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Loss of Mandibular Lymph Node Integrity Is Associated with an Increase in Sensitivity to HSV-1 Infection in CD118-Deficient Mice[1]

Christopher D. Conrady,* Manoj Thapa,* Todd Wuest,* and Daniel J. J. Carr[2†]

Type I IFNs are potent antiviral cytokines that contribute to the development of the adaptive immune response. To determine the role of type I IFNs in this process in an infectious disease model, mice deficient in the type I IFN receptor ($CD118^{-/-}$) were ocularly infected with HSV-1 and surveyed at times post infection in the nervous system and lymph node for virus and the host immune response. Virus titers were elevated in the trigeminal ganglia and brain stem with virus disseminating rapidly to the draining lymph node of $CD118^{-/-}$ mice. T cell and plasmacytoid dendritic cell infiltration into the brain stem was reduced in $CD118^{-/-}$ mice following infection, which correlated with a reduction in CXCL10 but not CXCL9 expression. In contrast, CXCL1 and CCL2 levels were up-regulated in the brainstem of $CD118^{-/-}$ mice associated with an increase in $F4/80^+$ macrophages. By day 5 post infection, there was a significant loss in T, NK, and plasmacytoid dendritic cell numbers in the draining lymph nodes associated with an increase in apoptotic/necrotic T cells and an appreciable lack of HSV-specific $CD8^+$ T cells. The adoptive transfer of HSV-specific TCR transgenic $CD8^+$ T cells into $CD118^{-/-}$ mice at the time of infection modestly reduced viral titers in the nervous system suggesting in addition to the generation of HSV-specific $CD8^+$ T cells, other type I IFN-activated pathways are instrumental in controlling acute infection. ***The Journal of Immunology,*** **2009, 182: 3678–3687.**

IFN-$\alpha\beta$ (type I IFNs) are cytokines produced by numerous cell types typically associated with antiviral or antiproliferative characteristics and more recently, appreciated for their potential application in controlling autoimmune processes including multiple sclerosis (1, 2). Type I IFNs signal through a single heterodimer receptor (CD118) composed of an α- and β-chain, which upon activation elicits a signaling cascade leading to the induction of a number of IFN-responsive genes (3). The absence of one of the components within the signaling cascade STAT1 increases susceptibility to viral pathogens in humans (4) and mice (5). Relative to HSV-1 infection, STAT1-deficient mice are profoundly sensitive to infection underscored by avirulent HSV-1 mutants that replicate and spread in STAT1-deficient animals but not in fully competent wild type (WT)[3] mice (6). Consequently, it is not surprising HSV-1 encodes for proteins that repress CD118 signaling (7) or target downstream effector pathways that promote an antiviral state in the host cell (8).

In addition to targeting the type I IFN pathway, HSV-1 interferes with the MHC class I (9–11) and class II (12) processing pathways, disrupts TCR signaling (13), attenuates CTL cytolytic activity (14), and induces $CD4^+$ T cell apoptosis (15). Active infection of immature dendritic cells (DCs) results in asynchronous down-regulation of costimulatory and adhesion molecules including CD40, CD54, and CD80, which may be driven by the HSV-encoded virion host shutoff protein (16–19). Similar to T cells, DCs also reportedly undergo apoptosis following HSV-1 infection (20, 21). Collectively, disruption in the capacity to process and present Ag, suppress the expression of appropriate costimulatory molecules, and block DC maturation all contribute to the success of HSV-1 in eluding detection and countering the host immune response. Type I IFNs provide direct support of the host adaptive immune system at the level of T cells (22, 23) and DCs (24–26) and, therefore, antagonize the action of HSV-1-encoded proteins.

Previous investigations have used CD118-deficient ($CD118^{-/-}$) mice to demonstrate the sensitivity of these animals to HSV-1 infection based on virus replication, dissemination of the virus in the host, or to illustration of the importance of virally encoded proteins (6, 27, 28). However, no studies have addressed the innate or adaptive immune response within organized lymphoid tissue or infected tissue in $CD118^{-/-}$ mice in response to acute HSV-1 infection. The current study has focused on characterizing changes within the draining lymph nodes (mandibular lymph nodes, MLN) and nervous system relating viral loads to infiltrating and resident leukocyte populations and the cytokines/chemokines expressed following infection.

Materials and Methods

Virus and mice

C57BL/6J (WT) mice were purchased from The Jackson Laboratory. Mice deficient in the type I IFN receptor ($CD118^{-/-}$) (29) or HSV glycoprotein B (gB)T-I.1 TCR transgenic mice (30) on a WT background were maintained at Dean McGee Eye Institute. Animal treatment was consistent with the National Institutes of Health Guidelines on the Care and Use of Laboratory Animals. All procedures were approved by the University of Oklahoma Health Sciences Center and

*Departments of Microbiology, Immunology, and †Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

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[2] Address correspondence and reprint requests to Dr. Daniel J. J. Carr, Department of Ophthalmology, Room 415, University of Oklahoma Health Sciences Center, 608 Stanton L. Young Boulevard, Oklahoma City, OK 73104. E-mail address: dan-carr@ouhsc.edu

[3] Abbreviations used in this paper: WT, wild type; DC, dendritic cell; MLN, mandibular lymph nodes; pi, post infection; TG, trigeminal ganglia; BS, brain stem; gB, glycoprotein B.

