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Deficiency of the B Cell-Activating Factor Receptor Results in Limited CD169$^+$ Macrophage Function during Viral Infection

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ABSTRACT

The B cell-activating factor (BAFF) is critical for B cell development and humoral immunity in mice and humans. While the role of BAFF in B cells has been widely described, its role in innate immunity remains unknown. Using BAFF receptor (BAFFR)-deficient mice, we characterized BAFFR-related innate and adaptive immune functions following infection with vesicular stomatitis virus (VSV) and lymphocytic choriomeningitis virus (LCMV). We identified a critical role for BAFFR signaling in the generation and maintenance of the CD169$^+$ macrophage compartment. Consequently, *Baffr*$^{-/-}$ mice exhibited limited induction of innate type I interferon production after viral infection. Lack of BAFFR signaling reduced virus amplification and presentation following viral infection, resulting in highly reduced antiviral adaptive immune responses. As a consequence, BAFFR-deficient mice showed exacerbated and fatal disease after viral infection. Mechanistically, transient lack of B cells in *Baffr*$^{-/-}$ animals resulted in limited lymphotoxin expression, which is critical for maintenance of CD169$^+$ cells. In conclusion, BAFFR signaling affects both innate and adaptive immune activation during viral infections.

IMPORTANCE

Viruses cause acute and chronic infections in humans resulting in millions of deaths every year. Innate immunity is critical for the outcome of a viral infection. Innate type I interferon production can limit viral replication, while adaptive immune priming by innate immune cells induces pathogen-specific immunity with long-term protection. Here, we show that BAFFR deficiency not only perturbed B cells, but also resulted in limited CD169$^+$ macrophages. These macrophages are critical in amplifying viral particles to trigger type I interferon production and initiate adaptive immune priming. Consequently, BAFFR deficiency resulted in reduced enforced viral replication, limited type I interferon production, and reduced adaptive immunity compared to BAFFR-competent controls. As a result, BAFFR-deficient mice were predisposed to fatal viral infections. Thus, BAFFR expression is critical for innate immune activation and antiviral immunity.

The B cell-activating factor (BAFF) receptor (BAFFR) is critical for B cell development (1, 2). Patients lacking the BAFFR have been identified within cohorts with common variable immunodeficiency, the most prevalent symptomatic primary immunodeficiency in adult patients. BAFFR-deficient humans exhibit severe B cell lymphopenia and impaired immunoglobulin production (3). Similarly, the lack of BAFF signaling in *Baffr*$^{-/-}$ mice is also associated with severe B cell lymphopenia (4). BAFF binds to three receptors, the BAFFR, transmembrane activator and CAML interactor (TACI), and B cell maturation antigen (BCMA) (5). While TACI and BCMA engage both BAFF and a proliferation-inducing ligand (APRIL), BAFFR binds BAFF exclusively. The BAFF-BAFFR association leads to recruitment and degradation of TRAF3 (6). TRAF3 negatively regulates NF-κB-inducing kinase (NIK), the upstream kinase for NF-κB2 activation (7). In the presence of BAFF, degradation of TRAF3 leads to stabilization of NIK and activation of NF-κB2, which triggers B cell survival (8–11). Furthermore, BAFF triggers activation of Akt signaling pathways, which increase the metabolic activity of B cells (12). Additionally, BAFF transmits survival signals via Erk activation, which triggers phosphorylation and degradation of the proapoptotic molecule Bim (13–15). Together, these signaling pathways promote BAFF-mediated survival of B cells. Whether innate immunity may be abnormal in *Baffr*$^{-/-}$ mice has not yet been investigated.

Successful defense against viral infections relies on effective innate and adaptive immunity. During infection, viral particles are captured in secondary lymphoid organs, such as the lymph node or the spleen (16). In mice, macrophages in the marginal sinus in the splenic white pulp or macrophages located in the subcapsular space of the lymph node filter pathogens from the blood and the lymph, respectively (16–18). Metallophilic macrophages and subcapsular sinus macrophages are characterized by expression of the C-type lectin CD169 (Siglec-1) (19). CD169$^+$ cells are critical for innate cytokine production and viral antigen

presentation to B cells and represent an important link between innate and adaptive immunity (18). In particular, *Usp18*-driven suppression of antiviral type I interferon signaling allows viral replication to occur in CD169$^+$ cells in close proximity to marginal-zone B cells (20, 21). Consequently, large quantities of viral antigen are made available in order to induce rapid and robust adaptive immunity (22–25). The rapid induction of adaptive immune responses as a result of early viral replication in the spleen guarantees virus elimination and survival of the virus-infected host (20, 21).

B cells are important for the generation of the splenic architecture, including maintenance of the marginal zone (26, 27). Following a recent report indicating that BAFF is produced by neutrophils in the marginal zone of the spleen (28), we chose to investigate the impact of BAFFR signaling on innate immune responses. We found that absence of BAFFR resulted in reduced lymphotoxin expression, decreased presence of CD169$^+$ cells, delayed and impaired innate and adaptive immune activation, and, consequently, promotion of fatal disease development after viral infection.

MATERIALS AND METHODS

Mice. Mice were infected intravenously with vesicular stomatitis virus (VSV) or lymphocytic choriomeningitis virus (LCMV) at the indicated doses. *Baffr*$^{-/-}$ mice were bred on a C57BL/6 genetic background (4). CD45.1$^+$ mice were purchased from Jackson Laboratory. *Ltb*$^{fl/fl}$ × *CD19-Cre*$^+$ and *Ifnar1*$^{-/-}$ mice were previously described (29, 30). All experiments were performed in single ventilated cages. During survival experiments, the health status of the mice was checked twice daily. Upon the appearance of clinical signs of VSV replication in the central nervous system (CNS), such as paralysis, mice were removed from the experiment. Animal experiments were carried out with the authorization of the Veterinäramt of Nordrhein Westfalen, Recklinghausen, Germany, in accordance with the German law for animal protection and the institutional guidelines of the Ontario Cancer Institute.

Viruses. VSV, Indiana strain (VSV-IND, Mudd-Summers isolate), was originally obtained from D. Kolakofsky (University of Geneva, Geneva, Switzerland). The virus was propagated on BHK-21 cells at a multiplicity of infection (MOI) of 0.01 and was then plaqued onto Vero cells. LCMV was used and virus titers were determined as previously described (21). Viruses were administered to mice through intravenous injection.

Poly(I·C) and recombinant interferon. Poly(I·C) (GE Healthcare Life Sciences) and mouse recombinant interferon alpha (IFN-α) A (PBL Biosciences) were administered to mice through intravenous injection at the indicated doses.

