The Transcriptional Repressor BLIMP1 Curbs Host Defenses by Suppressing Expression of the Chemokine CCL8


*J Immunol* published online 29 January 2014
http://www.jimmunol.org/content/early/2014/01/28/jimmunol.1301799

Supplementary Material
http://www.jimmunol.org/content/suppl/2014/01/28/jimmunol.130179
9.DCSupplemental

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The Transcriptional Repressor BLIMP1 Curbs Host Defenses by Suppressing Expression of the Chemokine CCL8


The transcriptional repressor B lymphocyte–induced maturation protein 1 (BLIMP1) is a master regulator of B and T cell differentiation. To examine the role of BLIMP1 in innate immunity, we used a conditional knockout (CKO) of Blimp1 in myeloid cells and found that Blimp1 CKO mice were protected from lethal infection induced by Listeria monocytogenes. Transcriptome analysis of Blimp1 CKO macrophages identified the murine chemokine (C-C motif) ligand 8, CCL8, as a direct target of Blimp1-mediated transcriptional repression in these cells. BLIMP1-deficient macrophages expressed elevated levels of Ccl8, and consequently Blimp1 CKO mice had higher levels of circulating CCL8, resulting in increased neutrophils in the peripheral blood, promoting a more aggressive antibacterial response. Mice lacking the Ccl8 gene were more susceptible to L. monocytogenes infection than were wild-type mice. Although CCL8 failed to recruit neutrophils directly, it was chemotactic for γδ T cells, and CCL8-responsive γδ T cells were enriched for IL-17F. Finally, CCL8-mediated enhanced clearance of L. monocytogenes was dependent on γδ T cells. Collectively, these data reveal an important role for BLIMP1 in modulating host defenses by suppressing expression of the chemokine CCL8.

The Journal of Immunology, 2014, 192: 000–000.

B lymphocyte–induced maturation protein 1 (BLIMP1) is a transcriptional repressor critical for early embryonic development in multiple species (1–3). BLIMP1 was first identified as a factor that bound to the positive regulatory domains I and III of the IFN-β enhancer, where it was shown to compete with IFN regulatory factors to attenuate IFN-β gene transcription (4, 5). BLIMP1 is best studied, however, in the context of terminal differentiation of B lymphocytes to plasma cells (6–8) and in the differentiation, homeostasis, and/or function of both CD4 and CD8 T cells (9, 10). It also plays a central role in the differentiation and function of keratinocytes (11) and sebocytes (12) and in the tolerogenic function of DCs (13). The role of BLIMP1 in macrophages and antimicrobial defenses, however, has not been examined in detail.

Macrophages are one of the major mediators of inflammation and critical effector cells of innate immunity. They serve as host cells for intracellular pathogens, including Listeria monocytogenes (14). L. monocytogenes is a Gram-positive facultative intracellular bacterium, which causes disease in humans as a result of ingestion of contaminated foods. L. monocytogenes infections are generally limited; however, lethal infections can occur in immunocompromised individuals, pregnant women, and neonates. Upon ingestion, bacteria invade the intestinal epithelium, enter the draining lymph node, and disseminate via the bloodstream to the liver and spleen (15). This rapid clearance of bacteria from the peripheral blood is generally attributed to the resident liver macrophages—the Kupffer cells, which line the liver sinusoids—and to the hepatocytes. Within the first 24 h, a coordinated interaction between neutrophils, γδ T cells, monocytes, and NK cells is instrumental in preventing the bacterium from spreading and ensuring the survival of the host. L. monocytogenes is an excellent model system to evaluate the role of innate immunity in antimicrobial defenses. Several classes of germline-encoded pattern recognition receptors (PRRs) have been implicated in the recognition of L. monocytogenes (14, 16). TLR-2 plays a key role in bacterial recognition at the cell surface (17–19). Upon escape of intracellular bacteria into the cytosol, L. monocytogenes engages nucleotide-binding oligomerization domain (NOD) 2, members of the NACHT-, LRR- and pyrin-domain–containing protein family, as well as the cytosolic DNA sensor AIM2 (absent in melanoma 2), all of which play a role in the recognition of intracellular L. monocytogenes and release of IL-1β through the activation of caspase-1 (20–23). In addition, L. monocytogenes triggers type I IFN gene transcription via STING (stimulator of IFN genes) (24–28), TBK1 (TANK-binding...
were obtained from The Jackson Laboratory, and CCR8
S. Lira (Mount Sinai School of Medicine, New York, NY). Specific pathogen–free conditions in the animal facilities at the University of
and MG132, both used at 40
100% of the IL-1 activity when used at 100 ng/ml. The inhibitors SB203580
IRF3
2
purchased from The Jackson Laboratory. TLR2
Jackson Laboratory, Bar Harbor, ME) to generate Prdm1flox/floxLysMCre
(34). Peritoneal macrophages were obtained from mice by washing their
Cell isolation
Bone marrow–derived macrophages (BMDMs) were generated as described (34). Peritoneal macrophages were obtained from mice by washing their
peritoneal cavities with 5 ml ice-cold PBS 3 d after i.p. injection with 3 ml thioglycollate (Remel, Lenexa, KS). Splenocytes were isolated by disrupting
sacrificed experimentally harvested spleens in RPMI 1640 10% FCS, using a 70-µm pore size mesh cell strainer (BD Biosciences) to obtain single-cell suspensions. Whole-blood samples were obtained by cardiac puncture using 1-ml syringes containing EDTA and from tails of sterile 5-ml polypropylene tubes containing PBS with 10 mM EDTA. Lysis of RBCs was performed using RBC lysis buffer (Sigma-Aldrich, St. Louis, MO). Neutrophils were isolated from the peritoneal cavity (4 h after thioglycollate injection) or from peripheral blood by negative selection (Miltenyi Biotec).
Isolation and chemotaxis of bone marrow neutrophils and γδ T cells
FRESHLY harvested bone marrow leukocytes from C57BL/6 mice were first stained with PE-conjugated Ly6G Ab (BD Biosciences), and neutrophils were then microfluorometrically isolated using EasySep PE selection kits (Stem Cell Technologies). Purity was assessed by flow cytometry (FACS Calibur). Pooled γδ T cells were purified from splenocytes of age–matched WT C57BL/6 and CCR8–/– mice. Splenocytes were labeled with PE-conjugated Hamster Anti-Mouse γδ TCR Ab (UC7–13D5; BD Biosciences) before preincubation with anti-Mouse CD16/CD32 (BD Pharmingen), and then magnetically purified with anti–PE-Ab (Miltenyi Biotec)–coated microbeads. Isoylated γδ T cells were rested in 0.5% FCS RPMI overnight prior to assays. Neutrophils (1 × 10⁶ per well) and γδ T cells (2.5 × 10⁶ per well) were stained in RPMI 1640 containing BSA were respectively placed on the top of a 3-µm and a 5-µm pore size 96-well ChemoTx chemotaxis apparatus (Neuroprobe) in chemotaxis assays. Neutrophils and γδ T cells were incubated for 1 h and 2 h, respectively, at 37°C. In the neutrophil assays, all nonmigrated cells were manually removed from the upper side of the filter, whereas the migrated cells remained adherent to the lower side of the filter. The filters were stained with 0.5% Fisher's crystal violet on glass slides, and cell counts were determined by manual counting using a light microscope. In the γδ T cell assays, cell counts were determined in the lower wells by manual counting using an inverted microscope. Chemokines were placed in the lower wells of the chemotaxis apparatus in triplicate in all assays. The number of cells migrating at each concentration of chemokine was normalized to the number of cells migrating in the presence of media alone (chemokinesis) to calculate the chemotactic index.
Flow cytometry analysis
The differentiation state of BMDMs and peritoneal macrophages was confirmed by staining for F4/80 and CD11b (BD Pharmingen, San Jose, CA), using mAbs as direct conjugates and their isotype controls. Splenocytes and peripheral blood cells were stained with the indicated combination of the following fluorochrome-conjugated mAbs: CD19-FITC, B220-APC, CD3–PE-Cy7, CD4-APC, CD8–PE-Cy7, F4/80-PE, Ly6C-FITC, CD11c-PE-Cy7, CD11b-PE-Cy7, and CD19-APC. Mouse splenocytes were isolated as described above, were labeled with a standard cocktail of fluorochrome-conjugated mAbs, and then magnetically purified with anti–PE-Ab (Miltenyi Biotec)–coated microbeads. NK1.1–/–, T cell–depleted splenocytes were labeled with a cocktail of fluorochrome-conjugated mAbs specific for B220, CD4, CD8, and Ly6C. All antibody–conjugated mAbs were from BioLegend, San Diego, CA, unless otherwise stated. CD11b+ Mac-1+ cells were gated as non–NK1.1+CD11b+Lyt-2– cells. All antibodies were used according to the manufacturer's instructions.
In vivo infections
Pretreated tryptic soy broth (TSB)–glycerol stocks of L. monocytogenes strain 10403S were stored at −80°C. Prior to infection, 1-mL bacterial aliquots were recovered for 1 h at 37°C in 9 ml TSB (BD Biosciences), washed, and resuspended to the desired CFU in PBS. Mice were injected i.v. with 3 × 10⁴ CFU L. monocytogenes (strain 10403S) in 0.3 ml PBS. At defined time points, infected animals were killed by CO₂ asphyxiation. Livers and spleens of infected animals were aseptically harvested, weighed, and homogenized in 0.02% Triton X-100. Prior to homogenization, samples of infected livers were taken for slide preparation and staining with H&E or Gram dye. Aliquots of serial 5-fold dilutions in sterile water were plated in duplicate on TSB agar (BD Biosciences) plates containing 10 mg/ml streptomycin. After overnight incubation, the number of bacteria per gram of tissue was determined by counting colonies at the appropriate dilution.
Microarray hybridization and analysis
RNA (5 µg) was isolated from WT and Blimp1 CKO BMDM untreated or infected for 2 h with L. monocytogenes (MOI = 5). cDNA and cRNA
kinase 1), and IFN regulatory factor (IRF) 3 (29–31). Bacterial DNA and the second messenger cyclic-di-AMP represent the L. monocytogenes products driving these responses (31–33).

