Prdm1 Regulates Thymic Epithelial Function To Prevent Autoimmunity

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Autoimmunity is largely prevented by medullary thymic epithelial cells (TECs) through their expression and presentation of tissue-specific Ags to developing thyocytes, resulting in deletion of self-reactive T cells and supporting regulatory T cell development. The transcription factor Prdm1 has been implicated in autoimmune diseases in humans through genome-wide association studies and in mice using cell type–specific deletion of Prdm1 in T and dendritic cells. In this article, we demonstrate that Prdm1 functions in TECs to prevent autoimmunity in mice. Prdm1 is expressed by a subset of mouse TECs, and conditional deletion of Prdm1 in either Keratin 14– or Foxn1-expressing cells in mice resulted in multisymptom autoimmune pathology. Notably, the development of Foxp3* regulatory T cells occurs normally in the absence of Blimp1. Importantly, nude mice developed anti-nuclear Abs when transplanted with Prdm1 null TECs, but not wild-type TECs, indicating that Prdm1 functions in TECs to regulate autoantibody production. We show that Prdm1 acts independently of Aire, a crucial transcription factor implicated in medullary TEC function. Collectively, our data highlight a previously unrecognized role for Prdm1 in regulating thymic epithelial function. The Journal of Immunology, 2017, 199: 1250–1260.
in epithelium are not due to defects in the development of CD4, CD8, or Foxp3+ Tregs. In fact, by performing thymus transplantation experiments into nude mice, we demonstrated that Prdm1-deficient mTECs are sufficient to induce autoimmuneity. Together, our findings implicate Prdm1 in the regulation of autoimmunity in TECs and are consistent with the identification of polymorphisms in Prdm1 associated with autoimmune diseases, such as systemic lupus erythematosus (SLE) (33, 34).

Materials and Methods

Animal use

All animals were housed and handled according to the institutional guidelines of Yale University. Keratin14-Cre (35), Prdm1-floxed (27), Prdm1-eGFP (36), Prdm1-YFP (37), mTom (38), Aire KO (39), and FoxN1-Cre (40) mice were previously described. MRL/Fas+ (MRL/MpJ-Fas+8922) animals were purchased from The Jackson Laboratory (41).

For Prdm1fl/fl;K14Cre, Prdm1fl/+;K14Cre, FoxN1Cre, Prdm1eGFP, and Prdm1YFP, mice were maintained in the inbred C57Bl/6J background, and age-matched Prdm1fl/fl;K14Cre littermates were used as controls. For the K14Cre:mTomG and FoxN1Cre;mTomG lineage tracing experiments, mice were in a mixed C57Bl/6;129X1/SvJ background. Control mice for all experiments were Prdm1fl/fl;K14Cre age-matched littermates because these mice did not develop the initial ventral alopecia and dermatitis.

Cell isolation

Thymocyte, splenocyte, and lymph node suspensions were obtained from adult organs by mechanical dissociation. Stromal cells from adult thymi were isolated, as previously described (42). In brief, thymus lobes were cut into small pieces, washed with R-5 medium (t-glutamine-supplemented RPMI 1640 [Sigma-Aldrich]), and 10 mM HEPES (Life Technologies). Enzymatic treatment was repeated for an additional 20 min, and remaining enzymatic treatment was repeated for an additional 10 min. Remaining tissue suspensions were mechanically dispersed by careful pipetting. Cell suspensions from each digestion were pooled and washed in ice-cold PBS containing 2% FCS and 2 mM EDTA to prevent aggregate formation. Stromal cells were enriched by MACS immunomagnetic depletion of CD45+ cells (Miltenyi Biotec), according to the manufacturer’s protocol. Cell surface Ab staining was performed in 2% FCS and 2 mM EDTA to prevent aggregate formation.

