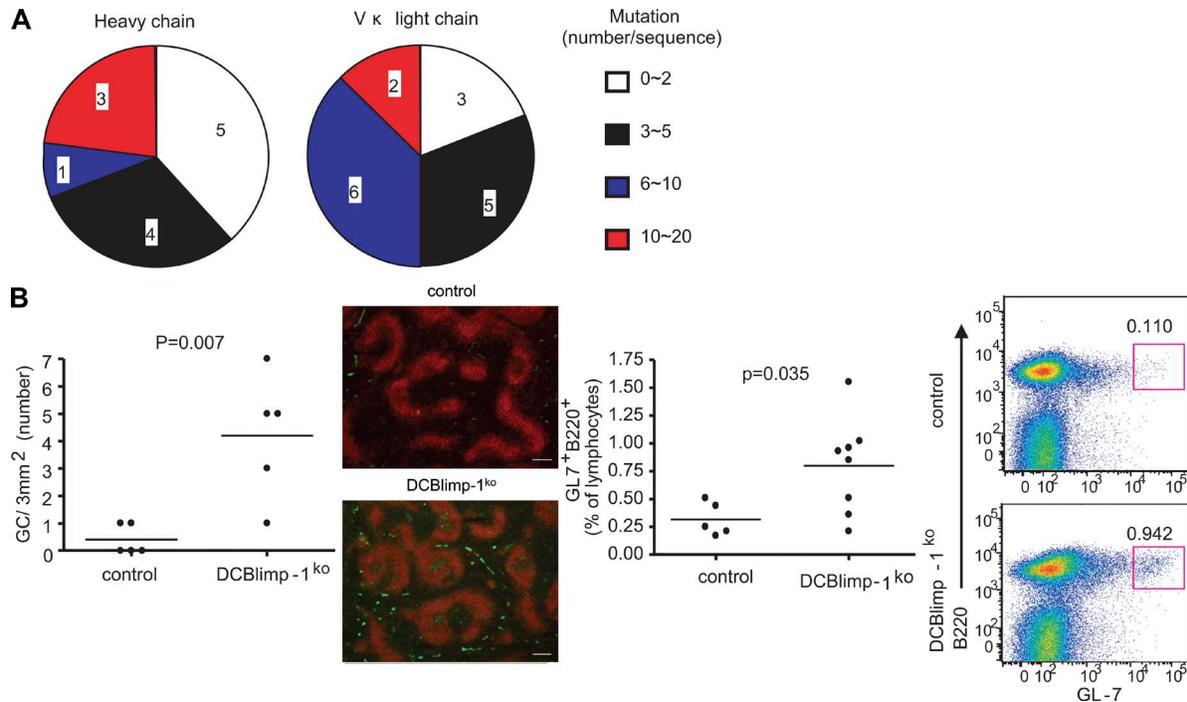


## Tolerogenic function of Blimp-1 in dendritic cells

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The authors regret that they included incorrect immunohistochemistry images in the original Fig. 3 B. The original Fig. 3 legend also lacked information defining the stains represented by red and green colors in Fig. 3 B. These errors have been corrected in the html and pdf versions of this paper. The corrected figure and legend are below.



**Figure 3. Characterization of ANA IgG and GC response.** (A) Sequence analysis of ANA IgG from hybridomas. Hybridomas were generated by splenocytes of 4-mo-old DCBlimp-1<sup>ko</sup> mice. Total heavy and light chains of ANA-positive IgG were amplified and sequenced. Mutations were determined by comparison with the mouse genomic sequence database. Numbers in each pie graph represent the number of clones categorized by the number of mutations ( $n = 4$ ). (B) Spontaneous GC formation in the spleen of 6–10-wk-old control and DCBlimp-1<sup>ko</sup> mice. On the left, GCs were analyzed by immunohistochemistry using FITC-PNA (green) and PE-conjugated anti-B220 (red). Graph shows quantification of GCs in the spleens of mice. Representative IHC images are shown (bars, 260  $\mu$ m). On the right, GL-7<sup>+</sup>B220<sup>+</sup> GC B cells were quantified by flow cytometry as depicted in representative dot plots. Each dot represents an individual mouse and horizontal bars indicate means of three independent experiments.

# Tolerogenic function of Blimp-1 in dendritic cells

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**Blimp-1 has been identified as a key regulator of plasma cell differentiation in B cells and effector/memory function in T cells. We demonstrate that Blimp-1 in dendritic cells (DCs) is required to maintain immune tolerance in female but not male mice. Female mice lacking Blimp-1 expression in DCs (DCBlimp-1<sup>ko</sup>) or haploid for Blimp-1 expression exhibit normal DC development but an altered DC function and develop lupus-like autoantibodies. Although DCs have been implicated in the pathogenesis of lupus, a defect in DC function has not previously been shown to initiate the disease process. Blimp-1<sup>ko</sup> DCs display increased production of IL-6 and preferentially induce differentiation of follicular T helper cells (T<sub>FH</sub> cells) in vitro. In vivo, the expansion of T<sub>FH</sub> cells is associated with an enhanced germinal center (GC) response and the development of autoreactivity. These studies demonstrate a critical role for Blimp-1 in the tolerogenic function of DCs and show that a diminished expression of Blimp-1 in DCs can result in aberrant activation of the adaptive immune system with the development of a lupus-like serology in a gender-specific manner. This study is of particular interest because a polymorphism of Blimp-1 associates with SLE.**

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Abbreviations used: ANA, anti-nuclear antibody; BM-DC, BM-derived DC; cDC, conventional DC; GC, germinal center; ICOS, inducible co-stimulator; IHC, immunohistochemistry; pDC, plasmacytoid DC; SLE, systemic lupus erythematosus.