**Materials and Methods**

**Reagents and bugs**

*L. monocytogenes* (clinical isolate 10403S) and its derivative mutant listeriolysin O (LLO), *Staphylococcus aureus* (ATCC 25923), and *Escherichia coli* (ATCC 27325 [W3110]) were used at multiplicity of infection (MOI) 5. Sendai virus (Cantrell strain) was from Charles River Laboratories (Boston, MA) and was used at 300 hemagglutinating units per milliliter. Recombinant IL-1β, murine CCL8, CCL2, and CXCL2 were from Pepro-tech (Rocky Hill, NJ). IL-1β was used at 100 ng/ml. The IL-1R antagonist Kineret (or anakinra; Amgen) was used at 150 µM/ml, a dose that inhibits 100% of the IL-1 activity when used at 100 ng/ml. The inhibitors SB30580 and MG1312, both used at 40 µM, were from Calbiochem (San Diego, CA).

**Mice**

Pdmllox/lox mice were generously provided by K. Calame (Columbia University, New York, NY) (3) and were crossed with LysMCre mice (The Jackson Laboratory, Bar Harbor, ME) to generate Pdmllox/lox;LysMCre and Pdmllox/lox;LysMCre mice. C57BL/6 mice and IL-1R–/– mice were purchased from The Jackson Laboratory, TLR2–/– and MyD88–/– mice were obtained from The Jackson Laboratory, and CCR8–/– mice were from S. Akira (Osaka University, Osaka, Japan). IRF3–/– mice were from T. Taniguchi, (University of Tokyo, Tokyo, Japan). Receptor-interacting serine-threonine kinase 2 (RIP2)–/– mice were from Genentech (San Francisco, CA). Ccl2/Ccl12 and Ccl12 knockout (KO) were obtained from I. Chao (Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, CA). In all experiments, 7- to 8-wk-old animals were used. All mouse strains were bred and maintained under specific pathogen–free conditions in the animal facilities at the University of Massachusetts Medical School. All experiments involving live animals were carried out in accordance with the guidelines set forth by the University of Massachusetts Medical School, Department of Animal Medicine and the Institutional Animal Care and Use Committee. γδ T cell–deficient mice were obtained from The Jackson Laboratory, and CCR8–/– mice were from S. Lira (Mount Sinai School of Medicine, New York, NY).