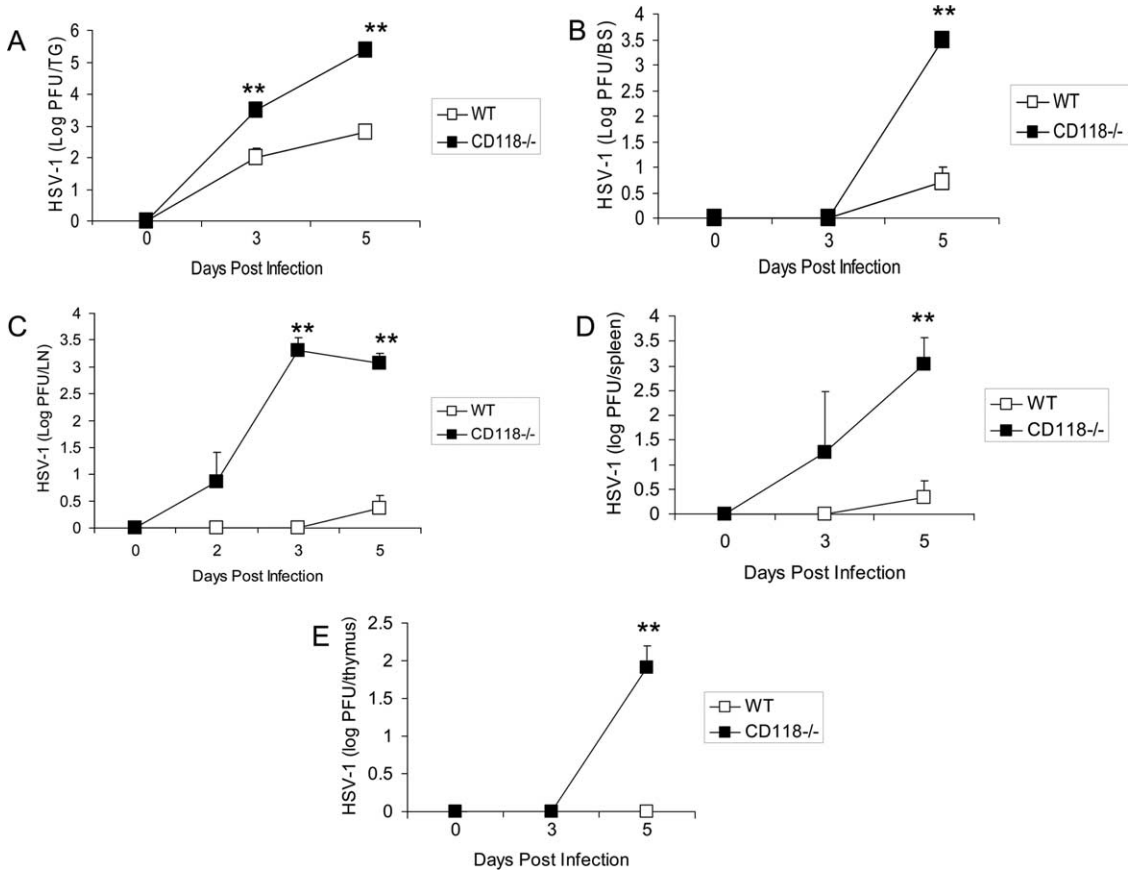

FIGURE 1. Elevated virus titers in tissues of HSV-1-infected CD118$^{-/-}$ mice. C57BL/6 (WT) and CD118-deficient (CD118$^{-/-}$) mice (n = 5–9 mice/group) were anesthetized and infected with 1,000 pfu/cornea. At the indicated time p.i., mice were euthanized, the tissue collected and processed, and resultant samples were assayed for virus content by plaque assay. *A*, Viral titer in TG. *B*, Viral titer in BS. *C*, Viral titer in mandibular lymph nodes (MLN). *D*, Viral titer in spleen. *E*, Viral titer in thymus. Each point represents the mean ± SEM. **, $p < 0.01$ comparing WT to CD118$^{-/-}$ mice for each time point evaluated.

Dean A. McGee Eye Institute Institutional Animal and Care Use Committee. HSV-1 (strain McKrae) was grown and maintained as previously described (31).

HSV-1 infection

Male and female WT and CD118$^{-/-}$ mice (6–10 wk of age) were anesthetized by i.p. injection with xylazine (6.6 mg/kg) and ketamine (100 mg/kg) followed by scarification of the cornea using a 25 5/8-gauge needle. The tear film was then blotted, and the cornea was topically inoculated with 1,000 PFU of HSV-1 in 3 μl of RPMI 1640 medium. HSV-1 viral titers were determined in the designated tissue at times post infection (p.i.) by plaque assay as previously described (32).

Flow cytometry

At the indicated time p.i., mice were exsanguinated and the MLN, thymus, spleen, trigeminal ganglia (TG), and brainstem (BS) were removed, processed, labeled with Abs, and analyzed using a Coulter Epics XL flow cytometer (BD Biosciences), and the absolute number of cells residing in the indicated tissue or organ was determined as previously described (33).

Tetramer staining

Single cell suspensions from the MLN were generated by passing the tissue through a 70-μm cell strainer (BD Falcon). One million cells were labeled with 1–2 μg of the PE-conjugated HSV peptide-specific $gB_{498-505}$ (SSIEFARL) MHC tetramer (MHC Tetramer Laboratory, Baylor College of Medicine) for 60 min on ice in the dark. The cells were washed (300 × g, 5 min at 4°C) and labeled with 1–2 μg FITC-conjugated anti-CD8 and PE-Cy5-conjugated anti-CD45. Following a 30-min incubation on ice in the dark, cells were washed again and resuspended in 1% paraformaldehyde. After a 60 min incubation at 4°C, the cells were washed again and resuspended in 1× PBS. Cells were subsequently analyzed by flow cytometry as described (33).

ELISA

At the indicated time before or p.i., the TG, BS, and MLN were removed from the exsanguinated mice and placed in 500 μl of 1× PBS containing a protease inhibitor mixture (Calbiochem) on ice. Following homogenization with a tissue miser (Fisher Scientific), the homogenates were clarified by centrifugation at 10,000 × g for 1 min. The levels of CXCL1, CXCL9, CXCL10, IFN-γ, and IL-2 were determined by ELISA according to the manufacturer's instructions (Quantikine immunoassay; R&D Systems).

Apoptosis

Apoptotic cells from the MLN of WT and CD118$^{-/-}$ mice were determined using a commercially available kit containing PE-conjugated annexin V (BD Pharmingen) and used according to the manufacturer's instructions. Uninfected MLN served as background controls.

In vitro culture and infection of mandibular lymph node cells

One million MLN cells from WT and CD118$^{-/-}$ mice were placed in 1.0 ml of RPMI 1640 containing 10% FBS and added to 24-well cultures plates containing 10 μl of HSV-1 (multiplicity of infection = 0.01). Twenty-four hours following incubation in 5% CO_2, 95% air at 37°C, the cultures were frozen/thawed twice, and clarified (10,000 × g, 1 min) supernatant was assayed for infectious virus by plaque assay.

Adoptive transfer of HSV glycoprotein B-specific TCR transgenic CD8$^+$ T cells or CD4$^+$ T cells into CD118$^{-/-}$ mice

Spleen cells from naive HSV gB-specific TCR transgenic mice (34) were highly enriched (>95%) for CD8$^+$ T cells using MACS columns (Miltenyi Biotec). At the time of infection, 3×10^6 enriched HSV gB-specific CD8$^+$ T cells were introduced i.v. into CD118$^{-/-}$ mice. Five days p.i., the mice were euthanized and assessed for viral content of infected TG and BS or

