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IFN-α Exerts Opposing Effects on T Cell Responses Depending on the Chronicity of the Virus Infection

Ichiro Misumi* and Jason K. Whitmire*†

IFN-α induces an antiviral state in many cell types and may contribute to the overall inflammatory environment after infection. Either of these effects may influence adaptive immune responses, but the role of type 3 IFNs in the development of primary and memory T cell responses to infection has not been evaluated. In this study, we examined T cell responses to acute or persistent lymphocytic choriomeningitis virus infection in IFN-λ1–deficient mice. Following acute infection, we find that IFN-λ1–deficient mice produced normal levels of IFN, robust NK cell responses, but greater than normal CD4+ and CD8+ T cell responses compared with wild type BALB/c mice. There were more T cells that were IL-7Rhi, and, correspondingly, the IFN-λ1 expression was independent of direct cytokine signaling into T cells. In contrast with acute infection, the IFN-λ1–deficient mice generated markedly diminished T cell responses and had greater weight loss compared with wild type mice when confronted with a highly disseminating variant of lymphocytic choriomeningitis virus. These data indicate that IFN-λ1 limits T cell responses and memory after transient infection but augments T cell responses during persisting infection. Thus, the immune-regulatory functions for IFN-λ1 are complex and vary with the overall inflammatory environment. The Journal of Immunology, 2014, 192: 3596–3606.

Interferons play a key role in limiting virus replication and stimulating adaptive immune responses against virus infections. The IFN-αs (also known as type III IFN; IL-28/29) are a new family of IFNs (1–3) that are found in many species, including humans, mice, bats, chickens, amphibians, and fish (4–7). There are three subtypes of IFN-α in humans (α1, α2, α3) and two in mice (α2 and α3; α1 is a pseudogene). IFN-α is highly conserved in human populations, implying strong evolutionary selection for these genes for protection against infections (8). Genetic polymorphisms in IFN-α are associated with either enhanced clearance of hepatitis C virus (HCV) or poor outcomes (9–13). Although several models demonstrate that IFN-α signals reduce virus replication in cell lines or in vivo, the role of type III IFNs in adaptive immune responses is less well understood.

IFN-α are induced by many cell types, including plasmacytoid dendritic cells (pDCs), conventional dendritic cells, peritoneal macrophages, T cells, B cells, eosinophils, hepatocytes, neuronal cells, and epithelial cells, after virus infections or after activation of TLR3, TLR4, TLR7, TLR8, stimulation of RIG-I, or Ku70

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Abbreviations used in this article: DC, dendritic cell; HCV, hepatitis C virus; ICS, intracellular cytokine staining; IRF, IFN regulatory factor; KO, knock out; LCMV, lymphocytic choriomeningitis virus; LCMV-Arm, Armstrong CA-1371 strain of LCMV; MPEC, memory precursor effector cell; pDC, plasmacytoid DC; AR-deficient, IFN-AR–deficient; SLEC, short-lived effector cell; Treg, regulatory T cell; WT, wild type.
functional (51), other evidence shows clear antiviral innate defense in some of these cells, and IFN-α signals stimulate monocytes and macrophages to produce IL-6, IL-8, and IL-10 (52). These findings suggest that IFN-α has an important role in innate immunity as a first-line defense against invading pathogens through skin and mucosal surfaces but may also function during systemic infections. Mice that lack both IFN-α/βR and IFN-λR show increased susceptibility against respiratory viruses compared with wild-type (WT), IFN-α/βR1-knock out (KO), or IL-28Rα-KO (36, 37). rIFN-λα given to mice protects against influenza infection because the IFN-λR is expressed on the epithelial cells targeted by the virus (37). At intestinal mucosal sites, IFN-λ shows antiviral functions that are independent of type 1 IFNs (50), and IFN-λ-deficient (λR-deficient) mice are more susceptible to rotavirus infection than IFN-λR–sufficient mice (50). Thus, the antiviral activities of IFN-λ largely depend on the type of virus and the route of administration of rIFN-λ (36, 37). IFN-λ may exert protective antiviral functions in multiple tissues. For example, pegylated IFN-λ treatment improves immunity to chronic HCV (9, 48), which indicates that it can improve immunity in the liver. Together, these data suggest that IFN-λ may function in immune defense against systemic infections, because numerous immune cell types respond to IFN-λ, IFN-λ improves T cell–based vaccination (53, 54), and IFN-λ reduces hepatic infection (9, 48).