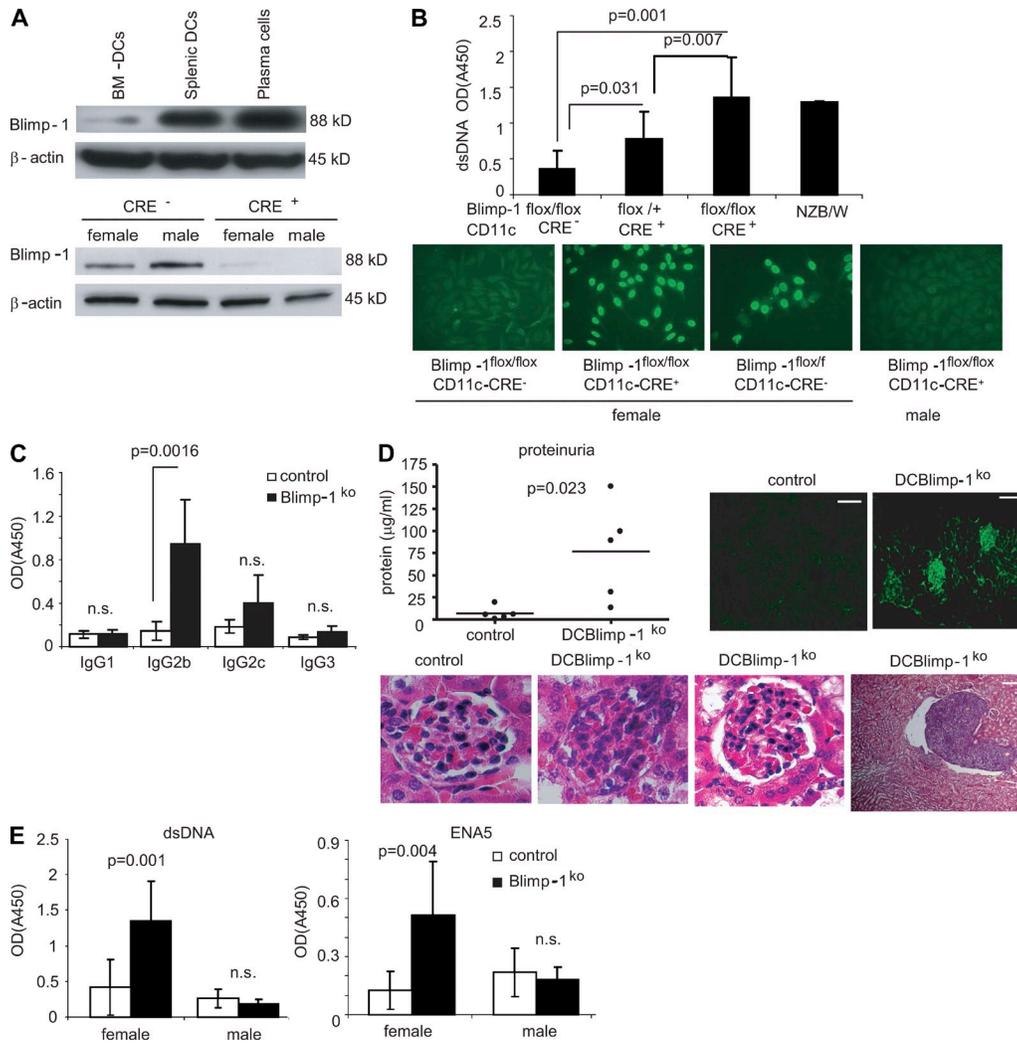
Tolerance to self-antigens is a key feature of the immune system. Systemic autoimmune diseases, such as systemic lupus erythematosus (SLE), result from dysregulation of B and T cell activation and altered function of macrophages and DCs (Shlomchik, 2009), leading to pathogenic autoantibodies, which are IgG isotype switched, high affinity to self-antigens, and somatically hypermutated. (Rothfield and Stollar, 1967; Diamond and Scharff, 1984). Although B cells are the proximal cells in the phenotypic manifestations of SLE, interactions with other immune cell types are dynamically involved (MacLennan, 1994; Shlomchik et al., 2001; Craft, 2011).

Recently, a polymorphism of Blimp-1 has been identified as a risk factor in SLE by genome-wide association studies, suggesting a critical function of Blimp-1 in SLE (Gateva et al., 2009; Han et al., 2009). Blimp-1 negatively regulates expression of IFN- $\beta$  in both humans and mice (Keller and Maniatis, 1991; Turner et al., 1994). In B cells, Blimp-1 is a key regulator of plasma cell development (Shapiro-Shelef et al., 2003). In T cells, Blimp-1 regulates the differentiation of T<sub>H</sub>1 and the function of regulatory T cells (Martins et al., 2006). Blimp-1 was suggested to be a survival factor in monocytes (Chang et al., 2000); however, a

more recent study using a Tie2-CRE system has suggested that Blimp-1 may regulate the differentiation and activation of DCs (Chan et al., 2009). In that study, IL-6 and MCP-1 were shown to be direct targets of Blimp-1 and the deletion of Blimp-1 resulted in increased expression of proinflammatory cytokines. The physiological importance of Blimp-1 specifically in DCs, however, could not be addressed in this mouse model in which Blimp-1 was deleted in all hematopoietic cell lineages.

