Histone Deacetylase Sirtuin 1 Deacetylates IRF1 Protein and Programs Dendritic Cells to Control Th17 Protein Differentiation during Autoimmune Inflammation*

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Background: The roles of Sirt1 in regulating dendritic functions are not known.

Results: Sirt1 deacetylates the IRF1 transcription factor to suppress IL-27 expression in dendritic cells, leading to elevated Th17 differentiation for inflammatory disease development.

Conclusion: Sirt1 programs DC functions to promote Th17 differentiation and inflammation.

Significance: This study defines a previously unappreciated inflammatory role of Sirt1 in dendritic cells.

The type III histone deacetylase Sirt1 has recently emerged as a critical immune regulator by suppressing T cell immunity and macrophage activation during inflammation, but its role in dendritic cells (DCs) remains unknown. Here, we show that mice with genetic Sirt1 deletion specifically in DCs are resistant to MOG-induced experimental autoimmune encephalomyelitis. Loss of Sirt1 functions in DCs enhances their ability to produce IL-27 and interferon β (IFN-β). Co-cultivation of Sirt1-null DCs with CD4⁺ T cells inhibited Th17 differentiation, which is reversed by anti-IL27 and anti-IFN-β neutralization antibodies. Sirt1 antagonizes acetylation of IRF1, a transcription factor that drives IL-27 production. Genetic deletion of IRF1 in Sirt1-null DCs abolishes IL-27 production and suppresses Th17 differentiation. Our results show that the histone deacetylase Sirt1 programs DCs to regulate Th17 differentiation during inflammation.

The silent information regulator 2 (SIR2) gene was first discovered in yeast as a transcriptional repressor (1). Its mammalian orthologs, the sirtuins, are a family comprising seven members, Sirt1 to Sirt7. The sirtuins function as NAD-dependent histone deacetylases, removing acetyl groups on histones and transcription factors to inhibit gene transcription, and act in an antagonistic fashion to the histone acetyltransferases (2). Sirt1 in particular is reported to be involved in various biological processes, such as aging, metabolism, and development (3, 4). We and others have demonstrated Sirt1 as a critical suppressor of T cell immunity and macrophage activation by suppressing the transcription factors NFκB and AP-1 (5–8). More recently, we discovered that Sirt1 is required for T cell immune tolerance, and IL-2 reverses T cell tolerance by down-regulating Sirt1 gene expression (8). Therefore, loss of Sirt1 functions results in the elevated T cell activation and a lupus-like autoimmune phenotype in mice (9).

IL-27 is a heterodimer consisting of the Epstein-Barr-induced gene 3 (EBI3) and p28, both of which are secreted by antigen-presenting cells, in particular DCs,² upon toll-like receptor (TLR) stimulation. IL-27 inhibits CD4⁺ T cell differentiation into Th1, Th2, and Th17, but promotes the IL-10-producing type I regulatory T cells (Tr1) (10–13). Because of its anti-inflammatory properties, especially inhibition of IL-17 expression, IL-27 could be a potential therapeutic agent against autoimmune disorders. However, studies also show IL-27 pro-inflammatory functions in colitis (25), suggesting that IL-27 suppression is beneficial for certain types of inflammatory diseases.

In this study, we show that Sirt1 functions as a negative regulator of IL-27 and IFN-β expression. At the molecular level, Sirt1 interacts with IRF1 and inhibits IRF1 acetylation to suppress IRF1 binding to the IL-27p28 promoter in DCs upon TLR stimulation. Because both IL-27 and IFN-β are anti-inflammatory cytokines by suppressing Th17 differentiation, DC-specific Sirt1 gene deletion protects mice from MOG-induced EAE, an experimental model of human autoimmune inflammatory disease, multiple sclerosis.

**EXPERIMENTAL PROCEDURES**

*Mice—Sirt1 gene floxed mice, IRF1 knock-out mice (14), CD11c-Cre transgenic mice (15), and OT-II TCR transgenic mice were purchased from The Jackson Laboratory. DC-specific Sirt1-null mice were generated by breeding Sirt1 floxed mice with CD11c-Cre transgenic mice. All mice used in this study were maintained and used at the Northwestern University mouse facility under pathogen-free conditions according to institutional guidelines and animal study proposals approved by the institutional animal care and use committees.

²The abbreviations used are: DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; TLR, toll-like receptor; BMDC, bone marrow-derived DC; Ab, antibody; MEF, mouse embryonic fibroblast; myelin oligodendrocyte glycoprotein; PMA, phorbol 12-myristate 13-acetate.