The effect of IFN-λ on adaptive immune responses is unclear because multiple groups working in different model systems have arrived at varying conclusions. For example, in a macaque DNA vaccination model, vaccine-induced CTL responses were improved when an additional IFN-λ–expressing plasmid was included to enhance granzyme-dependent killing (53). Similarly, an IL-28B–dependent antiviral response was observed in a human β-cell line (54). IFN-λ may have immunoregulatory functions in addition to the demonstrated antiviral functions. The IFN-λR β-chain is shared with several additional cytokine receptors, including IL-10, IL-19, IL-20, and IL-22, which have well-defined immunomodulatory functions after infection. Consistent with this, IFN-α–stimulated DCs induced the proliferation of regulatory T cells (Tregs) in vitro (55), although overexpression of IFN-α in vivo resulted in fewer Tregs in a DNA vaccination model (56). IFN-λ signals inhibit the in vitro differentiation of Th2 cells but stimulate Th1 cells (57, 58). Respiratory syncytial virus–infected monocyte-derived dendritic cells (DCs) secrete IFN-λ that limits the in vitro proliferation of CD4+ T cells (59). Thus, a mixture of in vitro and in vivo data show that IFN-λ–mediated signals can exert positive or negative effects on T cells.

The overall influence of IFN-α on innate and adaptive immune responses against systemic virus infections is not understood. In this study, we explored the role of IFN-α using AR-deficient mice (24) that were given either acute Armstrong CA-1371 strain of lymphocytic choriomeningitis virus (LCMV-Arm) infection or the highly disseminating variant, LCMV-Clone13. We evaluated the effects of AR deficiency on IFN induction, NK cell frequencies, virus-specific B cell responses, and primary and memory T cell responses. We found that AR-deficient mice efficiently induced type 1 IFNs and eliminated acute infection with kinetics indistinguishable from those of WT mice. Virus-specific memory B cell responses and Ab also appeared normal without IFN-α signals. However, AR-deficient mice showed a 3-fold increase in primary and memory T cell responses compared with WT mice. In contrast, AR-deficient mice were unable to sustain T cell responses when exposed to persistent virus infection. Thus, IFN-AR signals limit T cell responses during acute infection but support T cell responses during persisting virus infection.

Materials and Methods

Mice and infections

BALB/c mice were purchased from Jackson Laboratory (Bar Harbor, ME) and were used as controls for the AR-deficient mice. In some experiments, BALB/cBy.PL-Thyl/1aScrt mice from the Jackson Laboratory were used as recipients of BALB/c or AR-deficient cells. Mice deficient in IFN-αR1 (IL-28Rα−/−; AR-deficient) on the BALB/c background were originally purchased by ZymoGenetics (Seattle, WA). All animal experiments were performed in accordance with the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill. Adult mice (8–10 wk old) received an i.p. injection of 2 × 10⁶ PFU of LCMV-Arm. Some mice were given an i.v. injection of 2 × 10⁶ PFU of LCMV-Arm or LCMV-Clone13. Viral stocks of plaque-purified LCMV were prepared from infected BHK-21 monolayers. The virus titer in various organs was determined by plaque assay on Vero cell monolayers (60). Some mice were infected with 1 × 10⁷ CFU recombinant Listeria monocytogenes that expresses OVA (61, 62).

RT-PCR

A reverse transcriptase reaction was performed to identify viral RNA (63). RNA was extracted from 5 mg spleen using RNeasy mini kit (Qiagen; http://www.qiagen.com), and cDNA was synthesized using SuperScript II with random primers (Promega; http://www.promega.com). The cDNA was at 37˚C for 20 min followed by a denaturation step at 98˚C for 5 min. PCR was performed using NPS-001 (5′-ATCCTAGGTGCAAGCTGGCGG- GTGAT-3′) and NPS-001′ (5′-GTAGGGAAGACACCACAAATGATC-3′) primers to amplify the NP region of LCMV S RNA. The PCR conditions were 95˚C (15 s); 94˚C for 30 s; 60˚C for 30 s; 72˚C for 30 s × 35 cycles; 72˚C for 10 min. Ten microliters of the PCR product were run on gel electrophoresis on a 1.5% agarose gel using a 100-bp ladder as a size reference.

Flow cytometry and intracellular cytokine staining

Single-cell leukocyte suspensions were prepared from spleens, and erythrocytes were removed using ACK lysis buffer (Life Technologies-BRL, Grand Island, NY). Single-cell suspensions of spleenocytes were surface stained with combinations of fluorescently labeled mAbs that were specific for CD4 (clone RM4-5), CD8 (56-3.7), NK1.1 (PK136), CD200 (3F9), and B220 (RA3-6B2) and co-stained with CD11b or CD11c in some of these cells, and IFN-γ-deficient (γR-deficient) mice were used. To detect virus replication in the absence of type 1 IFNs (50), and IFN-γ–deficient mice were used. To detect virus replication in the absence of type 1 IFNs (50), and IFN-γ–deficient mice were used. To detect virus replication in the absence of type 1 IFNs (50), and IFN-γ–deficient mice were used. To detect virus replication in the absence of type 1 IFNs (50), and IFN-γ–deficient mice were used. To detect virus replication in the absence of type 1 IFNs (50), and IFN-γ–deficient mice were used. To detect virus replication in the absence of type 1 IFNs (50), and IFN-γ–deficient mice were used. To detect virus replication in the absence of type 1 IFNs (50), and IFN-γ–deficient mice were used. To detect virus replication in the absence of type 1 IFNs (50), and IFN-γ–deficient mice were used. To detect virus replication in the absence of type 1 IFNs (50), and IFN-γ–deficient mice were used.
that were coated with lysates from LCMV-infected BHK cells as described previously (69).