DCs are important in lupus pathogenesis, although a primary defect in DC function has not been reported. Because DCs were discovered by Steinman and Cohn (1973), they have been recognized as the key immune-regulating cells. DCs can mediate both immune tolerance and immune activation (Cools et al., 2007). DCs can acquire tolerogenic phenotype after phagocytosis of apoptotic cells (Qiu et al., 2009). They can also generate regulatory T (T<sub>reg</sub>) cells or cause immune suppression by secretion of cytokines (Yamazaki

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**Figure 1. Deletion of Blimp-1 in DCs and phenotype of DCBlimp-1<sup>ko</sup> mice.** (A) Expression of Blimp-1 in DCs and plasma cells by Western blotting. Cell lysates from 5 × 10<sup>5</sup> BM-DCs, splenic DCs, and plasma cells of control mice were loaded (top). DCs from spleens of control and DCBlimp-1<sup>ko</sup> mice were used. The level of Blimp-1 was compared in splenic DCs from control (CRE<sup>-</sup>) and Blimp-1<sup>ko</sup> (CRE<sup>+</sup>) female and male mice. Representative data from three independent experiments are shown. (B) Anti-dsDNA IgG in serum of control and DCBlimp-1<sup>ko</sup> mice by ELISA. Mean ± SD of three independent experiments is shown. *n* = 8. The bottom shows representative pictures of ANA. (C) Isotype-specific anti-dsDNA ELISA. Mean ± SD of three independent experiments is shown. *n* = 7. (D) 24-h urine samples were collected from 8–10-mo-old control and DCBlimp-1<sup>ko</sup> mice and protein was measured. IgG deposition and histology of kidneys from 8-mo-old control and DCBlimp-1<sup>ko</sup> mice. Pictures are representative of each strain. Bars: (IgG) 20 µm; (histology) 40 µm. Horizontal bars indicate mean. (E) Anti-dsDNA and anti-ENA5 IgG in serum from 6–8-mo-old control and DCBlimp-1<sup>ko</sup> female and male mice by ELISA. Mean ± SD of three independent experiments is shown. *n* > 10.

et al., 2008). Although differentiation of DCs into immunogenic or tolerogenic DCs has not been fully characterized, it is generally accepted that DC maturation status rather than DC lineage alone determines the functionality of DCs (Cools et al., 2007).

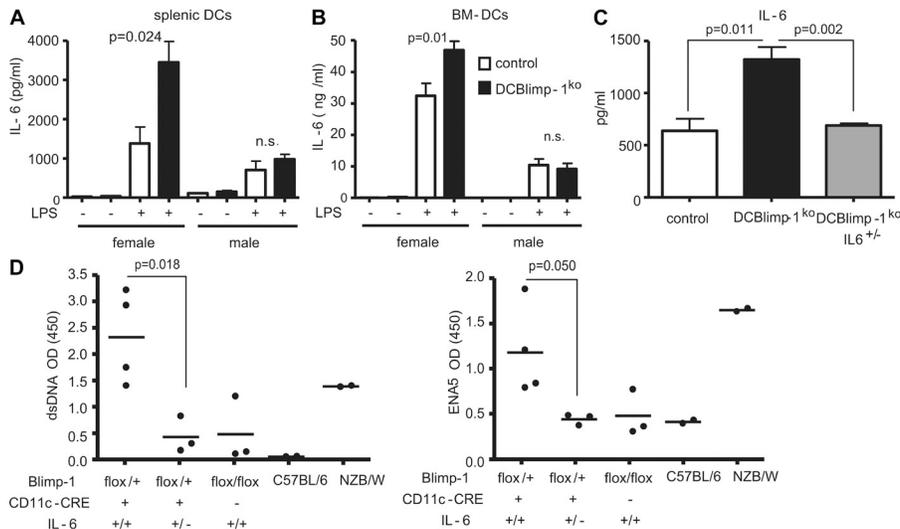
In this study, DCBlimp-1<sup>ko</sup> mice were generated by mating Blimp-1<sup>flox</sup> mice to CD11c-CRE<sup>+</sup> mice to identify the in vivo consequences of Blimp-1 deficiency to DC function. Female, but not male, DCBlimp-1<sup>ko</sup> mice developed autoantibodies with extensive mutations, suggesting their maturation in a germinal center (GC) response. Consistent with this observation, female mice display increased GC formation in the basal state and after immunization, accompanied by an increased frequency of T<sub>FH</sub> cells. Finally, DCs from female DCBlimp-1<sup>ko</sup>

mice produced increased IL-6 and preferentially induced differentiation of T<sub>FH</sub> cells. All aspects of the phenotype were abolished in DCBlimp-1<sup>ko</sup> mice haploid for IL-6. Together, these observations suggest that a defect restricted to DCs can alter T cell differentiation resulting in the production of high titers of lupus-like autoantibodies in a gender-specific fashion.