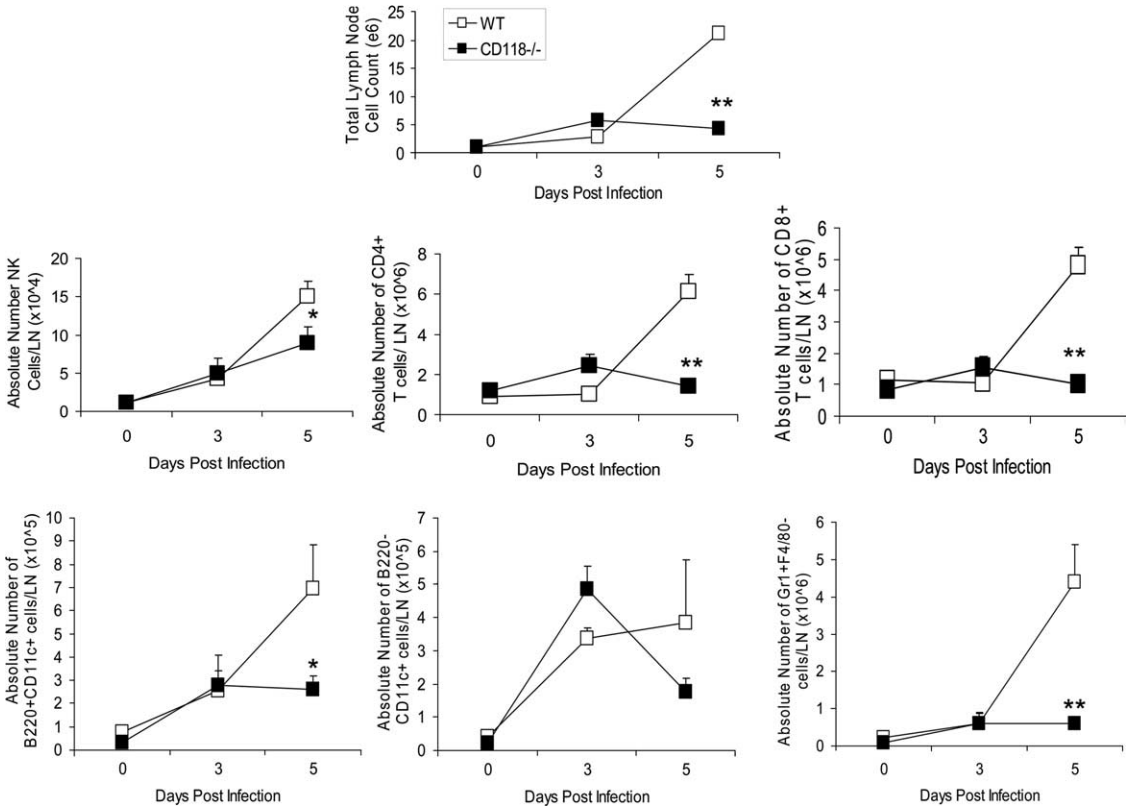

FIGURE 2. Phenotypic analysis of MLN leukocytes before and following HSV-1 infection. C57BL/6 (WT) and CD118-deficient ($CD118^{-/-}$) mice ($n = 6-8$ mice/group) were anesthetized and infected with 1,000 pfu/cornea. At 3 or 5 days p.i., mice were euthanized and cells from the MLN were removed and processed for flow cytometric analysis. Noninfected mice served as controls (day 0). Each point represents the mean number of phenotypic-defined cells ± SEM; **, $p < 0.01$; *, $p < 0.05$ comparing the WT to $CD118^{-/-}$ mice for each phenotype at the select time point.

gB-specific $CD8^+$ T cells in the MLN by tetramer staining and flow cytometry. $CD118^{-/-}$ and WT mice that did not receive cells served as negative and positive controls, respectively.

Statistics

Statistical analysis was conducted using the GBSTAT program (Dynamic Microsystems). Student's *t* test was used to determine significant ($p < 0.05$) differences between WT and $CD118^{-/-}$ groups. For adoptive transfer experiments, ANOVA and Tukey's *t* test were used to determine significant ($p < 0.05$) differences.

Results

Dissemination of HSV-1 to organized lymphoid tissue in $CD118^{-/-}$ mice

A previous study reported the dissemination of HSV-1 in $CD118^{-/-}$ mice following inoculation of mice in the footpad or cornea using a luciferase-engineered recombinant virus and bioluminescence imaging (28). To more fully understand the contribution of the type I IFN pathway in resistance to HSV-1 infection, we sought to evaluate the host innate and adaptive immune response relative to virus replication and spread. Following ocular infection, $CD118^{-/-}$ mice harbored significantly more virus in the TG and BS in comparison to WT controls at day 3 and 5 p.i. (Fig. 1, *A* and *B*). Moreover, virus rapidly disseminated to the MLN evident by detection as early as day 2 p.i. (Fig. 1*C*). Likewise, virus was detected in 50% of spleen samples from $CD118^{-/-}$ mice by day 3 p.i. with 7/8 $CD118^{-/-}$ spleen samples with detectable virus by day 5 p.i. in comparison to 0/8 WT spleen samples at day 3 p.i. and 1/5 WT spleen samples at day 5 p.i. (Fig. 1*D*). Thymus samples were equally telling with 7/7 thymus samples from $CD118^{-/-}$ mice possessing HSV-1 by day 5 p.i. compared with 0/7 thymus from WT mice (Fig. 1*E*). No virus was detectable in the thymus of $CD118^{-/-}$ mice at earlier time points.

Draining lymph node profile following HSV-1 infection is altered in $CD118^{-/-}$ mice

Because virus was detected early within the MLN of the $CD118^{-/-}$ mice following ocular infection, the draining lymph node was evaluated for predicted changes in the cellular constituency populating the organ. There was no significance change in the total number of $CD45^+$ leukocytes or subpopulations including NK cells, neutrophils, macrophages, DC, or T cells residing in the MLN before or 3 days p.i. comparing WT to $CD118^{-/-}$ mice (Fig. 2). However, by day 5 p.i., there was a massive loss of nearly all cell populations found in the MLN of $CD118^{-/-}$ mice in comparison to WT animals (Fig. 2). The loss was most pronounced in the $CD4^+$ and $CD8^+$ T cell populations. Consistent with this finding, there was a significant increase in the percentage of late apoptotic/necrotic $CD4^+$ and $CD8^+$ T cells in the MLN of $CD118^{-/-}$ mice by day 5 p.i. in comparison to WT animals (Fig. 3). At day 3 p.i., there were between 30 and 50% more T cells undergoing apoptosis in the $CD118^{-/-}$ mouse MLN in comparison to WT MLN T cells that may have contributed to the significant increase in late apoptotic cells observed by day 5 p.i. in $CD118^{-/-}$ MLN (Fig. 3). Even though there was a significant loss of DC in the MLN of HSV-1-infected $CD118^{-/-}$ mice by day 5 p.i. (Fig. 2), the percentage of cells positive for the costimulatory molecule CD80 was elevated in comparison to WT mice at this time point (Fig. 4*A*).

