Role of Interferon Regulatory Factor 7 in T Cell Responses during Acute Lymphocytic Choriomeningitis Virus Infection

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Type I interferons (IFNs), predominantly IFN-α and IFN-β, play critical roles in both innate and adaptive immune responses against viral infections. Interferon regulatory factor 7 (IRF7), a key innate immune molecule in the type I IFN signaling pathway, is essential for the type I IFN response to many viruses, including lymphocytic choriomeningitis virus (LCMV). Here, we show that although IRF7 knockout (KO) mice failed to control the replication of LCMV in the early stages of infection, they were capable of clearing LCMV infection. Despite the lack of type I IFN production, IRF7 KO mice generated normal CD4+ T cell responses, and the expansion of naïve CD8+ T cells into primary CD8+ T cells specific for LCMV GP33–41 was relatively normal. In contrast, the expansion of the LCMV NP396-specific CD8+ T cells was severely impaired in IRF7 KO mice. We demonstrated that this defective CD8+ T cell response is due neither to an impaired antigen-presenting system nor to any intrinsic role of IRF7 in CD8+ T cells. The lack of a type I IFN response in IRF7 KO mice did not affect the formation of memory CD8+ T cells. Thus, the present study provides new insight into the role of the innate immune system on viral pathogenesis and demonstrates the critical contribution of innate immunity in controlling virus replication in the early stages of infection, which may shape the quality of CD8+ T cell responses.

Induction of type I interferons (IFNs) is not only essential for host innate immunity against viral pathogens but also critically involved in the development of virus-specific adaptive immune responses, in particular, T cell responses. Activation of naïve T cells occurs through three signals: the peptide-major histocompatibility complex (MHC) (signal 1), costimulatory molecules on antigen-presenting cells (APCs) (signal 2), and immunoregulatory cytokines (signal 3) (16). Type I IFNs are involved in regulating all three signals and can also directly act on T cells by interacting with the IFN-α/β receptor on T cells to provide survival signals (7, 26, 29, 31). In addition, in vitro studies have shown that IFN-α coordinately regulates lymphocytic choriomeningitis virus (LCMV)-specific CD8+ T cell activation (38, 41).

Studies suggest that type I IFN production is controlled by a group of transcriptional factors, including nuclear factor κB (NF-κB) and the interferon regulatory factors (IRFs) (19, 21). Nine IRFs have been identified to date, and IRF3 and its close relative IRF7 are important in controlling the induction of type I IFNs during viral infections (20, 22, 50). IRF7 expression is restricted to certain cell types, such as B cells and dendritic cells (DCs) (42). In other cells, IRF7 expression is inducible in response to type I IFNs as well as viral infections (42, 50). IRF7 is localized in the cytoplasm in an inactive form. Once activated, IRF7 undergoes phosphorylation and translocation to the nucleus to induce expression of the genes responsible for the production of type I IFNs (50, 53). We, and others, demonstrated previously that IRF7 is required for LCMV-induced type I IFN production (28, 62). The role of IRF7 in regulating antiviral adaptive immunity remains largely unexplored.

We used the Armstrong strain of LCMV (LCMV-Arm) in a model of acute viral infection to further explore the role of IRF7 in viral pathogenesis. LCMV-Arm infection induces a strong CD8+ T cell response specific for both dominant epitopes (H-2Dβ-restricted NP396–404, H-2Dβ-restricted GP33–41, and H-2Kβ-restricted GP54–64) and subdominant epitopes (H-2Dβ-restricted GP276–286) (59). Acute LCMV infection is cleared 8 to 10 days postinfection in immunocompetent mice. Viral clearance is predominantly mediated by virus-specific CD8+ T cells. In addition, IRF7-deficient mice are extremely susceptible to cytopathic virus infections (10, 21, 22), with mice dying prior to the development of the adaptive immune response. In contrast, infection of mice with noncytopathic LCMV (through either the intravenous or the intraperitoneal route) is not lethal. Therefore, LCMV infection in mice is a suitable model for examining the influence of IRF7 on the activation of naïve CD8+ T cells specific for different epitopes. Interestingly, we found that IRF7 KO mice, despite lacking type I IFN production and harboring uncontrollable LCMV replication in the early stages of infection, are capable of clearing an acute LCMV infection. While IRF7 KO mice develop normal CD4+ T cell responses specific for the GP61–80 epitope and relatively normal GP33-specific CD8+ T cells, the activation of NP396-specific CD8+ T cells in IRF7 KO mice is significantly impaired compared to that in wild-type (WT) mice. However, when IRF7 KO mice were treated with recombinant type I IFN, the defective development of NP396-specific CD8+ T cells was corrected. Furthermore, generation of bone marrow chimeric mice with IRF7 KO and WT bone marrow cells demonstrated that the expansion and differentiation of the NP396-specific IRF7 KO CD8+ T cells was comparable to that of WT control CD8+ T cells in a WT host. Thus, IRF7 does not play an intrinsic role in the activation of T cell responses. Collectively, these studies provide new insight into the role of IRF7 in regulating the development of adaptive immune responses and viral pathogenesis during an acute LCMV infection.
MATERIALS AND METHODS