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Cell Lines, Antibodies, and Reagents—Human embryonic kidney (HEK) 293 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen). The medium was supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 200 μg/ml streptomycin, and 0.25 μg/ml amphotericin B. Polyclonal antibodies against the epitope tags (HA and Myc) and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Fluorescence-labeled Abs used for the flow cytometry analysis in this study, including CD11c, CD11b, CD4, CD8, CD45, F4/80, MHC I and II, CD80, CD86, IL-17, and IFN-γ, were purchased from eBioscience (San Diego). Abs used for ELISA, including IL-17, IL-2, and IFN-γ, were purchased from Biologend (San Diego).

Bone Marrow-derived DC Cultivation and Activation—Bone marrow cells were isolated from leg bones of 8–10-week-old mice and were cultured in RPMI 1640 medium containing 10% FCS and GM-CSF (20 ng/ml, Biologend). Cell cultures were fed on days 3, 6, and 8 and used on days 9 or 10. To isolate pure DCs, cells were purified by CD11c microbeads (Miltenyi Biotec) and stimulated with each TLR agonists, including LPS (Sigma, St. Louis, MO), for 24 h. Cells were then washed and visualized with an enhanced chemiluminescence detection system (ECL; Amersham Biosciences). When necessary, membranes were stripped by incubation in stripping buffer (Bio-Rad), washed, and then reprobed with other antibodies as indicated.

Chromatin Immunoprecipitation (ChIP)—ChIP assay were performed as described (19). Briefly, wild type and Sirt1-null DCs were stimulated with LPS (1 μg/ml) for 24 h. Cells were then washed with 1% formaldehyde, lysed, and sonicated for 15 min. 5% of the cell lysate was used to determine the total amount of target DNA in the input. The remaining cell lysates were diluted in ChIP dilution buffer. Immunoprecipitation was performed with 4 μg of polyclonal anti-IRF1 antibodies at 4 °C overnight. Immune complexes were then mixed with salmon sperm DNA/protein agarose, 50% slurry 4 °C for 1 h. Immunoprecipitates were then washed sequentially with low salt buffer, high salt buffer, LiCl wash buffer, and TE buffer. DNA-protein complexes were eluted with elution buffer, and cross-linking was reversed. Genomic DNA was extracted using phenol/chloroform, and ethanol-precipitated DNA was resuspended in Tris/EDTA buffer. PCR was performed with the following specific primers for mouse Il-27p28 or Ebi3 promoters: Il-27p28, 5′-GGGAGGGGTGGCACTGGGT-3′ and 5′-GGGAGACCGATGGGGGTGCGC-3′; Il-27Ebi3, 5′-CTGATGACCTTTTGGAAGGGCA-3′ and 5′-GCTGTGTGATGTGTTGGAGGCA-3′.

Luciferase Assay—Experiments were performed as described (20). Briefly, HEK293 cells in 12-well plates were transfected with pRL-TK (Promega, Madison, WI), and each of Sirt1 luc plasmids, along with EGR2, EGR3, or FoxO3a expression plasmids or both as indicated. Transfected cells were lysed 2 days after transfection. The luciferase activities in the cell lysates were analyzed using a Dual-Luciferase reporter assay kit (Promega, Madison, WI). Luciferase activity was measured using a luminometer (Turner BioSystems, Inc., Sunnyvale, CA) and expressed in relative light units.

EAE Induction—Six- to 8-week-old C57/Bl6 mice were immunized with MOG(35–55) peptide (emulsified with complete Freund’s adjuvant (200 μg per mouse)). Mice were also given pertussis toxin (200 ng per mouse) on day 0 and day 2 via tail vein injection. All mice were weighed and examined daily for clinical symptoms and assigned scores on a scale of 0–5 as follows: 0, no overt signs of disease; 1, limp tail; 2, limp tail and partial hindlimb paralysis; 3, complete hindlimb paralysis; 4, complete hindlimb and partial forelimb paralysis; 5, moribund state or death.

Immunohistochemistry and Isolation of Infiltrated Lymphocytes from Brain and Spinal Cord—The immunohistological analysis was performed as described (21). Briefly, brain tissue sections from MOG-immunized mice were immunostained with anti-CD45 (Biologend) RORγt (ab78007, Abcam, Cambridge, MA) followed by the secondary Ab labeled by fluorescence with Alexa 594 and Alexa 488, respectively. Tissue sections were visualized with a fluorescence microscopy (Nikon), and representative images are shown.

Following extensive transcardial perfusion with PBS, spinal cords and brains were removed from mice (tissues from three mice were pooled for each experiment) and pooled for mononuclear cell isolation. Briefly, tissues were incubated with collagenase D (300 μg/ml) and DNase I (20 μg/ml) in Hanks’ buffered saline solution. After 45 min at 37 °C, tissues were
mechanically dissociated through a 40-μm strainer and washed with PBS. The resultant pellet was fractionated on a discontinuous Percoll gradient. Infiltrating mononuclear cells were harvested from the interface, washed, counted, and cultured for 4 h in RPMI 1640 medium containing 10% FCS with PMA (10 ng/ml) and ionomycin (500 ng/ml) in the presence of brefeldin A (10 μg/ml) for flow cytometry.