**Cell isolation**

Bone marrow–derived macrophages (BMDMs) were generated as described (34). Peritoneal macrophages were obtained from mice by washing their...
quantitative real-time PCR

DNase I-treated total RNA from BMDMs and peritoneal exudate cells (PECs) was extracted with the RNaseasy kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. cDNA was synthesized, and quantitative real-time RT-PCR (qPCR) analysis was performed on a DNA engine Opticon 2 cycler (MJ Research, Watertown, MA), using the SuperScript III Two-Step qRT-PCR Kit with SYBR Green (Invitrogen, Carlsbad, CA). The specificity of amplification was assessed for each sample by melting curve analysis. Relative quantification was performed using standard curve analysis. The quantification data are presented as a ratio to the β-actin level. The SEs (95% confidence limits) were calculated using the Student t test. All gene expression data are presented as a ratio of gene copy number per 100 copies of β-actin ± SD. The following primer pairs have been used:

- β-Actin forward 5′-TGCACTAGGGTCTTACCGGA-3′
- β-Actin reverse 5′-TGGCATAGGGTCTTACCGGA-3′
- BLIMP1 forward 5′-GAGGATCTGGCCAGCAACTCA-3′
- BLIMP1 reverse 5′-GTGGTCTGGAGCAACACTCA-3′
- CCL8 forward 5′-TAAGGCTCCAGTCACCTGCT-3′
- CCL8 reverse 5′-TTCCAGGTGGGTGCTCTC-3′
- Atm forward 5′-CAACGGTCTAGAGCTGAAGG-3′
- Atm reverse 5′-TCACCAACAGGCACCTTGG-3′
- Mpu2l forward 5′-TGGAGGACGTCTGATATTGCTC-3′
- Mpu2l reverse 5′-GCCTTGGGTTGGTGCTACCT-3′
- Arhgap25 forward 5′-ATGCTTGCGTGAAGGTGGTG-3′
- Arhgap25 reverse 5′-ACCTCAAGAGGCTGCTTTT-3′
- Arhgap26 forward 5′-AAGACCATACGCAGCCGACAC-3′
- Arhgap26 reverse 5′-CTGGCTCTGCACAATCTGC-3′
- Pldn forward 5′-GGACCCTCTGGTGAGGACATA-3′
- Pldn reverse 5′-AGCTTGGGGATAGCTGCTT-3′
- Rasgrpl forward 5′-TGTCACAGCTCCATCTCCAG-3′
- Rasgrpl reverse 5′-TTCACCTTCCATCTCCAG-3′
- Tm3s4 forward 5′-CAAGAGTGCTGGCATGATT-3′
- Tm3s4 reverse 5′-GGCTGTGATGCTGTTTCTC-3′
- Tnem87a forward 5′-AATGGTGTTGACAACCTTCAAA-3′
- Tnem87a reverse 5′-AGGGCGAAATACTACAGCAGAC-3′

Cell extracts

BMDMs were infected with L. monocytogenes 10403s at MOI 5. After 1 h of infection, cells were washed once with 10% FCS/DMEM and then treated with 10 mg/ml gentamicin to kill extracellular bacteria. Bone marrow cells were then left for another 2 h, and nuclear lysates were prepared as previously described (34).

Western blot analysis

Nuclear cell extracts were separated by 7.5% SDS-PAGE gel and blotted onto nitrocellulose membranes. Blots were incubated with mouse mAbs against murine BLIMP1 (Novus Biological) or β-actin (Sigma-Aldrich), as indicated, and detected using an ECL system (Amersham, Piscataway, NJ).

ChIP assay

ChIP experiments were performed essentially as described (40). Briefly, ∼4 × 10^7 BMDMs were used to perform immunoprecipitation with rabbit polyclonal recognizing the C terminus of BLIMP1 (a kind gift of K.L. Calame, Columbia University, New York, NY) (41). qPCR was performed on immunoprecipitated and input fractions from the immunoprecipitation. The following set of primers was used to amplify the different regions of the Ccl8 promoter, as indicated:

- Unrelated (unr) for 5′-CATGTGCAAGTTCTGGCTA-3′
- urv 5′-GGCTCAATGCTGGCTTCCCT-3′
- +141 for 5′-ACCGTAGCTTACTCCTCAA-3′
- +141 for 5′-GTGGCTGTAACCATCCTC-3′
- −288 for 5′-GGGACCAAGCTAGGCTTT-3′
- −288 for 5′-GAAGGGCAAGCATCTGAAAC-3′
- −85 for 5′-CTGACCACTGGGCTGCTT-3′
- −85 for 5′-GAAGGCTAGCTGCTTCTCAAA-3′

Transwell chemotaxis and intracellular cytokine staining of γ/δ T cells

γ/δ T cells were first isolated, as described above, from pooled lymph nodes and spleens of 10–20 WT C57BL/6 mice and rested overnight in 0.5% FCS RPMI 1640 supplemented with HEPES, l-glutamate, nonessential amino acids, and Na pyruvate. The next day, γ/δT cells (5 × 10^6 per milliliter in 100 μl) were harvested and placed in the upper chamber of Transwells (5-μm pore size polycarbonate membrane, 12-mm diameter) (Costar, Fisher) and 600 μl chemokine or media was placed in the lower chamber. Migration was allowed to proceed for 2–3 h at 37°C and was monitored after 2 h with an inverted microscope and compared with media controls to determine the completion of the assay. A fraction of input total spleen and lymph node γ/δ T cells were set aside and simultaneously incubated at 37°C during the course of the assay. At the end of the assay, total input γ/δ T cells and CCL8- or CCL2-responsive migrated γ/δ T cells in the Transwells were harvested for intracellular staining and activated with 50 ng/ml PMA (Sigma-Aldrich) and 500 ng/ml ionomycin (Sigma-Aldrich) for 2 h in the presence of brefeldin A (BD Biosciences). At the end of the activation, cells were fixed and permeabilized with Fix & Perm Medium A & B (Invitrogen), and then stained with anti-IL-17A Alexa Fluor 647 (TC11-18H10.1, BioLegend) and anti-IL-17F Alexa Fluor 488 (eBioscience). Cells were acquired on a Becton-Dickinson FACSCalibur and analyzed with FlowJo 8.8 software.

Statistical analysis

Statistical analysis was calculated using a two-tailed Student t test, if not otherwise stated. A p value ≤ 0.05 was considered statistically significant.

