Correction

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>−/−</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>−/−</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 µm). 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 (DC(Blimp-1<sup>−/−</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>−/−</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.
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\(^{−/−}\) mice were generated by mating Blimp-1\(^{−/−}\) mice to CD11c-CRE\(^{+}\) mice to identify the in vivo consequences of Blimp-1 deficiency to DC function. Female, but not male, DCBlimp-1\(^{−/−}\) 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\(_{FH}\) cells. Finally, DCs from female DCBlimp-1\(^{−/−}\) mice produced increased IL-6 and preferentially induced differentiation of T\(_{FH}\) cells. All aspects of the phenotype were abolished in DCBlimp-1\(^{−/−}\) 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\(^{−/−}\) mice**

Blimp-1 expression was measured by Western blotting (Fig. 1 A). CD11c\(^{+}\) DCs were purified from spleens of age-matched DCBlimp-1\(^{−/−}\) (Blimp-1\(^{−/−}\) flox/fox, CD11c-CRE\(^{+}\)) and control
matory infiltrates were observed in 10-mo-old DCBlimp-1 mice. Deposition of IgG and mesangial cell proliferation and inflammation were shown. Proteinuria was developed, and kidney reactivity to dsDNA antibodies. An isotype-specific ELISA demonstrated a lupus-like phenotype in both genders. Overall, mice haploid for Blimp-1 also displayed IgG reactivity to both dsDNA and ENA5 stranded (ds) DNA, and anti-ENA5 by ELISA. All the ANA-positive immunoglobulin was IgG; IgM ANA was negligible.

Consistent with a previous study (Chan et al., 2009), development of DCs was normal in both conventional DCs (cDCs; CD11c+Siglec-H+) and plasmacytoid DCs (pDCs; CD11c+Siglec-H+) in the spleen or in BM-derived DCs (BM-DCs; Fig. S1 B). However, female DCBlimp-1ko mice developed autoantibodies as early as 4–5 mo of age. Sera from age-matched female DCBlimp-1ko 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-1ko 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-1ko mice (Fig. 1 D). In contrast to the results from female mice, serum from male DCBlimp-1ko 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-1ko mice (Fig. S2 A and Table S1). The gender disparity in DCBlimp-1ko 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, haplosufficient 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-1ko DCs**

To understand the alterations in Blimp-1ko 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-1ko 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-1ko 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 1 diabetes in women (Kristiansen et al., 2003). The level of expression of several genes also increased in Blimp-1ko DCs as measured by quantitative (q) PCR (Table S2). Bcl-6, a molecule negatively regulated by Blimp-1, was up-regulated in Blimp-1ko 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-1ko 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 TFH cells (Nuriya et al., 2009). To test the importance of increased IL-6 in autoantibody production in DCBlimp-1ko mice, we generated DCBlimp-1ko mice...
haploid for IL-6 (IL-6+/− DCBlimp-1ko). DCs from IL-6+/− DCBlimp-1ko 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+/− DCBlimp-1ko mice with NP-CGG showed that IL-6+/− DCBlimp-1ko mice mount an antibody response that is indistinguishable from that of control mice (Fig. S3 A), demonstrating that B cells from IL-6+/− DCBlimp-1ko mice are not defective in antibody production or affinity maturation. Although IL-6+/− DCBlimp-1ko 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-1ko mice.

Enhanced GC formation in DCBlimp-1ko 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-1ko 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-1ko mice (Fig. 3 B). In contrast to the response in IL-6+/− DCBlimp-1ko mice, there was an enhanced immune response in DCBlimp-1ko 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 (7/12 + GCs in DCBlimp-1ko mice and 1/3 + GCs in control 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-1ko 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-1ko mice. GC (PNA+B220+, asterisks) was analyzed by IHC. Pictures are representative images (bars, 100 µm). On the right, GL-7+B220+ 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.

Blimp-1ko DCs induce expansion of Tfh cells in vivo and in vitro

Tfh 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+ Tfh cells has been demonstrated in studies of lupus-prone sanroque mice (Vinuesa et al., 2005); moreover, ICOS blockade inhibits lupus in NZB/W F1 mice (Hu et al., 2009).