In Vitro Th17 Differentiation—Wild type and Sirt1-null bone marrow-derived DCs (2 × 10^6) were plated into 96-well plate and activated with LPS (100 ng/ml) for 16 h as described (22). After washing DCs with PBS twice, CD4^+ T cells (2 × 10^5) were added and activated with anti-CD3 (1 μg/ml), recombinant human TGFβ (2 ng/ml; Biolegend), recombinant mouse IL-6 (20 ng/ml, Biolegend), anti-IFN-γ, and anti-IL-4 (10 μg/ml, Biolegend). For IL-27 and IFN-β neutralization assay, anti-IL-27 and anti-IFN-β (10 μg/ml each, R&D Systems) were added to the culture. Five days after incubation, cells were cultivated with PMA/ionomycin for an additional 4 h in the presence of brefeldin A (10 μg/ml). T cells were collected, and the levels of IL-17 and IFN-γ were determined by intracellular staining.

RESULTS

Sirt1 Inhibits TLR-induced IL-27 and IFN-β Expression in DCs—Our recent studies discovered that Sirt1 plays important roles in T cell immune tolerance (8). To study the possible functions of Sirt1 in dendritic cells, we analyzed the expression levels of the Sirt1 gene in DCs upon TLR stimulation. Fig. 1A indicates that stimulation of DCs with TLR agonists, including LPS, Pam3, and poly(I:C) significantly induced Sirt1 mRNA expression, suggesting a possible role of Sirt1 in DC functions. We then generated Sirt1 conditional knock-out mice to elucidate Sirt1 functions in dendritic cells by breeding Sirt1^flox/flox mice with CD11c-Cre transgenic mice (C57/B6 genetic background). Cre expression under the control of the CD11c promoter sufficiently deleted Sirt1 expression in bone marrow-derived DCs as confirmed by Western blotting (Fig. 1B). Therefore, we used these Sirt1^flox/flox/CD11c-Cre^+ mice for our study.

Genetic deletion of the Sirt1 gene in CD11c^+ cells did not affect DC development. The percentages and total numbers of CD11c^+ DCs were comparable in the spleen and lymph nodes from wild type and Sirt1 conditional knock-out mice (data not shown). In addition, the percentages of CD11c^+ B220^− conventional DCs (including the CD8^-CD11b^-CD11c^+ B220^- mye-

FIGURE 1. Sirt1 inhibits IL-27 and IFN-β expression in DCs. A, BMDCs from wild type mice were stimulated with TLR agonists, including LPS, Pam3, and poly(I:C) for 6 h. The mRNA levels of Sirt1 were determined by real-time RT-PCR using β-actin as an internal control. The relative Sirt1 mRNA levels are shown.

B, wild type and Sirt1-null BMDCs were lysed, and Sirt1 protein expression levels were analyzed by Western blotting using Sirt1-specific Abs (top panel) using tubulin as a loading control (bottom panel). NS, nonspecific bands. C, wild type and Sirt1^−/− BMDCs were stimulated with LPS or Pam3 or quantitative poly(I:C) at each indicated concentration for 6 h. Total RNA in the stimulated DCs was extracted, and the levels of Il-27p28 (top panels) and Ebi3 (middle panels), as well as Ifn-β (bottom panels), were determined by real-time PCR. D, wild type and Sirt1^−/− BMDCs were stimulated with LPS or Pam3 or poly(I:C) for 6 h. Total RNA in the stimulated BMDCs was extracted, and the levels of Il-1β (top panels), Il-6 (middle panels), and Il-12p35 subunit (bottom panels) were determined by real-time PCR. Error bars represent data from three independent experiments. Error bars represent data from three independent experiments (A) or three pairs of mice (C and D) (mean ± S.D.). Student’s t test was used for the statistical analysis. *, p < 0.05; **, p < 0.01, and ***, p < 0.005.
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FIGURE 2. Sirt1-null DCs suppress Th17 polarization. A and B, wild type and Sirt1-null BMDCs were activated with LPS followed by co-cultivating with naive CD4⁺ T cells under Th17 polarization conditions (anti-CD3, 1 μg/ml; IL-6, 50 ng/ml; TGF-β1, 1 ng/ml; anti-IL-4, 10 μg/ml, and anti-IFN-γ, 10 μg/ml). C and D, Th17 differentiation assay was performed as described in A in the absence or presence of anti-IL-27 neutralization Abs (10 μg/ml). E and F, Th17 differentiation assay was performed as described in A in the absence or presence of anti-IL-27 (10 μg/ml) and anti-IFN-β (10 μg/ml) neutralization Abs. Five days after culture, CD4⁺ T cells were stimulated with 10 ng/ml phorbol 12-myristate 13-acetate plus 500 ng/ml ionomycin in the presence of 10 μg/ml of brefeldin A for additional 4 h. The production of IL-17 and IFN-γ was analyzed by intracellular staining. Representative data (A, C, and E) and data from four independent experiments (B, D, and F) are shown. Student’s t test was used for the statistical analysis. **, p < 0.01; ***, p < 0.005. NS, nonspecific.