Immunofluorescence of tissues

Wild-type (WT) tissues were embedded in OCT compound (Tissue-Tek, Sakura). Normal human thymus (use of human tissue approved by National Research Ethics Service/Research Ethics Committee reference: 07/Q1206/48). Fetal thymic organ culture and thymus grafting

Embryonic day (E) 15 fetal thymuses were isolated from Prdm1fl/fl;K14Cre and Prdm1fl/+;K14Cre mice or from nude hosts grafted with embryonic thymus isolated from Prdm1fl/fl;K14Cre and Prdm1fl/+;K14Cre mice for 30 min at RT on 12 spot anti-nuclear Ab (ANA) diagnostic HEp2 Slides (Bio-Rad). Serum isolated from MRL/Fas+ mice was used as an ANA-positive control (41). FITC anti-mouse secondary Ab (Southern Biotechnology) was used for detection. Coverslips were mounted with Fluoromount.

Supplemented RPMI 1640 (Sigma-Aldrich), 10 mM HEPES (Life Technologies). Aire KO (39), and FoxN1-Cre (40) mice were previously described. MRL/Fas+ mice were used as an ANA-positive control (41). FITC anti-mouse secondary Ab (Southern Biotechnology), diluted 1:50 in 0.1% BSA/PBS, was used for detection. Coverslips were mounted with Fluoromount.

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the top 50 Prdm1-associated features are displayed. Spearman-rank correlations for Prdm1 were obtained for each gene across all 200 cells, and resultant rho values were plotted as a bar graph. Statistical computations were performed using WinStat 2014 (R. Fitch Software, Bad Krozingen, Germany). The p values obtained from this analysis were tabulated and plotted in Plotly. All genes associated with Prdm1 with an r cutoff of 0.0005 are reported. Additional analysis was performed whereby Aire-dependent and Aire-independent gene sets identified by Sansom et al. (47) (GSE53111 within the National Center for Biotechnology Information Gene Expression Omnibus; http://www.ncbi.nlm.nih.gov/geo/) and Brennec et al. (45) were analyzed for associations with Prdm1. Box and whisker plots were generated to describe the median Spearman-rank rho values for Prdm1 to Aire-dependent, Aire-independent, and all gene sets.

Statistical analysis

Analyses were performed using GraphPad Prism version for Macintosh (GraphPad Software, La Jolla, CA). Categorical data were analyzed using a Fisher exact test, whereas noncategorical data between two groups were analyzed using a Mann–Whitney U test and a nonparametric test that does not assume a normal distribution; noncategorical data between multiple groups were compared using one-way ANOVA.

Results

Epithelial deletion of Prdm1 results in autoimmune

Previous studies have established a role for Prdm1 in barrier epithelial tissues, namely the skin and intestine (24, 27, 31, 32), but a role within thymic epithelium remained unexplored. Mice lacking Prdm1 in squamous epithelial cells were generated by crossing Prdm1<sup>cKO</sup> mice (27) to mice expressing Cre recombinase driven by the K14 promoter (Prdm1<sup>cKO<sub>K14</sub></sup>) (35) or the forkhead/winged-helix box protein N1 (FoxN1) promoter (Prdm1<sup>cKO<sub>FoxN1</sub></sup>) (40). Interestingly, in contrast with WT or heterozygous littermates, ~80% of Prdm1<sup>cKO<sub>K14</sub></sup> mice stochastically developed mandibular and accessory mandibular cervical lymphadenopathy as they aged (≥2 mo) (Fig. 1A–C). Similarly, 100% of Prdm1<sup>cKO<sub>FoxN1</sub></sup> mice analyzed developed lymphadenopathy, albeit at a slightly later time point (≥5 mo) (Supplemental Fig. 1A, 1B). Further investigation of the immunological phenotype in Prdm1<sup>cKO<sub>K14</sub></sup> mice revealed a significant infiltration of activated T lymphocytes in their cervical lymph nodes compared with littermate control mice (Fig. 1D, 1E), with Prdm1<sup>cKO<sub>FoxN1</sub></sup> mice following the same trend (Supplemental Fig. 1C, 1D). Interestingly, the lymphadenopathy observed in these mice was not systemic, as suggested by the absence of significant splenomegaly (Supplemental Fig. 1E–G).