Results

Regulation of Blimp1 gene expression following L. monocytogenes infection

While studying immune response genes in murine BMDMs infected with bacterial and viral pathogens, we found that expression of the Prdm1 gene (which encodes BLIMP1) was markedly increased upon infection with a variety of pathogens, including L. monocytogenes, E. coli, S. aureus, the paramyxovirus Sendai, and the Gram (−)-derived LPS. In all cases, the induction was strikingly rapid and transient, with expression peaking 2 h postinfection (hpi) (Fig. 1A). We also extended this time course to later time points, which revealed a second wave of inducible BLIMP1 expression at the 24-h time points (Supplemental Fig. 1A). We focused on L. monocytogenes as a model pathogen to understand both the molecular basis for BLIMP1 induction and the role of BLIMP1 in antimicrobial defenses. L. monocytogenes is readily phagocytosed by macrophages, and once inside a host vacuole, the essential virulence factor LLO, a pore-forming toxin encoded by the hemolysin (hly) gene, enables the bacterium to permeabilize the vacuolar membrane and enter the cytosol (42). Several classes of PRRs can sense L. monocytogenes−derived products at different stages of infection. These include TLR2 (17–19, 43), NOD2 (20), NLRP3 (21), AIM2 (22, 23), STING, well as an as yet unidentified DNA sensor that turns on IFN-β gene transcription (28, 31). To understand how Blimp1 gene induction was regulated, we monitored L. monocytogenes−induced Blimp1 transcript levels in macrophages...
derived from mice with targeted deletions in components of these signaling pathways. These included macrophages deficient in TLR2; in RIP2, the downstream effector of NOD1/2 signaling; in IL-1R chain 1, which fails to signal in response to IL-1β released upon the activation of caspase-1; and finally in IRF3, the key transducer of the cytosolic DNA sensing pathway. Induction of Blimp1 gene transcription upon *L. monocytogenes* infection was induced normally in macrophages lacking RIP2 and IRF3 but was reduced in both TLR2−/− and IL1R−/− macrophages (Fig. 1B).

Moreover, this response was completely lost in TLR2−/− macrophages pretreated with the IL-1R antagonist, showing that for a full induction of Blimp1 transcription, both these signaling pathways have to be activated by *L. monocytogenes* (Fig. 1C).

Indeed, *L. monocytogenes* infection of BMDMs lacking MyD88, a key adaptor downstream of both TLR2 and IL-1R intracellular cascades, fails to induce Blimp1 expression. Furthermore, inhibition of p38 MAPK and NF-κB signaling using the respective specific inhibitors SB203580 or MG132 prevented *L. monocytogenes*–induced expression of BLIMP1 protein in macrophages (Fig. 1D).

These data suggest that cell surface TLR2 and cytosolic PRRs, which regulate IL-1β production, cooperate in control of Blimp1 transcription. In support of this idea, an LLO-deficient strain of *L. monocytogenes*, which is not capable of entering the cytosol of the host cell and, as a consequence, is not able to trigger IL-1β or type I IFN production, was less potent than WT bacteria in inducing Blimp1 transcription. This response to LLO−/− bacteria was

**FIGURE 1.** Blimp1 is rapidly induced by *L. monocytogenes* via TLR2 and cytosolic sensors. (A) RNA was isolated from BMDMs left untreated (un) or infected with *L. monocytogenes* (LM), *E. coli*, *S. aureus* (MOI = 5), or Sendai virus (SV) (300 hemagglutinating units) or treated with LPS (100 ng/ml) for the indicated times, and Blimp1 gene expression was quantified by qPCR. Data were normalized with β-actin and are representative of three independent experiments. (B) BMDMs from C57BL/6, IRF3−/−, RIP2−/−, TLR2−/−, and IL1R−/− mice were stimulated with *L. monocytogenes* for 2 h. mRNA expression for Blimp1 was measured by qPCR. One of three different experiments conducted singularly is shown. (C) RNA was isolated from *L. monocytogenes*–infected or IL-1β–treated BMDMs from C57BL/6, TLR2−/−, IL1R−/−, or MyD88−/− mice or from TLR2−/− preincubated with IL-1R antagonist (IL1RA, 150 μg/ml). Blimp1 expression was quantified by real-time RT-PCR. Results are mean ± SD of triplicate values and are representative of at least four experiments. (D) Lysates were prepared from BMDMs treated for 3 h with *L. monocytogenes* (MOI = 5) either before or after pretreatment (30 min) with an inhibitor for p38 MAPK (SB203580, 40 μM) or an inhibitor for NF-κB (MG132, 40 μM). BLIMP1 and β-actin expression was detected by Western blotting using the respective specific Abs. (E) Blimp1 gene expression was evaluated by real-time RT-PCR in RNA derived from C57BL/6, TLR2−/−, and MyD88−/− macrophages and treated 2 h with MOI = 5 of either the WT or the LLO mutant *L. monocytogenes* strains.
entirely dependent on TLR2 because macrophages lacking TLR2 failed to upregulate Blimp1 (Fig. 1E).

Generation of a myeloid-specific Blimp1 CKO mouse

Direct targeted disruption of the gene encoding BLIMP1 (Prdm1) is lethal for mouse embryos (6). Therefore, to define the role of this protein in the host response to microbial challenge, we generated a myeloid-specific Blimp1 CKO mouse via lysozyme M-driven Cre-mediated recombination (44). Prdm1<sup>flox/flox</sup> M-lysozyme Cre-negative (6) and the Prdm1<sup>flox/flox</sup> M-lysozyme Cre-positive mice (hereafter referred to as WT and Blimp1 CKO mice, respectively) were developmentally normal and fertile. Efficient deletion of the floxed Prdm1 sequence in the Blimp1 CKO macrophages was confirmed by PCR analysis of BMDM genomic DNA (Fig. 2A, 2B). Both the mRNA and protein were undetectable in BMDMs from Blimp1 CKO mice either before or after infection with L. monocytogenes (Fig. 2C, 2D, respectively). Similar results were obtained when elicited PECs from WT and Blimp1 CKO mice were examined ex vivo for the mRNA expression of the Blimp1 gene (Fig. 2E). M-lysozyme is expressed in both macrophages and granulocytes, so we measured BLIMP1 in both of these cell types. Although BLIMP-1 was expressed in macrophages and dendritic cells, its expression was very low by comparison in neutrophils (Supplemental Fig. 1B). We did observe efficient deletion of the floxed Prdm1 DNA sequence by PCR in neutrophils isolated from the peritoneal cavity of Blimp1 CKO mice (Supplemental Fig. 1C). Although Blimp1 was poorly expressed in resting neutrophils, its expression was, however, inducible upon L. monocytogenes infection (Supplemental Fig. 1D).