Neutralizing antibodies were determined by a plaque reduction neutralization test (PRNT) as previously described (21). Serum was prediluted (1:40), and the complement system was inactivated (56°C for 30 min). The serum was titrated 1:2 over 12 steps and incubated with 1,000 PFU of VSV. After 90 min of incubation, the virus-serum mixture was plaqued on Vero cells. An overlay was added after 1 h. Plaques were counted 24 h later by crystal violet staining. The cutoff was 50% reduction of plaques compared to serum-free controls.

ELISA. Interferon alpha enzyme-linked immunosorbent assay (ELISA) (PBL Biosciences) was performed as instructed by the manufacturer.

Purification of B cells. For B cell purification, single-cell suspensions of splenocytes were enriched following the manufacturer's instructions with a CD45R (B220) MicroBeads mouse kit (Miltenyi).

RT-PCR analyses. RNA purification and real-time (RT)-PCR analyses were performed as previously described according to the manufacturer's instructions (Qiagen) (31). Gene expression of *Isg15*, *Mx1*, *Ifit2*, *Lta*, *Ltb*, and *Gapdh* was performed using kits from Applied Biosystems. For analysis, the expression levels of all target genes were normalized to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) expression levels (ΔC_T). Gene expression values were then calculated based on the $\Delta\Delta C_T$ method relative to naive wild-type (WT) controls. Relative quantities (RQ) were determined using the following equation: $RQ = 2^{-\Delta\Delta CT}$.

Histology. Histological analyses were performed on snap-frozen tissue as described previously by using anti-VSV-G monoclonal antibody (clone Vi10) (21). CD90.2, CD4, CD8, and B220 antibodies were purchased from eBioscience (San Diego, CA). CD169 (clone MOMA-1) antibody was obtained from Abcam (Cambridge, MA). Hematoxylin and eosin (H&E) staining was described previously (32).

Flow cytometry. Flow cytometry analyses were performed as previously described for LCMV tetramer staining and intracellular-cytokine staining (33). Different spleen immune populations were identified using anti-B220 (RA3-6B2), anti-major histocompatibility complex class II (MHC-II) (M5/114.15.2), anti-CD11c (N418), anti-GR-1 (RB6-8C5), anti-F4/80 (BM8), anti-CD3 (145-2C11), anti-CD8 (52-6.7), and anti-CD4 (GK1.5) antibodies (clone). Stem cell analyses were performed as previously described (34). The lineage (Lin) antibody mixture used for progenitor analysis contained CD3, CD11b (M1/70), Ly6C, Ter119 (TER-119), CD19, CD11c, MHC-II, interleukin 7 receptor (IL-7R) (A7R34), and NK1.1. Common myeloid progenitors (CMP) and granulocyte and macrophage progenitors (GMP) were determined using anti-CD34 (RAM34), anti-CD16/32 (93), anti-Sca1 (D7), and anti-CD117 (2B8) antibodies. All antibodies were obtained from eBioscience (San Diego, CA), except anti-CD169 (3D6.112), which was obtained from AbD Serotec (Dusseldorf, Germany).

Statistical analysis. Data are expressed as means and standard errors of the mean (SEM). Student's *t* test was used to detect statistically significant differences between two groups. Significant differences between several groups were detected by one-way analysis of variance (ANOVA) with Bonferroni or Dunnett *post hoc* tests or as mentioned specifically in the figure legends. The level of statistical significance was set at a P value of <0.05.

RESULTS

BAFFR is critical in overcoming viral infection. Murine BAFFR deficiency resulted in severe B cell lymphopenia but did not have a major impact on T cell, dendritic cell, or neutrophil numbers (Fig. 1A and B). As expected, *Baffr*$^{-/-}$ mice exhibited delayed virus-neutralizing antibody production after infection with the cytolytic VSV (Fig. 1C). Consistent with a requirement for rapid neutralizing antibody formation for elimination of VSV (21), *Baffr*$^{-/-}$ mice succumbed to VSV infection, while WT animals overcame the infection (Fig. 1D). When neuronal tissue was harvested from the sick animals (days 7 to 10), the presence of VSV could be detected (data not

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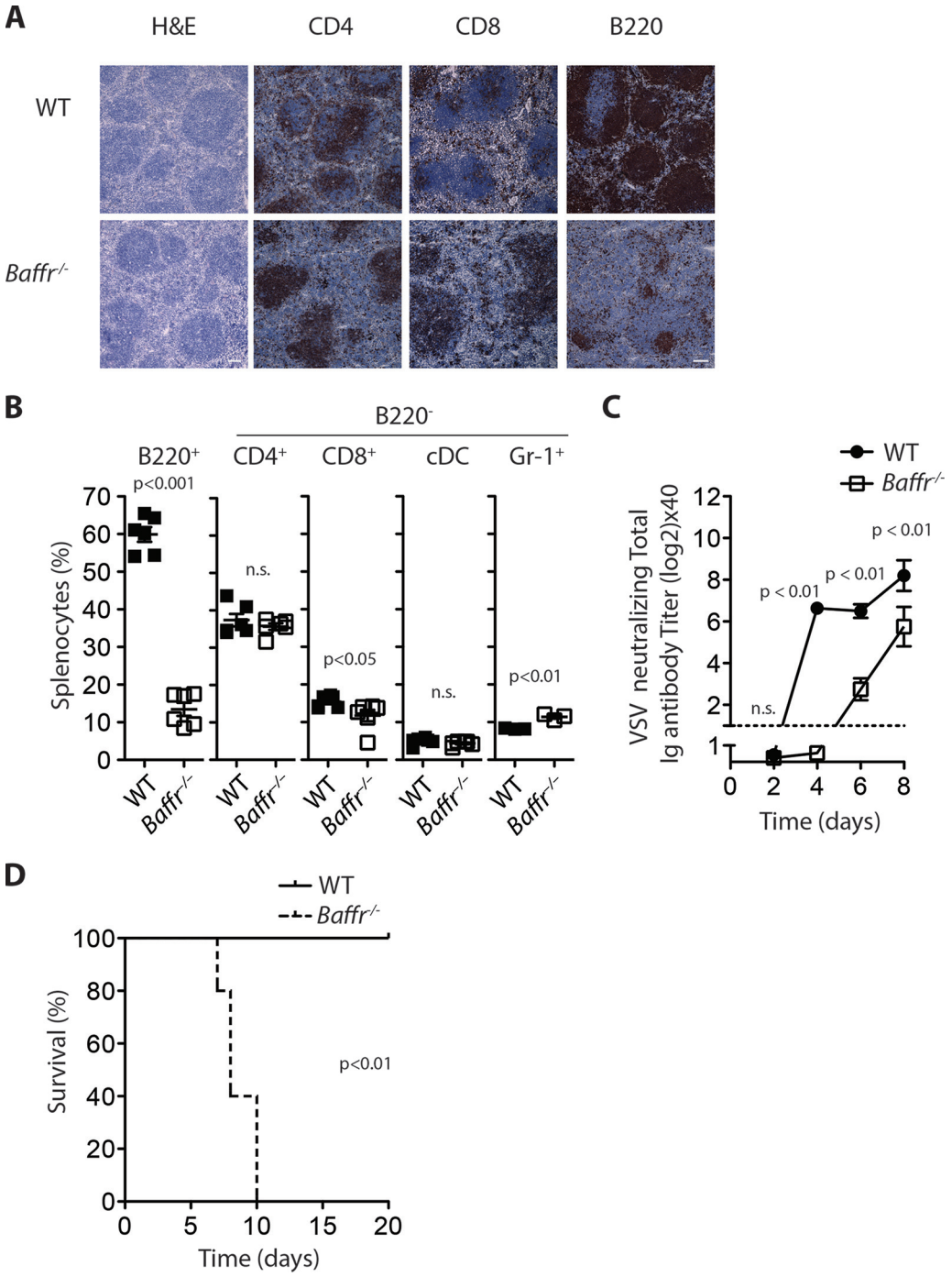