Statistics
The graphs show mean data ± SEM. The statistical significance was determined by Student two-tailed t test with Prism 5 software (http://www.graphpad.com). Comparisons were considered significantly different when p < 0.05.

Results
AR-deficient mice generate robust IFN, NK cell, and primary T cell responses to quickly resolve acute LCMV infection
Type 1 IFNs are induced early after LCMV infection. pDCs are responsible for production of IFN-α by day 1, and other cell types make lower amounts of IFN-α later (70). Type 1 IFNs directly inhibit virus replication, activate NK cell responses against infected cells, and stimulate adaptive T cell responses. Given the parallel relationship between IFN-λ signals and type 1 IFN expression, we considered that type 1 IFN expression might be perturbed in the AR-deficient mice. However, AR deficiency did not impact type 1 IFN levels after virus infection, because the AR-deficient mice generated normal levels of IFN-α in the serum (Supplemental Fig. 1A).

Type 1 IFNs and IFN-λ act on NK cells to increase their antiviral functions (71). Cohorts of WT or AR-deficient mice were given LCMV-Arm infection to determine whether IFN-λ affects NK cell responses after infection. Before infection, ~5% of cells in the blood were NK cells (DX5*NKp46+) in both groups of mice (Supplemental Fig. 1B). Three days after infection, 10–20% of blood cells were DX5*NKp46+ NK cells in both groups of Armstrong-infected mice, indicating that NK cell frequencies in the blood increased independently of IFN-λ signals. The number of NK cells in the spleen was similar in both groups of mice at day 3 postinfection (Supplemental Fig. 1C).

IFN-λs induce antiviral activity against a number of viruses, including influenza, poxvirus, herpesvirus, and HCV (14, 22, 35–37, 47, 50, 72). Early analyses showed that AR-deficient mice and WT mice replicate similar levels of virus in the spleen 3 d after LCMV-Arm infection (24), but the analyses did not extend to other tissues or later times to evaluate whether the mice eventually resolved the infection. Because early virus loads can influence T cell differentiation processes, we quantified the amount of infection at several times after infection with LCMV-Arm. At day 4, AR-deficient mice replicated virus in the liver, lung, and kidney to levels comparable with those found in WT mice (Fig. 1A), and viral RNA could be detected in the spleens of both groups by RT-PCR (Fig. 1B). By day 8, the AR-deficient mice and the WT mice reduced the infection to levels below detection by plaque assay, and the RT-PCR analyses showed no evidence of viral RNA at day 8 or later in the spleens (Fig. 1B). IFN-λs play a key role in limiting mucosal infections (36, 37, 50, 72, 73). We considered that the AR-deficient mice might show impaired immunity to LCMV when the virus was given through a mucosal route. However, the intranasal delivery of LCMV resulted in similar levels of infection in the spleen and lung in both groups of mice at day 4 (Supplemental Fig. 2A); by day 8, both groups of mice showed major reductions in the viral load, resulting in similar levels of infection. Tissue samples from long-term immune mice were also analyzed and found to have no infectious virus (data not shown), indicating that there is no virus recrudescence in these mice. Thus, AR signaling does not limit the early burden of virus and is not critical for the rapid resolution of LCMV-Arm when it is given parentally or mucosally.

The resolution of LCMV depends on the formation of large numbers of CD8+ CTL, so the earlier viral clearance data imply that AR signals are not needed to generate functional CTL. Type 1 and type 2 IFNs augment antiviral T cell responses (64, 74–77). These IFNs signal directly on responding T cells to increase their antiviral responses in WT and AR-deficient mice. The AR-deficient mice showed normal abundances of resting CD8+ T cells before infection (Supplemental Fig. 3A and data not shown), which implies that IFN-λ signals are not involved in naïve T cell development or seeding of peripheral organs. Upon infection, there was a tremendous increase in the frequency of activated, CD44hi, CD62Llo, and CD11a hi CD8+ T cells in the spleens of WT and AR-deficient mice (Supplemental Fig. 3; 3A); however, the frequencies of these cells were similar in the AR-deficient mice compared with the WT mice and corresponded to ~2-fold greater numbers of CD44hi, CD62Llo, and CD11a hi CD8+ T cells in the AR-deficient mice (Fig. 2A). The overall cellularity of the spleens was similar in the two groups before and after infection (Supplemental Fig. 3B), indicating that there was a selective increase in activated CD8+ T cells in the AR-deficient mice.