Because BLIMP1 is a key factor controlling the differentiation of many different cell types, we examined immune cell development in Blimp1 CKO mice. As expected, no significant differences in the numbers of B cells or T cells were observed when BLIMP1 was deleted only in myeloid cells (Fig. 3A). Previous studies using overexpression of a truncated version of BLIMP1 in a human monocytic cell line suggested that BLIMP1 was important in the differentiation of myeloid cells (45). Flow cytometry analysis revealed normal numbers of macrophages, neutrophils, and dendritic cells in the spleen of Blimp1 CKO mice (Fig. 3A). Similar numbers of elicited PECs were also obtained from WT and Blimp1 CKO mice after thioglycollate injection, and expression of CD11b and F4/80 on these cells was equivalent between WT and CKO mice (data not shown). We also examined the differentiation of Blimp1 CKO macrophages ex vivo from bone marrow. BMDMs from WT and Blimp1 CKOs expressed similar levels of CD11b and F4/80 (Fig. 3B) and induced equivalent levels of NO synthase in response to L. monocytogenes or after treatment with LPS (Fig. 3C). Moreover, L. monocytogenes grew at a comparable rate in WT and Blimp1 CKO BMDMs (Fig. 3D). Collectively, these data demonstrate that BLIMP1 is not required for the development or normal functions of macrophages.

Blimp1 deficiency in myeloid cells protects mice from L. monocytogenes infection

To examine the role of BLIMP1 in innate immunity, we performed in vivo infection experiments using L. monocytogenes. We first monitored bacterial numbers in the spleens and livers 48 hpi (3 × 10<sup>5</sup> CFU delivered i.v.). Both organs in the Blimp1 CKO mice had lower bacterial loads than did WT littermate controls (Fig. 4A). Whereas the bacterial load in both organs was comparable between WT and Blimp1 CKO mice 20 hpi, it progressively increased in WT, but not in Blimp1 CKO, spleens and livers at the later time points examined (Supplemental Fig. 1E). Furthermore, Blimp1 CKO mice had a greater rate of survival and quickly recovered from a dose of L. monocytogenes delivered i.v. that was lethal to most control mice (Supplemental Fig. 1F). Importantly, we found no differences in bacterial load in liver or spleen when C57BL/6 WT mice and C57BL/6 mice expressing M-lysozyme Cre only were examined (Supplemental Fig. 1G), suggesting that the Cre recombinase had no adverse effect in this system.

FIGURE 2. Generation of prdm1<sup>flox/flox</sup> M-lysozyme Cre<sup>+</sup> mice. (A) Schematic representation of the locus targeted by M-lysozyme Cre within the Prdm1 gene, with indicated primer sites used for screening the deletion (1–3). (B) Genomic DNA was isolated from BMDMs from C57BL/6, prdm1<sup>flox/flox</sup>Cre<sup>−</sup> (WT), or prdm1<sup>flox/floxCre<sup>+</sup></sup> (CKO) mice. PCR analysis for the Cre-targeted region on the Prdm1 gene was performed using a mix of primer 1, 2, and 3 on genomic DNA. Amplicon sizes are shown. (C–E) Lysozyme M-driven Cre-mediated deletion of Blimp1 was confirmed in untreated or L. monocytogenes-treated BMDMs from WT or Blimp1 CKO mice at the mRNA level by qPCR (C), at the protein level by Western blotting (D), or at the steady-state level in PECs from WT or Blimp1 CKO mice by qPCR (E). The p values were calculated by the two-tailed Student t test. *p < 0.001.
Histological analysis of liver sections revealed microabscesses containing neutrophils and macrophages by 48 hpi and a further increase in the number of these lesions by 72 hpi in WT animals (Fig. 4B [H&E staining], 4C), in line with bacterial counting described in Supplemental Fig. 1E. In contrast, very few micro-abscesses were present in the Blimp1 CKO livers. The lesions in the Blimp1 CKO mice, although present at higher numbers 20 hpi, did not increase in number during the course of the infection (Fig. 4B, right panels [H&E staining]). Notably, the lesions in Blimp1 CKO livers were morphologically distinct from those found in the WT mice. Neutrophils were present at what was presumably the site of infection, surrounded by a zone rich in the ghostlike remnants of dead hepatocytes, characteristic of coagulative necrosis, a pathological finding that results from a highly robust neutrophil response. Gram staining of liver sections revealed large numbers of bacteria in microabscesses of WT livers but uncovered no bacteria in the Blimp1 CKO lesions (Fig. 4B, right panels [Gram staining]). These observations suggested that there was a more robust and rapid clearance of bacteria from the livers of Blimp1 CKO mice. The presence of hepatocytes in a state of coagulative necrosis also supports this idea, because enzymes released from neutrophils are thought to control this process.

**The chemokine CCL8 is a target of BLIMP1-mediated transcriptional repression**

To elucidate the molecular mechanisms responsible for the heightened clearance of *L. monocytogenes* in Blimp1 CKO mice, we focused on macrophages and compared the gene expression profile of WT and Blimp1-deficient macrophages before and after infection with *L. monocytogenes*. Supplemental Fig. 2A and Supplemental Table I show genes with significantly different expression levels (*p* ≤ 0.05) between WT and Blimp1 CKO BMDMs for either untreated or infected cells, as evaluated by microarray analysis. The set of genes was significantly enriched in a number of GO terms (Supplemental Table II) related to macrophage activation and the immune system. Comparison of transcriptomes of untreated WT with untreated CKO macrophages identified a small number of genes that had significantly different expression levels. Importantly, these observations revealed that deletion of Blimp1 had surprisingly little effect on global gene expression in macrophages.
but rather identified a specific and restricted subset of genes that were differentially expressed in macrophages in the absence of this factor. One gene in particular, Ccl8, encoding the chemokine belonging to the C-C family, was expressed ∼17 times higher in Blimp1-deficient than in WT macrophages, as measured by qPCR (Fig. 5A). The expression profile of seven additional genes was also confirmed by qPCR (Supplemental Fig. 2B). Ccl8 transcription levels were also significantly elevated in macrophages isolated from the peritoneum (PECs) of Blimp1 CKO mice in the absence of infection, compared with the levels found in WT mice (Fig. 5B). Similarly, CCL8 protein levels were higher in the culture medium of untreated BMDMs and uninfected sera from Blimp1 CKO mice, compared with WT littermate controls (Fig. 5C).

Sequence analysis of a genomic region spanning 1000 bp upstream and 500 bp downstream of the Ccl8 transcription start site using the software MatInspector (http://www.genomatix.de/matinspector.html) (46) identified three BLIMP1 consensus binding sites, at positions −228, −85, and +141 (Fig. 5D, upper panel). ChIP analysis using an Ab to BLIMP1 (41) in macrophages derived from C57BL/6 mice revealed weak binding of BLIMP1 to both the −85 and +141 sites and a very strong binding to the −228 site (Fig. 5D, lower panel). Similar results were obtained when ChIP was performed on infected BMDMs (data not shown). No binding was observed when an unr sequence at position −618 of the Ccl8 promoter was examined (Fig. 5D, upper and lower panels). These data indicate that BLIMP1 directly binds to the Ccl8 promoter in vivo.