Mice and virus. IRF7 knockout (KO) and IFN-α/β receptor (IFNAR) KO mice were obtained from RIKEN (Tsukuba, Japan) and the Jackson Laboratory (Bar Harbor, ME). All mice used in this study were 8-10 weeks old. LCMV-Arm/53b (LCMV or LCMV-Arm) and clone 13 (C13) were kindly provided by Raymond Welsh (University of Massachusetts Medical School, Worcester, MA) and were propagated on BHK-21 cells (American Type Culture Collection [ATCC]) at a low multiplicity of infection (0.01). Viral titers were determined with an immunological focus assay (4). Rat anti-LCMV NP antibody was kindly provided by Demetrius Moskophidis (Georgia Health Sciences University Medical College, Augusta, GA). For infection, mice were injected intravenously (i.v.) or intraperitoneally (i.p.) with 105 PFU of LCMV-Arm/53b. For intracranial (i.c.) injection, mice were lightly anesthetized with isoflurane and injected with 30 μL (200 PFU) of LCMV-Arm diluted with phosphate-buffered saline (PBS) (63).

To test if exogenous type I IFNs could restore CD8+ T cell responses in IRF7 KO mice, mice were first infected with LCMV-Arm/53b on day 0 and then given three injections (days 0, 1, and 2 postinfection [p.i.]) with 106 U/mouse recombinant universal alpha interferon A/D (also known as hybrid alpha interferon A/D; PBL Biomedical Laboratories). For ribavirin treatment, mice were first infected with 2 × 105 PFU of LCMV-Arm on day 0. Mice were treated with 300 mg/kg ribavirin (i.p.) at 4 h p.i. and once daily thereafter (45, 55). The specificity and function of CD8+ T cell responses in the spleens were analyzed using intracellular cytokine staining. LCMV GP33–41 and NP396–404-specific CD8+ T cells were also directly visualized using tetramer reagents.

Viral peptide. LCMV-specific H-2Db-restricted CD8 epitope peptides used in this study were GP33–41, GP276–286 and NP396–404 (40). The LCMV-specific CD4+ T cell epitope peptide used in this study was H-2Dd-restricted tetramer complexed with the T-cell receptor beta/delta (TCR-β/δ, TCR-Tg/Thy1.1) was performed as described previously (4). Briefly, splenocytes were incubated with uninfected or LCMV-CI13-infected DC2.4 cells as described previously (65). After 5 h of stimulation, the cells were harvested, washed once with PBS, and surface stained with allophycocyanin-conjugated monoclonal rat antibody specific to mouse CD8 (clone 53–6–72) or CD4 (clone RM4–5). After a washing, the cells were stained for intracellular cytokines by using a CytoTox/Cytoperm kit according to the manufacturer’s instructions. Phycoerythrin (PE)-conjugated monoclonal rat antibody specific to murine IFN-γ (clone XMG1.2) or fluorescein isothiocyanate (FITC)-conjugated monoclonal rat antibody to murine TNF-α (clone MP6-XT22) and the isotype control (IgG1) were used to identify cytokine-positive cells. All antibodies were purchased from BD Pharmingen (San Diego, CA). Samples were acquired on a BD-LSR-II flow cytometer (Becton, Dickinson). Data were analyzed with Flowjo software (Tree Star Inc.).

Enrichment of CD8+ T cells and adoptive transfer experiment. Naïve polyclonal CD8+ T cells were enriched from the spleens and mesenteric lymph nodes of either naive WT (B6-Thy1.1) or IRF7 KO mice using magnetic beads for negative selection, according to the manufacturer’s instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). Naïve P14 TCR-Tg/Thy1.1 T cells were purified from P14 TCR-Tg/Thy1.1 mice using the same strategy. The purity of CD8+ T cells was assessed (>85%) by staining with antibodies specific for mouse CD3 and CD8 or CD4 and CD8. Adoptive transfer experiments were carried out as indicated. Briefly, enriched naïve polyclonal WT, IRF7 KO, and IFNAR KO CD8+ T cells (5 × 106 enriched CD8+ T cells per mouse) were transferred into sex-matched TCR-β/δ KO recipient mice. One day after transfer, mice were infected with LCMV-Arm/53b (2 × 105 PFU, i.p.). CD8+ T cell responses in the spleens were examined between days 7 and 9 postinfection (97). The proliferative capacity of IRF7 KO CD8+ T cells in vivo, enriched naïve polyclonal WT and IRF7 KO CD8+ T cells were labeled with carboxyfluorescein diacetate N-succinimidyl ester (CFSE; Molecular Probes) as described previously (30, 63). The proliferation of CD8+ T cells was monitored using the CFSE dilution method.

