Ly49H Engagement Compensates for the Absence of Type I Interferon Signaling in Stimulating NK Cell Proliferation During Murine Cytomegalovirus Infection

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NK cells vigorously proliferate during viral infections, resulting in an expanded pool of innate lymphocytes that are able to participate in early host defense. The relative contributions of cytokines and activation receptors in stimulating NK cell proliferation during viral infections are not well characterized. In this study, we demonstrated that signaling through the NK cell activation receptor Ly49H was able to compensate for the absence of cytokine stimulation in the preferential phase of viral-induced proliferation during murine cytomegalovirus infection. In the absence of type I IFN stimulation, NK cell proliferation was strongly biased toward cells expressing the Ly49H receptor, even at early time points when minimal preferential Ly49H-mediated proliferation was observed in wild-type mice. In the absence of effective Ly49H signaling or following infection with virus that did not express the ligand for Ly49H, no difference was observed in the proliferation of subsets of NK cells that either express or lack expression of Ly49H, although the overall proliferation of NK cells in IFNαβR−/− mice was substantially reduced. These results highlight the contribution of NK cell activation receptors in stimulating proliferation and subsequent expansion of NK cells that are able to recognize virally infected cells. The Journal of Immunology, 2009, 183: 5830–5836.
mice (as well as IL-12-deficient mice) underwent normal preferential Ly49H+ NK cell proliferation and expansion (11). We recently demonstrated that preferential proliferation of Ly49H NK cells during MCMV infection resulted from Ly49H signaling through DAP12 augmenting proliferative cytokine (e.g., IL-15) stimulation and that the impact of Ly49H-mediated signaling could be masked in the presence of high concentrations of proliferative cytokines (11). However, the ability of Ly49H stimulation to initiate and sustain NK cell proliferation in the context of blunted proliferative cytokine stimulation secondary to the absence of type I IFN signaling has not been evaluated. Therefore, we proceeded to characterize the relative contributions of cytokines and NK cell activation receptors to NK cell proliferation during MCMV infection using IFNαR−/− and DAP12KI mice on a C57BL/6 background.

Materials and Methods

Mice

C57BL/6 (B6) mice were obtained from National Cancer Institute (Charles River, MA). 129:IFNαR-deficient mice (22) were backcrossed onto a C57BL/6 background for ten generations, and the completeness of the backcross was confirmed with speed congenic markers (>99% B6 with 136/137 B6 microsatellite markers) in the Rheumatic Diseases Core Center at Washington University. C57BL/6.DAP12 loss-of-function knock-in (DAP12KI; Ref. 23) mice were a generous gift from Eric Vivier (CNRS-INSERM-Université de la Méditerranée, France). C57BL/6.BXD8 (BXD8; Ref. 24) mice that lack the Ly49H receptor were a kind gift from Wayne Yokoyama (Washington University, St. Louis, MO). Mice were maintained under specific pathogen-free conditions and used between 8 and 16 wk of age. All experiments were conducted in accordance with institutional guidelines for animal care and use.

Antibodies

Biotinylated anti-Ly49H Ab (3D10) was a gift from Wayne Yokoyama. PerCP-conjugated anti-CD3 (145–2C11), allophycocyanin-conjugated anti-NK1.1 (PK136), allophycocyanin-conjugated anti-Ly49G2 (4D11), allophycocyanin-conjugated anti-Ly49D (4E5), and FITC-conjugated anti-BrdU Abs as well as PE-streptavidin were all purchased from BD Pharmingen or eBioscience. 5831The Journal of Immunology

MCMV and infection of mice

A salivary gland stock of Smith strain MCMV was prepared from young BALB/c mice that had been i.p. injected with 1×106 pfu of tissue culture propagated MCMV, and the titer of the stock was determined via standard plaque assay (4) using permissive NIH3T12 fibroblasts (American Type Culture Collection). MCMV m157-deficient isolate AT1.5 (G881A mutation) was propagated MCMV, and the titer of the stock was determined via standard plaque assay (4).