In addition to an increase in apoptotic/necrotic events in the MLN as a means to explain the reduction in leukocyte numbers in

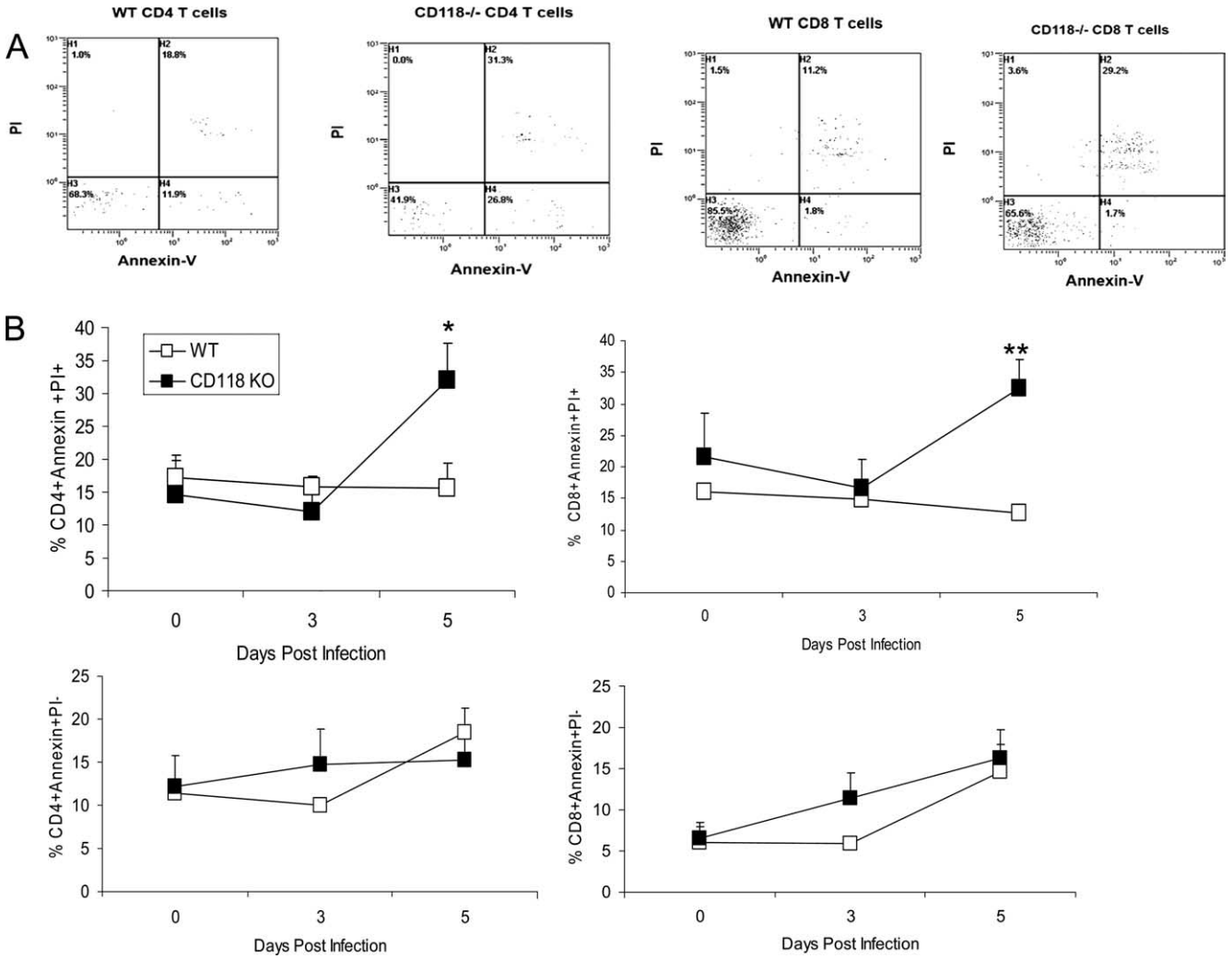

FIGURE 3. Apoptosis/necrosis of $CD4^+$ and $CD8^+$ T cells. Single cell suspensions were prepared from MLN of C57BL/6 (WT) and CD118 deficient ($CD118^{-/-}$) mice ($n = 6$ mice/group) at the indicated time before or following infection with HSV-1 (1,000 pfu/cornea). $CD4^-$ or CD8-labeled cells were incubated with Annexin V and propidium iodide (PI) according to the manufacturer's instructions and analyzed by flow cytometry. *A*, Representative flow plot of $CD4^+$ and $CD8^+$ T cells from WT and $CD118^{-/-}$ mice stained for PI and annexin-V at day 5 p.i. *B*, The summarized results of three experiments are expressed as the mean percent ± SEM of $CD4^+$ or $CD8^+$ T cells undergoing apoptosis (Annexin V^+PI^-) and late apoptosis/necrosis (Annexin V^+PI^+).

HSV-1-infected $CD118^{-/-}$ mice, a number of other aspects may factor into the mechanism associated with cell loss including soluble mediators that contribute to the antiviral state in the lymph node environment or facilitate growth and differentiation of lymphocytes. To address the first possibility, single cell suspensions of MLN cells were evaluated for susceptibility to HSV-1 infection in culture. MLN cell cultures from WT and $CD118^{-/-}$ mice produced similar levels of HSV-1 (3.59 ± 0.14 HSV-1 log PFU/ml compared with 3.81 ± 0.13 HSV-1 log PFU/ml, respectively) negating the possibility that MLN cells from $CD118^{-/-}$ mice were more susceptible to infection due to the absence of the type I IFN pathway. To address the second point, MLN from infected and uninfected mice were assayed for cytokine content. Contrary to the predicted outcome, MLN from $CD118^{-/-}$ contained significantly more IFN-γ compared with the WT MLN at times p.i. (Fig. 4*B*). There was also an increase in IFN-responsive chemokines including CXCL9 and CXCL10 (Fig. 4*B*). In contrast, IL-2 levels were reduced in the MLN from $CD118^{-/-}$ mice commensurate with the loss of T cells by day 5 p.i. (Fig. 4*B*). Consequently, the crash in T cell numbers in the MLN of $CD118^{-/-}$ mice may be due to a combination of virus present in the tissue resulting in an increase in apoptotic T cells and a reduction in IL-2 levels which exacerbate the disruption.

Alterations in chemokine and leukocyte migration in the TG and BS of HSV-1-infected $CD118^{-/-}$ mice

Because $CD118^{-/-}$ mice were found to possess significantly more virus in the nervous system at times p.i. and chemokines are expressed within the nervous system in response to infection (35, 36), levels of select chemokines were evaluated in the TG and BS of WT and $CD118^{-/-}$ mice. In the TG, CXCL1 levels were elevated whereas CXCL10 levels were reduced in $CD118^{-/-}$ mice at day 5 p.i. In uninfected mice or at day 3 p.i., the chemokine levels were found to be similar comparing the two genotypes (Fig. 5*A*). In contrast, no significant change in the levels of two other prominent chemokines CCL2 or CXCL9 were found to be different (Fig. 5*A*). By comparison, CXCL1 and CCL2 levels were elevated in the BS of $CD118^{-/-}$ mice by day 5 p.i. whereas at day 3 p.i. and in uninfected mice, similar levels were found comparing WT to $CD118^{-/-}$ mice (Fig. 5*B*). The results measuring CXCL9 and CXCL10 levels showed a striking difference in BS CXCL10 with reduced expression in $CD118^{-/-}$ mice by day 5 p.i. (Fig. 5*B*).