All virus-specific T cells are contained within the emergent CD44hi and CD11a hi populations of cells (78–80), so the data in Fig. 2A and Supplemental Fig. 3A suggest that there are more virus-specific CD8+ T cells in the AR-deficient mice. Therefore, ICCS was used to quantify epitope-specific CD8+ T cells in WT and AR-deficient mice before and at the peak of the T cell response. There was a vigorous NP118-specific CD8+ T cell response in the AR-deficient mice that was ~4-fold greater than that seen in the WT mice (Fig. 2B). Combined with total spleen cells counts,
FIGURE 2. Greater accumulation of virus-specific CD8+ T cells in AR-deficient mice. Groups of WT and AR-deficient (KO) mice were infected with LCMV-Arm. (A) At day 8 postinfection, spleen cells were analyzed for CD8, CD44, CD62L, and CD11a by flow cytometry. Bar graphs show cumulative data for the total number of activated CD8+ T cells that were CD44hi (left panel), CD62Llo (middle panel), CD11ahi (right panel) in WT and AR-deficient mice (means ± SEM). Data represent two to five experiments with 5–11 mice/group at each time point. (B) NP118-specific CD8 T cell responses in the spleen were quantified by ICCS followed by flow cytometry analysis. Dot plots show examples of CD8+ T cell production of IFN-γ in response to NP11 peptide on days 0 (uninfected) and 8 postinfection; numbers indicate the percentage of spleen cells in each quadrant. Bar graph shows cumulative data for the number of NP118-specific CD8+ T cells per spleen from 11–13 mice analyzed in four independent experiments (mean ± SEM). (C) The representative dot plots are gated on CD8+ T cells and show the percentage of cells that coexpressed IFN-γ with TNF, as assessed by ICCS. Bar graphs show cumulative data from 11–13 mice. The left graph shows the average (± SEM) number of NP118-specific CD8+ T cells per spleen that were TNF+IFN-γ+; the right graph shows the number that was IL-2+IFN-γ+. Data represent two to five experiments with 5–11 mice/group at each time point. A two-tailed Student t test was used to evaluate significance: **p < 0.01, ***p < 0.001.

this frequency corresponded to >10 × 10^6 more NP118-specific CD8+ T cells in the AR-deficient mice than in the AR-sufficient mice (Fig. 2B, bar graph).

Differentiating virus-specific T cells acquire the ability to make large amounts of TNF and IL-2 as they progress into effector and memory cells. Among NP118-specific CD8+ T cells in both groups of mice, ~60% of IFN-γ+ve cells also made TNF (Fig. 2C, left panel), and 10–15% of IFN-γ+ve cells also made IL-2 (data not shown). There was no significant difference in the amount of IFN-γ, TNF, or IL-2 produced by NP118-specific CD8+ T cells at a per-cell level, as indicated by geometric mean fluorescence intensity (data not shown). However, the AR-deficient mice generated 3-fold greater numbers of IFN-γ+TNF+ and IFN-γ+IL-2+ CD8+ T cells at day 8 compared with AR-sufficient mice (Fig. 2C, right panel). Thus, AR signals do not impact the cytokine output of virus-specific CD8+ T cells but restrict T cell number. In contrast with acute LCMV infection, we observed no difference in the expansion of Listeria monocytogenes–specific CD8+ T cells in WT and AR-deficient mice (Supplemental Fig. 3C), and the mice cleared the infection in the liver as determined by colony counts on BHI agar (data not shown). Thus, the expression of IFN-λAR leads to reduced T cell responses to some acute infections, perhaps correlating with the magnitude of the T cell expansion, which is far greater after LCMV than Listeria monocytogenes. Therefore, we focused our analyses on T cell responses induced by LCMV infection.

The increased T cell response in the AR-deficient mice suggests that IFN-λ is suppressive in WT mice. This effect might be mediated by direct signaling into T cells or could be an indirect consequence of IFN-λ signaling into other cell types. To determine whether T cells need to express IFN-λAR, splenocytes from WT or AR-deficient mice were adoptively transferred to separate congenic recipient mice that were subsequently given acute LCMV infection (Fig. 3A). At day 9 postinfection, the NP118-specific donor cells were identified by ICCS assay in the recipient mice. The overall number of WT and AR-deficient CD8+ T cells was similar in the WT recipient mice (Fig. 3B). These data imply that IFN-λ signaling into other cell types accounts for the difference in the virus-specific T cell number when comparing WT and AR-deficient mice.