Sirt1 Programs DCs to Suppress Th17 Differentiation through IL-27 and IFN-β Expression—Because both IL-27 and IFN-β inhibit Th17 differentiation (23–27), we then determined whether the impaired ability of Sirt1-null DCs in inducing Th17 differentiation was due to the elevated IL-27 and IFN-β production by adding neutralization Abs. We tested this by adding neutralization Abs in the in vitro co-culture system using Sirt1-null DCs. The anti-IL-27 neutralizing was insufficient to fully rescue Th17 differentiation (Fig. 2, C and D). However, when both anti-IL-27 and anti-IFN-β Abs were added, Th17 differentiation was fully rescued during co-culture with Sirt1-null DCs (Fig. 2, E and F), proving the principle that the elevated IL-27 and IFN-β produced by Sirt1-null DCs are responsible for suppressing Th17 differentiation.

Sirt1 Interacts with and Deacetylates IRF1 in DCs upon TLR Stimulation—As a deacetylase, Sirt1 may suppress IL-27 production by deacetylating the transcription factors that mediate

CD11c⁺ B220⁻ lymphoid DCs and the CD11c⁺mPDCA-1⁺ plasmacytoid DCs in the spleen were also similar in Sirt1 conditional knock-out mice and wild type littermate controls (data not shown), indicating that Sirt1 gene is dispensable for DC differentiation. In addition, flow cytometry analysis detected comparable levels of MHC class I and II, as well as CD80 and CD86 between wild type and Sirt1-null DCs either in naive conditions or after LPS stimulation, suggesting that Sirt1 is dispensable for cell surface expression of MHC and co-stimulatory molecules (data not shown). Interestingly, upon TLR stimuli with either LPS, Pam3, or poly(I:C), the expression levels of IL-27, both of its subunits p28 and EBI3, and IFN-β were significantly increased (Fig. 1C), indicating that Sirt1 inhibits TLR-induced IL-27 and type I interferon production. We also observed that Sirt1-null DCs produced significantly greater amounts of cytokines, including IL-1β, IL-6, and IL-12p35 upon TLR stimulation (Fig. 1D). Therefore, Sirt1 appears to be a negative regulator for the production of a broad spectrum of both inflammatory and anti-inflammatory cytokines by DCs when stimulated by TLR agonists.

Sirt1⁻/⁻ DCs Suppress Th17 Polarization through IL-27 and IFN-β Expression—Because both IL-27 and IFN-β inhibit Th17 differentiation (23, 24), we speculated that Sirt1-null DCs might impair Th17 differentiation. To test this hypothesis, we isolated CD4⁺ naïve T cells from wild type mice and co-cultivated them with pre-activated wild type or Sirt1-null BMDCs in the presence of anti-CD3 antibody under Th17 polarization conditions. The production of IL-17 and IFN-γ was determined by intracellular staining. Fig. 2, A and B, show that Sirt1-null DCs were less efficient in Th17 polarization when compared with wild type DCs, but the production of Th1 cytokine IFN-γ was not affected. Therefore, loss of Sirt1 functions in DCs impairs CD4⁺ T cell differentiation toward IL-17-producing Th17 cells.