## RESULTS AND DISCUSSION

### Gender-dependent development of autoantibodies in DCBlimp-1<sup>ko</sup> mice

Blimp-1 expression was measured by Western blotting (Fig. 1 A). CD11c<sup>hi</sup> DCs were purified from spleens of age-matched DCBlimp-1<sup>ko</sup> (Blimp-1<sup>flox/flox</sup>, CD11c-CRE<sup>+</sup>) and control



**Figure 2. Increased expression of IL-6 in Blimp-1<sup>ko</sup> DCs from female mice and IL-6-dependent autoantibody generation.**

Splenic DCs (A) and BM-DCs (B) were prepared as described in Materials and methods and cultured with or without LPS stimulation. IL-6 in the supernatant was measured by ELISA. Mean  $\pm$  SD of three independent experiments is shown.  $n = 6$ . (C) IL-6 secretion by purified splenic DCs of control, DCBlimp-1<sup>ko</sup>, and IL-6<sup>+/-</sup> DCBlimp-1<sup>ko</sup>. Mean  $\pm$  SD of three independent experiments is shown.  $n = 5$ . (D) Serum from 4-mo-old mice was obtained, and the level of dsDNA and ENA5 IgG was measured as described in Materials and methods. Each dot represents an individual mouse and horizontal bars indicate mean.

(either Blimp-1<sup>+/+</sup>; CD11c-CRE<sup>+</sup> or Blimp-1<sup>flox/flox</sup>; CD11c-CRE<sup>-</sup>) mice. Splenic DC expression of Blimp-1 in DCBlimp-1<sup>ko</sup> mice was barely detectable compared with control DCs in both female and male. DCs from Blimp-1<sup>flox/+</sup>; CRE<sup>+</sup> mice displayed an intermediate level of expression. Deletion of Blimp-1 is DC specific because the Blimp-1 level was unaffected in B cells, NK cells, macrophages, and  $\alpha$ -CD3/CD28-activated T cells (Fig. S1 A).

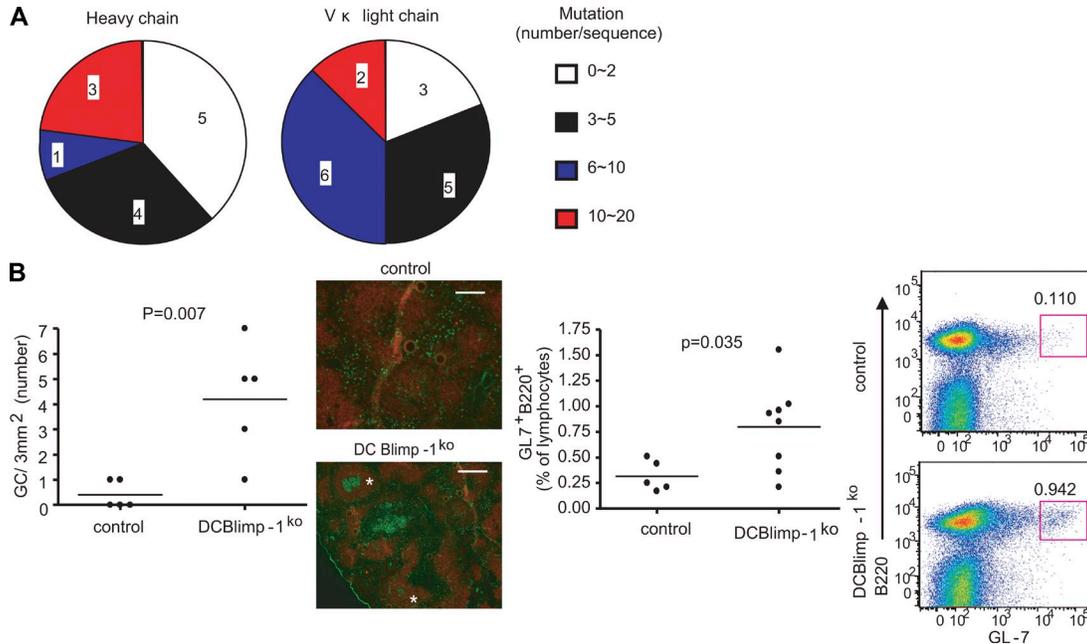
Consistent with a previous study (Chan et al., 2009), development of DCs was normal in both conventional DCs (cDCs; CD11c<sup>hi</sup>Siglec-H<sup>-</sup>) and plasmacytoid DCs (pDCs; CD11c<sup>lo</sup>Siglec-H<sup>+</sup>) in the spleen or in BM-derived DCs (BM-DCs; Fig. S1 B). However, female DCBlimp-1<sup>ko</sup> mice developed autoantibodies as early as 4–5 mo of age. Sera from age-matched female DCBlimp-1<sup>ko</sup> mice and control mice were assayed for anti-nuclear antibody (ANA), anti-double-stranded (ds) DNA, and anti-ENA5 by ELISA. All the ANA-positive immunoglobulin was IgG; IgM ANA was negligible (Fig. 1 B and not depicted). Moreover, sera from DCBlimp-1<sup>ko</sup> mice displayed IgG reactivity to both dsDNA and ENA5 (Fig. 1 E). Mice haploid for Blimp-1 also displayed IgG reactivity to dsDNA antibodies. An isotype-specific ELISA demonstrated that IgG2b is the major isotype of anti-dsDNA antibodies (Fig. 1 C). Proteinuria was developed, and kidney deposition of IgG and mesangial cell proliferation and inflammatory infiltrates were observed in 10-mo-old DCBlimp-1<sup>ko</sup> mice (Fig. 1 D). In contrast to the results from female mice, serum from male DCBlimp-1<sup>ko</sup> mice displayed no anti-dsDNA or ENA5 reactivity (Fig. 1 E). Similarly, total serum immunoglobulin levels and number of splenocytes are significantly increased in female but not in male DCBlimp-1<sup>ko</sup> mice (Fig. S2 A and Table S1). The gender disparity in DCBlimp-1<sup>ko</sup> mice is interesting because most lupus mouse models demonstrate a lupus-like phenotype in both genders. Overall, these data suggest that expression of Blimp-1 in DCs plays a critical role in tolerance against self-reactivity. Moreover, haploinsufficient expression is not sufficient for immune tolerance,

and, most surprisingly, downstream effects of Blimp-1 deficiency are gender specific.