Critical role of CCL8 in antibacterial resistance to L. monocytogenes

Neutrophils play a key role in the early control of L. monocytogenes growth, appearing in the liver within the first 24–36 hpi (15). First generated in the bone marrow, neutrophils are elicited into the peripheral blood and attracted to the liver, where they contribute to antibacterial resistance by killing bacteria, lysing infected host cells, stimulating antimicrobial activity of infected macrophages, and inducing hepatocyte apoptosis (47). We speculated that the elevated expression of CCL8 in Blimp1 CKO mice could encourage egress of neutrophils from the bone marrow and promote their infiltration into the livers of L. monocytogenes-infected mice. Consistent with this hypothesis, both the bone marrow and peripheral blood of uninfected Blimp1 CKO mice showed a higher percentage of CD11b<sup>+</sup>/Ly6G<sup>+</sup> neutrophils than did WT mice, whereas the percentage of CD11b<sup>+</sup>/Ly6C<sup>hi</sup> inflammatory monocytes remained constant in both compartments (Fig. 6A, 6B). Accordingly, in infected spleens of Blimp1 CKO
mice, a marked and statistically significant increase was observed in the percentage of neutrophils following *L. monocytogenes* infection, compared with that observed in WT organs, whereas no differences were observed for monocytes or macrophages (Fig. 6C).

To test whether CCL8 would be responsible for this increased mobilization of neutrophils in *Blimp1* CKO mice, we injected C57/Bl6 mice i.v. with two increasing doses of recombinant CCL8 for 2 days consecutively and monitored CD11b+/Ly6G+ neutrophil numbers in the peripheral blood. The number of CD11b+Ly6G+ cells in mock-treated mice was very low, but this percentage significantly increased in a dose-dependent manner with the administration of CCL8 (Fig. 6D). Both CCL8- and mock-treated mice were then infected with *L. monocytogenes*, and bacterial loads in the spleen and liver were examined 48 hpi. The bacterial burden in both organs analyzed was much lower in the CCL8-treated than in the mock-treated mice, and the numbers of bacteria decreased proportionally with the increase of the chemokine dose (Fig. 6E). Histological analysis of liver sections by Gram staining confirmed these findings, revealing fewer bacteria-laden microabscesses in CCL8-treated mice than mock-treated animals (Fig. 6F, lower panels). Most interestingly, lesions present in the livers of CCL8-injected mice looked very similar to those we observed in *Blimp1* CKO mice: zones of hepatocytes in a state of coagulative necrosis surrounded by infiltrating neutrophils (Fig. 6F, upper panels). We also administered CCL2 (also called MCP1) i.v., a chemokine known to selectively induce migration of monocytes (47–49), and monitored the percentage in the blood of both CD11b+Ly6G+ neutrophils and CD11bint/Ly6Chi monocytes (Supplemental Fig. 2C). As expected, we found a significant increase in the monocyte numbers, but no effect on the neutrophil population.

To further define the role of CCL8 in antimicrobial resistance, we infected mice lacking Ccl8 with *L. monocytogenes*. Ccl8 single KOs are not available; however, mice lacking Ccl8 and Ccl12 (also called MCP5) or mice lacking Ccl12 alone were studied (48). Consistent with a role for CCL8 in the clearance of bacteria during infection, the bacterial load was much higher in mice lacking Ccl8/Ccl12 than in Ccl12 single KOs or C57BL/6 WT mice (Fig. 7A). CCL8 was recently found to be highly expressed in the skin, where it acts via the chemokine receptor CCR8. CCL8 responsiveness defined a population of highly differentiated, CCR8-expressing inflammatory Th2 cells enriched for IL-5 (50, 51). The ability of CCL8 to signal via CCR8 rather than CCR2 distinguishes CCL8 from all other MCP chemokines. In an effort to understand the role of CCL8 in innate immune responses to *L. monocytogenes*, we first examined whether CCL8 was chemotactic for neutrophils.
FIGURE 6. Role of CCL8 in antibacterial resistance and host defense against *L. monocytogenes*. (A and B) Bone marrow and peripheral blood were harvested from naive WT or *Blimp1* CKO mice. Cells were stained for CD11b, Ly6G, or Ly6C, and their expression was evaluated in a large gate drawn in the live granulocyte/lymphocyte/monocyte population. Cytometric analysis of CD11b/Ly6G neutrophils (A) and CD11b<sup>int</sup>/Ly6C<sup>hi</sup> monocytes (B) from bone marrow (upper panels) or peripheral blood cells (lower panels) is shown. A representative experiment of three conducted separately is depicted. (C) CD11b<sup>int</sup>/Ly6C<sup>hi</sup> monocytes, CD11b<sup>+</sup>F<sub>480</sub><sup>+</sup> macrophages, and CD11b<sup>+</sup>Ly6G<sup>+</sup> neutrophils derived from three WT or three CKO infected spleens were analyzed by FACS 20, 48, and 72 hpi. Mean percentages are shown. Error bars represent SEM. *p < 0.001 (two-tailed Student *t* test). (D) Percentages of CD11b/Ly6G-positive cells evaluated by FACS analysis in peripheral blood from WT mice mock treated or injected i.v. with 400 or 800 ng of recombinant CCL8 for 2 d consecutively are shown. Each bar represents an average of three to four mice from two independent experiments. Error bars represent SEM. *p = 0.012, **p < 0.001, as compared with mock controls (two-tailed Student *t* test). (E) Bacterial burdens from spleens and livers were calculated in mice mock treated or injected with 400 or 800 ng of recombinant CCL8 and then infected with *L. monocytogenes* i.v. (n = 3 mice per genotype). *p < 0.05 versus mock (two-tailed Student *t* test). (F) Representative H&E staining (original magnification ×40) and Gram staining (original magnification ×100) of livers derived from mock-treated or CCL8-injected mice subsequently infected with *L. monocytogenes* i.v. Mice were sacrificed 48 hpi.
by monitoring the migration of neutrophils in response to this chemokine using an in vitro chemotaxis assay. Neutrophils were purified from bone marrow and tested for their ability to migrate in response to CCL8 (Fig. 7B, 7C). We found that in contrast to MIP2 (also called CXCL2, an agonist of CXCR2 expressed on neutrophils), CCL8 was not chemotactic for neutrophils (52) (Fig. 7B). Neutrophils can also be mobilized to the site of infection through IL-17–mediated events that stimulate granulopoiesis-inducing cytokines and granulocyte-attracting chemokines (53). The major IL-17 producers at the early stage of Listeria infection in the liver are γδ T cells (54), which also express CCR8 on their surface (55). We therefore next investigated whether γδ T cells were the target of CCL8 action and found that CCL8 was chemotactic for these cells (Fig. 7D, 7E). The migration of γδ T cells to CCL8 was similar to that seen with CCL2, a known chemotactic factor for these cells (56). We next wanted to examine if the enhanced clearance of bacteria in animals treated with exogenous CCL8 was dependent on γδ T cells. In agreement with published studies, γδ T cell–deficient mice had significantly higher bacterial loads in liver and spleen compared with their WT counterparts following L. monocytogenes infection (Fig. 7F). Although WT animals treated with exogenous CCL8 cleared bacteria efficiently, bacterial loads in γδ T cell–deficient mice treated with CCL8 were unaltered.