Splenocyte preparation, intracellular staining, and flow cytometry

To minimize the complication of bromodeoxyuridine (BrdU)− NK cells from the bone marrow emigrating to the spleen, we used an acute pulse of BrdU (2 mg/mouse) injected i.p. 3 h before euthanizing the mice at various time points after infection rather than prolonged BrdU exposure in the drinking water (11). Single-cell suspensions of splenocytes were prepared using standard techniques (10). Splenocytes were incubated in 2.4G2 (anti-Fcγ II/III receptor) supernatants (hybridoma from ATCC) before staining with labeled Abs to block nonspecific binding of Abs to Fc receptors. Cells were fixed and permeabilized using a Cytofix/Cytoperm kit (BD Pharmingen), treated with DNase, and then stained with FITC-conjugated anti-BrdU Abs. Analysis was performed with a FACScalibur flow cytometer (BD Pharmingen), and the data were analyzed with CellQuest (BD Pharmingen). A selectivity index to assess the extent of preferential Ly49H+ NK cell proliferation was used as previously defined (11).

Quantitative RT-PCR for IL-15

Total RNA of naive or MCMV-infected spleens was isolated using an RNeasy Mini kit (Qiagen), digested with RQ1 DNase (Fisher Scientific), and reverse transcribed with Oligo dT12–18 (Invitrogen) and SuperScript II reverse transcriptase (Invitrogen). Quantitative real-time PCR was performed on an ABI 7500 Fast Real Time system using AmpliTaq Gold polymerase (ABI) with Taqman probes and primer sets for IL-15 (previously described in Ref. 26; Integrated DNA Technologies) and β-actin (β-actin gene expression assay from ABI). After normalization with β-actin, relative expression levels of IL-15 were calculated using the ΔΔCT method.

Statistical analysis

Unpaired, two-tailed t test was used to determine statistically significant differences between experimental groups. Error bars in figures represent SDs from the mean value.

Results

Viral-induced NK cell proliferation during MCMV infection

Peripheral NK cells in naive wt mice were relatively quiescent, with only ~1% of splenic NK cells dividing (i.e., taking up BrdU) over a 3-h period (Fig. 1A; Ref. 10, 11). The first two phases of MCMV-induced NK cell proliferation are illustrated in the wt B6 column in Fig. 1A where equivalent nonspecific NK cell proliferation was observed in both the Ly49H+ and Ly49H+− subsets of splenic NK cells (17 and 22% BrdU+, respectively, with n = 6) early during MCMV infection on day 1.5 postinfection (p.i.) and preferential proliferation of Ly49H+ NK cells compared with Ly49H− NK cells was evident at day 3 p.i. (22 and 39% BrdU+ in Ly49H+ and Ly49H+− subsets, respectively, with n = 6). Preferential NK cell proliferation during MCMV infection was restricted to the Ly49H+ subset of NK cells (supplemental Fig. S1A; Ref. 10, 11). Consistent with prior studies (11), splenic NK cells from DAP12KI mice underwent normal early, nonspecific viral-induced NK cell proliferation during MCMV infection, but they failed to display any preferential Ly49H+ NK cell proliferation at later time points (Fig. 1A). In contrast, early proliferation was severely deficient in the IFNαR−/− mice (Fig. 1A, day 1.5 p.i.) compared with both B6 and DAP12KI mice. The overall percentage of splenic NK cells that were BrdU+ in the IFNαR−/− mice was 3.6% compared with 20% in the wt B6 mice and 15% in the DAP12KI mice (n = 6–8). At day 3 p.i., NK cell proliferation in IFNαR−/− mice (19% with n = 15) was more vigorous but still lower than observed in wt B6 mice (32% with n = 10). Interestingly, NK cell proliferation in the IFNαR−/− mice was strongly biased toward the Ly49H+ subset of NK cells, demonstrating that these mice were able to undergo preferential Ly49H+ NK cell proliferation even in the absence of cytokine stimulation elicited by type I IFNs.