Given the similarities in phenotypes between the deletion of Prdm1 with K14- and FoxN1-Cre mice, we sought to confirm that Cre recombinase was active in the thymus of K14-Cre mice. To this end, we crossed K14-Cre mice with the membrane-tomato membrane GFP (mTom/mG) reporter line, which induces mG expression after Cre-mediated recombination in K14-expressing cells (38). Flow cytomteric analysis of mTEC and cTEC cell populations (Supplemental Fig. 2A) revealed the expression of mG in both mTEC and cTECs (Supplemental Fig. 2B–E), indicating the activity of Cre recombinase within the thymus of K14-Cre mice. Although the absolute cell number of GFP<sup>+</sup> cells tended to be greater in mTECs compared with cTECs, this was not significantly different (Supplemental Fig. 2D), and on average the proportion of GFP<sup>+</sup> cells in mTECs and cTECs was comparable (Supplemental Fig. 2E). Because both K14-Cre and FoxN1-Cre are active in TECs (40), we hypothesized that activation of T cells observed in Prdm1<sup>cKO<sub>K14</sub></sup> and Prdm1<sup>cKO<sub>FoxN1</sub></sup> mice may be because of a breakdown in central tolerance. Consequently, we sought to examine whether Prdm1<sup>cKO<sub>K14</sub></sup> displayed characteristics of autoimmunity, such as the presence of autoantibodies (48, 49). The sera of 80% of Prdm1<sup>cKO<sub>K14</sub></sup> (Fig. 1F, 1G) and 100% of Prdm1<sup>cKO<sub>FoxN1</sub></sup> (Supplemental Fig. 1H) mice positively stained HEp2 cells with a nuclear pattern, whereas only ~20% of control mice displayed ANA staining (Fig. 1G). The positive staining in Prdm1<sup>cKO<sub>K14</sub></sup> mice exhibited a range of antinuclear patterns (50) with homogeneous and speckled patterns being most prevalent (Fig. 1H). Interestingly, this was observed in this particular model only. Analysis of total serum Ig levels by ELISA revealed normal levels of IgM and IgG3 in control versus Prdm1<sup>cKO<sub>K14</sub></sup> mice, whereas IgG1 and IgG2b levels were significantly higher in the blood of Prdm1<sup>cKO<sub>K14</sub></sup> mice (Fig. 1I). Moreover, Prdm1<sup>cKO<sub>K14</sub></sup> mice displayed deposits of IgG in their kidneys (Fig. 1J, 1K). Together, these data demonstrate that the absence of Prdm1 in epithelial cells leads to the development of spontaneous autoimmune-like disease and T cell activation.

Prdm1 is expressed in TECs

Because the conditional deletion of Prdm1 from TECs results in autoimmune-like pathology and TECs are pivotal in establishing central tolerance, we next sought to determine whether Prdm1 was expressed in TECs. Indeed, published microarray and RNA sequencing experiments demonstrated the presence of Prdm1 mRNA expression in purified mTECs (GDS1655 and GSE70798) (51, 52).

To quantify the percentage of TECs that express Prdm1, we used flow cytometry of isolated TECs from a mouse model expressing YFP driven by the Prdm1 promoter (37). Thymi from Prdm1<sup>YFP</sup> mice (6–8 wk of age) were enzymatically dissociated, and the resulting single-cell suspensions were stained with Abs against EPCAM1, Ly51, MHCII, and CD80 and analyzed by flow cytometry (42, 53) (Fig. 2A–E). mTECs were identified as CD45<sup>−</sup>, EPCAM1<sup>+</sup>, Ly51<sup>lo</sup>, MHCII<sup>−</sup>, and CD80<sup>−</sup> epithelial cells, whereas cTECs were identified as CD45<sup>+</sup>, EPCAM1<sup>+</sup>, Ly51<sup>hi</sup>, and CD80<sup>+</sup> cells (Fig. 2B). The mTEC population was dissected further as mTEC<sup>lo</sup> and mTEC<sup>hi</sup> based on MHCII and CD80 expression levels (Fig. 2C). Although control mice displayed no detectable YFP expression (data not shown), a small percentage of cTECs were YFP<sup>+</sup> (Fig. 2D). Interestingly, YFP was not detected in mTEC<sup>lo</sup> cells, whereas YFP was detected in a proportion of mTEC<sup>hi</sup> cells. Quantification of the percentage of YFP<sup>+</sup> cells in multiple mice revealed a significantly higher percentage of mTEC<sup>hi</sup> cells (~9%) were YFP<sup>+</sup>, compared with ~2% of YFP<sup>+</sup> cTECs (Fig. 2E).