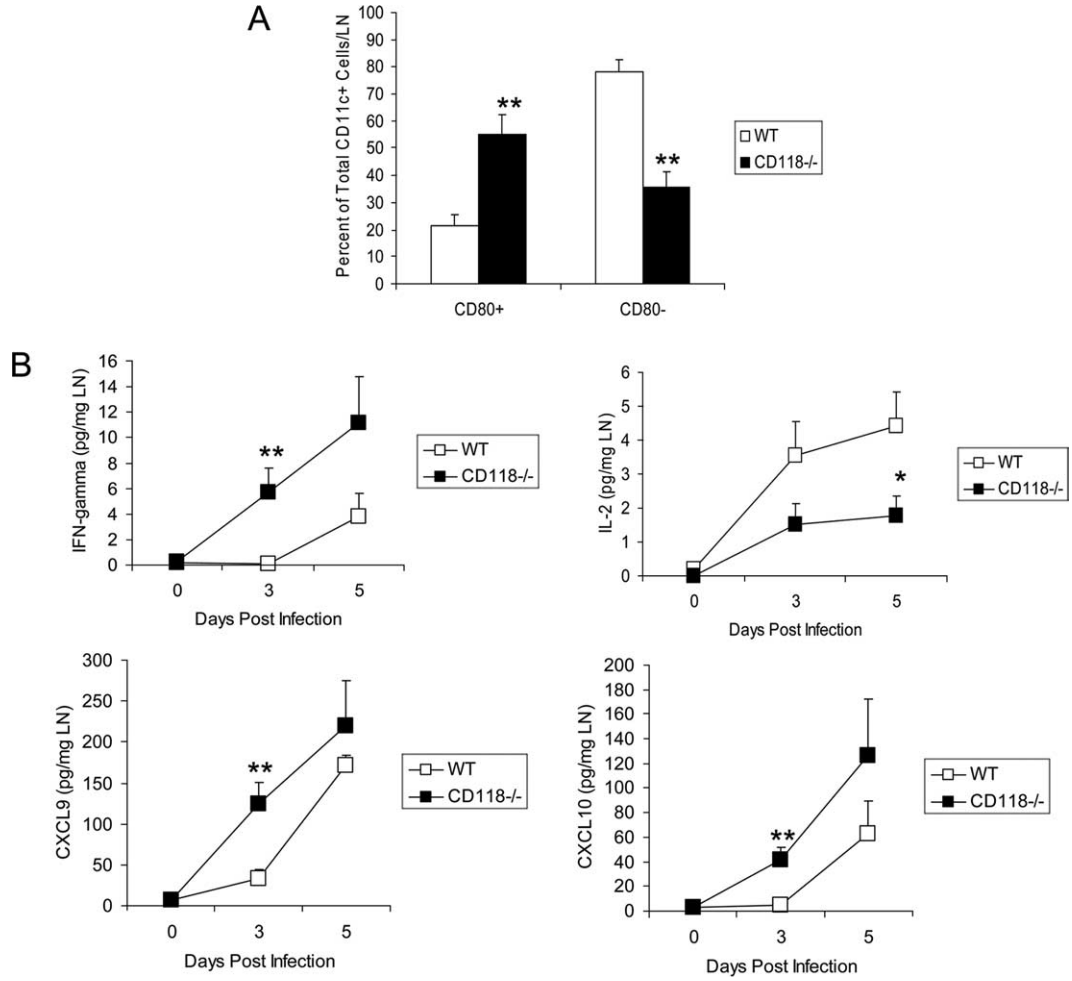

FIGURE 4. CD80, cytokine, and chemokine profile in the MLN. *A*, Single cell suspensions from MLN of C57BL/6 (WT) and CD118-deficient ($CD118^{-/-}$) mice ($n = 6$ mice/group) were generated day 5 p.i. The cells were labeled with PE conjugated anti-CD11c Ab and FITC-conjugated anti-CD80 Ab and assessed by flow cytometry. The results are expressed as mean ± SEM; **, $p < 0.01$ comparing WT to $CD118^{-/-}$ groups. Uninfected WT and $CD118^{-/-}$ mice showed similar levels of $CD11c^{+}CD80^{+}$ cells ranging from 3.9–4.1%. *B*, MLN were removed from C57BL/6 (WT) or CD118-deficient ($CD118^{-/-}$) mice ($n = 5$–8 mice/group/time point) before or at the indicated times p.i. The MLN were weighed, homogenized, and the clarified (10,000 × *g*, 1 min) supernatants were assessed for cytokine/chemokine content by ELISA. The results are expressed as mean pg/mg MLN ± SEM; **, $p < 0.01$; *, $p < 0.05$ comparing WT to $CD118^{-/-}$ samples at the indicated time point.

Similar levels were found at day 3 p.i. or in uninfected mice comparing WT to $CD118^{-/-}$ animals (Fig. 5*B*). Likewise, there were no significant differences in CXCL9 expression in the BS comparing WT to $CD118^{-/-}$ mice before or following infection (Fig. 5*B*).

Because the results show selective changes in specific chemokines in the peripheral and CNS comparing WT to $CD118^{-/-}$ mice following HSV-1 infection, the influence these changes may have on the recruitment of leukocytes was next investigated. Analysis of leukocyte populations (gated on $CD45^{high}$ expressing cells) residing in the TG including DC (both $B220^{+}CD11c^{+}$ and $B220^{-}CD11c^{+}$), macrophages ($F4/80^{+}Gr1^{-}$), neutrophils ($F4/80^{-}Gr1^{+}$), NK cells ($NK1.1^{+}CD3^{-}$), $CD4^{+}$ T cells ($CD3^{+}CD4^{+}$), and $CD8^{+}$ T cells ($CD3^{+}CD8^{+}$) found no difference in the total influx of leukocytes before or after infection comparing WT to $CD118^{-/-}$ mice (data not shown) but showed a trend ($p = 0.06$) in an increase in macrophages and a decrease in $CD8^{+}$ T cells by day 5 p.i. in the $CD118^{-/-}$ mice (Fig. 6*A*). Similar to the results in the TG, there was no significant difference in the total leukocyte population residing in the BS of WT or $CD118^{-/-}$ mice before or after infection (data not shown). However, there was a significant increase in the number of macrophages and a decrease in the number of plasmacytoid DC (pDC, $B220^{+}CD11c^{+}$) and $CD8^{+}$ T cells recruited or retained in the BS of $CD118^{-/-}$ mice by day 5 p.i. (Fig. 6*B*). $CD4^{+}$ T cell numbers were also found to be reduced in the BS of $CD118^{-/-}$ mice but similar to TG samples, the difference did not reach significance ($p > 0.05$).