To address whether there were more activated CD4+ T cells in DCBlimp-1ko mice, we measured ICOS expression on T cells. There was increased expression of ICOS in CD4+ T cells from DCBlimp-1ko mice compared with control mice (Fig. S4 A). In addition, the percentage and the number of Tfh cells were also increased in DCBlimp-1ko 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 and others, 2006).
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 ko DCs into T FH (Fig. S5). These observations suggest that the increased generation of T FH cells results from increased IL-6 production by DCs in DCBlimp-1 ko mice. Interestingly, there was no significant increase in T H17 cells, another subset for which IL-6 is critical (unpublished data), suggesting that additional requirements for the generation of T H17 must exist and are not provided by Blimp-1 ko DCs.

IL-6–dependent generation of GC B cells and T FH cells
Because increased T FH cells and GC response are important mechanisms for autoantibody production in DCBlimp-1 ko mice and the phenotype was impaired in IL-6+/− DCBlimp-1 ko mice, we compared GC and T FH cells in IL-6+/− DCBlimp-1 ko mice. IL-6+/− DCBlimp-1 flox/+ mice showed a reduced number of T FH 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 ko DCs is a
major molecular mechanism responsible for the expansion of T<sub>H</sub> 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<sub>H</sub> 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<sub>H</sub> cells, and increased IL-6 levels (Swaak et al., 1989).

MATERIALS AND METHODS

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

T<sub>H</sub> cell in vitro differentiation. 10<sup>5</sup> purified DCs (CD11c<sup>+</sup>) from control and DCBlimp-1<sup>−/−</sup> mice and 5 × 10<sup>5</sup> naive T cells (CD4<sup>+</sup>, CD62L<sup>hi</sup>, and CD44<sup>lo</sup>) were co-cultured with anti-CD3ε antibody precoated (145-2C11: 5 µg/ml) 96-well plates. To induce T<sub>H</sub> cell differentiation, T<sub>H</sub> medium (10 µg/ml anti-IL-4 [B11B1], 10 µg/ml anti–IFN-γ [XM1G12], 10 µg/ml anti–TGF-β [1D11], 30 ng/ml IL-6 [PeproTech], and 50 ng/ml IL-21 [PeproTech]) was added. PBS alone and anti-CD3ε alone without T<sub>H</sub> medium were used as a negative control. T cells were cultured for 4 d and T<sub>H</sub> 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>−/−</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>−/−</sup> mice. Fig. S4 shows analysis of CD4<sup>+</sup> T cells in the spleen of control and DCBlimp-1<sup>−/−</sup> mice. Fig. S5 shows blocking of T<sub>H</sub> 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.

We thank K. Calame (Columbia University) for Blimp-1 floxed mice, and P. Gregersen, J. Cohen-Solal, and P.M. Osorio for valuable discussions and critical reading of the manuscript. The animal experimental procedure was approved by Ping Wang, a chairperson of the Feinstein Institute for Medical Research Institutional Animal Care and Use Committee.

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The authors have no financial conflicts of interest to declare.

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REFERENCES


SUPPLEMENTAL MATERIAL

Kim et al., http://www.jem.org/cgi/content/full/jem.20110658/DC1

ELISAs. EIA/RiIA high-binding 96-well plates, (Costar, Corning, NY) were coated with 1 µg/ml goat anti-mouse IgG, IgM, or isotype-specific antibodies (SouthernBiotech) overnight at 4°C. The next day, plates were blocked with 1% BSA in PBS for 2 h at 37°C incubator, and serum was incubated for 1 h at 37°C. Alkaline phosphatase-conjugated anti-mouse IgG or anti-mouse isotype-specific antibodies (SouthernBiotech) were incubated for 1 h. For NP-specific ELISA, 10-µg/ml NP (4)–BSA– and NP (23)–BSA (Bioresource Technologies)–coated plates were prepared. Each plate was developed with p-nitrophenyl phosphate (Sigma-Aldrich), and OD was monitored at 405 nm.