Bone marrow chimeras. B6-Thy1.1 mice were lethally gamma-irradiated (950 rads). Four hours later, equal amounts (5 × 106) of bone marrow cells from sex-matched IRF7 KO (CD45.2) and B6-SJL-PtprcaPep3b/Bayl mice (CD45.1; The Jackson Laboratory) were adoptively transferred (i.v.) into irradiated B6-Thy1.1 mice, which were given water containing antibiotic. At 8 weeks posttransplantation, mice were infected with LCMV-Arm/53b at 2 × 106 PFU/mouse (i.v.). At day 8 p.i., mice were sacrificed, and the CD8+ T cell responses were analyzed using both tetramer reagents and ICS approaches.

Quantitative analysis of virus-specific CD8+ and CD4+ T cells by tetramer staining. H-2Dd-restricted tetramers complexed with the LCMV GP33–41 or NP396–404 peptide and I-Ab-restricted tetramer com-
plexed with the LCMV CD4 epitope GP61–80 or control were kindly provided by the NIH Tetramer Core Facility (Emory Vaccine Center, Atlanta, GA). Single-cell suspensions were prepared from spleens. Cells were stained with H-2Db tetramer following the protocol described previously (40). I-Ab tetramer staining was performed following the protocol described previously (60,63). After staining at 37°C for 3 h in RPMI/2%FCS, cells were washed once with PBS and stained with anti-CD4 allophycocyanin-conjugated rat monoclonal antibody with 1% bovine serum albumin and 0.2% sodium azide. After staining for 30 min at 4°C, cells were washed twice with PBS, fixed in PBS containing 4% formaldehyde, and analyzed on a BD-LSR-II flow cytometer (Becton, Dickinson). Data were analyzed with Flowjo software.

RESULTS
IRF7 KO mice are capable of clearing an acute LCMV infection. Our previous study, and those of others, demonstrated that IRF7 is essential for the LCMV-induced type I IFN response (28, 62). LCMV infection in IRF7 KO mice did not induce type I IFNs in peripheral blood (Fig. 1A) (62) or in spleen (Fig. 1B). Type I IFNs have strong antiviral activity (5,37, 40, 46, 51, 52). Thus, we evaluated the ability of IRF7 KO mice to clear LCMV infection. Consistent with previous results, WT mice effectively cleared LCMV infection by day 8 postinfection (p.i.) (40, 59). In contrast, IRF7 KO mice failed to control LCMV replication in the early phase of infection and harbored significantly higher levels of LCMV on day 3 (Table 1 and Fig. 2A) as well as day 8 (Fig. 2B) p.i.. Surprisingly, by day 12 p.i., IRF7 KO mice were able to clear LCMV infection (Fig. 2C). In line with previous studies (40), LCMV-Arm persisted longer in mice deficient in IFNAR. Type I IFNs play a critical role in the initial control of LCMV replication; however, the clearance of LCMV from the host is dependent on the functionality of virus-specific CD8+ T cells (39,66). Thus, these data raise the possibility that despite lacking type I IFN production following LCMV infection, IRF7 KO mice are capable of mounting a functional CD8+ T cell response.

IRF7 KO mice develop relatively normal CD8+ T cell responses compared to IFNAR KO mice. To evaluate the potential of CD8+ T cells in IRF7 KO mice, we first took advantage of the well-established murine model of LCMV intracerebral (i.c.) infection. Infection of immunocompetent mice with LCMV by the i.c. route induces a well-characterized fatal neurological disease, which is CD8+ T cell dependent (1,33, 47). In the absence of

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a WT and IRF7 KO mice (n = 3 for each group) were infected (i.v.) with 2 × 10^6 PFU of LCMV-Arm/53b. At day 3 p.i., the indicated tissues were collected, and virus titers were determined by an immunological focus assay. ND, not detected.

**FIG 2 IRF7 KO mice are able to clear LCMV infection.** Mice were infected (i.v.) with 2 × 10^6 PFU of LCMV-Arm/53b. At 3 (A), 8 (B), and 12 (C) days p.i., virus titers in spleens were determined using an immunological focus assay (limit of detection, 200 PFU/g tissue, corresponding to 2.3 log_{10} PFU). Data are representative of at least three independent experiments.
CD8\(^+\) T cells (depletion of CD8\(^+\) T cells or T cell deficiency), mice survive following i.c. LCMV infection but develop CD4\(^+\) T cell-mediated weight loss or wasting syndrome (18, 24, 63). Consistent with the literature (2, 12, 33, 47, 63, 67), WT mice succumbed to i.c. LCMV infection within 9 days (Fig. 3A). Interestingly, IRF7 KO mice also developed typical lethal neurological illness characterized by convulsive seizures and eventually succumbed to i.c. LCMV infection, although the onset of the lethal disease was slightly delayed (median survival for WT was 7 days; that for IRF7 KO mice was 8 days). Together with their ability to clear an acute LCMV infection, these data suggest that IRF7 KO mice have the potential to generate a functional CD8\(^+\) T cell response following an acute LCMV infection. Therefore, we next examined the CD8\(^+\) T cell response in IRF7 KO mice.