Up-regulation of IL-15 mRNA is disrupted in IFNαR−/− mice

The up-regulation of IL-15 transcripts observed in both wt and DAP12KI mice during MCMV infection was nearly abrogated in the IFNαR−/− mice at days 1.5 and 3 p.i. (Fig. 1B), corroborating what Biron and colleagues (13) had previously observed with semiquantitative RT-PCR in IFNαR−/− mice on a 129 background. This blunted up-regulation of IL-15 mRNA provides a plausible explanation for the near absence of nonspecific, early NK cell proliferation and the minimal proliferation of Ly49H+ NK cells on day 3 during MCMV infection in the IFNαR−/− mice (Fig. 1A).

NK cell cytokine production is independent of type I IFN stimulation

In contrast to the deficits observed in viral-induced NK cell proliferation in IFNαR−/− mice, no defects were noted in IFN-γ

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production early during MCMV infection (Fig. 1C). When stained for intracellular IFN-γ immediately ex vivo on day 1.5 p.i., 19 ± 9%, 46 ± 11%, and 25 ± 11% of the splenic NK cells from wt B6, IFNαβR−/−, and DAP12K1 mice, respectively, were producing IFN-γ (cumulative results from four independent experiments; n = 11–15 mice/group). Consistent with a previous report in B6 mice (10), no differences in intracellular IFN-γ production were observed between the Ly49H+ and Ly49H− subsets of NK cells at day 1.5 p.i. in wt B6, IFNαβR−/−, or DAP12K1 mice. The increased percentage of NK cells producing IFN-γ in the IFNαβR−/− mice may reflect more intense stimulation elicited in the context of less efficient early viral control. Regardless of the mechanism responsible for elevated levels of IFN-γ in the IFNαβR−/− mice, these results demonstrated that IFN-γ production proceeds independently of IFNαβ stimulation.

These experiments using a standard inoculum dose of MCMV (5 × 10^4 pfu/mouse) provided initial insight into the relative impact of the signaling mediated by type I IFNs and Ly49H receptors on viral-induced NK cell proliferation; however, these studies were limited by the inability of the IFNαβR−/− and DAP12K1 mice to effectively control MCMV infection at this inoculum dose. In addition to a paucity of viable splenic NK cells at later time points, we encountered considerable variability in the proliferative response as early as day 3 p.i. For example, the percentage of splenic NK cells in the IFNαβR−/− mice that were BrdU+ at day 3 p.i. with 5 × 10^4 pfu MCMV/mouse was 19.2 ± 11.6% (n = 15). Five × 10^4 pfu MCMV/mouse is one-fifth the LD_{50} in wt B6 mice and is commonly used as an inoculum dose because it provides a strong stimulus without significant morbidity in wt B6 mice (11). To facilitate a more detailed investigation of the relative influence of signaling through type I IFN and Ly49H receptors on viral-induced NK cell proliferation, we subsequently used an inoculum dose of MCMV (1 × 10^4 pfu MCMV/mouse) that was approximately one-fifth the LD_{50} in IFNαβR−/− mice. This dose allowed assessment of NK cell proliferation in IFNαβR−/− mice without causing significant morbidity, while still stimulating a robust proliferative response in wt B6 mice (Figs. 2 and 3).

Decreased and Ly49H-biased NK cell proliferation in IFNαβR−/− mice early during MCMV infection

The early NK cell proliferative response at day 1.5 p.i. was more robust in IFNαβR−/− mice following infection with 1 × 10^4 pfu MCMV/mouse, although it was still deficient compared with either wt B6 or DAP12K1 mice (Fig. 2, A and B). Moreover, the NK cell proliferation present in the IFNαβR−/− mice at day 1.5 p.i. was strongly biased to the Ly49H+ subset of NK cells (Fig. 2C) with a selectivity index (ratio of the fraction of Ly49H+ NK cells that are proliferating compared with the fraction of Ly49H− NK cells that are proliferating)