FIG 1 BAFFR is essential for antiviral immunity. (A) Sections from snap-frozen spleen tissues of WT and *Baffr*$^{-/-}$ mice were stained with H&E (scale bar = 100 μm) or stained with anti-CD4, anti-CD8, and anti-B220 antibodies (scale bar = 50 μm). One representative out of 5 is shown. (B) Single-cell suspensions from splenocytes derived from WT and BAFFR-deficient mice were analyzed for immune cell populations with monoclonal antibodies specific for the indicated markers ($n = 6$). n.s., not significant. Except for B220$^+$ cells, the percentages are related to B220$^-$ splenocytes). (C and D) WT and *Baffr*$^{-/-}$ mice were infected with 10^5 PFU of VSV. (C) Serum was taken at the indicated time points and analyzed for antiviral neutralizing antibodies by PRNT assay (n [initial] = 8). (D) Survival was monitored over the indicated period (n [initial] = 5). The error bars show SEM; n.s., not significant.

shown). These data suggest that absence of BAFFR resulted in fatal disease development during infection with VSV.

BAFFR mediates enforced viral replication during viral infection. Despite detection of VSV replication in the CNS in the later phase of infection, VSV titers were below the detection limit in spleen tissues of *Baffr*$^{-/-}$ animals 8 h and 24 h after infection with VSV, while virus was readily detectable in WT controls (Fig. 2A). Overcoming VSV infection is not only dependent on production of neutralizing antibodies, but also on innate type I interferon production (30, 35, 36). Consistent with decreased replication of

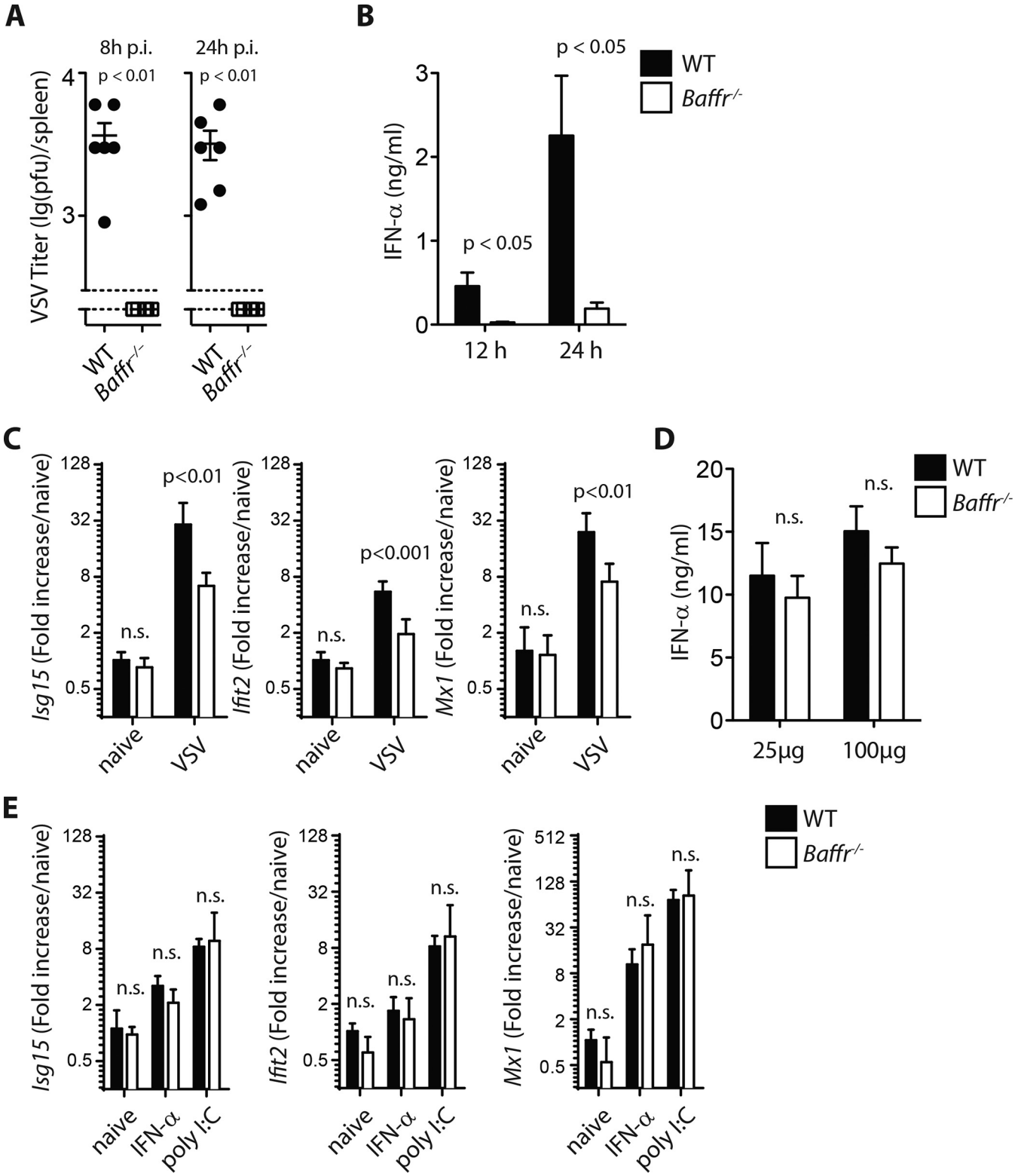
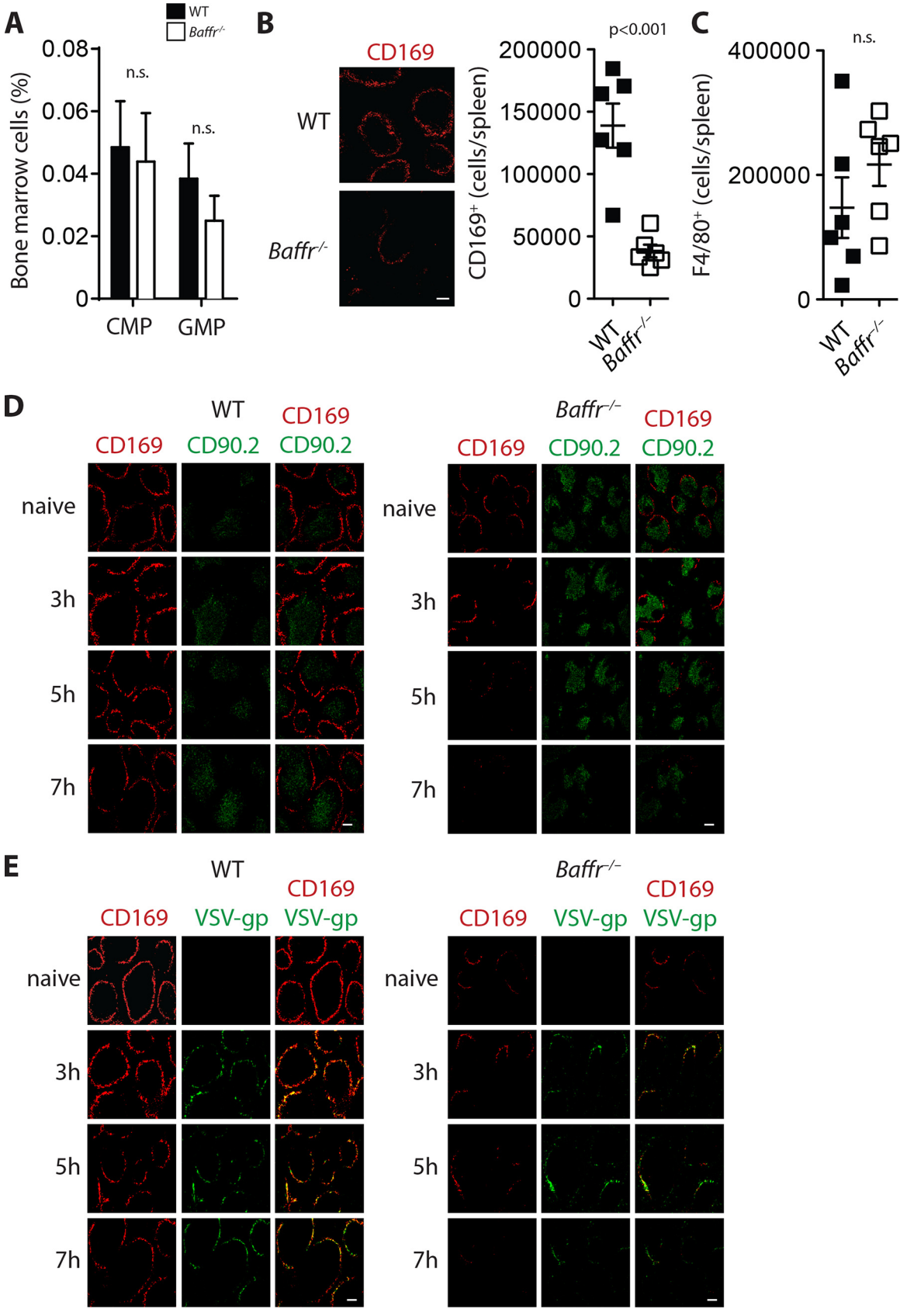