To further determine the expression of Prdm1 within TECs, we analyzed thymi from a reporter mouse line that expressed membrane-localized GFP (mGFP) driven by Prdm1 promoter (36). Immunofluorescence staining of adult thymi from the Prdm1-mGFP reporter line with Abs against K5, or involucrin, revealed expression of GFP in a subset of TECs (Fig. 2F, 2G); both K5<sup>+</sup> and involucrin<sup>+</sup> cells expressing mGFP were identified (54).

To explore whether Prdm1 was expressed in human TECs, we immunostained sections of human thymus with Abs against PRDM1. PRDM1<sup>+</sup> nuclei were identified in human Hassall’s corpuscles (Fig. 2H, arrows) (55) with a comparable staining pattern to that seen within human epidermis used as the staining control (Fig. 2I) (27). Together, these data are consistent with the expression of Prdm1 in K5<sup>+</sup> cells in mouse thymus, within Hassall’s corpuscles in mouse and human thymus, and suggest that Prdm1 is expressed in mature mTECs.

Prdm1 does not regulate TEC or T cell development

Given the autoimmune pathology in Prdm1<sup>cKO<sub>K14</sub></sup> mice, the expression of Prdm1 in TECs, and the Cre activity in K14-Cre mice, we hypothesized that Prdm1 may regulate TEC development or function. To identify whether TECs developed normally in Prdm1<sup>cKO<sub>K14</sub></sup> mice, we analyzed control and Prdm1<sup>cKO<sub>K14</sub></sup> TECs by flow cytometry for the expression of EPCAM1, Ly51, CD80, and MHCII to examine TEC maturation (42, 53) (Fig. 3A). cTECs (EPCAM1<sup>+</sup>, Ly51<sup>lo</sup>) are immature mTECs (EPCAM1<sup>+</sup>, Ly51<sup>hi</sup>).
FIGURE 1. Conditional deletion of Prdm1 in K14+ epithelium results in spontaneous development of autoimmunity. (A) Quantification of cervical lymphadenopathy presentation in controlK14 (n = 35) and Prdm1 cKOK14 (n = 51). (B) Images of mandibular cervical lymphadenopathy in Prdm1 cKOK14 mice compared with the normal nodes of control mice. Scale bar, 10 mm. (C) Total lymphocyte numbers are elevated in Prdm1 cKOK14 and Prdm1 cKOPMNT1 mandibular and accessory mandibular cervical lymph nodes. (D) Flow cytometric analysis of CD4 T cells isolated from cervical (mandibular and accessory mandibular) lymph nodes shows an increase in proportion of CD44+CD62L− activated CD4 T cells. (E) An increase in CD44+CD62L− activated CD4 and CD8 T cells from Prdm1 cKOK14 cervical (mandibular and accessory mandibular) lymph nodes compared with control. (F) Serum isolated from Prdm1 cKOK14 mice contains ANAs indicated by positive staining of HEp2 cells. Positive control is MRL/FasL−/− mouse serum; negative control is secondary Ab alone. Scale bar, 50 μm. (G) The presence of ANAs is significantly more frequent in Prdm1 cKOK14 than in control. (H) Quantification of nuclear patterns identified in sera derived from Prdm1 cKOK14 mice. (I) ELISA of serum Ab isotypes. Note IgG2b and IgG1 are significantly increased in Prdm1 cKOK14 serum. (J) Representative images of Prdm1 cKOK14 kidney sections exhibit more intense IgG staining than those of control mice. Scale bar, 25 μm. (K) The presence of IgG is significantly more frequent in Prdm1 cKOK14 mice than in control. Data are mean ± SEM. n ≥ 8 (C), n ≥ 5 (D and E), n ≥ 13 (F–H), n ≥ 15 (I), and n ≥ 3 (J) for each genotype. *p < 0.05, **p < 0.01, ***p < 0.001.
MHCI$^{\text{lo}}$ CD80$^{\text{lo}}$), and mature mTECs (EPCAM$^+$ Ly51$^{\text{lo}}$) were present in Prdm1 cKO$^{\text{K14}}$ at similar cell numbers compared with littermate control mice (Fig. 3B–D). Similarly, thymi from Prdm1 cKO$^{\text{K14}}$ mice and control mice displayed similar numbers of cTEC and mTEC populations (Supplemental Fig. 2F–I). Crucially, the levels of MHCI expression were comparable between control and Prdm1 cKO$^{\text{K14}}$ mTECs (Fig. 3A, 3E, 3F). Furthermore, the gross thymic architecture was unaltered in the absence of Prdm1 (Fig. 3E).