Western blotting. Whole cell extracts were prepared from purified DCs resolved in RIPA buffer (Tris 50 mM, NaCl 150 mM, SDS 0.1%, sodium deoxycholate 0.5%, and Triton X 100) with 10° cells/50 µl protease inhibitor (Roche). Proteins of 2–5 × 10° cells/lane were loaded and separated in 4–12% SDS-PAGE gels (Invitrogen) and then transferred to polyvinylidene fluoride membrane. For immunoblotting, membrane was blocked with 5% milk in TBS-Tween overnight and incubated with 1 mg/ml α-Blimp-1 in 5% BSA in TBS-Tween (Cell Signaling Technology) and HRP-conjugated anti-mouse IgG antibodies.

ANA. ANA antibody test system (Bion) was used to detect autoantibodies. Serum obtained from age-matched control and DCBlimp-1ko mice and positive and negative serum were diluted in PBS (1:40 dilution) and applied to Hep-2 cell slides for 30 min at room temperature. After incubation, slides were washed with PBS three times, and FITC-conjugated anti-mouse IgG or IgM antibodies diluted in PBS containing 0.001% Evans Blue were added for 30 min at room temperature. Slides were washed with PBS and ANA was detected by a fluorescence microscope (AxioCam II).

Antibodies. Anti–mouse CD11c-APC, CD4-FITC, CD8α-PE, B220-PerCP, CD3ε, CD69, TLR4/MD2-PE, CXCR5, MHCII (I-Aβ), hamster anti–mouse CD3, hamster anti–mouse IFN-γ, and rat anti–mouse IL-4 were purchased from BD. Monoclonal anti–TG-β antibodies were purchased from R&D Systems. Anti–mouse CD11b–eFluor450, PD-1–PE, Gr-1–eFluor450, TCR-β–PE-Cy5, CD4–APC, CXCR5-unlabeled, anti–rat IgG-biotinylated, SA–PE-Cy7, and PD-1–PE were purchased from ebioscience.

NP immunization. 8–12-wk-old DC Blimp-1ko, IL-6+ DCBlimp-1ko, and control mice were immunized with 100 µg 4-hydroxy-3-nitropheny lacetyl conjugated to chicken gamma globulin (NP(16)-CGG) in alum (Thermo Fisher Scientific) i.p. Blood was drawn at days 0, 7, 14, and 28 to analyze anti-NP antibodies.

Proteinuria and IgG deposition in kidney. Urine samples were collected from 8-mo-old female mice for 24 h of isolation. Protein concentration in the urine sample was measured by NanoDrop and strip (Bayer). After 60 s, protein level was measured based on color changes.

Western blotting. Whole cell extracts were prepared from purified DCs resolved in RIPA buffer (Tris 50 mM, NaCl 150 mM, SDS 0.1%, sodium deoxycholate 0.5%, and Triton X 100) with 10° cells/50 µl protease inhibitor (Roche). Proteins of 2–5 × 10° cells/lane were loaded and separated in 4–12% SDS-PAGE gels (Invitrogen) and then transferred to polyvinylidene fluoride membrane. For immunoblotting, membrane was blocked with 5% milk in TBS-Tween overnight and incubated with 1 mg/ml α-Blimp-1 in 5% BSA in TBS-Tween (Cell Signaling Technology) and HRP-conjugated anti-mouse IgG antibodies.

Supplemental material

JEM
Figure S1. Blimp–1 expression in hematopoietic lineages and additional phenotypes of DCBlimp−1−/− mice. (A) The level of Blimp–1 of purified DCs from spleens of control, Blimp−1−/− CD11c−/−; CD11c-CRE, and Blimp−1−/− CD11c-CRE− mice [top graph]. The level of Blimp–1 of purified NK cells, B cells, macrophages, and α-CD3/CD28–activated T cells was measured by qPCR. Mean ± SD of three independent experiments is shown (n = 6). (B) Development of DCs in spleen and BM-DCs. Spleen and bone were harvested from 6–8-wk-old DCBlimp−1−/− and control mice. mDCs (top, CD11c−/− Siglec-H−) and pDCs (bottom, CD11c−/−Siglec-H+) were analyzed by flow cytometry. Each dot represents an individual mouse and horizontal bars indicate mean. Representative dot plots are shown.