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**FIGURE 1.** Viral-induced NK cell proliferation during the course of MCMV infection. A, Representative scatterplots gated on splenic NK cells from wt B6, IFNαβR−/−, and DAP12K1 mice are shown with Ly49H expression on the x-axis and BrdU uptake on the y-axis. The upper row includes scatterplots of naive mice, while the next two rows illustrate representative scatterplots of mice infected with 5 × 10^5 pfu MCMV at days 1.5 and 3 p.i., respectively. The results are representative of groups of three to eight mice in four independent experiments. B, Up-regulation of IL-15 mRNA is blunted in IFNαβR−/− on days 1.5 p.i. (1 × 10^4 pfu MCMV; black) and 3 p.i. (5 × 10^5 pfu MCMV; white) (n = 4 mice/group). C, Intracellular staining for IFN-γ in NK cells immediately ex vivo from mice infected with 5 × 10^6 pfu MCMV at day 1.5 p.i. (n = 6–8 mice/group from two independent experiments). Representative scatterplots gated on NK cells from wt B6, IFNαβR−/−, and DAP12K1 mice are shown with IFN-γ expression on the x-axis and Ly49H expression on the y-axis.
that are proliferating) of 2.1. This contrasted with equivalent levels of proliferation observed in the Ly49H⁺ and Ly49H⁻ subsets of NK cells in wt and DAP12KI mice at this early time point. The selectivity index in wt mice was 1.2, demonstrating that little preferential proliferation was observed in these mice at day 1.5 p.i. (*, p < 0.01).

**FIGURE 2.** Decreased but Ly49H-biased NK cell proliferation observed in IFNαβR⁻/⁻ mice early during infection with $1 \times 10^6$ pfu MCMV. A, Representative scatterplots gated on splenic NK cells from wt B6, IFNαβR⁻/⁻, and DAP12KI mice on day 1.5 p.i. with $1 \times 10^5$ pfu MCMV are shown with Ly49H expression on the x-axis and BrdU uptake on the y-axis. These results are representative of two independent experiments with six mice per group. Cumulative results (12 mice/group) from these two experiments are quantified in B, the percentage of splenic NK cells that are proliferating (i.e., BrdU⁺) and C, the percentage of Ly49H⁺ (gray) and Ly49H⁻ (white) splenic NK cells that are proliferating at day 1.5 p.i. (*, p < 0.01).

**FIGURE 3.** Strongly Ly49H-biased NK cell proliferation in IFNαβR⁻/⁻ mice at day 3 postinfection with $1 \times 10^6$ pfu MCMV is abrogated during infection with m157-deficient MCMV. A, Representative scatterplots gated on splenic NK cells from wt B6, IFNαβR⁻/⁻, and DAP12KI mice on day 3 p.i. with $1 \times 10^6$ pfu wt MCMV (top row) or m157-deficient MCMV (bottom row) are shown with Ly49H expression on the x-axis and BrdU uptake on the y-axis. These results are representative of three independent experiments with four to six mice per group. Cumulative results (12–17 mice/group) from these experiments are quantified in the remaining figures with gray (and black) bars indicating infection with wt MCMV and white (and striped) bars indicating infection with m157-deficient MCMV: the percentage of splenic NK cells that are proliferating (B), the percentage of Ly49H⁺ (gray or white) and Ly49H⁻ (black or striped) splenic NK cells that are proliferating where gray and black bars represent infections with wt MCMV while white and striped bars represent infections with m157-deficient MCMV (C), selectivity index (D), and the fraction of splenic NK cells that were Ly49H⁺ at day 3 p.i. (E) (*, p < 0.01).
during infection, signaling through the NK cell activation receptor was able to partially compensate for the absence of efficient cytokine stimulation in eliciting NK cell proliferation.

**Strongly Ly49H-biased NK cell proliferation in IFNγR−/− mice at day 3 p.i. is abrogated during infection with m157-deficient MCMV**