Primary CD4+ T cell responses are increased in AR-deficient mice after acute infection

Earlier studies using in vitro–stimulated T cell cultures indicated that IFN-λ stimulates Th1 cells and inhibits Th2 cells (57, 58, 81, 82). Therefore, we examined the effects of IFNλ on CD4+ T cell responses to infection. At day 8 postinfection, modest levels of

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CD4+ T cell activation were revealed by slight increases in the proportion of CD4+ T cells that were either CD44hi or CD62Llo (data not shown). Several H-2d–restricted epitopes have been identified (66), which enables the quantitation of epitope-specific T cells in BALB/c mice using ICCS. In WT mice, ~0.25% of CD4+ T cells were specific for GP176 or NP6, 0.4% for NP116 and 0.13% for Z31 (Fig. 4A). These frequencies corresponded to 3–10^4 epitope-specific T cells per spleen. Data represent four mice/group for each analysis. Based on two-tailed Student t test, there was no significant difference in the expansion of WT and λR-deficient CD8+ T cells in the WT mice.

CD4+ T cell responses drive strong antiviral B cell responses after LCMV (69, 83). We examined whether the greater CD4+ T cell responses in the λR-deficient mice affected the humoral response after LCMV-Arm infection. WT and λR-deficient mice showed comparable numbers of CD19+ B cells before and several times postinfection (Supplemental Fig. 4A and data not shown). The percentage of cells with phenotypic markers of GC B cells (CD19+GL7+) at day 15 was higher in λR-deficient mice versus WT mice (20 ± 1 versus 15 ± 1%); however, when measured by an in vitro memory B cell assay (67, 68) at day 120, there was no statistically significant difference in memory B cell numbers between WT and λR-deficient mice (Supplemental Fig. 4B). Thus, the elevated GC-phenotype B cells seen at day 15 did not result in more LCMV-specific memory B cell responses. Correspondingly, LCMV-specific serum Ab levels were similar in both groups of mice at days 14 and 49 (Supplemental Fig. 4C). These data indicate that λR signals are not involved in differentiating memory B cell or plasma cells after acute infection.

**λR-deficient mice sustain increased numbers of LCMV-specific memory CD8+ T cells**

The earlier data (Figs. 2, 4) show that IFN-λR deficiency leads to an increase in virus-specific CD8+ and CD4+ T cells. For CD8+ T cells, IFNs influence the formation of short-lived effector cells (SLECs) and long-lived memory precursor effector cells (MPECs) (84). We explored whether λR signals affect the development of SLECs (KLRG1hiIL-7Rlo) or MPECs (KLRG1loIL-7Rhi). Among activated CD11a+ T cells, the λR-deficient mice showed normal frequencies of SLEC but 2-fold higher frequencies of MPECs at day 8 (Supplemental Fig. 3D), which corresponded to a 1.7-fold increase in SLEC number and a 4-fold increase in MPEC number (Supplemental Fig. 3D). These data suggest that the expression of IFN-λR restricts the accumulation of cells with memory potential, which implies there could be long-term effects on T cell memory. Therefore, cohorts of mice were infected with LCMV-Arm, and the virus-specific CD8+ T cell number was quantified at various times after infection. The λR-deficient mice continued to have 2- to 3-fold greater numbers of NP118-specific CD8+ T cells after the peak response (Fig. 5A, top panel). The proportions of memory T cells capable of making IFN-γ with TNF (Fig. 5A, middle panel) or IFN-γ with IL-2 (Fig. 5A, bottom panel) were also increased in λR-deficient mice compared with WT mice. At day 180, the λR-deficient mice continued to have nearly 3-fold more NP118-specific CD8+ T cells and more CD8+CD11a+ T cells that expressed the IL-7Rhi MPEC phenotype (Fig. 5B) that is associated with memory.

No information is available about the number and epitope specificity of memory LCMV-reactive CD4+ T cells in BALB/c mice. In this study, we followed CD4+ T cell memory responses in WT (BALB/c) and λR-deficient mice. Virus-specific memory IFN-γ+ CD4+ T cells were quantified by ICCS at late times postinfection. At day 180, the NP118-specific CD4+ T cells were the largest population of epitope-specific T cells in the WT mice; that population was 2-fold greater in the λR-deficient mice (Fig. 5C). Approximately 10^4 CD4+ T cells per spleen were specific for GP176, NP6, and Z31 in both groups of mice. Among the NP118-reactive CD4+ T cells, a higher percentage made IL-2 in the λR-deficient mice compared with the WT mice (Fig. 5D). These data indicate that IFN-AR signals limit the abundance and cytokine content of immune-dominant memory CD4+ and CD8+ T cells.