Because both IL-27 and IFN-β were increased in Sirt1-null DCs and both of them inhibit Th17 differentiation (23–27), we then determined whether the impaired ability of Sirt1-null DCs in inducing Th17 differentiation was due to the elevated IL-27 and IFN-β production by adding neutralization Abs. We tested this by adding neutralization Abs in the in vitro co-culture system using Sirt1-null DCs. The anti-IL-27 neutralizing was insufficient to fully rescue Th17 differentiation (Fig. 2, C and D). However, when both anti-IL-27 and anti-IFN-β Abs were added, Th17 differentiation was fully rescued during co-culture with Sirt1-null DCs (Fig. 2, E and F), proving the principle that the elevated IL-27 and IFN-β produced by Sirt1-null DCs are responsible for suppressing Th17 differentiation.
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**FIGURE 3. Sirt1 interacts with and deacetylates IRF1.** A, FLAG-tagged Sirt1 (F-Sirt1) expression plasmid was co-transfected with an empty control vector or with each of Myc-tagged IRFs into HEK293 cells. The interactions of Sirt1 with each IRF were determined by co-immunoprecipitation with anti-Myc Abs and Western blotting with anti-Sirt1 Abs (top panel). The expression of Sirt1 in the whole cell lysates was confirmed by Western blotting (bottom panel). B, interaction of HA-Sirt1 with Myc-IRF1 was confirmed in transiently transfected HEK293 cells as described in A. C, IRF1 expression plasmid was transfected with the full-length (FL) Sirt1, or each of the Sirt1 mutants including the N terminus (N), N terminus with the histone deacetylase domain (NH), or the histone deacetylase domain with the C terminus (HC) into HEK293 cells. The interaction of IRF1 with Sirt1 or its truncated mutants was determined as indicated in A. D, mouse BMDCs were stimulated with LPS (1 μg/ml) for the indicated hours and lysed. The interactions of IRF1 with Sirt1 were analyzed by co-immunoprecipitation of IRF1 and Western blotting with anti-Sirt1 Abs (top panel). The expression levels of Sirt1 (middle panel) and IRF1 (bottom panel) in the whole cell lysates were confirmed by Western blotting. E, IRF1 was co-expressed with Sirt1 as indicated. IRF1 acetylation in the transfected cells was determined by immunoprecipitation of IRF1 with anti-IRF1 Abs and Western blotting with anti-acetyl-lysine Abs (top panel). The expression levels of IRF1 were confirmed by Western blotting with anti-IRF1 Abs (bottom panel). F, BMDCs from Sirt1+/− and Sirt1−/− mice were stimulated with LPS (1 μg/ml) for each indicated hour. The acetylation of IRF1 was determined as described in D (top panel). The expression levels of IRF1 (middle panel) and Sirt1 (bottom panel) were confirmed in the whole cell lysates.

**IL-27 transcription.** To test this hypothesis, we analyzed the interactions of Sirt1 with the IRF family transcription factors, including IRF1, IRF3, IRF7, and IRF8, which have been found to regulate transcription of either IL-27 or Ifn-β, or both (12, 28–33). Strong interactions of Sirt1 with IRF1 and IRF7 and a weak interaction of Sirt1 with IRF3 were detected in transiently transfected HEK293 cells (Fig. 3A). IRF1 has been found as a critical transcription factor for IL-27p28 because genetic deletion of IRF1 almost completely abolishes IL-27 expression (12). Therefore, we tested whether Sirt1 functions as a deacetylation of IRF1. Data in Fig. 3B confirms that Sirt1 interacts with IRF1 in the transiently transfected HEK293 cells, as a strong Sirt1 band was detected in the anti-IRF1 immunoprecipitate when IRF1 was co-transfected. The N terminus of Sirt1 protein mediates its interaction with IRF1 because truncated Sirt1 mutation carrying the N terminus alone was sufficient to mediate their interaction (Fig. 3C). To further confirm Sirt1 interaction with IRF1, BMDCs were stimulated with or without LPS at different time points, and the interaction of endogenous Sirt1 with IRF1 was determined by co-immunoprecipitation and Western blotting. Only background levels of the Sirt1 band were detected in the anti-IRF1 immunoprecipitate from BMDCs without stimulation. Interestingly, LPS stimulation dramatically enhanced Sirt1 interaction with IRF1 (Fig. 3D), indicating that Sirt1 interacts with IRF1 in DCs, and their interaction is regulated by TLR4 stimulation.

Acetylation of IRF family transcription factors by the acetyltransferase p300, cAMP-response element-binding protein, and p300/CBP-associated factor has been shown to be critical for their functions (34). We then reasoned whether Sirt1 regulates IL-27 productions through suppressing IRF1 acetylation. In fact, expression of Sirt1 significantly inhibited IRF1 acetylation (Fig. 3E), suggesting that Sirt1 is a deacetylase of IRF1. To further investigate the role of Sirt1 in regulating IRF1 acetylation, we analyzed IRF1 acetylation in BMDCs from wild type and Sirt1 conditional knock-out mice. Weak IRF1 acetylation was detected in untreated wild type BMDCs but was enhanced by LPS stimulation. Importantly, we detected a significant increase in IRF1 acetylation in Sirt1−/− BMDCs compared with the IRF1 acetylation levels in wild type BMDCs. In contrast, the total protein levels of IRF1 were not affected by the loss of Sirt1 (Fig. 3F). Therefore, Sirt1 deacetylates IRF1 in DCs and mediates suppression of IRF1 acetylation after TLR signaling.

