and Rad50 genes (45). Horizontal arrows above the genes indicate the transcriptional orientation. **Lower panel,** Semi-quantitative PCR analysis of chromatin immunoprecipitated with an HDAC1 Ab from sorted primary F and Δ CD4+ T cells. The location of the 11 amplicons within known HSs spanning the Il4 and Il13 genes are indicated by the lines. The precipitation is compared with the 1:80 dilution of input DNA. One representative PCR amplification (from experiment 1) of three independent ChIP assays is shown, and the summary for all experiments is shown below. Boxed amplicons indicate similar results observed in all three ChIP experiments. B. Quantitative real-time PCR analysis of chromatin immunoprecipitated with an HDAC1 Ab from sorted primary Hdac1WT (F) and Hdac1ΔT (Δ) CD4+ T cells. The location of the 11 amplicons is shown in A. Data represent the summary of two independent experiments. +, binding; −, no binding; Δ, Hdac1ΔT; F, Hdac1WT; nd, not done.
The differentiation of CD4+ T cells into Th1 or Th2 effector cells is a complex process that is accompanied by several epigenetic changes at the Ifng and Il4 cytokine loci (3). TCR/CD28-mediated triggering of CD4+ T cells leads to a rapid increase in histone acetylation at the Ifng and Il4 cytokine loci and low-level, unbiased transcription of IL-4 and IFN-γ (30, 31). Subsequently, cytokine signaling and the activities of the transcriptional regulators T-bet and GATA-3 are required to increase and maintain lineage-specific epigenetic modification at the Ifng and Il4 loci, respectively, to allow high-level lineage-specific Th1 and Th2 cytokine expression. In parallel, histone acetylation of the lineage-inappropriate cytokine loci decreases in the presence of the polarizing cytokines, leading, together with de novo DNA methylation, to complete transcriptional silencing (3, 5). Therefore, the increase in lineage-specific cytokine expression in lung CD4+ T cells in Hdac1−/− mice and in HDAC1-deficient Th1 and Th2 cells indicates an essential role for HDAC1 in controlling cytokine-expression levels in differentiated effector T cells. Our finding is substantiated by a recent study showing a genome-wide association of HDAC1 with promoters of active genes in CD4+ T cells, suggesting that HDAC1 fine-tunes gene expression instead of keeping silent genes repressed (42). We observed increased expression of IL-4 already at day 3 in Th2-polarizing cultures. Thus, the demonstration that HDAC1 bound to the Il4 gene locus in nonactivated naive CD4+ T cells suggests a critical role for HDAC1 during the early phase of effector differentiation in restricting cytokine transcription to low levels. In support of our data, it was shown that treatment of differentiating T cells with the HDAC inhibitor Trichostatin A induced Th1 and reduced Th2 cytokine responses. BMC Cancer 3: 30.

Moreover, our data show that activation of the Th1 and Th2 cytokine locus triggers lineage-inappropriate cytokine expression. This suggests a functional hierarchy of HDAC1 and MB2 in the regulation of the Th2-cytokine loci. Taken together, our study provides genetic evidence that HDAC1 activity in T cells regulates the extent of allergic airway inflammation. Moreover, our data show that activation of the Th1 and Th2 cytokine loci is counterbalanced by HDAC1, indicating a specific role for HDAC1 in modulating the cytokine-expression program during effector differentiation. This may indicate a potential therapeutic usability of specific HDAC1 inhibitors in clinical settings that require enhanced immune responses and inflammation.

Disclosures

The authors have no financial conflicts of interest.

References