Robust NK cell proliferation was observed in the preferential phase of viral-induced NK cell proliferation in the IFNγR−/− mice following infection with 1 × 10^4 pfu MCMV/mouse. Indeed, nearly identical overall proliferative responses (i.e., the percentage of splenic NK cells that were BrdU^+) were observed in the IFNγR−/− and wt B6 mice on day 3 p.i., while lower levels of proliferation were observed in the DAP12^−/− mice (Fig. 3A and gray bars in 3B). The proliferative response in the IFNγR−/− mice was even more strongly biased to preferential Ly49H^+ NK cell proliferation than that observed in wt B6 mice (Fig. 3C with gray and black bars representing Ly49H^− and Ly49H^+ subsets of NK cells, respectively). This was reflected in the selectivity index of 2.3 (n = 17) in the IFNγR−/− mice and 1.5 (n = 16) in the wt B6 mice at day 3 p.i. with 1 × 10^4 pfu MCMV/mouse (gray bars in Fig. 3D). In contrast, no preferential proliferation of Ly49H^+ NK cells was observed in the DAP12^−/− mice (Fig. 3, C and D). The strongly Ly49H-biased NK cell proliferation in the IFNγR−/− mice contributed to an earlier shift in the percentage of splenic NK cells that were Ly49H^+ in the IFNγR−/− mice compared with that observed in wt B6 mice (Fig. 3E and supplementary Fig. S2).

We previously proposed that preferential viral-induced NK cell proliferation occurs as activation receptor signaling augments low levels of proproliferative cytokine stimulation (11). Given the blunted up-regulation of IL-15 mRNA observed during MCMV infection in the IFNγR−/− mice (Fig. 1B), we hypothesized that the IFNγR−/− mice would undergo minimal MCMV-induced NK cell proliferation in the absence of Ly49H stimulation. We directly tested this prediction by infecting mice with m157-deficient MCMV (25) to remove the influence of Ly49H stimulation. Following infection with 1 × 10^4 pfu m157-deficient MCMV/mouse, we observed significantly lower levels of overall NK cell proliferation in the IFNγR−/− mice compared with wt B6 or DAP12^−/− mice (Fig. 3A and white bars in Fig. 3B). As would be expected, no preferential Ly49H^+ proliferation was observed following infection with MCMV deficient for the ligand for Ly49H (Fig. 3C). Indeed, the selectivity indexes in all three strains of mice were nearly one (Fig. 3D), and no increases were observed in the percentage of Ly49H^+ NK cells (Fig. 3E).

Nearly identical proliferation was observed in Ly49H^+ and Ly49H^− subsets of NK cells in wt B6 and DAP12^−/− mice following infection with m157-deficient MCMV (white and striped bars in Fig. 3C). Interestingly, the percentages of proliferating Ly49H^+ and Ly49H^− NK cells observed in these mice were very similar to the percentages of proliferating Ly49H^+ NK cells in B6 mice and Ly49H^+ and Ly49H^− NK cells in DAP12^−/− mice following infection with wt MCMV (Fig. 3C), suggesting that similar levels of MCMV-induced proproliferative cytokine stimulation were present in the wt B6 and DAP12^−/− mice following infection with either wt or m157-deficient MCMV. In contrast, the percentages of Ly49H^+ and Ly49H^− NK cells in the IFNγR−/− mice were lower, consistent with lower levels of proproliferative cytokine stimulation in these mice (Fig. 3C). Overall, these results illustrated that MCMV-induced NK cell proliferation in the IFNγR−/− mice occurred predominantly in the Ly49H^− subset of NK cells and was severely reduced following infection with MCMV deficient in the expression of the ligand for Ly49H.

One caveat to using DAP12^−/− mice as Ly49H signaling-deficient controls was that DAP12 is also expressed in other immune cells including dendritic cells and monocytes (23). In light of this potential confounding factor, we used B6.BXD8 mice, which lack Ly49H (4, 24), as an alternative Ly49H signaling-deficient control. Although the absence of Ly49H expression on the NK cells in BXD8 mice precluded comparisons of proliferative responses in Ly49H^+ and Ly49H^− NK cell subsets, the overall proliferative responses observed in these mice were similar to those seen with DAP12^−/− mice following infection with either wt or m157-deficient MCMV (data not shown).