To determine the functionality of LCMV-specific CD8\(^+\) T cells in IRF7 KO mice, we compared the phenotypes and function of CD8\(^+\) T cells in WT, IRF7 KO, and IFNAR KO mice infected with LCMV by direct tetramer staining and intracellular cytokine staining (ICS) for IFN-\(\gamma\) and TNF-\(\alpha\) (Fig. 3B). The specificity and function of virus-specific CD8\(^+\) T cells in spleen were determined by direct visualization using tetramer reagents (C) and intracellular staining for IFN-\(\gamma\) (D). Cells were gated on total CD8\(^+\) T cells. The data are the means and standard deviations (SD) of the numbers of LCMV-specific CD8\(^+\) T cells per spleen and are representative of at least three separate experiments. *P < 0.05 compared to WT mice. (E) WT (n = 3) and IRF7 KO (n = 3) mice were infected with LCMV-Arm for 10 days. The function of CD8\(^+\) T cell response in the spleen was determined using intracellular IFN-\(\gamma\) staining after in vitro restimulation with the LCMV-specific CD8 epitope peptide GP\(_{276-286}\). One representative of three separate experiments is shown. *P < 0.05.

IRF7 KO mice have a defective LCMV NP\(_{396}\)-specific CD8\(^+\) T cell response but develop a normal memory CD8\(^+\) T cell pool. We next performed detailed analyses of the CD8\(^+\) T cell response in LCMV-infected IRF7 KO mice to further elucidate the observed differences. Interestingly, although the total number of LCMV-specific CD8\(^+\) T cells was significantly reduced in IRF7 KO mice during the initial stage of infection (day 8 p.i.) (Fig. 4A), IRF7 KO mice showed a pattern of GP\(_{33}\)-specific CD8\(^+\) T cell responses similar to that in WT mice, as determined by direct tetramer staining (Fig. 4B) and ICS-IFN-\(\gamma\) staining (Fig. 4D) during a time course experiment. In contrast, the LCMV NP\(_{396}\)-specific CD8\(^+\) T cell responses in IRF7 KO mice during the effector phase were significantly lower than that of WT CD8\(^+\) T cells, as indicated by direct tetramer staining (Fig. 4C) or production of IFN-\(\gamma\) following restimulation with LCMV NP\(_{396}\) peptide (Fig. 4E). The reduced expansion of LCMV NP\(_{396}\)-specific CD8\(^+\) T cells in IRF7 KO mice is not due to an increase in apoptosis, because both GP\(_{33}\)- and NP\(_{396}\)-specific CD8\(^+\) T cells in WT and IRF7 KO mice expressed annexin V (an early apoptotic marker [56]) at similar levels (Fig. 4F and G).