#### Increased expression of IL-6 in Blimp-1<sup>ko</sup> DCs

To understand the alterations in Blimp-1<sup>ko</sup> DCs responsible for the generation of autoantibodies, we analyzed the characteristics of the DCs. Because the expression of IL-6 is regulated by Blimp-1 (Chan et al., 2009) and enhanced expression of IL-6 is associated with SLE, and possibly related to DC activation (Colonna et al., 2006; Jeon et al., 2010), we examined IL-6 production. We observed an increased production of IL-6 by splenic Blimp-1<sup>ko</sup> DCs compared with control DCs (Fig. 2 A) and by BM-DCs after LPS stimulation (Fig. 2 B). In male mice, however, there was no significant difference in the level of IL-6 produced by either splenic DCs or BM-DCs from control or DCBlimp-1<sup>ko</sup> mice (Fig. 2, A and B). In fact, production of IL-6 was higher in female than in male control DCs, implicating a sex difference in cytokine production even in wild type DCs. This observation is consistent with data in patients showing an association of an estrogen-sensitive polymorphism of the IL-6 promoter with susceptibility to type I diabetes in women (Kristiansen et al., 2003). The level of expression of several genes also increased in Blimp-1<sup>ko</sup> DCs as measured by quantitative (q) PCR (Table S2). Bcl-6, a molecule negatively regulated by Blimp-1, was up-regulated in Blimp-1<sup>ko</sup> DCs. Expression of XBP, which has been demonstrated to be a survival factor for DCs (Iwakoshi et al., 2007), was equivalent in control and Blimp-1<sup>ko</sup> DCs, supporting our observation that activation not development is affected by Blimp-1 deficiency.

IL-6 overexpression was of particular interest because IL-6 has been shown to affect several B cell functions, including GC formation and antibody secretion by plasma cells (Kopf et al., 1998; Cassese et al., 2003), and to participate in the differentiation of T<sub>H</sub> cells (Nurieva et al., 2009). To test the importance of increased IL-6 in autoantibody production in DCBlimp-1<sup>ko</sup> mice, we generated DCBlimp-1<sup>ko</sup> mice



**Figure 3. Characterization of ANA IgG and GC response.** (A) Sequence analysis of ANA IgG from hybridomas. Hybridomas were generated by splenocytes of 4-mo-old DCBlimp-1<sup>ko</sup> mice. Total heavy and light chain of ANA-positive IgG was amplified and sequenced. Mutations were determined by comparison with the mouse genomic sequence database. Numbers in each pie graph represent the number of clones categorized by the number of mutation ( $n = 4$ ). (B) Spontaneous GC formation in the spleen of 6–10-wk-old DCBlimp-1<sup>ko</sup> mice. GC (PNA<sup>+</sup> B220<sup>+</sup>, asterisks) was analyzed by IHC. Pictures are representative images (bars, 100  $\mu$ m). On the right, GL-7<sup>+</sup>B220<sup>+</sup> GC B cells were quantified by flow cytometry as depicted in representative pictures. Each dot represents an individual mouse and horizontal bars indicate means of three independent experiments.

haploid for IL-6 (IL-6<sup>+/-</sup> DCBlimp-1<sup>ko</sup>). DCs from IL-6<sup>+/-</sup> DCBlimp-1<sup>ko</sup> mice express the same level of IL-6 as DCs from control mice after LPS stimulation (Fig. 2 C). Immunization of control and IL-6<sup>+/-</sup> DCBlimp-1<sup>ko</sup> mice with NP-CGG showed that IL-6<sup>+/-</sup> DCBlimp-1<sup>ko</sup> mice mount an antibody response that is indistinguishable from that of control mice (Fig. S3 A), demonstrating that B cells from IL-6<sup>+/-</sup> DCBlimp-1<sup>ko</sup> mice are not defective in antibody production or affinity maturation. Although IL-6<sup>+/-</sup> DCBlimp-1<sup>ko</sup> mice had a normal antibody response to immunization, they did not develop autoantibodies (Fig. 2 D). These data suggest that the increased expression of IL-6 by DCs contributes to the generation of autoantibodies in female DCBlimp-1<sup>ko</sup> mice.

### Enhanced GC formation in DCBlimp-1<sup>ko</sup> mice

Because we observed only IgG, and not IgM, autoantibodies, we asked whether the autoantibodies were derived from GC-experienced B cells. We generated hybridomas of splenocytes from 4-mo-old DCBlimp-1<sup>ko</sup> mice. There were 27 ANA-positive clones from 304 IgG-secreting clones (~10%). From the 27 clones, 13 and 16 clones were successfully sequenced for heavy and light chain, respectively. Sequence analysis revealed a high incidence of mutation in most clones (Fig. 3 A). Interestingly, 3 out of 13 clones contained an arginine residue acquired by point mutation in the complementary determining region 3 in the heavy chain, which is often seen in high-affinity anti-DNA antibodies. These data suggest that autoantibodies are produced by GC-matured plasma cells.