**Increased abundance of m157 may contribute to the strongly biased Ly49H^+ NK cell proliferation observed in the IFNγR−/− mice**

The absence of selective Ly49H^+ NK cell proliferation following infection with m157-deficient MCMV demonstrated that Ly49H-mediated NK cell proliferation was dependent on the interaction of the receptor and its ligand. We hypothesized that the magnitude of preferential Ly49H^+ NK cell proliferation would be altered by the relative abundance of m157 encountered by NK cells in the spleen, and we, therefore, investigated m157 levels following infection with MCMV in wt B6 and IFNγR−/− mice. Unfortunately, currently available anti-m157 Abs have not proven useful in flow cytometry studies of infected cells ex vivo (27). One surrogate approach to assess the relative abundance or “concentration” of m157 in the splenic environment during MCMV infection is to measure the decrement in the mean fluorescence intensity (MFI) of Ly49H expression (7, 27). Interactions of Ly49H with its ligand, m157, result in decreased MFI of the receptor due to internalization of the activated receptor, although interference to binding of the anti-receptor Ab may also be contributing. Decreased Ly49H MFI can be seen in vivo in wt B6 mice (Fig. 1A, day 1.5 p.i. B6 mice), although it is more pronounced during in vitro coculture with transfected cells expressing high levels of m157 (7, 27). We consistently observed a greater decrement in Ly49H^+ MFI on NK cells from IFNγR−/− mice than from wt B6 mice following infection with MCMV (at inoculum doses of either 1 × 10^4 or 5 × 10^4 pfu/mouse), suggesting more frequent or persistent interactions between Ly49H and m157 in IFNγR−/− mice (Figs. 4, A and B). In contrast, no decrease in the MFI of Ly49H was observed in IFNγR−/− (or wt B6) mice following infection with m157-deficient MCMV (Fig. 4B).

The apparent higher relative “concentrations” of m157 (reflected in the greater decrement in Ly49H MFI) suggested that the viral loads in the IFNγR−/− mice would be higher than in the B6 mice. We evaluated splenic viral titers in wt B6, IFNγR−/−, DAP12^−/−, and BXD8 mice after infection with 1 × 10^4 pfu/mouse of wt or m157-deficient MCMV (Fig. 4C). Following infection with wt MCMV, we observed comparable elevated splenic titers in IFNγR−/− and DAP12^−/− mice that were significantly higher than the viral titers seen in wt B6 mice. Type I IFNs have broad antiviral functions beyond their impact on NK cells (28), and it was not surprising to observe elevated splenic titers following infection with MCMV despite the presence of Ly49H^+ NK cells in these mice. Infection with m157-deficient MCMV circumvented the impact of Ly49H^+ NK cells and resulted in viral titers that were >100-fold higher in wt B6 mice and nearly 50-fold higher in the IFNγR−/− mice than observed following infection with wt MCMV. No difference in viral titers was observed in the DAP12^−/− mice following infection with either wt or m157-deficient MCMV, as would be expected because Ly49H signaling is deficient in these
mice. BXD8 mice were included as an alternative Ly49H signaling-deficient control, and no difference in splenic titers was observed following infection with either wt or m157-deficient MCMV, although the titers were modestly higher than those observed in DAP12KI mice. The elevated viral titers together with the significantly decreased Ly49H MFI in the IFNαβR−/− mice provided evidence that an environment enriched in m157 (the ligand for Ly49H) may contribute to the robust Ly49H-biased NK cell proliferation observed in the IFNαβR−/− mice during wt MCMV infection.

Discussion

NK cells are stimulated to vigorously proliferate during viral infections, resulting in increased NK cell numbers early during infection before an effective adaptive immune response has been mustered (9–13). In addition, the proliferative response sculpts the make-up of the NK cell pool through the preferential proliferation of NK cells that are able to recognize infected cells (11). We have demonstrated, for the first time, the ability of NK cell activation receptors to compensate for the absence of type I IFN stimulation in mediating in vivo viral-induced NK cell proliferation. The results in this study strongly corroborate the hypothesis that preferential, viral-induced NK cell proliferation occurs when NK cell activation receptor signaling amplifies proliferative cytokine stimulation, including low levels of cytokines that alone are insufficient to stimulate significant NK cell proliferation (11).