**Sirt1 Inhibits IL-27p28 Production by Suppressing IRF1 Transcriptional Activity in DCs**—Because IRF1 has been shown to be a critical transcription factor responsible for production of the p28 subunit of IL-27 (35), we speculated that Sirt1-mediated deacetylation of IRF1 may be responsible for suppressing IL-27p28 expression in DCs. To test this hypothesis, we subcloned a 3-kb fragment of the IL-27p28 promoter region into a luciferase plasmid. As shown in Fig. 4A, this 3-kb promoter region shows a modest reporter activity, which is further
enhanced by IRF1 co-expression. Interestingly, co-expression of Sirt1 significantly suppressed IRF1-mediated IL-27p28 reporter activity, suggesting that Sirt1 inhibits IL-27p28 expression through IRF1. Conversely, the IL-27 p28-luciferase activity was dramatically increased in Sirt1-/- MEFs compared with that in wild type MEFs. Reconstitution of Sirt1 inhibited IL-27p28 luciferase activity in Sirt1-/- MEFs (Fig. 4B). In addition, chromatin immunoprecipitation using anti-IRF1 Abs detected a significant increase in the binding activity of IRF1 to IL-27p28 DNA promoter in Sirt1-null DCs compared with that in wild type DCs (Fig. 4C). As a negative control, only background levels of IL-27p28 promoter binding by IRF1 were detected in Irf1-null DCs. These results imply that Sirt1 inhibits IL-27p28 gene transcription through IRF1 suppression.

To further confirm that Sirt1 suppresses IL-27p28 expression in an IRF1-dependent manner, we generated Sirt1/IRF1 double knock-out mice by breeding Sirt1<sub>flx/flx</sub>CD11c-Cre<sup>+</sup> mice with Irf1 knock-out mice. Bone marrow cells were isolated and differentiated into DCs. Consistent with previous discoveries (35), loss of IRF1 function dramatically impaired IL-27p28 expression (Fig. 4D). Interestingly, genetic deletion of IRF1 completely abolished the elevated IL-27p28 gene transcription in Sirt1-null DCs (Fig. 4D), clearly indicating that Sirt1 inhibits IL-27p28 expression in an IRF1-dependent manner. To support this notion, we discovered that the suppressive activity of Sirt1-null DCs in Th17 differentiation was largely reversed by further Irf1 gene deletion, because the percentages of Th17 cells were significantly increased when naive CD4<sup>+</sup> T cells were co-cultivated with Sirt1-/-Irf1-/- DCs than those with Sirt1-/- DCs (Fig. 4E). Collectively, our results indicate that Sirt1 suppresses IRF1 transcriptional activity on the IL-27p28 gene possibly through deacetylating IRF1, providing a possible molecular explanation for the increased IL-27 expression in Sirt1-/- DCs.

Both IL-27 subunits, p28 and EBI3, are significantly higher expressed in Sirt1-null DCs upon TLR stimulation (Fig. 1C), indicating that Sirt1 suppresses the expression of both IL-27 subunits. To investigate whether IRF1 is also a transcription factor of EBI3, we generated Ebi3-luciferase expression plasmids and tested the effect of IRF1 overexpression on its reporter activity. A dramatically increased Ebi3 reporter activity was detected in Sirt1-null MEFs when compared with the wild type controls, confirming that Sirt1 is a transcription suppressor of the Ebi3 gene (Fig. 4F). However, IRF1 expression did not affect Ebi3 luciferase activity and anti-IRF1 ChIP analysis did not show any IRF1 binding activities to the Ebi3 promoter (Fig. 4, G and H). Instead, co-expression with the NF-κB transcription factor p50 dramatically enhanced Ebi3 promoter-driven reporter activity, and Sirt1 expression significantly inhibited the p50-driven Ebi3 reporter activity (Fig. 4, I and J). These results indicate that NF-κB but not IRF1 is involved in EBI3 gene transcription. To support this conclusion, further deletion of IRF1 in Sirt1-null DCs inhibited IL-27p28, but not Ebi3 gene transcription in mouse bone marrow-derived DCs upon TLR stimulation (Fig. 4K). Together with the previous
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FIGURE 5. Genetic deletion of Sirt1 gene in DCs protects mice from MOG-induced EAE. A, 6–8-week-old C57/B6 mice were immunized with MOG(35–55) peptide (200 μg per mouse, emulsified with CFA). Mice were also given pertussis toxin (200 ng per mouse) on day 0 and day 2 via tail vein injection. All mice were weighed and examined for clinical symptoms. Error bars represent data from nine pairs of mice (mean ± S.D.). B, spinal cords of MOG-immunized mice were fixed, and tissue sections were analyzed by H&E staining. Representative images are shown. C, wild type and Sirt1 conditional knock-out mice were used for EAE induction. Mice were euthanized during the disease peak. Spinal cord tissue sections were immunostained for ROR-γT and our discovery here that Sirt1 expression in CD11c+ cells promotes inflammation, we used an experimental model of human multiple sclerosis and mouse EAE, these results suggest that the reduced Th17 differentiation is responsible, at least partially, for the attenuated EAE in DC-specific Sirt1 knock-out mice. Consistent with our observations, the clinical symptoms of EAE were significantly reduced in DC-specific Sirt1 knock-out mice, and a significant reduction in the total numbers of infiltrated lymphocytes was detected in the brain and spinal cords of Sirt1−/− mice (Fig. 5E). Although the percentage of IFN-γ production of Th1 cells was slightly increased, their total numbers were statistically reduced (Fig. 5, D and F); this is possibly due to the total infiltrated lymphocytes that were reduced in Sirt1−/− mice. We also detected an increase in the percentages of IL-10-producing and FoxP3+ CD4 T cells from the brain and spinal cord of Sirt1 conditional knock-out mice. However, their total numbers are indistinguishable from those of wild type mice because the total number of infiltrating lymphocytes was reduced (Fig. 5, D and F). Therefore, it is likely that loss of Sirt1 in DCs in mice causes reduced Th17 infiltration into the central nervous system to suppress EAE development after MOG immunization.