FIG 2 BAFFR mediates enforced viral replication during viral infection. (A to C) WT and *Baffr*$^{-/-}$ mice were infected with 10^5 PFU of VSV. (A) Virus titers were measured in the spleen 8 h (left) and 24 h (right) after infection (p.i.) ($n = 6$). The dashed line indicates the detection limit. (B) IFN-α concentrations were measured 12 h and 24 h after infection with 10^5 PFU of VSV in the sera of WT and BAFFR-deficient mice ($n = 6$). (C) *Isg15*, *Ifit2*, and *Mx1* mRNA expression was determined from brain tissues of infected WT and *Baffr*$^{-/-}$ mice 24 h p.i. ($n = 5$). (D) IFN-α concentrations were examined in the sera of WT and *Baffr*$^{-/-}$ mice 3 h after challenge with 25 μg or 100 μg poly(I·C) ($n = 6$). (E) *Isg15*, *Ifit2*, and *Mx1* mRNA expression was determined from brain tissues of WT and *Baffr*$^{-/-}$ mice 6 h after treatment with 10,000 units of mouse recombinant IFN-α or 100 μg poly(I·C) ($n = 6$). The error bars show SEM; n.s., not significant.


A
WT
Baffr-/-
Bone marrow cells (%)
0.08
0.06
0.04
0.02
0
n.s.
n.s.
CMP
GMP
B
CD169
WT
Baffr-/-
p<0.001
CD169+ (cells/spleen)
200000
150000
100000
50000
0
WT
Baffr-/-
C
n.s.
F4/80+ (cells/spleen)
400000
200000
0
WT
Baffr-/-
D
WT
CD169
CD169
CD90.2
CD169
CD90.2
Baffr-/-
CD169
CD169
CD90.2
CD169
CD90.2
naive
3h
5h
7h
naive
3h
5h
7h
E
WT
CD169
CD169
VSV-gp
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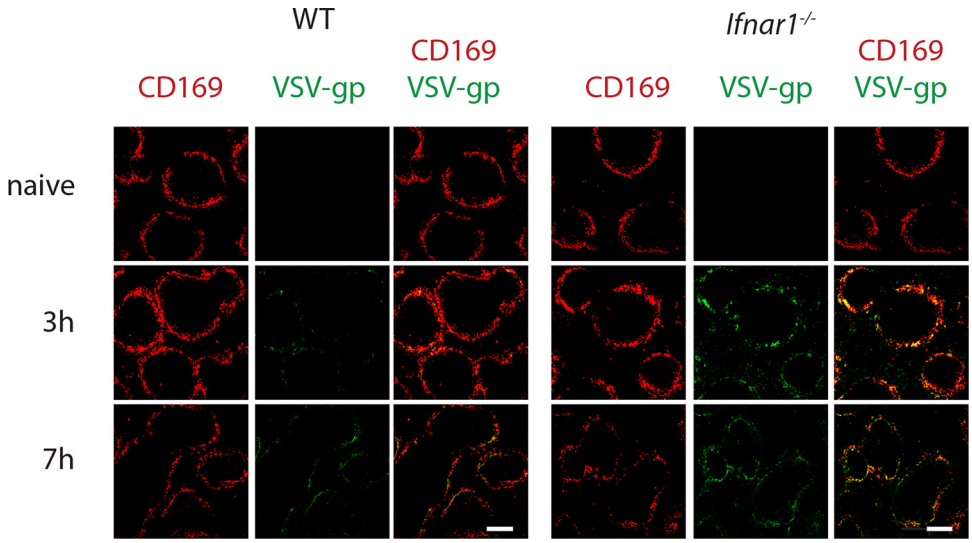

FIG 4 CD169$^+$ cell survival following VSV infection in *Ifnar1*$^{-/-}$ mice. Sections from snap-frozen spleen tissues obtained from WT and *Ifnar1*$^{-/-}$ mice after the indicated periods following VSV infection were stained with anti-CD169 and anti-VSV-G protein (clone Vi10) (1 representative out of 6 is shown; scale bars = 100 μm).