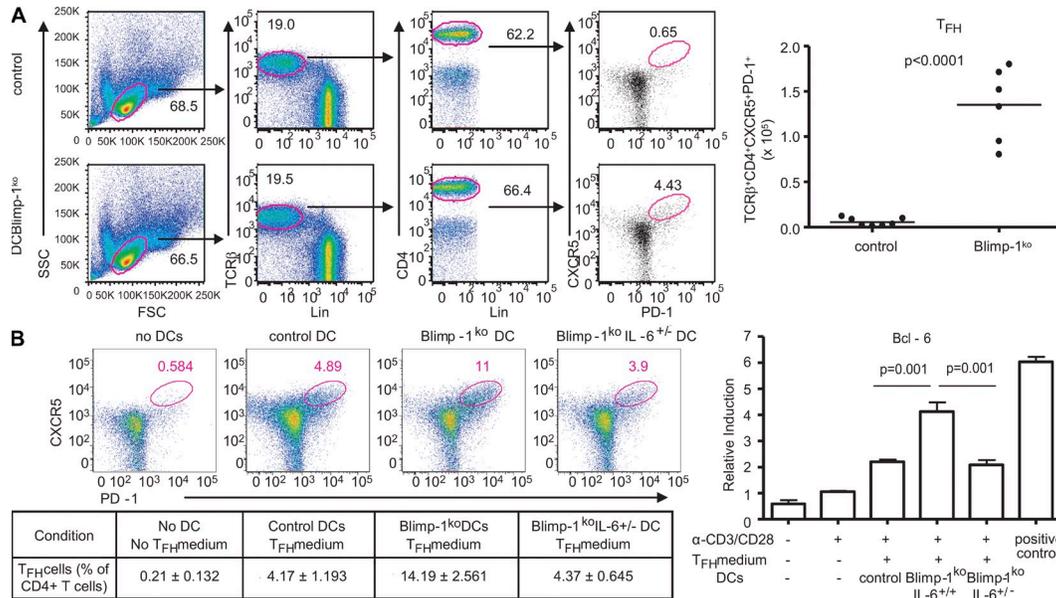
Flow cytometry and immunohistochemistry (IHC) demonstrated an enhanced GC response in young mice (8–12 wk old); many more spontaneous GC B cells, as well as GCs in spleens, were present in DCBlimp-1<sup>ko</sup> mice (Fig. 3 B). In contrast to the response in IL-6<sup>+/-</sup> DCBlimp-1<sup>ko</sup> mice, there was an enhanced immune response in DCBlimp-1<sup>ko</sup> mice after NP-CGG immunization with an increased high-affinity anti-NP IgG response (Fig. S3 B). There was an increased number of GC B cells (Fig. S3 C) and an increased number of total GCs (Fig. S3 D) as well as antigen-specific GCs (7/12  $\lambda^+$  GCs in DCBlimp-1<sup>ko</sup> mice and 1/3  $\lambda^+$  GCs in control mice).

### Blimp-1<sup>ko</sup> DCs induce expansion of T<sub>FH</sub> cells in vivo and in vitro

T<sub>FH</sub> cells in GCs provide direct help to antigen-specific B cells (Garside et al., 1998). The importance of tight regulation of expression of the costimulatory molecule inducible co-stimulator (ICOS) on CD4<sup>+</sup> T<sub>FH</sub> cells has been demonstrated in studies of lupus-prone sanroque mice (Vinueza et al., 2005); moreover, ICOS blockade inhibits lupus in NZB/W F<sub>1</sub> mice (Hu et al., 2009).

To address whether there were more activated CD4<sup>+</sup> T cells in DCBlimp-1<sup>ko</sup> mice, we measured ICOS expression on T cells. There was increased expression of ICOS in CD4<sup>+</sup> T cells from DCBlimp-1<sup>ko</sup> mice compared with control mice (Fig. S4 A). In addition, the percentage and the number of T<sub>FH</sub> cells were also increased in DCBlimp-1<sup>ko</sup> mice (Fig. 4 A and Fig. S4 B).

Several studies have suggested that specific subsets of DCs preferentially induce different helper T cells (Maldonado-López



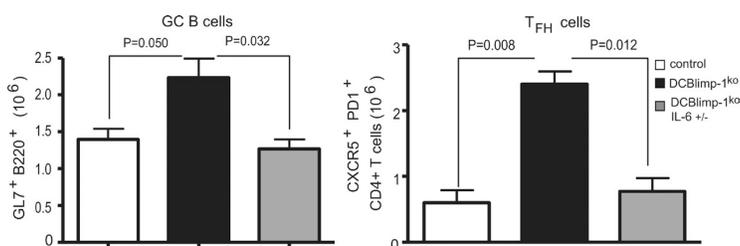
**Figure 4. Increased T<sub>FH</sub> cells in DCBlimp-1<sup>ko</sup> mice in vivo and in vitro.** (A) 6–10-wk-old control and DCBlimp-1<sup>ko</sup> mice were sacrificed and T<sub>FH</sub> (Lin (B220/CD11b/Gr-1)<sup>-</sup>, TCR-β/CD4/CXCR5/PD-1<sup>+</sup>) were analyzed by flow cytometry. Total T<sub>FH</sub> cell number was calculated and graphed on the right. Each dot represents an individual mouse and horizontal bars indicate means of three independent experiments. (B) In vitro differentiation of T<sub>FH</sub> cells. Naive CD4<sup>+</sup> T cells and splenic DCs were co-cultured and activated as described in Materials and methods. After 4 d, cells were harvested and the frequency of T<sub>FH</sub> cells was analyzed by flow cytometry. The top panel displays representative flow cytometry and the bottom is a summarized table (five independent experiments, n = 9). Total RNA was prepared from purified CD4<sup>+</sup> T cells from each culture conditions, and the Bcl-6 level was measured by qPCR. The level of Bcl-6 of in vivo T<sub>FH</sub> cells (T<sub>FH</sub> cells sorted 12 d after NP immunization) was used as a positive control. Mean ± SD of three independent experiments is shown (n = 6).