Notably, the IL-17 cytokine family includes different members, and three of them—IL-17A, IL-17F, and IL-17E (or IL-25)—have been best characterized both in vivo and in vitro and have been shown to be proinflammatory in nature (57). In particular, whereas IL-17E induces Th2 cytokine production and eosinophilia, both IL-17A and IL-17F are involved in the recruitment of neutrophils. In models of mucocutaneous bacterial infections and autoimmunity, IL-17A and IL-17F were both shown to play important roles in host defense but to have distinct activity in inflammatory processes and to be produced by different cell types (58). However, γδ T cells are able to produce both of these cytokines, amplifying Th17 responses (59). Hence, we evaluated in our model which type of IL-17 was produced by CCL8-attracted γδ T cells. To this end, we first compared by intracellular staining IL-17A and IL-17F expression in γδ T cells that migrate in response to CCL8 after Transwell migration assays, compared with the total input population of γδ T cells isolated from WT spleen and lymph nodes (Fig. 8A, 8B). Our experimental setting shows that CCL8–responding γδ T cells are the main producers of IL-17F, even if IL-17A was also expressed, in contrast to what was observed for γδ T cells responding to CCL2, which only weakly induced IL-17F. We conclude from these studies, therefore, that the enhanced antibacterial effects associated with CCL8 are dependent on IL-17F–producing γδ T cells.

Discussion

The role of BLIMP1 as a transcriptional repressor in embryonic development (1–3), as well as in the differentiation of B and T lymphocytes, is well established (9, 10, 60, 61). In this study we have identified a novel role for BLIMP1 in the regulation of early host defenses in macrophages. Using myeloid-specific Blimp1 CKO mice, we found that mice lacking Blimp1 in myeloid cells had greater survival than their WT counterparts following infection with L. monocytogenes. Analysis of the Blimp1 CKO–infected organs revealed few or no bacteria in the liver and spleen of these animals compared with WT mice. The livers of KO animals had lesions with considerable coagulative necrosis, a pathological finding that results from a highly robust neutrophil response. Both the bone marrow and the peripheral blood of uninfected Blimp1 CKO mice had a higher percentage of CD11b+Ly6G+ neutrophils than did that of WT mice.

Genome-wide transcriptional profiling of WT and Blimp1-deficient macrophages identified the gene encoding the chemokine CCL8 as differentially modulated in the presence or absence of BLIMP1. Even in the absence of infection, Ccl8 was expressed at much higher levels in Blimp1 CKO macrophages both at the transcriptional and at the protein levels. ChIP analysis indicated that BLIMP1 occupied the Ccl8 promoter in WT cells. Basal expression of BLIMP1 in unstimulated macrophages could bind ISGF3 or IRF transcription factors for these sites, resulting in basal repression of these genes. BLIMP1 binding sites present within the CCL8 promoter bound by IRF factors, such as IRF1 or IRF7, would induce its transcription. Because IRF1 and IRF7 are themselves regulated via ISGF3 binding sites, their transcription might also be held in check by BLIMP1. In the context of Blimp1 KO mice, therefore, these transcription factors are likely also expressed at elevated levels, which could lead to elevated CCL8 transcription, explaining this high expression of CCL8 in uninfected Blimp1 KO BMDMs.

In an effort to link elevated CCL8 levels to the enhanced neutrophil response and heightened ability of Blimp1 CKO mice to clear Listeria, we administered CCL8 in vivo to C57BL/6 mice to mimic the elevated levels of the chemokine observed in the Blimp1 CKO mice. CCL8-treated C57BL/6 mice upon infection in the liver had significantly lower bacterial loads in the infected organs. The heightened clearance of this bacterium in response to CCL8 correlated with a higher percentage of neutrophils in the peripheral blood. Conversely, Ccl8 KO mice were considerably more susceptible to L. monocytogenes infection, having higher bacterial loads in both spleen and liver, than their WT counterparts.

On the basis of these observations, we hypothesized that under normal circumstances, BLIMP1 acts to suppress the production of CCL8 as a means to limit neutrophil responses and excess inflammation, which can be deleterious to the host. We speculate that the elevated expression of CCL8 in Blimp1 CKO mice could either directly or indirectly promote neutrophils to egress from the bone marrow and infiltrate the livers of L. monocytogenes–infected mice. Our study, however, demonstrates that neutrophils do not directly migrate in response to CCL8. Rather, we propose an indirect mechanism involving CCL8 in neutrophil mobilization via an IL-17–mediated feedback loop. The IL-17 cytokine family comprises six isoforms, with IL-17A and its closest homolog IL-17F best characterized in vivo and in vitro (57). Cellular sources of IL-17 are mainly the innate immune cells γδ, γδ, and NKT cells (62), and the primary function of this cytokine is to curb infection by bacterial and fungal pathogens (63, 64). Both IL-17A and IL-17F play a particularly significant role in regulating neutrophil recruitment and granulopoiesis (65–67) by inducing expression of factor-CSF and IL-6. In addition, the most strongly induced IL-17 target genes are neutrophil-recruiting chemokines, such as CXCL1, CXCL5, and CXCL2 (68–70).