VSV in spleen tissue, *Baffr*$^{-/-}$ mice had reduced type I interferon levels in the serum compared to infected WT mice (Fig. 2B). Moreover, the increase of interferon-regulated genes (IRGs), such as *Isg15*, *Mx1*, and *Ifit2*, 24 h after VSV infection was reduced in brain tissue harvested from BAFFR-deficient animals compared to their corresponding controls (Fig. 2C). However, competent production of type I interferon was detectable in *Baffr*$^{-/-}$ mice following injection of the Toll-like receptor 3 (TLR3) agonist poly(I·C) *in vivo* (Fig. 2D). Furthermore, IRG expression levels in the brain tissues from *Baffr*$^{-/-}$ mice and their WT controls following treatment with poly(I·C) were similar (Fig. 2E). This indicates that the capacity to induce type I interferon was normal in *Baffr*$^{-/-}$ mice. Moreover, when animals were treated with mouse recombinant IFN-α, similar brain IRG expression levels were observed in BAFFR-deficient and WT mice (Fig. 2E). Collectively, reduced type I interferon levels in *Baffr*$^{-/-}$ mice following virus infection was likely explainable by a different mechanism than defective pathogen recognition receptor (PRR) signaling.

BAFF signaling is required for maintenance of metallophilic macrophages in the spleen. We have recently demonstrated that early virus replication in the spleen depends on CD169$^+$ metallophilic macrophages and is triggered by *Usp18*-mediated resistance to type I interferon in these cells (21). In mice, CD169$^+$ cells in the spleen are in direct contact with the bloodstream and remove virus particles and apoptotic cells from circulation (21, 37). Lack of CD169$^+$ macrophages blocks virus replication early after infection, causing limited antigen amplification and reduced virus-induced immune activation (21, 38). Since BAFFR-deficient animals exhibited lower viral replication during early infection, we investigated potential mechanisms by which BAFFR signaling might control enforced viral replication. Myeloid progenitor populations in the bone marrow, such as the CMP (Lin$^-$, Sca1$^-$, IL-7R$^-$, CD117$^+$, CD34$^+$, and CD16/32$^-$) or the GMP (Lin$^-$, Sca1$^-$, IL-7R$^-$, CD117$^+$, CD34$^+$, and CD16/32$^+$) did not differ between *Baffr*$^{-/-}$ and WT mice (Fig. 3A). However, CD169$^+$ metallophilic macrophages, which have a critical role during early viral replication (21), were highly reduced in spleen tissue of *Baffr*$^{-/-}$ mice compared to WT animals (Fig. 3B), while red-pulp macrophages were present at similar frequencies (Fig. 3C). Taken together, the data show that BAFFR appears to be required for maintenance of CD169$^+$ cells.

Next, we investigated the presence of CD169$^+$ cells following infection. CD169$^+$ cells are present at reduced numbers in BAFFR-deficient animals, but a residual population is still detectable in the naive state (Fig. 3D and E). However, shortly after infection with VSV, CD169$^+$ cells rapidly disappear in *Baffr*$^{-/-}$ mice, in contrast to WT animals (Fig. 3D). As a consequence of reduced CD169$^+$ macrophages, VSV protein (detected by the VSV-specific monoclonal antibody Vi10) was reduced in *Baffr*$^{-/-}$ mice in spleen sections following VSV infection, while it was readily detectable in spleen sections from WT animals (Fig. 3E). Next, we wondered whether type I interferon directly affected CD169$^+$ cell survival. However, the distributions of CD169$^+$ cells in spleen tissues harvested from *Ifnar1*$^{-/-}$ mice and WT animals were similar (Fig. 4). Taken together, these data indicate that lack of BAFFR expression is associated with a decreased presence of splenic

FIG 3 BAFFR signals are critical for maintenance of CD169$^+$ cells and viral replication in spleen tissue early after infection. (A) CMP and GMP in the bone marrow of WT and *Baffr*$^{-/-}$ mice were analyzed by flow cytometry (n = 6; n.s., not significant). (B) (Left) Sections of snap-frozen spleen tissues of WT and *Baffr*$^{-/-}$ mice were stained with anti-CD169 and analyzed by fluorescence microscopy (clone MOMA-1; 1 representative out of 6 is shown; scale bar = 100 μm). (Right) CD169$^+$ cells were measured in splenocytes of WT versus *Baffr*$^{-/-}$ mice by flow cytometry (n = 6). (C) F4/80$^+$ cells were analyzed in spleen tissues from WT and BAFFR-deficient animals by flow cytometry (n = 6). (D) Snap-frozen spleen sections were stained with an anti-CD169 antibody 0, 3, 5, and 7 h after VSV infection of WT versus BAFFR-deficient mice (1 representative out of 6 is shown; scale bars = 100 μm). (E) Sections from snap-frozen spleen tissues obtained from WT and *Baffr*$^{-/-}$ mice after the indicated time periods following VSV infection were stained with anti-CD169 and anti-VSV-G protein (clone Vi10) (1 representative out of 6 is shown; scale bars = 100 μm).