Figure S2. Gender-dependent serology and secretion of IL-6 from B cells. (A) Serum immunoglobulin in female and male mice. Serum was obtained from 6-mo-old control and DCBlimp−1−/− mice and isotype-specific ELISAs were performed. Each dot represents an individual mouse and horizontal bars indicate mean. (B) Expression of IL-6 in B cells. B cells were purified from spleens of control and DCBlimp−1−/− mice and cultured with or without LPS stimulation. The next day, supernatant was collected and IL-6 was measured by ELISA. Mean ± SD of two independent experiments is shown (n = 6),
Figure S3. Enhanced antibody and GC response in DCBlimp-1<sup>−/−</sup> mice. 6–8-wk-old control, DCBlimp-1<sup>−/−</sup>, and IL-6<sup>−/−</sup> DCBlimp-1<sup>−/−</sup> mice were immunized with 100 µg NP (16)–CGG in alum i.p. (A and B) Antigen-specific antibody response was measured by ELISA. Blood was drawn at days 0, 14, and 28 after immunization. NP-specific IgG was measured by ELISA, and high-affinity (NP(4)–BSA) and low-affinity (NP(23)–BSA) IgG was quantified compared with a standard. Each dot represents an individual mouse and horizontal bars indicate means from two independent experiments. (C) GC B cells were analyzed by flow cytometry from control and DCBlimp-1<sup>−/−</sup> mice on day 12 after immunization. The percentage of GL−7–positive cells was calculated within the total B220-positive cells (two independent experiments, n = 5). (D) PNA<sup>+</sup>B220<sup>+</sup> GCs were visualized by IHC of spleens from immunized mice. Spleens were harvested from control and DCBlimp-1<sup>−/−</sup> mice (day 12 after immunization) and prepared for IHC. Images were analyzed by fluorescent microscopy and the number of GCs was determined by counting. Each dot represents an individual mouse and horizontal bars indicate mean.
Figure S4. Analysis of CD4⁺ T cells in the spleen of control and DCBlimp-1⁻/⁻ mice. (A) The level of ICOS on CD4⁺ T cells was measured by flow cytometry. A representative figure is on the top and the graph is on the bottom. Each dot represents an individual mouse and horizontal bars indicate mean. (B) Strategy of flow cytometry for Tfh cells.
Figure S5. Blocking of T\textsubscript{FH} by anti–IL-6 antibodies in vitro. T\textsubscript{FH} cells were differentiated from naive T cells co-cultured with purified DCs from control and DCBlimp-1\textsuperscript{ko} mice in the absence or presence of various concentrations of anti–IL-6 antibodies. T\textsubscript{FH} cells were quantified by IL-21–positive CD4\textsuperscript{+} T cells (top graph) and confirmed by Bcl-6 expression of CD4\textsuperscript{+} T cells. Mean ± SD of two independent experiments is shown. n = 6.
Table S1. Absolute number of lymphocytes in the spleen (×10^6)