A reduction in the generation of HSV gB-specific $CD8^{+}$ T cells in the MLN is associated with increased sensitivity to HSV-1 infection in $CD118^{-/-}$ mice

$CD8^{+}$ T cells have previously been reported to participate in the clearance of HSV-1 during acute infection or prevention of latent virus from reactivation (37–40). Because type I IFNs have previously been reported to facilitate $CD8^{+}$ T cell clonal expansion in a STAT-4-dependent manner (41), the level of HSV gB-specific $CD8^{+}$ T cell numbers was evaluated in the MLN of WT and $CD118^{-/-}$ mice following ocular HSV-1 infection. The day 5 p.i. time point was chosen since an earlier time point (i.e., day 3 p.i.) found no detectable HSV gB-specific $CD8^{+}$ T cells in the MLN. At day 5 p.i., the results show the presence of the HSV-specific $CD8^{+}$ T cells in the MLN of infected $CD118^{-/-}$ mice was noticeably reduced in comparison to the population residing in the MLN of WT mice (Fig. 7*A*). Specifically, between 3.5–5.1% of the

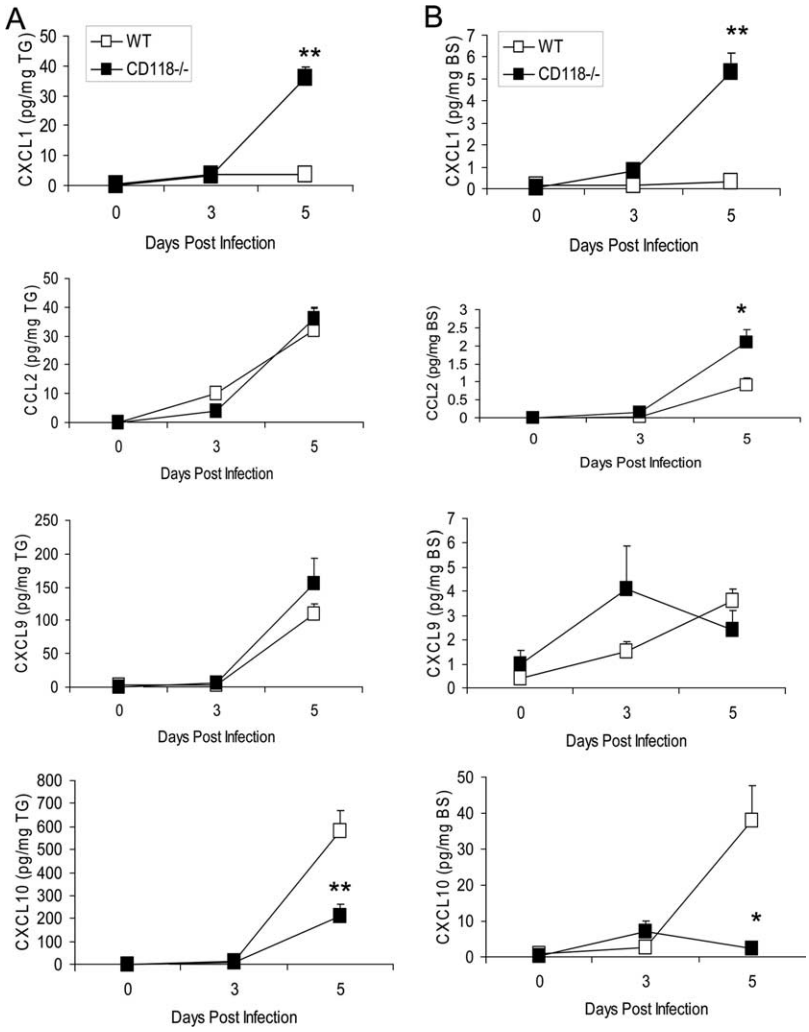

FIGURE 5. Chemokine levels in the nervous system in WT and CD118$^{-/-}$ mice. TG (*A*) and BS (*B*) (n = 6–9/group/time point) were harvested from perfused WT and CD118$^{-/-}$ mice before (day 0) and at times p.i. The tissue was weighed and homogenized, and clarified (10,000 × g, 1 min) supernatants were assessed for chemokine content by ELISA. The results are expressed as mean pg/mg tissue ± SEM; **, $p < 0.01$; *, $p < 0.05$ comparing WT to CD118$^{-/-}$ samples at the indicated time points.

CD8$^+$ T cells recovered from the MLN of WT mice were specific for HSV gB compared with 0.6–0.7% of the CD118$^{-/-}$ MLN population.

To further define the importance of this population of cells relative to HSV-1 infection in CD118$^{-/-}$ mice, adoptive transfer experiments were undertaken in which HSV gB-specific TCR transgenic CD8$^+$ T cells were transferred into CD118$^{-/-}$ mice and the recipients were evaluated for sensitivity to infection. The transfer of transgenic TCR T cells into CD118$^{-/-}$ significantly reduced the viral load in the TG and BS in comparison to CD118$^{-/-}$ mice that did not receive cells (Fig. 7*B*). However, the virus titer was still significantly elevated in comparison to WT mice (Fig. 7*B*). It should also be noted the number of HSV gB-specific CD8$^+$ T cells, total CD4$^+$ T cells, and total CD8$^+$ T cells were restored in the CD118$^{-/-}$ mouse recipients of the HSV gB-specific CD8$^+$ T cells (Fig. 7, *C* and *D*). Collectively, while HSV-specific CD8$^+$ T cells do contribute toward resistance to HSV-1 infection in CD118$^{-/-}$ mice, additional factors are also required to restore the phenotype consistent with that of WT mice.

Discussion

Type I IFNs contribute to the development of the immune response to infectious pathogens at various levels. Regarding the innate immune response, IFN-α promotes NK cell proliferation and cytotoxicity (42) in a STAT-4-independent, STAT-1-dependent fashion requiring IL-15 (43–45). In the present study, there was a modest loss of NK cell numbers by day 5 p.i. in the MLN of CD118$^{-/-}$ mice. However, at day 3 p.i., equivalent numbers of NK cells were measured in the MLN comparing WT to CD118$^{-/-}$ mice. At that time point, significant levels of HSV-1 were recovered in the MLN associated with the expression of IFN-γ in the CD118$^{-/-}$ mice. Although not studied, it is tempting to speculate the likely source of the IFN-γ was NK cells (46). Because IFN-α drives the expression of IL-10 thru IFN regulatory factor-1 and the ISGF3 complex (47, 48) and IL-10 is a feedback inhibitor of IFN-γ production (49), the absence of an intact type I IFN pathway would alleviate negative pressure on IFN-γ expression by NK cells. One likely outcome in this scenario is the up-regulation of chemokines responsive to IFN-γ including CXCL9 and CXCL10. Indeed, these chemokines were significantly elevated in the MLN of CD118$^{-/-}$ mice but there was not a corresponding increase in the number of cells responsive to these chemokines including NK cells and pDCs. It is possible HSV-1 within the MLN is capable of lysing the cells at a steady-state rate and thus, maintaining a level similar to that in WT mice.