We next investigated whether the lack of type I IFN production in IRF7 KO mice affected the formation of memory CD8\(^+\) T cells. CD127 is highly expressed in activated effector and memory T
IRF7 KO mice exhibit defective LCMV NP\textsubscript{396}–specific CD8\textsuperscript{+} T cell responses despite developing a normal memory CD8\textsuperscript{+} T cell pool. WT (n = 4) and IRF7 KO (n = 4) mice were infected with LCMV-Arm/53b as described for Fig. 3. Total numbers of LCMV-specific CD8\textsuperscript{+} T cells were determined using intracellular IFN-γ staining after stimulation for 5 h with uninfected or LCMV-infected DC2.4 cells in the presence of brefeldin A (A). The CD8\textsuperscript{+} T cell responses were characterized using both H-2Db–restricted tetramers complexed with LCMV GP\textsubscript{33–41} and NP\textsubscript{396–404} peptides (B and C) and intracellular IFN-γ secretion (D and E) after stimulation of lymphocytes with LCMV-specific CD8 epitope peptides. Data are means ± SD of the numbers of LCMV-specific CD8\textsuperscript{+} T cells per spleen and are representative of at least three separate experiments. *, P < 0.05. (F and G) Mice (n = 2 for both WT and IRF7 KO mouse) were infected with LCMV/53b for 8 days. The phenotypes of CD8\textsuperscript{+} T cells were examined by GP\textsubscript{33} tetramer reagent together with annexin V (an early apoptotic marker) and 7AAD (to exclude late apoptotic cells); cells were gated on CD8\textsuperscript{+} GP\textsubscript{33} Tet\textsuperscript{-} 7AAD\textsuperscript{+} cells. Histograms show the expression of annexin V on WT (F) and IRF7 KO (G) LCMV GP\textsubscript{33}– and NP\textsubscript{396}–specific CD8\textsuperscript{+} T cells. Gray areas represent CD8\textsuperscript{+} T cells from uninfected mice (gated on total CD8\textsuperscript{+} T cells); gray lines represent GP\textsubscript{33}–Tet\textsuperscript{-} cells (gated on CD8\textsuperscript{+} GP\textsubscript{33}–Tet\textsuperscript{-} 7AAD\textsuperscript{+} cells); black lines represent NP\textsubscript{396}–Tet\textsuperscript{-} cells (gated on CD8\textsuperscript{+} NP\textsubscript{396}–Tet\textsuperscript{-} 7AAD\textsuperscript{+} cells). (H and I) WT and IRF7 KO mice (n = 2 for both strains) were infected with LCMV-Arm for 35 days, and peripheral blood samples were collected and monitored by tetramer staining together with anti-CD127 antibody (WT mice [H] and IRF7 KO mice [I]). Cells were gated on CD8\textsuperscript{+} GP\textsubscript{33}–Tet\textsuperscript{-} cells. Histograms show the expression of CD127 (black lines) on CD8\textsuperscript{+} GP\textsubscript{33}–Tet\textsuperscript{-} cells; gray areas represent the isotype control. (J and K) At 380 days after LCMV-Arm infection, the CD8\textsuperscript{+} T cell responses in the spleens of WT (n = 3) and IRF7 KO (n = 2) mice were characterized using both H-2Db–restricted tetramers complexed with LCMV GP\textsubscript{33–41} and NP\textsubscript{396–404} peptides (J) and intracellular IFN-γ secretion (K) after stimulation of lymphocytes with LCMV specific CD8 epitope peptides. At day 35 p.i., mice (n = 2 for both strains) were rechallenged with LCMV-Cl13 (2 × 10\textsuperscript{6} PFU i.v.). At day 4 after Cl13 challenge, LCMV-specific CD8\textsuperscript{+} T cells in peripheral blood were monitored by tetramer staining, and results are expressed as numbers of LCMV-specific CD8\textsuperscript{+} T cells/ml of blood. *, P < 0.05 (day 4 after Cl13 challenge versus before rechallenge).
cells and has been used as one of the memory CD8$^+$ T cell markers \(13, 23, 27\). GP$\text{33}^+$ and NP$\text{396}^+$-specific CD8$^+$ T cells in IRF7 KO mice expressed similar levels of CD127 compared to their counterpart in WT control mice (Fig. 4H and I). Remarkably, despite the reduction in LCMV NP$\text{396}^+$-specific CD8$^+$ T cells during the effector phase, the lack of type I IFN production in IRF7 KO mice did not affect the formation of memory CD8$^+$ T cells specific for either GP$\text{33}^+$ or NP$\text{396}^+$. The numbers of both GP$\text{33}^+$ and NP$\text{396}^+$-specific CD8$^+$ T cells in WT and IRF7 KO mice were equalized at around 40 days p.i. and remained relatively stable for over 1 year (Fig. 4B to E, J, and K). Additionally, IRF7 KO CD8$^+$ T cells were capable of responding to secondary infection with Cl13 (Fig. 4L and M), and protecting LCMV-immune IRF7 KO mice from the otherwise persistent Cl13 infection (data not shown).

**IRF7 KO DCs undergo normal maturation following infection with LCMV-Arm.** Type I IFNs play an important role in DC maturation upon virus infection, and LCMV infection rapidly induces DC maturation \(34\). Additionally, splenic DCs are critical for LCMV-induced activation of naïve T cells \(43\). To determine if the defective NP$\text{396}^+$-specific CD8$^+$ T cell response in IRF7 KO mice is due to impaired DC maturation, we analyzed the upregulation of DC activation markers. Twenty-four hours after infection with LCMV, splenic DCs were enriched and their phenotypes were analyzed. The surface activation markers (CD40, CD80, and CD86) in IRF7 KO DCs exhibited upregulation patterns similar to those of WT control cells (Fig. 5), demonstrating that the maturation of DCs is not impaired in IRF7 KOs following LCMV infection.

**FIG 5** IRF7 KO DCs undergo normal maturation in response to LCMV infection. WT and IRF7 KO mice \((n = 2 \text{ for both strains})\) were infected with LCMV-Arm/53b, and CD11$^+$ DCs were isolated from spleens 24 h p.i. The expression of CD40, CD80, and CD86 on CD11$^+$ DCs was determined for isotype controls (filled histogram), CD11$^+$ DCs enriched from uninfected mice (gray line), and CD11$^+$ DC enriched from LCMV-Arm infected mice (black line). Results are representative of two independent experiments.