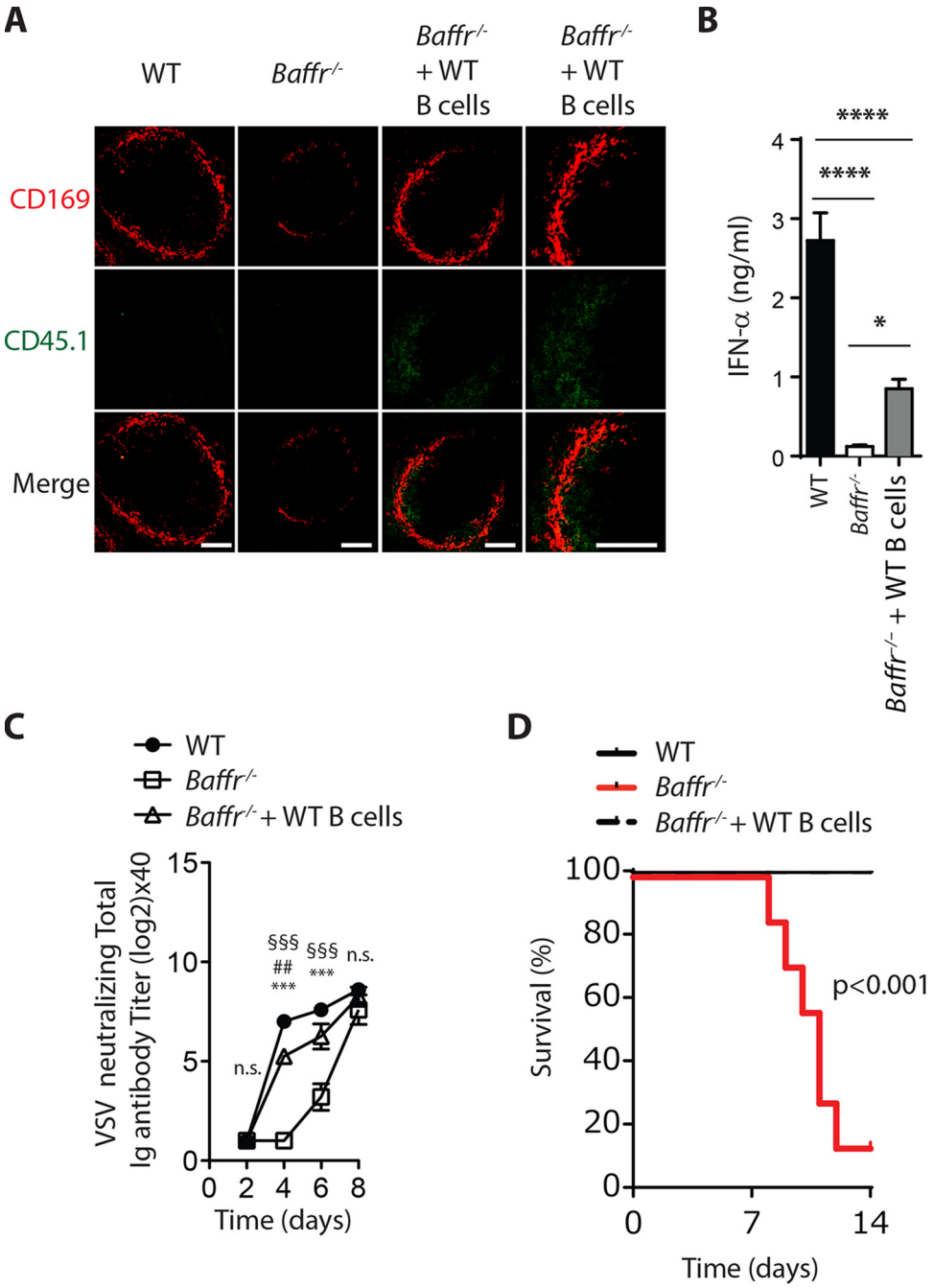

FIG 5 B cell-dependent maintenance of CD169$^+$ cells. (A) *Baffr*$^{-/-}$ mice were reconstituted with sorted WT B cells; 40 days later, spleen tissue was harvested and compared to that of WT and *Baffr*$^{-/-}$ mice. CD169$^+$ (top row) and transferred B cells (CD45.1; middle row) are shown in WT and *Baffr*$^{-/-}$ mice and *Baffr*$^{-/-}$ mice with transferred WT B cells ($n = 5$; one representative is shown; scale bars = 100 μm). (B to D) *Baffr*$^{-/-}$ mice were reconstituted with sorted WT B cells; 40 days later, the animals were infected with 10^5 PFU of VSV and compared to WT and *Baffr*$^{-/-}$ mice. (B) The IFN-α concentration was measured 24 h after infection in WT and *Baffr*$^{-/-}$ mice and *Baffr*$^{-/-}$ mice with transferred WT B cells. *, $P < 0.05$; ****, $P < 0.0001$; the Holm-Sidak test was used for *post hoc* testing. (C) Neutralizing Ig titers were determined at the indicated time points after infection ($n = 4$ or 5). ***, $P < 0.001$ between WT and *Baffr*$^{-/-}$ mice, and ##, $P < 0.01$ between WT and *Baffr*$^{-/-}$ mice after WT B cell transfer; §§§, $P < 0.001$ between *Baffr*$^{-/-}$ mice and *Baffr*$^{-/-}$ mice with transferred B cells. (D) Survival was monitored in WT and *Baffr*$^{-/-}$ mice and *Baffr*$^{-/-}$ mice 40 days after WT B cell transfer following infection with 10^5 PFU of VSV ($n = 7$ or 8). The error bars show SEM; n.s., not significant.

CD169$^+$ cells after virus infection, which consequently diminishes early viral replication.

BAFFR deficiency results in reduced B cell-mediated maintenance of CD169$^+$ cells. Next, we addressed whether the reduction of CD169$^+$ cells in *Baffr*$^{-/-}$ mice occurred due to severe B cell lymphopenia (39, 40). We transferred WT B cells into *Baffr*$^{-/-}$ mice and monitored CD169$^+$ cells in spleen tissue after 40 days. Interestingly, CD169$^+$ cells were readily detectable in spleen tissue