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Female Control</th>
<th>DC Blimp-1&lt;sup&gt;–/–&lt;/sup&gt;</th>
<th>Statistics</th>
<th>Male Control</th>
<th>DC Blimp-1&lt;sup&gt;–/–&lt;/sup&gt;</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total splenocytes</td>
<td>71.657 ± 7.53</td>
<td>122.428 ± 19.561</td>
<td>P &lt; 0.0001</td>
<td>70.225 ± 10.15</td>
<td>73.857 ± 6.75</td>
<td>n.s.</td>
</tr>
<tr>
<td>CD11c&lt;sup&gt;+&lt;/sup&gt; DCs</td>
<td>3.538 ± 0.606</td>
<td>5.970 ± 0.743</td>
<td>P &lt; 0.0001</td>
<td>3.052 ± 0.521</td>
<td>3.653 ± 0.627</td>
<td>n.s.</td>
</tr>
<tr>
<td>B cells</td>
<td>17.197 ± 1.807</td>
<td>30.117 ± 4.812</td>
<td>P &lt; 0.0001</td>
<td>16.152 ± 2.80</td>
<td>18.169 ± 1.66</td>
<td>n.s.</td>
</tr>
<tr>
<td>Immature B cells</td>
<td>2.579 ± 0.271</td>
<td>2.559 ± 0.409</td>
<td>P = 0.917</td>
<td>4.585 ± 1.665</td>
<td>4.167 ± 1.484</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mature B cells</td>
<td>12.898 ± 1.355</td>
<td>23.7927 ± 3.807</td>
<td>P &lt; 0.0001</td>
<td>11.207 ± 1.46</td>
<td>12.745 ± 1.66</td>
<td>n.s.</td>
</tr>
<tr>
<td>MZ B cells</td>
<td>1.289 ± 0.135</td>
<td>4.283 ± 0.684</td>
<td>P &lt; 0.0001</td>
<td>1.0957 ± 0.304</td>
<td>1.497 ± 0.605</td>
<td>n.s.</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>4.729 ± 0.497</td>
<td>9.549 ± 1.526</td>
<td>P &lt; 0.0001</td>
<td>5.255 ± 1.186</td>
<td>5.888 ± 0.520</td>
<td>n.s.</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>3.264 ± 0.343</td>
<td>5.056 ± 0.807</td>
<td>P &lt; 0.0001</td>
<td>3.264 ± 0.343</td>
<td>3.050 ± 0.279</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Spleens were collected from 6–10-wk-old female and male control and DC Blimp-1<sup>–/–</sup> mice. Red blood cells (RBCs) were removed by RBC-lysing buffer, and total viable cell number were counted by Trypan blue exclusion protocol. To get a specific cell types in the spleen, splenocytes were stained with antibodies, anti-CD11c (CD11c<sup>+</sup> DCs), anti-CD19, anti-AA4.1, anti-CD21, anti-CD23 (CD19<sup>+</sup> total B cells; CD19<sup>+</sup>AA4.1<sup>+</sup>, immature B cells; CD19<sup>+</sup>AA4.1<sup>+</sup><sup>–</sup>, mature B cells; CD19<sup>+</sup>AA4.1<sup>+</sup>CD23<sup>+</sup>CD21<sup>+</sup>, FO B cells; and CD19<sup>+</sup>AA4.1<sup>+</sup>CD23<sup>–</sup>CD21<sup>+</sup>, MZ B cells), anti-TCR-δ, anti-CD4, and anti-CD8 (CD19<sup>–</sup>CD11c<sup>–</sup>TCR-δ<sup>+</sup>CD4<sup>+</sup>, CD4<sup>+</sup>T cells; and CD19<sup>–</sup>CD11c<sup>–</sup>TCR-δ<sup>+</sup>CD8<sup>+</sup>, CD8<sup>+</sup>T cells). Absolute number of each cell type was calculated by multiplication of total splenocytes and percentage of specific cells. Data are presented as mean ± SD of three independent experiments (n = 15).

Table S2. Gene expression in splenic dendritic cells by real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control DC</th>
<th>Blimp-1&lt;sup&gt;–/–&lt;/sup&gt; DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blimp-1</td>
<td>1.50 ± 0.22</td>
<td>0.22 ± 0.17 (P = 0.0004)</td>
</tr>
<tr>
<td>BAFF</td>
<td>2.11 ± 1.5</td>
<td>5.12 ± 0.08 (P = 0.040)</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.21 ± 0.24</td>
<td>1.84 ± 0.02</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.19 ± 0.27</td>
<td>1.18 ± 0.1</td>
</tr>
<tr>
<td>IL-27</td>
<td>1.27 ± 0.38</td>
<td>3.07 ± 0.95 (P = 0.032)</td>
</tr>
<tr>
<td>IFN-β</td>
<td>1.19 ± 0.21</td>
<td>5.06 ± 0.57 (P = 0.030)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1.2 ± 0.2</td>
<td>11.5 ± 2.23 (P = 0.0031)</td>
</tr>
<tr>
<td>XBP-1</td>
<td>1.32 ± 1.46</td>
<td>1.22 ± 0.05</td>
</tr>
<tr>
<td>Bcl-6</td>
<td>0.73 ± 0.1</td>
<td>1.49 ± 0.23 (P = 0.021)</td>
</tr>
</tbody>
</table>

DCs were prepared from the spleen of 6–10-wk-old female control or DC Blimp-1<sup>–/–</sup> mice. CD11c<sup>+</sup> DCs were sorted, and total RNA was prepared. 1 µg of total RNA was converted to cDNA and each gene of interest was amplified by specific primer. Relative expression of each gene was calculated normalized by expression of the housekeeping gene Polr2a using the ∆∆ method. n = 3.