IL-2 signals are involved in both the establishment of memory and in the recall response (85–89). CD8+ T cells in λR-deficient mice made slightly more IL-2 per cell than did CD8+ T cells from WT mice at days 40, 120, and 180 postinfection (data not shown). The effect was small but significant based on an unpaired Student t test p value of 0.01 to 0.03 at each time, with three to five mice per group. A similar pattern was observed for CD4+ T cells (Fig. 5D). This suggests that λR-deficient cells are qualitatively improved.
FIGURE 4. Improved expansion of LCMV-specific CD4+ T cells in the absence of IFN-αR interactions. Epitope-specific CD4+ T cell responses were measured by ICCS assay using spleen cells from WT (BALB/c) and IFN-αR−KO mice 8 d postinfection. (A) The representative dot plots show IFN-γ production by CD4+ T cells in response to the indicated LCMV peptides; numbers indicate the percentage of cells in each quadrant. (B) The bar graphs show the average (± SEM) number of IFN-γ+ epitope-specific CD4+ T cells per spleen in WT and IFN-αR−KO mice. (C) The bar graphs show the average (± SEM) number of IL-2+ epitope-specific CD4+ T cells per spleen in WT and IFN-αR−KO mice. Data are representative of two experiments with seven mice/group. A two-tailed Student t test was used to evaluate significance: **p < 0.01, ***p < 0.001.

compared with cells in WT mice. Therefore, cohorts of WT and αR-deficient mice were immunized, and 4 mo later some were rechallenged with a higher dose of LCMV-Arm. As expected, WT mice mounted a robust recall response by 6 d, with ~6% of CD8+ T cells specific for NP118 and able to make IFN-γ (Fig. 5E); the αR-deficient mice generated 2-fold higher frequencies of these cells, and the same pattern was apparent when TNF and IL-2 production were quantified (Fig. 5E). These percentages corresponded to 5–10×10^6 more NP118-specific cytokine-producing CD8+ T cells per spleen in the rechallenged αR-deficient mice compared with the WT mice. The larger response in the αR-deficient mice upon rechallenge corresponded to the greater number of memory cells in these mice before challenge. Thus, there was no significant increase (Student t test, p = 0.07) for the αR-deficient mice compared with the WT mice when the ratio of effector cells after challenge was normalized to the number of memory cells before challenge. These data indicate that although αR-deficient mice establish more memory cells than WT mice, those memory cells are not inherently better at proliferative responses compared with memory cells in WT mice.

The recall response in both groups of immune mice led to efficient control of the infection (Fig. 5F; triangles). By comparison, both groups of naive mice given the challenge dose continued to show high levels of infection at day 6 (Fig. 5F; circles). Cumulatively, these data show that αR-dependent signals are dispensable for generating protective primary and memory T cell and B cell responses after acute infection and act to restrict the size of the overall response.

Exaggerated loss of virus-specific T cells during disseminated virus infection

The earlier data indicate αR signals are dispensable for primary and memory T cell formation and protection against LCMV-Arm infection. The immunobiology of persisting virus infection is often very different from acute infection, and is largely impacted by the detrimental effects of sustained T cell stimulation, inhibitory molecule expression on T cells, and the presence of immune-suppressive cytokines. Consequently, T cells undergo exaggerated deletion or functional inactivation during these conditions. T cell exhaustion is observed in mice with persisting LCMV infection and in people who are persistently infected with HIV or HCV. To better understand the role of IFN-α signals on the resolution of chronic virus infection, WT and αR-deficient mice were given LCMV-Clone13, which disseminates and persists. Both groups of mice showed an initial weight loss followed by partial recovery, although the αR-deficient mice endured greater weight loss than WT mice (Fig. 6A). Both groups of mice showed similar levels of virus across time (Fig. 6B). At days 9–20, there was 10^5 PFU/ml in the serum and >10^7 PFU/g in the liver, lung, and kidneys in both groups. By day 40, there was a reduction in viremia, and eventually both groups reduced the virus burden to the limits of detection, likely through a combination of T cell and neutralizing Ab-mediated mechanisms. Thus, there is no apparent effect of αR expression on peak viral titers or the longevity of the infection.