Type I IFNs are also instrumental driving the production of chemokines through TLR-dependent and independent pathways (50–52). The present study found a correlation between T cell infiltration in the nervous system and local expression of CXCL10. Specifically, CD118$^{-/-}$ mice were found to express significantly less CXCL10 but not CXCL9 in the TG and BS by day 5 p.i., and such expression was associated with reduced

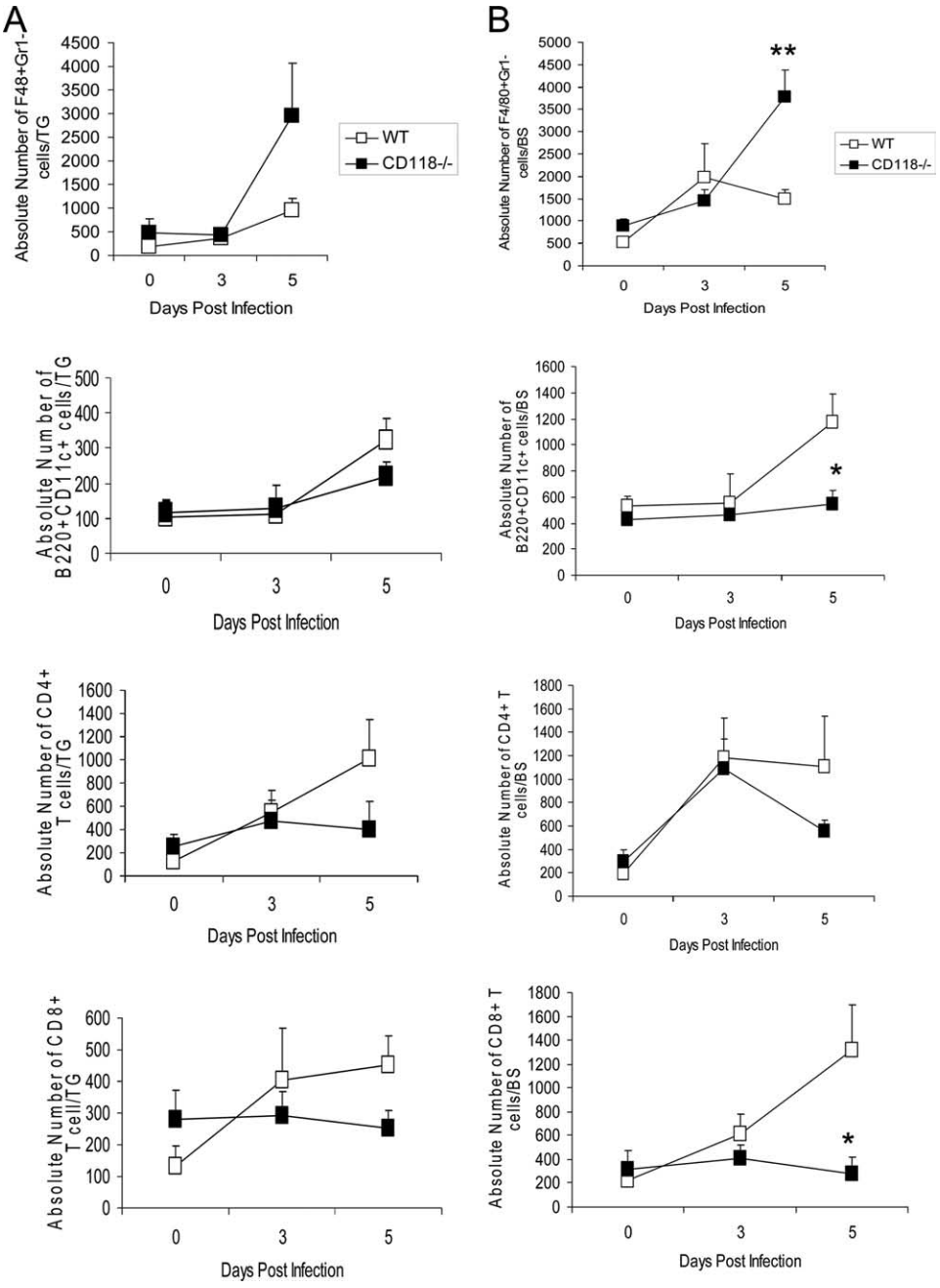

FIGURE 6. Loss of T cell and increase in macrophage recruitment to the brain stem of CD118$^{-/-}$ mice following HSV-1 infection. C57BL/6 (WT) and CD118-deficient (CD118$^{-/-}$) mice (n = 6–9 mice/group) were anesthetized and infected with 1,000 pfu/cornea. At 3 or 5 days post infection, mice were perfused and single cell suspensions from TG (*A*) and BS (*B*) were generated and processed for flow cytometric analysis. Noninfected mice served as day 0 controls. Each point represents the mean number of phenotypic-defined cells ± SEM; **, $p < 0.01$; *, $p < 0.05$ comparing the WT to CD118$^{-/-}$ mice for each phenotype at the select time point.

CD4$^+$ and CD8$^+$ T cell infiltration. Because a recent study by our group has found no deficiency in T cell recruitment to the nervous system in HSV-1-infected mice deficient in CXCL10 expression (33), it is more likely the deficiency in T cell recruitment to the nervous system in CD118$^{-/-}$ mice is due to the massive loss of T cells proliferating in the MLN of these animals. Of interest, elevated expression of CXCL1 and CCL2 in the BS was associated with an increase in macrophage influx in CD118$^{-/-}$ mice at a time when the animals present with edematous heads and eyes. In fact, CD118$^{-/-}$ mice rarely live pass 5 days p.i., which is likely due to the inflammatory response elicited by the pathogen including cytokines and proteases emanating from tissue macrophages and neutrophils that infiltrate the brain following infection (53).