et al., 1999). We reasoned that the increased number of T<sub>FH</sub> was dependent on DC function in DCBlimp-1<sup>ko</sup> mice; therefore, we assayed to assess in vitro differentiation of T<sub>FH</sub> cell with purified DCs. To avoid the possibility of indirect effects on T cells from DCBlimp-1<sup>ko</sup> mice, we used naive T cells from control mice. Naive T cells were cultured with DCs from either control or DCBlimp-1<sup>ko</sup> mice in the presence of differentiation factors. Fig. 4 B shows that the generation of T<sub>FH</sub> cells was highly enhanced in cultures with Blimp-1<sup>ko</sup> DCs compared with control DCs. We confirmed the phenotype of T<sub>FH</sub> cells by demonstrating expression of Bcl-6, which is a principle transcription factor for T<sub>FH</sub> differentiation and is expressed exclusively in T<sub>FH</sub>. To test whether the increased T<sub>FH</sub> was a result of the increased level of IL-6 from DCs, DCs from IL-6<sup>+/-</sup> Blimp-1<sup>ko</sup> mice were assayed in culture. There was no increased differentiation of T<sub>FH</sub> in the presence of DCs from IL-6<sup>+/-</sup> Blimp-1<sup>ko</sup> mice, suggesting a direct involvement of IL-6 from DCs in T<sub>FH</sub> differentiation (Fig. 4 B).

The importance of IL-6 was further demonstrated in vitro as α-IL-6 neutralizing antibody inhibited the differentiation of T cells co-cultured with control DCs and Blimp-1<sup>ko</sup> DCs into T<sub>FH</sub> (Fig. S5). These observations suggest that the increased generation of T<sub>FH</sub> cells results from increased IL-6 production by DCs in DCBlimp-1<sup>ko</sup> mice. Interestingly, there was no significant increase in T<sub>H</sub>17 cells, another subset for which IL-6 is critical (unpublished data), suggesting that additional requirements for the generation of T<sub>H</sub>17 must exist and are not provided by Blimp-1<sup>ko</sup> DCs.

#### IL-6-dependent generation of GC B cells and T<sub>FH</sub> cells

Because increased T<sub>FH</sub> cells and GC response are important mechanisms for autoantibody production in DCBlimp-1<sup>ko</sup> mice and the phenotype was impaired in IL-6<sup>+/-</sup> DCBlimp-1<sup>ko</sup> mice, we compared GC and T<sub>FH</sub> cells in IL-6<sup>+/-</sup> DCBlimp-1<sup>ko</sup> mice. IL-6<sup>+/-</sup> DCBlimp-1<sup>fllox/+</sup> mice showed a reduced number of T<sub>FH</sub> cells as well as a reduced number of GC cells in the spleen (Fig. 5). These data suggest that the increased expression of IL-6 in Blimp-1<sup>ko</sup> DCs is a



**Figure 5. Decreased GC and T<sub>FH</sub> cells in IL-6<sup>+/-</sup> DCBlimp-1<sup>ko</sup> mice.** Spleens were harvested from 6–8-wk-old mice, and GC B cells and T<sub>FH</sub> cells were enumerated by flow cytometry. Number of cells was calculated as percentage of positive cells × total splenocytes. Mean ± SD of three independent experiments is shown (n = 5).

major molecular mechanism responsible for the expansion of  $T_{FH}$  cells and enhanced GC formation, leading to the generation of autoantibodies in DCBlimp-1<sup>ko</sup> mice.

In summary, we propose a new mechanism for the development of a lupus-like phenotype mediated by Blimp-1, which is required to maintain tolerogenic function in DCs in a gender-dependent manner. The loss of function of Blimp-1 in female DCs results in increased secretion of the critical proinflammatory cytokine IL-6, and in increased differentiation of  $T_{FH}$  cells and increased GC responses. It is of considerable interest that a polymorphism of the Blimp-1 gene has now been implicated in both rheumatoid arthritis and SLE and that the phenotype of the DCBlimp-1<sup>ko</sup> mouse is analogous to human SLE with a female bias, enhanced  $T_{FH}$  cells, and increased IL-6 levels (Swaak et al., 1989).

## MATERIALS AND METHODS

**Mice.** Blimp-1<sup>fllox</sup> mice were provided by K. Calame (Columbia University, NY, NY) and backcrossed with C57BL/6 for eight generations. CD11c-CRE mice were generated in the Reizis laboratory. DCBlimp-1<sup>ko</sup> mice and control mice were bred in the animal facility of The Feinstein Institution for Medical Research (FIMR) in specific pathogen-free conditions. IL-6<sup>ko</sup> mice (The Jackson Laboratory) were bred with DCBlimp-1<sup>ko</sup> mice in the animal facility of FIMR.