**FIG 6** LCMV infection in IRF7 KO mice induces normal CD4$^+$ T cell responses. WT \((n = 4)\) and IRF7 KO mice \((n = 3)\) were infected i.v. with $2 \times 10^5 \text{ PFU of LCMV-Arm/53b. At day 9 p.i., LCMV-specific CD4}^+$ T cells in spleen were directly visualized using I$\text{A}^+$-restricted CD4 tetramer complexed with LCMV GP$\text{66}$ peptide or control peptide (A and B). Cells were gated on total CD4$^+$ T cells. The functional responsiveness of virus-specific CD4$^+$ T cells in spleen were determined by intracellular IFN-γ secretion after stimulation of lymphocytes with the LCMV-specific CD4 epitope peptide GP$\text{61−80}$ (C and D). Results are averages for three mice per group and are representative of at least three separate experiments.
**IFR7 KO mice have normal CD4⁺ T cell responses.** Although CD4⁺ T cells are not necessary for activation of naïve CD8⁺ T cells to acute LCMV infection, they are required for maintaining long-term memory in CD8⁺ T cell function (32, 58). In addition, it has been suggested that type I IFNs and the type I IFN receptor are involved in virus-specific CD4⁺ T cell development (17). To examine CD4⁺ T cell responses in IFR7 KO mice following LCMV infection, we used both tetramer staining (Fig. 6A and B) and measurement of intracellular IFN-γ on CD4⁺ T cells (Fig. 6C and D). Surprisingly, IFR7 KO mice developed a fully functional CD4⁺ T cell response following acute LCMV infection despite a lack of type I IFN production. Therefore, unlike the type I IFN receptors (IFNAR), deficiency of type I IFNs does not have a major impact on the activation of CD4⁺ T cell response following LCMV infection. This result is consistent with previous findings that type I IFNs have a more pronounced role in survival and activation of CD8⁺ T cells than in CD4⁺ T cells (26, 31).

**Addition of exogenous recombinant type I IFN restores CD8⁺ T cell responses in IFR7 KO mice.** The importance of type I IFNs in regulating CD8⁺ T cell responses is well documented (9, 26, 29). It is possible that the abnormal CD8⁺ T cell response in IFR7 KO mice is due to the lack of type I IFNs. To test this hypothesis, IFR7 KO mice were infected with LCMV followed by three injections of recombinant universal interferon-α-A/D (hybrid interferon-α-A/D), which is functional in mice (14, 38). On day 9 p.i., CD8⁺ T cells were analyzed by flow cytometry. (D) Lymphocytes were stimulated with LCMV-specific CD8⁺ epitope peptides, and the functional responsiveness of splenic CD8⁺ T cells was analyzed by intracellular IFN-γ staining. Results are means and SD of the numbers of LCMV-specific CD8⁺ T cells per spleen. *, P < 0.05 compared to the untreated group. (E to G) IFR7 KO mice were infected with 2 × 10⁶ PFU of LCMV-Arm/53b and then left untreated (n = 3) or treated with ribavirin (100 mg/kg) 4 h p.i. (day 0 p.i.) followed by daily ribavirin treatment for 9 days. Virus titers in spleens were determined.

**FIG 7** Treatment of IFR7 KO mice with either type I IFNs or ribavirin enhances the LCMV NP₃₉₀-specific CD8⁺ T cell response. (A) Both IFR7 KO and WT mice (n = 3 for each group) were infected (i.p.) with LCMV and followed by three injections (i.p.) with 10⁴ U/mouse recombinant universal IFN-α-A/D. (B) At day 9 p.i., the functional responsiveness of CD8⁺ T cells in the spleens was analyzed by intracellular IFN-γ production after restimulation of lymphocytes with LCMV-CD8 epitope peptides. Results are means and SD of the numbers of LCMV-specific CD8⁺ T cells per spleen. *, P < 0.05 (NP₃₉₀-specific CD8⁺ T cell responses between recombinant universal IFN-α-A/D-treated IFR7 KO mice and untreated IFR7 KO mice). (C) IFR7 KO mice (n = 3 for each group) were infected with LCMV-Arm/53b and treated daily with ribavirin (100 mg/kg) starting at 4 h (d0) or 2 days (d2) p.i. At day 9 p.i., splenic CD8⁺ T cells were visualized with H-2D⁺-restricted tetramers complexed with LCMV GP₃₃₋₄₁ and NP₃₉₀₋₄₀₄ peptides and the number of epitope-specific splenic CD8⁺ T cells was analyzed by flow cytometry. (D) Lymphocytes were stimulated with LCMV-specific CD8⁺ epitope peptides, and the functional responsiveness of splenic CD8⁺ T cells was analyzed by intracellular IFN-γ staining. Results are means and SD of the numbers of LCMV-specific CD8⁺ T cells per spleen. *, P < 0.05 compared to the untreated group. (E to G) IFR7 KO mice were infected with 2 × 10⁶ PFU of LCMV-Arm/53b and then left untreated (n = 3) or treated with ribavirin (100 mg/kg) 4 h p.i. (day 0 p.i.) followed by daily ribavirin treatment for 3 days (n = 4). At day 3 p.i., virus titers in spleens (E) and kidneys (F) were determined. (G) IFR7 KO mice were uninfected (n = 3) or infected with 2 × 10⁶ PFU of LCMV-Arm and then treated with ribavirin (100 mg/kg) starting at 4 h p.i. (day 0 p.i.; n = 3) or 2 days p.i. (n = 3), followed by daily ribavirin treatment for 9 days. Virus titers in spleens were determined.