At the crossroads between innate and adaptive immunity resides the DC, the principal Ag presenting cell that greatly influences the direction of the T cell response through the production of soluble factors including IFN-α (54). Different DC subtypes are known to promote Th1 or Th2 responses (55) in which type I IFNs can contribute toward Th1 (16) or Th2 (56) development. In the case of the infected CD118$^{-/-}$ mice, CD11c$^+$ cells expressed elevated levels of the costimulatory molecule, CD80. In contrast with our results, HSV-1 has been found to block DC maturation and stimulatory capacity (57, 58). However, mice deficient in IFN-β reportedly up-regulate CD80 expression (59). The mechanism driving the up-regulation of CD80 expression is unknown but could include the increase in IFN-γ (60) observed in the MLN of CD118$^{-/-}$ mice. Ironically, the elevated IFN-γ level in the MLN may be counterproductive to the overall immune status of the host. Specifically, it has recently been shown that IFN-γ-induced NO generated in response to apoptotic cells leads to cell-mediated immunosuppression (61). As an increase in apoptotic T cells is noted in the CD118$^{-/-}$ mice that manifest in significantly elevated levels of late apoptotic/necrotic T cells by day 5 p.i., apoptotic-driven

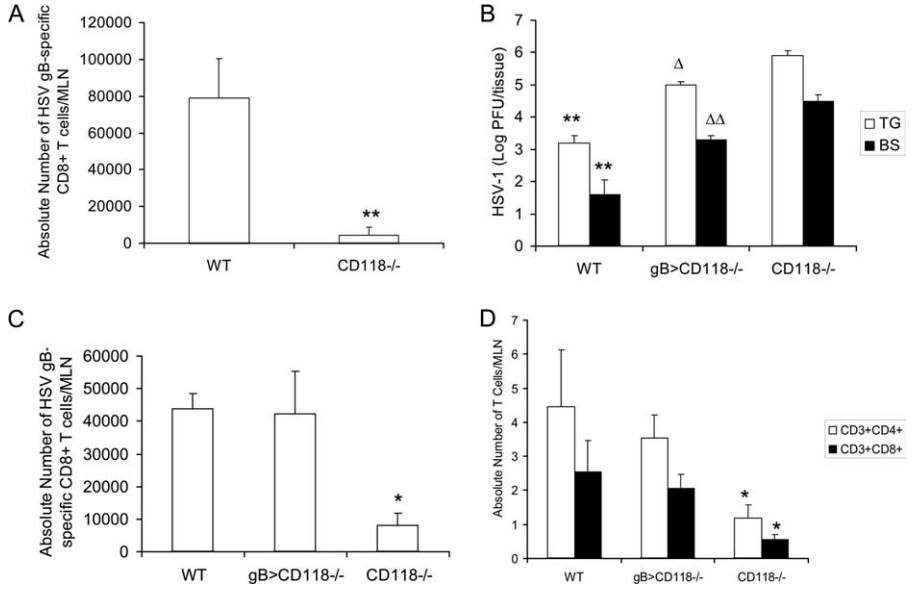

FIGURE 7. Partial restoration of resistance to HSV-1 infection in CD118$^{-/-}$ mice following adoptive transfer of HSV gB-specific CD8$^+$ T cells. *A*, WT and CD118$^{-/-}$ mice ($n = 6$ mice/group) were infected with HSV-1 (1,000 pfu/cornea) and euthanized 5 days p.i. The MLN were removed, processed, and analyzed for HSV gB-specific CD8$^+$ T cells by flow cytometry. The results are expressed in mean number of cells ± SEM; **, $p < 0.01$ comparing WT to CD118$^{-/-}$ mice. *B–D*, CD118$^{-/-}$ mice ($n = 5$) received highly enriched HSV gB-specific TCR transgenic CD8$^+$ T cells (gB>CD118$^{-/-}$) from naive HSV gB-specific TCR transgenic mice. The mice were then infected as were C57BL/6 (WT) and CD118$^{-/-}$ mice with HSV-1 (1,000 pfu/cornea). Five days p.i., the mice were euthanized and virus titer in the TG and BS (*B*), the number of gB-specific CD8$^+$ T cells residing in the MLN (*C*), and the number of CD4$^+$ and CD8$^+$ T cells in the MLN was determined (*D*). The results are expressed as the mean ± SEM; **, $p < 0.01$; *, $p < 0.05$ comparing the indicated group to the other group(s). ΔΔ, $p < 0.01$; Δ, $p < 0.05$ comparing the gB>CD118$^{-/-}$ to the CD118$^{-/-}$ mice.

suppression of HSV-specific T cell clonal expansion may explain the deficiency in HSV gB-specific CD8$^+$ T cells in the MLN of CD118$^{-/-}$ mice.

Type I IFNs facilitate the development of T cell immunity including TAP expression (62), which would greatly benefit the host and CD8$^+$ T cell responses through cross-priming (63). In mice infected with lymphocytic choriomeningitis virus, the loss of type I IFN signaling greatly diminishes clonal expansion of CD8$^+$ T cells as a result of a defect in cell survival (64). These results were found to be more pronounced in lymphocytic choriomeningitis virus-infected mice as opposed to other pathogens including vaccinia virus, vesicular stomatitis virus, or listeria monocytogenes, suggesting the dependence on type I IFNs by CD8$^+$ T cells is influenced by the insulting agent (65). In the present study, CD8$^+$ T cells were significantly affected by the absence of CD118 in terms of a reduction in cell number associated with an increase in late apoptosis/necrosis recovered in the MLN, lack of or diminished clonal expansion in the MLN, and a reduction in recruitment to the nervous system. The relevance of HSV-specific CD8$^+$ T cells in the resistance to HSV-1 infection is underscored by the reduction in infectious virus recovered in the TG and BS of CD118$^{-/-}$ recipients of HSV-specific TCR transgenic CD8$^+$ T cells and a partial restoration in the number of CD4$^+$ and CD8$^+$ T cells residing in the MLN. However, the viral titers did not achieve levels found in the immunocompetent WT mice, suggesting other pathways independent of type I IFN contribute to the control of HSV-1 which may include IFN-γ-activated mechanisms (32).

Unlike beta- and gamma-herpesviruses, it was originally thought primary HSV-1 infection does not cause viremia in healthy patients (66, 67). With the advent of PCR, a more recent study reported 34% of young patients presenting with primary herpetic gingivostomatitis had detectable viral transcripts in their PBMCs as determined by PCR (68). In comparing WT to CD118$^{-/-}$ mice, infectious virus was recovered in 20% of WT spleen samples screened in comparison to 88% of spleen samples screened from CD118$^{-/-}$ mice. The dissemination of virus to the spleen strongly suggests a blood-born route. In fact, we have previously reported CD11b$^+$ cells are actively infected with HSV-1 following ocular infection (69). If passenger leukocytes disseminate HSV-1 to various tissues and the incidence of spread is more pronounced in the host with a compromised type I IFN pathway, it is likely such a route may significantly contribute to the incidence of HSV-mediated encephalitis and meningitis (70).

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Disclosures

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