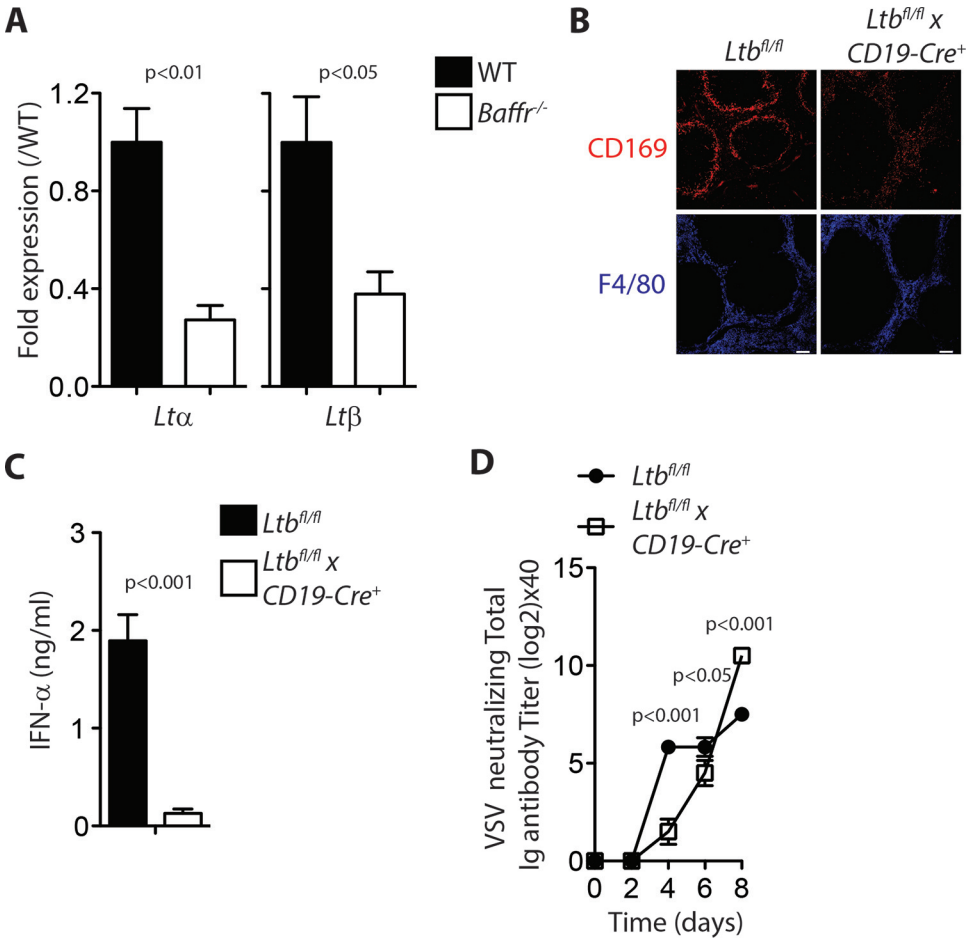

FIG 6 B cell-dependent maintenance of CD169$^+$ cells depends on lymphotoxin beta expression. (A) Lymphotoxin alpha (left) and beta (right) mRNA expression levels were analyzed from spleen tissue harvested from WT and *Baffr*$^{-/-}$ mice ($n = 5$ to 7). (B) Snap-frozen spleen sections from *Ltb*$^{fl/fl}$ × *CD19-Cre*$^+$ mice and control animals were stained with anti-CD169 and anti-F4/80 antibody (1 representative out of 3 is shown). (C) The IFN-α concentration was determined 24 h after infection with 10^5 PFU of VSV from *Ltb*$^{fl/fl}$ × *CD19-Cre*$^+$ and control animals ($n = 4$ to 6). (D) Neutralizing antibody titers were measured in sera harvested from *Ltb*$^{fl/fl}$ × *CD19-Cre*$^+$ and control animals at the indicated time points after infection ($n = 4$ to 6). The error bars show SEM; n.s., not significant.

of BAFFR-deficient mice that were supplemented with B cells (Fig. 5A). Furthermore, type I interferon production following VSV infection could be partially rescued by the transfer of WT B cells into *Baffr*$^{-/-}$ mice (Fig. 5B). Moreover, neutralizing antibody titers of the *Baffr*$^{-/-}$ animals that received WT B cells was increased compared to *Baffr*$^{-/-}$ animals (Fig. 5C), and the animals could overcome the VSV infection (Fig. 5D). These data indicate that B cells mediate maintenance of CD169$^+$ cells and consequently contribute to innate immunity during infection.

Lymphotoxin signaling is critical for CD169$^+$ cell development in spleen and lymph node tissues (32, 38, 39, 41). Moreover, it has been shown that lymphotoxins are derived from B cells, which are important for maintenance of CD169$^+$ cells (29, 39). Consistently, BAFFR-deficient animals exhibited lower lymphotoxin alpha (Ltα) and lymphotoxin beta (Ltβ) expression levels than their corresponding controls (Fig. 6A). These data suggest that impaired B cell numbers in *Baffr*$^{-/-}$ mice may contribute to insufficient lymphotoxin expression to maintain normal levels of CD169$^+$ cells (29, 38, 41, 42). To investigate the role of lymphotoxin beta during enforced viral replication, we infected *Ltb*$^{fl/fl}$ × *CD19-Cre*$^+$ animals and compared them to their corresponding controls. As expected, these animals exhibited fewer CD169$^+$ cells than the WT controls (Fig. 6B) (29, 42). Consistent with previous reports and our data obtained in *Baffr*$^{-/-}$ mice, we observed reduced type I interferon production shortly after infection in *Ltb*$^{fl/fl}$ × *CD19-Cre*$^+$ mice compared to *Ltb*$^{fl/fl}$ × *CD19-Cre*$^-$ animals (Fig. 6C) (39). Furthermore, low-dose infection resulted in reduced production of neutralizing antibodies (Fig. 6D). These data indicate that lack of B cells or B cell-derived expression of Ltβ results in limited innate immune activation and delayed adaptive immune priming.

BAFFR deficiency results in limited innate immune activation following LCMV infection. To further analyze the importance of BAFFR in viral replication and the induction of antiviral immunity, we examined *Baffr*$^{-/-}$ mice following LCMV infection. Consistent with the VSV infection, we observed reduced LCMV replication in the spleen tissue of *Baffr*$^{-/-}$ mice in comparison to WT controls at 72 h after infection (Fig. 7A). Furthermore, type I interferon levels were highly reduced in the sera of *Baffr*$^{-/-}$ animals compared to WT controls (Fig. 7B). Tetramer-positive LCMV-specific T cells were highly reduced following LCMV infection of *Baffr*$^{-/-}$ mice compared to WT