Biron and colleagues (13) previously demonstrated that IFNαβR−/− mice on a 129 background (which lack expression of the NK cell activation receptor Ly49H) displayed a deficit in MCMV-induced NK cell proliferation compared with wt 129 mice. Our IFNαβR−/− mice on a C57BL/6 background also had a deficit in viral-induced NK cell proliferation particularly during early MCMV infection (i.e., day 1.5 p.i.) when proliferation was primarily cytokine driven. This deficiency was most evident at higher inoculum doses of MCMV (e.g., 5 × 10⁴ pfu/mouse) where minimal NK cell proliferation was observed in the IFNαβR−/− mice at day 1.5 p.i. However, the present studies demonstrated that signaling through the NK cell activation receptor Ly49H in IFNαβR−/− mice was able to substantially compensate for the absence of type I IFNs in mediating NK cell proliferation particularly during the second, preferential phase of viral-induced NK cell proliferation (e.g., day 3 p.i.). Even at earlier time points where wt B6 mice underwent minimal preferential Ly49H+ NK cell proliferation (e.g., day 1.5 p.i.), NK cell proliferation in the IFNαβR−/− mice occurred predominantly in the Ly49H+ subset. The robust Ly49H+ NK cell proliferative response in the IFNαβR−/− mice was augmented by an increased abundance or elevated "concentration" of m157 in the splenic environment in these mice compared with wt B6 mice. The ability of Ly49H signaling in the IFNαβR−/− mice to compensate for the absence of type I IFN stimulation was completely abrogated during infection with MCMV that lacked expression of the ligand for the Ly49H receptor.

The strongly Ly49H-biased NK cell proliferation in the IFNαβR−/− mice resulted in an earlier shift in the percentage of splenic NK cells that were Ly49H+ in the IFNαβR−/− mice compared with that observed in wt B6 mice. A similar increase in the percentage of splenic NK cells that were Ly49H+ was observed in B6 mice at later time points (e.g., 79% of B6 splenic NK cells were Ly49H+ on day 6 p.i. with 1 × 10⁴ pfu MCMV/mouse; supplementary Fig. S2). The early perturbation in relative abundance of Ly49H+ and Ly49H− NK cell subsets in the spleens of IFNαβR−/− mice may reflect increased survival of Ly49H+ NK cells in addition to their preferential proliferation, and this issue is the subject of ongoing investigation.

We demonstrated that up-regulation of IL-15 mRNA observed in wt B6 mice was nearly absent during MCMV infection in the IFNαβR−/− mice. Although the regulation of IL-15 is complex and may involve a number of posttranslational mechanisms, the blunted up-regulation of IL-15 transcripts provides a plausible explanation for the near absence of nonspecific, early NK cell proliferation and diminished proliferation of Ly49H− NK cells on day 3 during MCMV infection in the IFNαβR−/− mice. The presence of preferential proliferation of Ly49H+ NK cells in the
IFNαβR−/− mice on day 3 p.i. (as well as the Ly49H-biased although limited proliferation on day 1.5 p.i.) in the context of blunted up-regulation of IL-15 supports our hypothesis that preferential proliferation occurs when NK cell activation receptor signaling augments low levels of proliferative cytokine stimulation that in isolation elicits little NK cell proliferation (11).

Although the regulation of NK cell proliferation and homeostasis remains incompletely characterized, the present study highlights the relative importance of NK cell activation receptors in mediating viral-induced proliferation even in the absence of type I IFN stimulation. Understanding the regulation of viral-induced NK cell proliferation and homeostasis is relevant to human health, as illustrated in the recent reports of a substantial NK cell expansion during a CMV infection in an infant with an unrecognized IL-7R deficiency (29), the dysregulated expansion of NK cells in patients with chronic NK cell lymphocytosis (30), and the inadequate NK cell-mediated antiviral responses in patients with functional NK cell defects (1). We propose that a more complete characterization of NK cell proliferation and homeostasis following in vivo stimuli, such as viral infections, will have broad translational implications and may lead to novel therapeutic interventions, either to stimulate more effective NK cell responses (e.g., during intractable viral infections or solid organ malignancies) or to down-regulate over-exuberant or inappropriate NK cell responses (e.g., during NK cell lymphoproliferative disorders such as chronic NK cell lymphocytosis or autoimmune diseases).

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