Compared with the WT mice, there was a significantly greater decline in the total spleen size in the αR-deficient mice by 8 d postinfection (Fig. 6C). The overall cellularity continued to decline across time to 6×10^9 cells by day 40 in the αR-deficient mice, whereas the WT mice had ~3-fold more splenocytes. Virus-specific T cell responses were analyzed by ICCS assay at days 8, 40, and 90 after LCMV-Clone13 infection. At day 8, the overall abundance of IFN-γ+ CD8+ T cells and CD4+ T cells was similar for the two groups (data not shown). In the αR-deficient mice, a smaller percentage of activated CD8+ T cells were KLRG1hi, fewer expressed the inhibitory molecule PD-1, and more were CD127hi (data not shown), which suggested that more T cells might survive to contribute to immune control in the αR-deficient mice. However, by day 40, there were 4-fold fewer virus-specific NP118-specific CD8+ T cells (Fig. 6D) and 3-fold fewer GP116-specific or NP6-specific CD4+ T cells (Fig. 6E) in the αR-deficient mice compared with the WT mice. The reduced T cell responses were near or below the limits of detection, and this pattern was sustained to day 90 (data not shown). Much of this reduction in virus-specific T cell number was due to the lower number of spleen cells in the αR-deficient mice (Fig. 6C). In contrast with Clone13, LCMV-Arm led to 2- to 3-fold greater numbers of virus-specific CD8+ and CD4+ T cells in the αR-deficient mice compared with WT mice (Fig. 6D, 6E). These data show that IFN-α functions vary with the chronicity of the infection. During persisting infection, IFN-α signals protect against infection-induced weight loss and sustain IFN-γ+ T cell responses. Following acute infection, IFN-α limits the size of the overall T cell response and memory.

Discussion

Earlier analyses of IFN-α focused on its role in limiting virus infection or replication, but much less is known in terms of how this pathway influences adaptive immunity. In this article, we examined primary and memory T cell responses after acute and persisting virus infection in αR-deficient mice. We found IFN-αR is not essential for mounting innate and adaptive antiviral defense, and appears to regulate peak effector T cell responses and memory cell number after acute infection. In contrast, T cell responses to disseminating infection were reduced in the αR-deficient mice, implying that IFN-α signals maintain T cell responses during persisting infection. The αR-deficient mice also showed greater weight loss that was prolonged compared with WT mice.
Although the main effect of IFN-λR deficiency was on T cells, we observed a modest trend toward increased NK cell frequencies in the blood after infection in the λR-deficient mice (Supplemental Fig. 1B, 1C). The increase achieved significance after Clone13 infection but not after Armstrong infection. NK cells are induced by type 1 and type 2 IFNs, so the increased NK cell response in the λR-deficient mice suggests that IFN-λ signals may counterbalance the inducing effects of these other IFNs. Other investigators have shown that the antitumor activity of IFN-λ is partly mediated by positive effects on NK cell recruitment into the liver and increased tumor killing by NK cells (71). IFN-λ is being tested in clinical trials to treat HCV infection (9, 48, 90); in ad-
dution to directly limiting HCV replication in liver cells, IFN-α may stimulate antiviral activity in hepatic NK cells to reduce HCV. NK cells restrain virus-specific T cell responses after LCMV-Clone13 infection and contribute to the formation of functionally exhausted T cells (91–94). Thus, the increased NK cell response in the Clone13-infected AR-deficient mice might contribute to greater NK cell activity and the eventual decline in virus-specific T cell responses during the chronic stage.

The levels of infectious virus early after LCMV-Arm infection were comparable in AR-deficient and WT mice (Fig. 1A, Supplemental Fig. 2), consistent with an earlier report (24) and another study showing that pretreating mice with 10 μg rIFN-λ does not reduce LCMV levels at day 2 postinfection (14). This implies that AR-deficient mice do not have new cellular targets of infection. IFN-AR signals do not limit the replication of the arenavirus, Lassa virus, in macrophages or DCs in vitro (95) and mice lacking IFN-AR did not show increased levels of Lassa virus replication compared with mice with the IFN-AR following intranasal infection (36). IFN-AR is important for the resolution of other infections at mucosal or liver sites; however, our analyses showed no difference in the lung or liver when LCMV-Arm was given intranasally (Supplemental Fig. 2). LCMV causes a systemic infection that induces robust levels of IFN-αβ and IFN-γ. In this context, direct IFN-αβR and IFN-γR signals may induce sufficient antiviral activity to impair early virus replication, thus overshadowing any antiviral effects mediated by IFN-λ (24).

We found that the overall CD8+ T cell response to acute infection was increased in the absence of IFN-AR signals. There are several potential explanations for our findings. First, pDCs express IFN-AR and are a primary source of IFNαβ immediately after LCMV infection. It is plausible that pDCs (55, 96) or a monocyte-derived lineage (52) were less active in the AR-deficient mice, leading to slightly higher Ag loads that stimulate T cells. Such slight differences in Ag load would not be revealed by the plaque assay or RT-PCR analysis but may be sufficient to induce more T cell accumulation. Second, IFN-AR may affect Treg activity. IFN-α is increased during the chronic stage of HCV and act on DCs to stimulate CD4+Foxp3+ Tregs (55, 97), perhaps suppressing immune responses to HCV. A novel population of CD4+Foxp3+CD25+ T cells makes IFN-λ and induce tolerance in a mouse experimental autoimmune encephalomyelitis model (98); thus, potentially these tolerance-inducing populations of cells are induced in the infected WT mice but are diminished in the AR-deficient mice. Finally, it has been shown that IFN-λ signals can induce SOCS1 and SOCS3 (99) that suppress inflammatory processes. All of these potential mechanisms are likely to be indirect because it does not appear that IFN-λ signals act directly on T cells. In some models, activated T cells express λR (58, 100,
infection of FRCs or DCs without significantly changing the overall viral burden in the spleen.