γδ T cells are specialized innate T cells that signal through a TCR formed by TCR-γ and -δ-chains (71, 72). These cells are rapidly activated, producing IFN-γ, TNF-α, and IL-17 in several mouse models of bacterial infection, including those of Mycobacterium bovis, Salmonella enterica, and L. monocytogenes (54, 73, 74). It has been also recently shown that the negative regulation of γδ T cell–mediated IL-17 production by type I IFN induced by primary influenza virus infection is of key importance for the increased susceptibility to secondary Streptococcus pneumoniae superinfection (75). Evidence confirms how the IL-17–γδ T cell axis plays a pivotal role in resistance to bacterial infection. In particular, for L. monocytogenes it was reported that IL-17 is expressed in the liver of infected mice from an early stage of infection and that the major IL-17–producing cells were γδ T cells.
FIGURE 7. \( \gamma \delta \) T cells are the cellular target of CCL8 chemotactic activity. (A) \textit{L. monocytogenes} in vivo infection was performed in six different \textit{Ccl8/12} double KO or six \textit{Ccl12} single KO mice. Six sex- and age-matched C57BL/6 mice were used as controls. Infected spleens and livers were harvested 48 hpi, and bacterial loads were calculated. Data represent means ± SD. *\( p = 0.001 \) (two-tailed Student \( t \) test). (B) Neutrophils purified from the bone marrow of WT mice were assayed for migration to mCCL8 and the positive control mCXCL2 in a Neuroprobe chemotaxis chamber. (C) Purity of neutrophils determined by Ly6G staining. (D) \( \gamma \delta \) T cells isolated from WT and \textit{Ccr8} \( 2/2 \) mouse spleens were assayed for migration to mCCL8 and the positive control mCCL2 in a Neuroprobe chemotaxis chamber. (E) Purity of WT and \textit{Ccr8} \( 2/2 \) \( \gamma \delta \) T cells determined by \( \gamma \delta \) TcR staining. Data in (B) through (E) are representative of three independent experiments. (F) \textit{L. monocytogenes} in vivo infection was performed in \( \gamma \delta \) T cell KO or C57BL/6 WT mice that had received CCL8 i.v. Infected spleens and livers were harvested 48 hpi, and bacterial loads were calculated. Data are from two different experiments conducted separately. The \( p \) values were calculated by two-tailed Student \( t \) test. *\( p < 0.001 \) untreated versus CCL8-treated WT mice, **\( p < 0.05 \) untreated WT versus untreated \( \gamma \delta \) KO mice.
expressing TCR Vγ4 or Vγ6 (54). Hamada et al. (54) also stated that IL-17A–deficient mice fail to control bacterial growth in vivo. IL-17, therefore, is an effector molecule produced by TCR γδ T cells, which is important in innate immunity against L. monocytogenes in the liver. Although murine CCL8 is a member of the MCP family, until recently it has been poorly characterized. Members of the C-C family of chemokines, such as CCL2 (also called MCP-1) and CCL7 (also called MCP3), are known to attract monocyte lineage cells bearing CCR2 on their surfaces (47, 48, 76). Luster and colleagues (51) identified CCR8 rather than CCR2 as the receptor for CCL8. Thus, in our study we finally demonstrated that the real target of CCL8 in the context of L. monocytogenes infection is γδ T cells, known to express CCR8 on their surface (55). γδ T cells are able to produce IL-17A and IL-17F, both involved in neutrophil recruitment, amplifying Th17 responses (59). Indeed, in this article we show that γδ T cells directly migrate in response to CCL8 and through the release of IL-17, and in particular IL-17F, likely encourage mobilization of neutrophils to the site of infection to promote a rapid innate immune response in situ. Even if the effects of removing γδ T cells or injecting CCL8 are milder on CFU counts than those seen in both Ccl8/12 double KOs and Blimp1 CKOs, we believe that our findings together support our model of CCL8-driven IL-17–γδ T cell–dependent resistance to bacterial infection.

The importance of neutrophils in the early resistance of mice to Listeria infection is still somewhat unclear. Early studies based primarily on cell depletions with anti–Gr-1 (clone RB6-8C5), an Ab that binds to the cell surface markers Ly6C and Ly6G suggested that neutrophils played a critical role in reducing bacterial burden in the liver and spleen early during infection. Ly6C is expressed on neutrophils, monocytes, and subsets of CD8+ T cells, whereas Ly6G is expressed solely on neutrophils. Recent studies using highly selective neutrophil depletion strategies indicated that neutrophils are important for control of L. monocytogenes in the liver, but not the spleen, during infection (77). Additional studies, however, indicate that they are dispensable for control of this bacterium and instead proposed that inflammatory monocytes (which were also depleted in the early anti-Gr1 studies) were essential for bacterial clearance (78). These disparate observations may suggest that the role of neutrophils may depend on the dose of bacteria. At lower doses of bacteria, at which the bacteria are primarily intracellular, neutrophils may be less important, whereas at higher doses of bacteria, which cause host cells heavily infected to lyse and release extracellular bacteria, this scenario may necessitate neutrophil cleanup. In the context of Blimp1 deletion, neutrophil-mediated bacterial clearance, rather than inflammatory monocyte-dependent mechanisms, appears to control enhanced bacterial clearance.

Our results describe a previously unknown mechanism involving BLIMP1-dependent control of CCL8 in macrophages used by the host to control L. monocytogenes infection. Our data demonstrate that BLIMP1 normally acts as a gatekeeper to curb CCL8–induced inflammation. Importantly, because BLIMP1 is also inducible in neutrophils and because LyzM Cre would also modulate BLIMP1 levels in neutrophils, our data cannot exclude additional roles for neutrophil-expressed BLIMP1 in this model. In the macrophage scenario, however, it is not clear whether induction of Blimp1 transcription early during infection acts to minimize the release of CCL8 to control indiscriminant cytolytic activity of neutrophils and minimize tissue damage or whether this is a result of the strategy of the pathogen to defeat host innate defenses. A common feature of pathogens is their ability to evade or suppress innate immunity, to avoid being eliminated and establish a successful infection. The ability of multiple pathogens to induce BLIMP1 suggests that this may be a common strategy of immune evasion.

Therapeutic manipulation of Blimp1 gene expression levels or BLIMP1 protein function may therefore represent a novel means to boost protective immunity in an early microbial infection. Furthermore, dysregulation of BLIMP1 could exacerbate autoimmunity by tipping the balance toward uncontrolled inflammation. In this regard, it is noteworthy that single-nucleotide polymorphisms in the Blimp1 gene have been linked to systemic lupus erythematosus. Enhancing Blimp1 expression under these circumstances could therefore also represent a novel means of alleviating persistent inflammatory and autoimmune diseases. Our discovery of the contribution of BLIMP1 to immune function reveals novel intervention points in the treatment of inflammatory diseases.

Acknowledgments
We thank Kathryn Calame for providing Prdm1fl/fl mice and Anna Cerny for backcrossing these animals as well as for animal husbandry and genotyping.

Disclosures
The authors have no financial conflicts of interest.
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