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Interestingly, naïve IRF7 KO CD8 T cells in Fig. 3B to D, we showed that enriched IFNAR KO CD8 T cells were directly compared in the same recipient using TCR-specific CD8 activation of CD8 T cells failed to expand and differentiate into functional CD8 NP396-specific effector CD8 T cells, when transferred into WT control CD8 T cells (Fig. 8A to C). In line with published results (26) and data in Fig. 3B to D, we showed that enriched IFNAR KO CD8 T cells failed to expand and differentiate into functional CD8 T cells (Fig. 8A to C).

Because the impaired CD8 T cell response specific for LCMV NP396 in IRF7 KO hosts can be corrected by the addition of exogenous recombinant type I IFN, we wished to determine if IRF7 expression in CD8 T cells has an intrinsic role in the development of virus-specific CD8 T cell responses. To more precisely test the impact of the expression of IRF7 in CD8 T cells on the activation of CD8 T cell responses and to minimize the impact of differences in antigen load on the development of CD8 T cell responses during an acute infection with LCMV-Arm, a mixed bone marrow chimeric approach was used (Fig. 9A). In this approach, equal amounts of BM cells from both IRF7 KO (CD45.2 or Thy1.2) and WT donors (CD45.1) were adoptively transferred into gamma-irradiated B6-Thy1.1 WT recipients. Eight weeks following reconstitution, recipients were infected with LCMV-Arm. CD8 T cells responses specific for both GP33 and NP396 epitope peptides were directly compared in the same recipient using tetramer staining (Fig. 9D) and ICS-IFN-γ staining (Fig. 9E). Both IRF7 KO and WT CD8 T cells comparably expanded (Fig. 9B and C) and differentiated into GP33- and NP396-specific CD8 T cells following infection with LCMV-Arm (Fig. 9D). Upon in vitro restimulation with GP33 and NP396 epitope peptides, both IRF7 KO and WT CD8 T cells equally expressed IFN-γ (Fig. 9E). Collectively, these data further demonstrate that the reduced CD8 T cell activity seen in IRF7 KO mice following infection with LCMV-Arm is not due to the intrinsic expression of IRF7 in CD8 T cells.

While the intrinsic expression of IRF7 on CD8 T cells is not necessary for their function, the lack of IRF7 could cause a defect in CD8 T cell proliferation. We used carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled T cells to examine if IRF7 KO CD8 T cells exhibit proliferation defects in response to LCMV infection. We demonstrated that naïve IRF7 KO CD8 T cells, when transferred into WT hosts which were then challenged by LCMV infection, proliferated as well as WT control CD8 T cells (data not shown). Because the defect in the development of NP396-specific effector CD8 T cells from IRF7 KO mice can be overcome by giving IFN to the mice (Fig. 7B), these results support the notion that the expression of IRF7 in CD8 T cells does not play a major role in the development of LCMV-specific effector CD8 T cells.

**DISCUSSION**

IRF7 is a critical regulator in the production of type I IFNs (22). Studies from our group and others have demonstrated that IRF7 is required for LCMV-induced type I IFN production (28, 62). In the present study, we further investigated the physiological role of IRF7 in virus clearance and in regulation of the activation of naïve T cells during acute LCMV infection. We made the following observations. First, although LCMV-Arm replication is enhanced in IRF7 KO mice in the early stages of infection, within 12 days p.i., IRF7 KO mice are capable of clearing LCMV-Arm. Second, although IRF7 KO mice did not produce type I IFNs following LCMV-Arm infection, they did mount a normal CD4 T cell response and a relatively normal CD8 T cell response specific for the LCMV GP33 and GP276 epitopes. In contrast, the development
FIG 9 IRF7 expression in CD8+ T cells is not required for the development of CD8+ T cell responses. (A) Equal numbers (5 × 10^6) of BM cells from sex-matched IRF7 KO (CD45.2) and WT (CD45.1) mice were adoptively transferred to lethally gamma-irradiated recipients (B6-Thy1.1; n = 5). At 8 weeks after BM reconstitution, three mice were infected with LCMV-Arm/53b (i.v.). Two mice were left uninfected. At day 8 p.i., flow cytometry was performed on splenic CD8+ T cells stained with the surface marker CD45.2 to gate on IRF7 KO CD8+ T cells (B) and with the surface marker CD45.1 to gate on WT CD8+ T cells (C) in the mixed chimera. The primary data are shown. (D) Epitope-specific CD8+ T cells of uninfected and infected WT and IRF7 KO mice were visualized with H-2Db-restricted tetramers complexed with LCMV GP33–41 and NP396–404 peptides and analyzed by flow cytometry. (E) Lymphocytes were restimulated with LCMV-CD8 epitope peptides GP33–41 and NP396–404 and analyzed by intracellular IFN-γ staining. The data are means and SD for three mice.
of CD8+ T cells for the LCMV NP396 epitope in IRF7 KO mice was impaired. Third, despite the lack of systemic (Fig. 1A) (62) and local type I IFN production (Fig. 1B), IRF7 KO mice were able to develop normal-memory-phenotype CD8+ T cells specific for both GP33 and NP396, which lasted for over 1 year p.i. (Fig. 4B to E, J, and K).