To further confirm that development of the mTEC compartment was normal in the absence of Prdm1, we investigated Aire expression in Prdm1 cKO$^{\text{K14}}$ mice. Similar numbers of K5$^+$, Aire$^+$ cells were present in sections of Prdm1 cKO$^{\text{K14}}$ thymi compared with littermate controls (Fig. 3G, 3H). In addition, the level of Aire mRNA expression was comparable between littermate control and Prdm1 cKO$^{\text{K14}}$ mice mTECs (Fig. 3I).

To investigate whether T cell development was impaired in the absence of Prdm1, we isolated thymocytes from control and Prdm1 cKO$^{\text{K14}}$ mice or Prdm1 cKO$^{\text{FoxN1}}$ mice, stained them for CD4, CD8, and TCR$^\beta$, and analyzed them by flow cytometry. Both control and Prdm1 cKO$^{\text{K14}}$ mice displayed typical CD4 and CD8 profiles (Fig. 3J) and cell numbers (Fig. 3K), suggesting

**FIGURE 2.** Expression of Prdm1 in the mouse thymus. (A) Representative flow cytometry plots indicating the gating strategy for the CD45$^-$ EPCAM$^+$ epithelium, (B) cTEC (Ly51$^+$EPCAM$^+$), mTEC (Ly51$^+$ EPCAM$^+$), (C) mTEC$^{\text{lo}}$ (Ly51$^+$ EPCAM$^+$ CD80$^{\text{lo}}$ MHCI$^{\text{lo}}$), and mTEC$^{\text{hi}}$ (Ly51$^+$ EPCAM$^+$ CD80$^+$ MHCI$^{\text{hi}}$) for Prdm1-YFP mice. (D) Representative flow cytometry plots indicating the YFP expression within the populations shown in (A). (E) Quantification of the percentage of YFP$^+$ cells for each of the indicated epithelial cell populations. Data are mean ± SEM, n = 5 mice. (F and G) Sections from human skin epithelium illustrating positive stain for Prdm1 in the granular layer of the epidermis and negative staining below. Scale bars, 5 μm (G); 10 μm (F); 20 μm (H and I). ****p ≤ 0.0001.
T cell development was not grossly impaired in these mice. In addition, TCR expression in CD4+ and CD8+ T cells from Prdm1 cKO(K14) mice was as seen in control mice (Fig. 3L), suggesting normal TCR expression. Normal T cell development also occurred in Prdm1 cKO(FoxN1) mice (Supplemental Fig. 2J–L).

Tregs develop within the thymus and require mTECs (4, 56–58). To define whether the loss of epithelial Prdm1 expression resulted in Treg developmental defects, we isolated thymocytes from control, Prdm1 cKO(K14), and Prdm1 cKO(FoxN1) mice, stained them for CD44, CD25, CD4, and Foxp3, and analyzed them by flow cytometry (Fig. 4). Tregs (CD4+, CD44+, CD25+, Foxp3+) were present in similar numbers in thymi from control, Prdm1 cKO(K14), and Prdm1 cKO(FoxN1) mice (Fig. 4A, 4B). In addition, the levels of CD25 and Foxp3 were comparable among control, Prdm1 cKO(K14), and Prdm1 cKO(FoxN1) Tregs, suggesting normal development of this population. Importantly, Tregs were also identified within peripheral lymphoid tissues of Prdm1 cKO(K14), Prdm1 cKO(FoxN1), and control mice (Fig. 4C, 4D). Taken together, these data suggest that in the absence of Prdm1, the thymic epithelium develops normally and is able to support a normal T cell developmental program including Tregs.