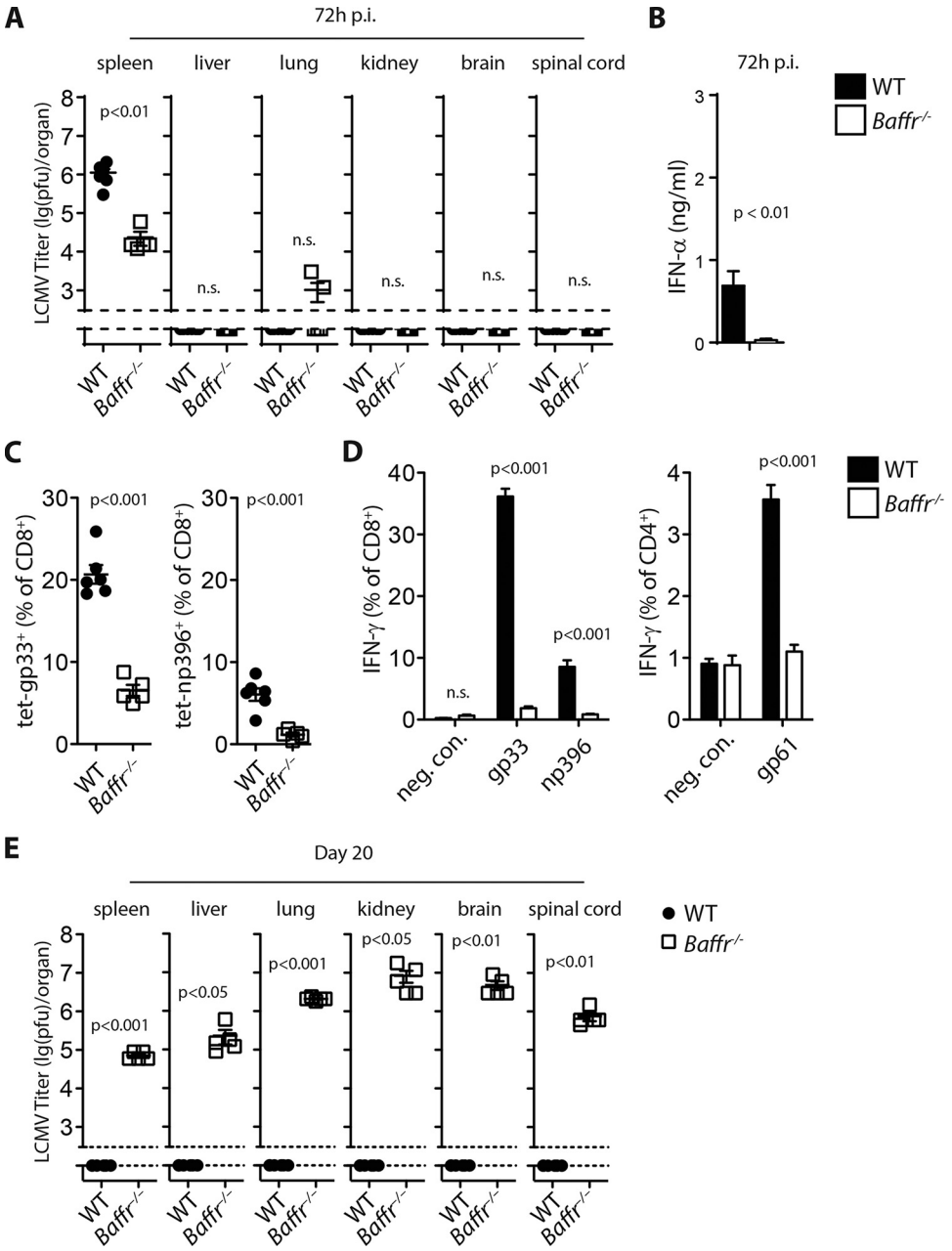

FIG 7 Impaired innate and adaptive immunity in BAFFR-deficient mice during LCMV infection. WT and *Baffr*$^{-/-}$ mice were infected with 200 PFU of LCMV-Docile. (A) Virus titers were measured in spleen, liver, lung, kidney, brain, and spinal cord tissues 72 h after infection (n = 5 or 6). (B) IFN-α concentrations were determined in the sera of infected animals 72 h following infection (n = 5 or 6). (C) Twenty days after infection, virus-specific CD8$^+$ T cells were examined by tetramer (tet) staining in spleen tissues of mice (n = 5 or 6). (D) IFN-γ production of T cells as assessed by intracellular-cytokine staining and flow cytometric analysis was measured after *in vitro* restimulation with the MHC-I peptides gp33 and np396 (left) and the MHC-II peptide gp61 (right) (n = 5 or 6). (E) Virus titers were measured in spleen, liver, lung, kidney, brain, and spinal cord tissues 20 days after infection (n = 5 or 6). The error bars show SEM; n.s., not significant; the dashed line indicates the detection limit.

mice (Fig. 7C). Moreover, IFN-γ production after *in vitro* restimulation with LCMV peptides was reduced in both CD8$^+$ and CD4$^+$ T cells harvested from *Baffr*$^{-/-}$ mice in comparison to WT controls (Fig. 7D). When viral titers were determined 20 days after infection, WT animals had eliminated the virus from all organs tested (Fig. 7E). In sharp contrast, LCMV-infected *Baffr*$^{-/-}$ mice displayed high virus titers in all organs tested (Fig. 7E). Collectively, these data suggest that the absence of BAFFR signaling causes impaired generation of the marginal-zone compartment and impaired induction of innate and adaptive immune responses during viral infection.

DISCUSSION

In this study, we have identified a critical role for BAFFR in the maintenance of CD169$^+$ macrophages. *Baffr*$^{-/-}$ mice showed limited innate immune activation and reduced adaptive immune

priming associated with fatal disease outcome. Mechanistically, impaired B cell development in *Baffr*$^{-/-}$ mice resulted in limited lymphotoxin expression and, likely as a consequence, reduced presence of CD169^{+} cells.

BAFF can be produced by a variety of immune cells, including dendritic cells, macrophages, and neutrophils (5). Interestingly, a recent report indicated BAFF-producing neutrophils were located in the marginal zone of the spleen (28). These neutrophil B helper cells contribute to marginal-zone B cell activation and antibody production against pathogens (28). Based on our results, BAFF production by neutrophil B helper cells, by promoting B cell-mediated lymphotoxin production, may also affect CD169^{+} cell survival and subsequently enforce antigen amplification and presentation. Furthermore, BAFF overexpression has been linked to a variety of autoimmune diseases, such as rheumatoid arthritis, lupus erythematosus, and Sjögren syndrome (5, 43–45). A clinically used BAFF-blocking antibody, belimumab, is effective in treating some lupus patients (46), and potentially, some clinical efficacy of BAFF neutralization in lupus patients may be due to effects on CD169^{+} macrophages.

Viral infections are potent activators of the immune system and can trigger autoimmunity (47) through several mechanisms, including molecular mimicry and bystander activation (48). Increased BAFF levels may affect not only B cell-mediated autoimmunity, but also B cell-mediated effects on CD169^{+} macrophages to increase bystander activation. Furthermore, replication of low-affinity antigens in CD169^{+} macrophages may contribute to development of virus-mediated autoimmunity induced by molecular mimicry (49). Considering our data, altered BAFF expression levels may lead to increased immune responses during viral infections. These mechanisms could also contribute to induction of autoantibodies observed during viral infections (50, 51).

As we show here, defects in BAFFR expression may limit innate immunity during infection. This may be triggered by reduced lymphotoxin beta production by B cells, as lack of lymphotoxin beta resulted in reduced presence of CD169^{+} macrophages (29, 38, 39). Furthermore, lymphotoxins trigger innate type I interferon production during viral infection (52, 53). This may be in part triggered by enforced viral replication in CD169^{+} cells, which is also critical for induction of adaptive immune priming (21, 49). Considering our data, deletions in BAFFR may not only affect B cell-driven immunity, but also trigger defects in innate immunity. Future studies may analyze the role of BAFFR deficiency during innate immunity in human patients.

In conclusion, we have determined that BAFFR deficiency mediates reduced presence of B cells, impacting the maintenance of CD169^{+} macrophages and innate immunity.

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