IRF7 expression is restricted to certain cell types, such as B cells and dendritic cells (DCs) (42). Whether IRF7 is expressed in T cells is currently unclear (42). There are several potential reasons for the compromised CD8+ T cell responses in IRF7 KO mice during LCMV infection. Our studies rule out a possible intrinsic role of IRF7 expression in CD8+ T cells for the differentiation of naive T cells. Another possibility is that IRF7 KO mice may have defects in APC maturation and thus fail to present LCMV-derived antigenic peptides to activate T cells. However, our results do not appear to support this mechanism. IRF7 KO DCs comparably upregulate surface activation markers (CD40, CD80, and CD86) upon LCMV infection and are able to support the activation of CD4+ T cells and CD8+ T cells specific for the LCMV GP33 epitope. In addition, IRF7 KO mice are able to support the differentiation and expansion of adoptively transferred either naive polyclonal WT naive CD8+ T cells or p14 Tg T cells into LCMV GP33-specific CD8+ T cells (data not shown).

IRF7 KO mice are unable to produce type I IFNs following challenge with LCMV (28, 62); therefore, it is likely that the absence of type I IFNs could contribute to the impaired CD8+ T cell response. Type I IFNs are critical for the initial control of virus infections and regulation of antiviral immune responses (37). The significantly high viral load during the initial phase of infection could trigger dysfunction or partial clonal exhaustion of NP396-specific CD8+ T cells (35, 36, 59, 61). A high NP396 load on APC has been shown to drive loss of function of CD8+ T cells (59). In contrast, CD8+ T cells specific for LCMV GP33 are more resistant to the impact of high viral load (56, 59, 64). NP396-specific CD8+ T cells are activated earlier than CD8+ T cells specific for other epitopes, such as GP33, and play an important role in control of LCMV infection (44). The NP396-specific CD8+ T cell response was enhanced when IRF7 KO mice were treated with either recombinant type I IFNs or ribavirin, supporting our hypothesis that the significantly higher viral load in the early stages of infection may contribute to the defective development of the NP396-specific CD8+ T cell response in the absence of type I IFN production. This is also true in the context of HIV infection, where the initial high antigen load causes dysfunctional development of CD8+ T cells (48). Our previous study demonstrated that IRF7 KO mice produced nearly undetectable levels of type IFN and significantly lower levels of RANTES in response to LCMV infection (62) (data not shown), IRF7 KO mice generated levels of IL-12 comparable to those in WT control mice (data not shown). IL-12 has been suggested to be able to activate CD8+ T cells in the absence of type I IFN (7). This may explain why IRF7 KO mice generate a normal CD4+ T cell response and a relatively normal CD8+ T cell response specific for GP33 and GP276 (7, 54). Interestingly, the pattern of CD8+ T cell responses in IRF7 KO mice following acute LCMV infection is similar to that in RANTES KO mice (8). Whether the lack of RANTES in IRF7 KO mice contributes to the compromised CD8+ T cell responses during an acute LCMV infection is under investigation.

Until the present study, evidence demonstrating the important role of type I IFNs in regulating T cell responses came from studies using IFNAR KO mice (17, 26, 38), model antigens (such as ovalbumin), or TCR-transgenic T cells (such as LCMV GP33 TCR, p14, and CD8+ T cells) specific for a single CD8+ epitope (17, 26). Those studies clearly demonstrated that IFNAR KO mice have broader impairments of both innate and adaptive immunity. Notably, the expression of the type I IFN receptor on both CD8+ and CD4+ T cells has an intrinsic role for T cell activation and survival (17, 26). The present study highlights the differences between type I IFN and the type I IFN receptor in regulating T cell responses during virus infection. Given the essential role of IRF7 in the regulation of type I IFN production, our data demonstrate that, unlike the global impact of the type I IFN receptor on the activation of both CD8+ T and CD4+ T cells during LCMV infection, lack of type I IFN production in IRF7 KO mice following infection with LCMV does not lead to a universal collapse in T cell responses.

In summary, we demonstrated that lack of IRF7, and thus type I IFNs, does not result in complete collapse of the T cell responses to LCMV; however, it does lead to a selective decrease in the number of LCMV-specific CD8+ T cells. Thus, our study reinforces the important biological roles of the critical innate immune transcription factor IRF7 in regulating viral clearance and the development of the adaptive immune responses.

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REFERENCES