Overall, our data indicate that IFN-α improves T cell responses during chronic LCMV infection. Recent data from clinical trials show that rIFN-α enhances protection against HCV (48). Although IFN-α synergizes with IFN-β to reduce viral genomes in infected hepatocytes, our data in Fig. 6 suggest an alternative hypothesis: rIFN-α improves antiviral T cell number or function during persisting infection to reduce virus levels.

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Disclosures

The authors have no financial conflicts of interest.

References

17. Marukian, S., L. Andrus, T. P. Sheehan, C. T. Jones, E. D. Charles, A. Plass, C. M. Rice, and L. B. Dustin. 2011. Hepatitis C virus induces interferon-α and infection, 101); however, we could not detect IFN-αR expression on naive or activated T cells using flow cytometry, and mRNA levels for IFN-AR appeared to be very low based on RT-PCR (data not shown), consistent with other findings for human T cells (102). Moreover, AR-deficient T cells did not respond better than WT T cells when the cells were placed in acutely infected WT mice (Fig. 3). Thus, the effects we observe on antiviral T cell number likely occurred as a result of IFN-α signaling in other cell types.

Interestingly, the expression of IFN-AR was associated with sustained T cell responses during persisting infection, which contrasts with the restraining effects of IFN-α on T cells during acute infection. We do not know why antiviral T cell responses collapsed in the chronically infected AR-deficient mice, nor do we know why the effects of IFN-α are opposite following acute and chronic infection. The effects of cytokines on T cell responses can differ between acute and chronic infections. For example, IL-10 and type I IFN also can stimulate or inhibit T cell responses to LCMV, depending on the duration of the infection. IL-10 signals during acute LCMV infection increase the formation of MPECs and long-term memory cells (103), yet limit T cell responses during persisting infection (104–108). Because IL-10 has been implicated in restricting T cell responses after LCMV infection (104, 107–109), we considered that IFN-AR deficiency might impact the amount of IL-10 that is present in the infected mice. Serum IL-10 levels rapidly increased on day 1 and declined in both WT and AR-deficient mice after Armstrong or Clone13 infection (Supplemental Fig. 1D). An interesting rebound of serum IL-10 levels on day 15 after LCMV-Clone13 infection was observed, consistent with an earlier report (104), but there was no significant difference in serum levels of IL-10 in the WT or AR-deficient mice at any time during LCMV-Clone13 infection. Type 1 IFNs contribute to the vigorous T cell responses after acute infection but diminish T cell responses during the chronic stage of LCMV-Clone13 infection (110–112) and can induce lymphopenia after virus infections (113, 114). We observed that the Clone13-infected, AR-deficient mice had somewhat smaller spleens than WT mice (Fig. 6C), and we considered that IFN-α may affect type 1 IFN levels but found that serum levels of IFN-α were similar between WT and AR-deficient mice (Supplemental Fig. 1).

An alternative hypothesis is that IFN-α inhibits T cell responses to both acute and chronic infections, but the differential effects observed on T cell number after LCMV-Arm and LCMV-Clone13 (Fig. 5) are linked to the tropism of these strains. Thus, LCMV-Clone13 more efficiently infects fibroblastic reticular cells (FRCs) (115, 116) and DCs and their progenitors (117, 118). Once these cells are infected, they become targets for CTL and are destroyed, leading to generalized immune suppression and sustained T cell loss during chronic infection (115–118). FRCs provide factors that support the retention and expansion of T cell responses to acute infection. However, LCMV-clone13 infection of FRCs leads to the disintegration of this important stromal cell network and a subsequent loss of cell populations (115, 116). Thus, a greater CTL response early on in the Clone13-infected AR-deficient mice may explain the exaggerated loss of spleen cell number in these mice (Fig. 6C). Alternatively, it may be that IFN-α acts on FRCs to protect them from destruction by CTL in the WT mice. Analogous effects could occur for DCs: LCMV-Arm efficiently activates DCs to stimulate T cells and IFN-α restrains T cell responses. In contrast, LCMV-Clone13 targets DCs and leads to their rapid destruction by CTL (117, 118), and IFN-α acts to limit CTL-mediated destruction of these DCs. Thus, in the presence of IFN-α, there could be lengthier DC-driven T cell responses during chronic infection. Finally, it is possible that IFN-α supports T cell responses during chronic infection by limiting Clone13 infection of FRCs or DCs without significantly changing the overall viral burden in the spleen.