Prdm1 functions in the thymus to regulate autoimmunity
To definitively ascertain whether autoimmunity associated with epithelial deletion of Prdm1 was due to a role of Prdm1 within TECs, we determined whether transplanted thymic epithelial tissue...
Our study has revealed a novel mechanism for TEC function via the transcription factor Prdm1. It is intriguing to speculate that Prdm1 regulates TEC function by regulating TSA expression in a subset of TECs either in coordination or independently from the transcription factor Aire. Perhaps, Prdm1 creates some of the epigenetic markers that Aire is thought to recognize to enable Aire’s subsequent activity (61–63). Our in silico analysis of gene expression patterns in Aire+ mTECs, compared with previously defined Aire-dependent or Aire-independent genes derived from three TSA-encoding genes: Tspln8, Ceacam1, and Klk5 (38, 39). Interestingly, we found that Prdm1 was more significantly linked with Aire-independent TSA-associated genes than Aire-dependent TSA-associated genes or all expressed genes (Supplemental Fig. 3D, Supplemental Table I). In addition, Prdm1 and Aire did not correlate with each other (rho = 0.07, p = 0.162). Together, these data suggest that Prdm1+ mTECs represent a unique subset of mTECs important in the prevention of autoimmune.

### Discussion

- **Prdm1 and its human ortholog PRDI-BF1 elicit their transcriptional modulation by several different mechanisms**, including direct competition for DNA binding (66) and recruitment of transcription factors. The data presented here suggest that Prdm1 regulates TEC function by regulating TSA expression in a subset of mTECs critical for the prevention of autoimmune disease. Further studies are needed to clarify the mechanisms by which Prdm1 regulates TEC function and how it interacts with other transcription factors like Aire.

### Supporting Evidence

- [Supplemental Fig. 3A](#), supporting the expression of Prdm1 expression with FoxN1.
- [Supplemental Fig. 3B](#), demonstrating that Prdm1 expression was detected in both WT and Aire KO mTECs, which is consistent with similar levels of Prdm1 expression in Aire KO mice (Supplemental Fig. 3B).
- [Supplemental Fig. 3C](#) Global analysis of gene expression in Prdm1+ mTECs revealed distinct patterns of genes compared with Prdm1- mTECs (Supplemental Fig. 3A). Spearman-rank correlation analysis between Prdm1+ and all expressed genes within the Meredith RNA-sequencing dataset indicated that a predominant number of transcripts were associated with Prdm1 expression (Supplemental Fig. 3C). We further filtered this gene set using a stringent cutoff of p ≤ 0.0005 to identify robust Prdm1-associated genes (Supplemental Fig. 3D, Supplemental Table I). To determine whether Prdm1 expression was correlated with TSA-associated gene expression patterns in Aire+ mTECs, we compared Prdm1 expression with previously defined Aire-dependent or Aire-independent genes derived from three TSA-encoding genes: Tspln8, Ceacam1, and Klk5 (38, 39). Interestingly, we found that Prdm1 was more significantly linked with Aire-independent TSA-associated genes than Aire-dependent TSA-associated genes or all expressed genes (Supplemental Fig. 3E, 3F, Supplemental Table I). In addition, Prdm1 and Aire did not correlate with each other (rho = 0.07, p = 0.162). Together, these data suggest that Prdm1+ mTECs represent a unique subset of mTECs important in the prevention of autoimmune.
FIGURE 5. Transplantation of Prdm1 cKO<sup>x14</sup> thymic stroma leads to autoimmunity. Representative flow cytometry analysis of blood CD4 and CD8 T cells in WT and Nude mice prior to transplantation (A) or 6 wk after grafting of control or Prdm1 cKO<sup>x14</sup> embryonic (E15) thymus lobes under the kidney capsule (B). (C) Analysis of CD4 and CD8 T cell development in the grafted thymic epithelial tissue after 5 mo revealed T cell profiles similar to those in WT thymus. (D) Quantification of percentage of T cell subsets between control and Prdm1 cKO<sup>x14</sup> grafts. Each bar represents three or more independently analyzed thymic grafts harvested from a single host. (E) Compared with serum isolated from control host mice, Prdm1 cKO<sup>x14</sup> host serum contains ANAs as indicated by positive staining of HEp2 cells. Representative images from sera derived from one host, engrafted with thymi from control, or two hosts engrafted with thymi from Prdm1 cKO<sup>x14</sup> mice. Scale bars, 50 μm. (F) The presence of ANAs is more frequent in Prdm1 cKO<sup>x14</sup> host mice compared with control. (G) Homogenous and speckled nuclear staining patterns were observed from sera isolated from mice engrafted with thymi from Prdm1 cKO<sup>x14</sup> mice. (H) ELISA of serum Ab isotypes after 5 wk of engraftment. Note the increase in IgG2b and IgG1 in Prdm1 cKO<sup>x14</sup> serum compared with control serum. Data are mean. n = 4 grafted mice for each genotype of transplanted thymi. *p < 0.05.
corepressor proteins such as the Groucho family that function in part through association with HDACs (12, 67). Prdm1 has been shown to interact with several chromatin-modifying proteins, for example, G9a lysine methyltransferase (10), HDAC1/2 (13), protein arginine methyltransferase 5 (68), and lysine deacetylase 1 (69). Thus, it seems possible that PRDI-BF1 might act as a DNA-binding scaffold protein capable of directly recruiting multiple chromatin-modifying enzymes to targeted promoters (10). Interestingly, Prdm1 is associated with HDAC5 expression in individual mTECs, and although further validation of this interaction remains an opportunity for future study, this may provide an additional epigenetic mechanism for regulation of TSAs and a novel interaction for Prdm1.