MOG-specific IL-17 Production by CD4 T Cells Is Reduced in CD11c-specific Sirt1 Conditional Mice during EAE—To elucidate the impact of DC-specific Sirt1 deletion on EAE develop-
ment in mice, we analyzed MOG-specific T cell immune responses in the spleen during EAE development. Consistent with the observation from immunohistological analysis of brain tissues, a significant reduction in the Th17 population was detected in the spleen of DC-specific Sirt1 knock-out mice (Fig. 6, A and B). We also observed a modest but statistically significant increase in FoxP3-positive Treg population in the spleens of Sirt1 conditional knock-out mice (Fig. 6, D and E). However, the percentages of the IFN-γ-positive Th1 cells, IL-4-positive Th2 cells, and the TNF-α-positive CD4+ T cells were not affected (Fig. 6, C, F, and G). When the splenocytes were co-cultivated with the MOG(35–55) peptide, a significant reduction in the production of IL-17 (Fig. 6H) was observed. In addition, the production of IL-2 (Fig. 6I), IFN-γ (Fig. 6J), and IL-6 (Fig. 6K) in the culture supernatants was also slightly reduced. Collectively, our studies here demonstrate that deletion of Sirt1 in DCs suppresses IL-17 production but facilitates Treg differentiation when mice were immunized with the MOG(35–55) peptide, leading to the reduction of the clinical disease severity of EAE. It has been shown that IL-27 controls Th17

**FIGURE 6.** Analysis of MOG-specific T cell immune responses in mouse spleens during EAE development. MOG-immunized mice were euthanized during the peak of disease (day 15 after immunization). A–G, expressions of IL-17 (A and B), IFN-γ (C), FoxP3 (D and E), TNF-α (F), and IL-4 (G) were analyzed by intracellular staining. H–L, splenocytes were cultured with MOG peptide for 3 days. The concentrations of IL-17 (H), IL-2 (I), IFN-γ (J), IL-6 (K), and TNF-α (L) in the supernatants were determined by ELISA. Error bars represent data from six pairs of mice. Student’s t test was used for statistical analysis. *, p < 0.05; **, p < 0.01, and ***, p < 0.005.
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production by inducing PD-L1 expression (42). However, the PD-L1 expression in CD4^+ T cells from MOG-immunized mice was not affected by DC-specific Sirt1 gene deletion (data not shown).

In summary, this study reveals a previously unrecognized mechanism by which dendritically specific Sirt1 regulates inflammatory immune response (Fig. 7). Sirt1 inhibits the production of anti-inflammatory cytokines, including IL-27 and IFN-β by DCs during inflammation through suppressing the acetylation of IRF1, which is possibly catalyzed by the acetyltransferase p300. Because both IL-27 and IFN-β suppress the pathogenic Th17 differentiation, dendritic Sirt1 seems to play a pathogenic role in inflammatory disease. Indeed, loss of Sirt1 specifically in CD11c-expressing DCs resulted in reduced EAE in mice. At the molecular level, Sirt1 interacts with and deacetylates IRF1 to suppress IRF1 binding to the promoter DNA of the p28 subunit of IL-27. Our study demonstrates that the histone deacetylase Sirt1 programs DCs to regulate Th17 differentiation during inflammation.