**Purification of splenic DCs and in vitro generation of BM-DCs.** CD11c<sup>+</sup> splenic DCs were enriched with an EasySep kit (STEMCELL technologies) according to the manufacturer's protocol. Then, CD11c<sup>hi</sup> Siglec-H<sup>-</sup> DCs were further purified by cell sorter (FACSaria; BD). Cell purity was routinely >95%.

To generate BM-DCs, BM cells were harvested from the femur with PBS. T cells and B cells were depleted by incubation with antibodies from hybridoma cell lines (American Type Culture Collection; TIB-120, TIB-211, TIB 207, and TIB-146) with rabbit complement (Pel-Freeze Biologicals). The remaining cells were cultured in RPMI 1640 with 10% FCS and 200 ng/ml Flt3L (PeproTech) for 8 d. The nonadherent cells were collected. To measure cytokines in the supernatants, 10<sup>6</sup> DCs/ml were cultured overnight in medium with or without 1  $\mu$ g/ml LPS (Sigma-Aldrich).

**ELISAs.** Anti-dsDNA and anti-ENA5 (Sm, RNP, SS-A, SS-B, and Scl-70) antibodies were measured by a QUANTA Lite ELISA kit (INOVA Diagnostics). Assays were performed as described in the protocol provided by manufacturer. In brief, serum samples were diluted in sample diluent at 1:101 and incubated in antigen-precoated plates for 30 min at room temperature (rt). Horse radish peroxidase (HRP)-conjugated isotype-specific anti-mouse IgG (1:2,000 SouthernBiotech) was added for 30 min at rt. Plates were washed after incubation, and TMB substrate was added for development. Absorbance (OD) was read for each well at 450 nm. L-6 ELISAs were performed with specific cytokine kits according to the manufacturer's protocol (BD).

**Histology of spleen and kidney.** Spleens from 6–10-wk-old DCBlimp-1<sup>ko</sup> and control mice were fixed with 4% PFA and transferred to a 30% sucrose solution. The fixed spleens were snap frozen in Tissue-Tek O.C.T. compound (Sakura) and sliced to 7  $\mu$ m. On the day of staining, sections were fixed with ice-cold acetone and blocked with blocking buffer. After blocking, samples were incubated with fluorochrome-conjugated antibodies diluted in dilution buffer for 1 h at room temperature. After incubation with antibodies, slides were washed with PBS three times.

Kidneys were harvested from 8-mo-old mice and fixed with formaldehyde and 70% ethanol. Fixed tissues were paraffin embedded and sliced to 5- $\mu$ m thickness. Tissue was stained with standard hematoxylin and eosin. Images were visualized using a fluorescence microscope (AxioCam II; Carl Zeiss) and analyzed by OpenLab software (PerkinElmer).

**qPCR.** Total RNA was extracted from purified DCs with RNeasy kit (Invitrogen) according to the manufacturer's instructions and subjected to reverse transcription with iScript cDNA synthesis kit (Bio-Rad Laboratories). cDNA was analyzed by qPCR using LightCycler 480 probes master with various primers (Applied Biosystems). Relative induction of each gene of interest was calculated by  $\Delta\Delta C_t$ .

**$T_{FH}$  cell in vitro differentiation.** 10<sup>5</sup> purified DCs (CD11c<sup>hi</sup>) from control and DCBlimp-1<sup>ko</sup> mice and 5  $\times$  10<sup>5</sup> naive T cells (CD4<sup>+</sup>, CD62L<sup>hi</sup>, and CD44<sup>lo</sup>) were co-cultured with anti-CD3 $\epsilon$  antibody precoated (145-2C11; 5  $\mu$ g/ml) 96-well plates. To induce  $T_{FH}$  cell differentiation,  $T_{FH}$  medium (10  $\mu$ g/ml anti-IL-4 [11B11], 10  $\mu$ g/ml anti-IFN- $\gamma$  [XMG1.2], 10  $\mu$ g/ml anti-TGF- $\beta$  [1D11], 30 ng/ml IL-6 [PeproTech], and 50 ng/ml IL-21 [PeproTech]) was added. PBS alone and anti-CD3 $\epsilon$  alone without  $T_{FH}$  medium were used as a negative control. T cells were cultured for 4 d and  $T_{FH}$  cells were analyzed by flow cytometry as described in Fig. S4 B. In some experiments, anti-IL-6 neutralizing antibody (eBioscience) was added during the culture. CD4<sup>+</sup> T cells were purified by anti-CD4 microbeads (Miltenyi Biotec) for Bcl-6 expression.

**Additional methods.** Information on serum immunoglobulin ELISA, Western blotting, ANA, antibodies, immunization with NP(16)-CGG, proteinuria and IgG deposition in kidney, hybridoma generation, and Ig sequencing are available in the [supplemental Materials and methods](#).

**Statistics.** Unpaired two-tailed Student *t* tests were used for statistical analysis with Prism software (GraphPad Software). *P* < 0.05 was considered to be significantly different.

**Online supplemental material.** Fig. S1 shows Blimp-1 expression in hematopoietic lineages and additional phenotypes of DCBlimp-1<sup>ko</sup> mice. Fig. S2 shows gender-dependent serology and secretion of IL-6 from B cells. Fig. S3 shows enhanced antibody and GC response in DCBlimp-1<sup>ko</sup> mice. Fig. S4 shows analysis of CD4<sup>+</sup> T cells in the spleen of control and DCBlimp-1<sup>ko</sup> mice. Fig. S5 shows blocking of  $T_{FH}$  by anti-IL-6 antibodies in vitro. Additional information is provided in the supplemental Materials and methods. Online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20110658/DC1>.

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