Furthermore, although Prdm1 is most commonly known as a transcriptional repressor, it has been identified in transcriptional activation (16, 70). Therefore, it is possible that Prdm1 could be directly promoting TSA expression; the exact mechanism by which Prdm1 controls TEC function will be an interesting area of future study.

Mice lacking Prdm1 in epithelial cells develop chronic skin inflammation, which is thought to be due to defects in epidermal barrier immunity (31, 32). Until now, these observations have precluded the analysis of Prdm1’s role in the thymic epithelium. We find that Prdm1 is expressed within the medullary thymic epithelium and Hassall’s corpuscles, which are thought to represent the terminally differentiated epithelial compartment within the thymus (55). This is consistent with Prdm1’s expression in the most differentiated layers of the skin epidermis (26). Although the function of Hassall’s corpuscles has not been fully explored, they are thought to be involved in removing dead thymocytes, maturation of medullary thymocytes (71, 72), and education of dendritic cells during Treg development (73). Because thymus tissue from immunodeficient patients lacks Hassall’s corpuscles (74, 75), it is exciting to speculate that these structures play a role in establishing tolerance. It will be interesting to determine whether Prdm1 could be a therapeutic target to maintain thymic function in these patients.

Intriguingly, Prdm1 does not affect mTEC development, as indicated by its expression within the most differentiated cell types within the thymus and by the absence of a defect in the epithelial compartment of the Prdm1 cKO14 and Prdm1 cKOfoxN1 thymus. Together with the absence of an identified association with HDAC3 in the global analysis of gene expression, these data suggest that Prdm1 works independently of HDAC3, which has recently been reported to be a master regulator of mTEC development and important for epigenetic regulation of Prdm1 in B lymphocytes (76, 77).

Slightly different phenotypes are observed between the Prdm1 cKO14 and cKOfoxN1 mouse models. Although the reasons for these differences remain unclear, it is important to note that the timing and pattern of expression of Cre in the K14Cre mouse is not well defined, despite both strains being frequently used as models to delete genes from total thymic epithelium. Furthermore, FoxN1 is comprised of eight coding exons and utilizes two alternative first exons in a tissue-specific fashion (78, 79); therefore, it is conceivable that FoxN1cre activity remains absent from the skin after birth (80), whereas it is possible that the K14cre is active within the skin.