DISCUSSION

Sirt1 has emerged as a critical immune regulator by suppressing T cell immunity and macrophage functions (5, 7, 16, 43, 44), providing a rationale for Sirt1 activators in autoimmune and inflammatory disease therapy. Our findings here show that Sirt1 suppresses DC production of IL-27 and IFN-β, two anti-inflammatory cytokines that negatively regulate CD4^+ T cell differentiation toward Th17, indicating that Sirt1 may tune or function as a balance keeper for the immune response during inflammation. On the one hand, Sirt1 inhibits IL-27 and IFN-β production in dendritic cells to facilitate T cell differentiation into Th17. On the other hand, Sirt1 expression in T cells suppresses their activation and differentiation when T cells recognize antigens provided by DCs. Sirt1 activators, such as resveratrol, have been widely studied in the treatment of autoimmune diseases in rodent models because Sirt1 inhibits T cell activation, promotes T cell tolerance, and suppresses macrophage activation (6, 8). However, due to both anti-inflammatory and proinflammatory natures of IL-27, a simple enhancement of Sirt1 activation, which leads to suppression of IL-27 production, could be a double-edged sword for the treatment of some autoimmune diseases. For example, as IL-27 is an anti-inflammatory cytokine during EAE development, Sirt1 activators might be less effective for EAE treatment. In contrast, because IL-27 is an inflammatory cytokine in colitis (23), Sirt1 activators could be a better choice for colitis treatment than other types of inflammatory diseases such as EAE because Sirt1 activation inhibits IL-27 production by DCs and T cell activation. Therefore, results from this study provide useful insights for the clinical use of the Sirt1 activator in autoimmune therapy.

Accumulated evidence suggests that acetylation of IRF family transcription factors is required for their optimal transcriptional activity (45–47). Our discoveries here reveal that Sirt1 inhibits Il-27p28 subunit expression through suppressing IRF1 transcriptional activity in dendritic cells. This suppressive activity of Sirt1 is achieved by modulating the IRF1 acetylation status. First, Sirt1 interacts with and inhibits IRF1 acetylation in transiently transfected cells. Second, Sirt1 interacts with IRF1 in DCs, and their interaction is enhanced by TLR stimulation. Third, loss of Sirt1 function results in elevated IRF1 acetylation and binding activity to Il-27p28 promoter DNA. Therefore, our studies here suggest that the deacetylase Sirt1 antagonistically regulates the transcriptional activity of IRF1. TLR-mediated signaling transduction appears to be required for Sirt1 to modulate IRF1 acetylation and transcriptional activity, because their interactions with IRF1 can only be detected in DCs upon TLR stimulation.

In addition to IRF1, Sirt1 appears to bind to IRF7 and IRF3 at least in transiently transfected HEK293 cells. Because both IRF3 and IRF7 have been found to regulate cytokine production, including type I IFN and possibly IL-27, it is likely that Sirt1 regulates IL-27 and IFN-β through multiple targets in DCs. In particular, it is well established that IRF3 is predominately responsible for TLR-mediated type I IFN expression in the antigen-presenting cells, including DCs. Further investigations are necessary to confirm how Sirt1 regulates the type I IFN expression in DCs. One additional interesting observation is that Sirt1 interacts with IRF1, IRF3, and IRF7 but not with all IRF family members, implying the specificity of Sirt1 in regulating IRF family transcription factors.

Although CD11c-specific deletion of the Sirt1 gene attenuates MOG-induced inflammatory demyelinating disease in mice, we also observed that inflammatory cytokine production, including IL-1β, IL-6, and TNF-α, by Sirt1-null DCs was dramatically increased upon TLR stimulation in vitro. This observation provided additional evidence to support our conclusion that Sirt1 is a double-edged sword during inflammation. Recent studies, including ours, demonstrated that Sirt1 functions as a deacetylase and negative regulator of the transcription factors NF-κB and AP-1 (7, 16), and it is speculated that Sirt1 may suppress the production of inflammatory cytokines by inhibiting NF-κB and AP-1, both of which are involved in inflammatory cytokine transcription. Moreover, our observations here indicate that Sirt1 inhibits the Ebi3 subunit of Il-27 transcription in DCs upon TLR stimulation through NF-κB. We showed NF-κB p50 expression activates Ebi3 luciferase activity and Sirt1 inhibits the NF-κB-mediated Ebi3 reporter. Because
NF-κB transcriptional activity is involved in regulating the transcription of inflammatory cytokines, including IL-1β and IL-6. It is speculated that Sirt1-mediated NF-κB inhibition may serve as a molecular mechanism in regulating their expression in DCs during inflammation. A recent study discovered that the kinase p38 programs DCs to drive Th17 differentiation during autoimmune inflammation (48). Unlike Sirt1, p38 promotes IL-6 and the co-stimulatory molecule CD86 expression to facilitate Th17 differentiation. Therefore, it is likely that Sirt1 and p38 regulate cytokine production in DCs through different pathways.

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