Importantly, Prdm1 cKO14 thymus transplantation demonstrates that Prdm1 deficiency in thymic epithelium is sufficient to elicit a breakdown in thymic tolerance because this model enabled the role of Prdm1 in thymic epithelium to be separated from its known role within skin epidermis (27, 32). With functional Prdm1 in the epidermis, hosts that received Prdm1-deficient thymus lobes developed ANAs. However, it was intriguing that, despite this, other phenotypes observed in the cKO14, such as cervical lymphadenopathy, were not observed. Perhaps this could be attributable to a previously undetermined role for Prdm1 in other barrier epithelial tissues, promoting their functional integrity and homeostasis, such as the tongue and esophagus, where the activity of the K14 promoter has been identified (81); further investigation would be required to clarify this. In addition, impaired containment of the commensal microflora at these sites could explain the local inflammation resulting in enlarged cervical lymph nodes.

Because it is known that Prdm1 negatively regulates the expression of CIITA, a transcription factor that regulates MHCII gene expression (82), and that TSA expression is dependent on MHC expression levels, it was critical to check the protein and mRNA expression levels of MHCII; these did not differ between control and Prdm1 cKO14 mice, ruling out a dysregulation of MHCII in the autoimmune phenotype presented by these mice. In addition, it was important to decipher whether Prdm1 was an Aire-regulated gene. To do this, we analyzed mTECs from both WT and Aire KO for Prdm1 expression, which was detected at similar levels in both WT and Aire KO mTECs (Supplemental Fig. 3F), implying that Prdm1 is not an Aire-dependent gene.

Despite the ability of mTECs and Hassall’s corpuscles to direct Treg development (4, 56–58, 73), loss of Prdm1 in epithelial cells did not alter Treg development or export to the periphery because comparable numbers within both the thymus and the peripheral lymph nodes were identified in control and Prdm1 cKO14 and Prdm1 cKOfoxN1 mice. This suggests that the autoimmune phenotype that presented in Prdm1 cKO mice was not due to an absence of immune regulation and that Prdm1 null mTECs are unable to efficiently delete self-reactive T cells.

Interestingly, polymorphisms in Prdm1 are associated with autoimmune diseases, such as SLE (33, 34). Importantly, dermatitis (83, 84), hypergammaglobulinemia, and kidney immune complex deposition are frequently used as diagnostic markers for SLE. Furthermore, ANAs (50), particularly the homogenous and speckled patterns, are prevalent within patients with SLE (85). Importantly, we show that Prdm1 cKO14 mice develop SLE-associated symptoms, including ANAs, hypergammaglobulinemia, and kidney immune complex deposition, suggestive of glomerulonephritis (33, 83, 84, 86), and previous studies reported the dermatitis using the K14-Cre and K5-Cre mouse models (31, 32). Together, these data raise the interesting possibility that SLE may be driven by a breakdown in thymic tolerance, and the data are consistent with a role for Prdm1 in SLE. Of relevance, we show that the autoantibodies produced in Prdm1 cKO14 and Prdm1 cKOfoxN1 mice are the T cell-dependent isotypes, IgG2b and IgG1 (60). This highlights the requirement of T cell help for Ab production in this model, consistent with a T cell component in activating autoreactive B cells in SLE.

Collectively, our findings show that despite Prdm1 being expressed within the medullary thymic epithelium, particularly the Hassall’s corpuscles, it does not regulate mTEC development and does not alter T cell development including intrathymic Treg development. The absence of Prdm1 from the thymic epithelium does, however, result in an autoimmune-like phenotype that manifests as the presentation of ANAs and hypergammaglobulinemia that is transferable by transplantation of Prdm1 cKO thymus lobes into Nude hosts, uncoupling the role of Prdm1 in the skin epithelium from being the sole reason for the phenotype presented by cKO mice. Furthermore, our data present the possibility that Prdm1 is involved in the regulation of Aire-independent TSA expression within the thymus, thus adding another dimension to the association of Prdm1 mutations with autoimmune disorders such as SLE (33), rheumatoid arthritis (87), and Crohn’s disease (88).
Disclosures
The authors have no financial conflicts of interest.

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