Cutting Edge: IL-15-Independent NK Cell Response to Mouse Cytomegalovirus Infection

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NK cells respond rapidly during viral infection. The development, function, and survival of NK cells are thought to be dependent on IL-15. In mice lacking IL-15, NK cells are found in severely decreased numbers. Surprisingly, following infection of IL-15- and IL-15Rα-deficient mice with mouse CMV, we measured a robust proliferation of Ly49H-bearing NK cells in lymphoid and nonlymphoid organs capable of cytokine secretion and cytolytic function. Remarkably, even in Rag2−/− × Il2rg−/− mice, a widely used model of NK cell deficiency, we detected a significant number of NK cells 1 wk after mouse CMV infection. In these mice we measured a >300-fold expansion of NK cells, which was dependent on recognition of the m157 viral glycoprotein ligand and IL-12. Together, these findings demonstrate a previously unrecognized independence of NK cells on IL-15 or other common γ signaling cytokines during their response against viral infection. The Journal of Immunology, 2009, 183: 2911–2914.

Interleukin-15 and its receptor (IL-15Rα) are important in the homeostasis of NK cells and memory CD8+ T cells (1–10). IL-15 bound to the receptor IL-15Rα on the surface of dendritic cells is "trans-presented" to IL-15-responsive cells bearing the shared IL-2 and IL-15 receptor common β-chain (CD122) (11–17). During infection, dendritic cells respond to inflammatory cytokines, leading to the production of IL-15 and IL-15Rα (13–15, 18–20). Expression of IL-15 and IL-15Rα on activated myeloid cells has thus been thought to contribute to NK cell responses against pathogens. Although mice deficient in IL-15 or the IL-15 receptor severely lack peripheral NK cells, a small population of NK cells (<0.1%) is detectable in the spleen (3, 5). We sought to determine whether these NK cells that arise in the absence of IL-15 signals can mount effector responses against viral infection.

Materials and Methods

Mice and infections

C57BL/6 (B6) and Rag2−/− × Il2rg−/− B6 mice were purchased from the National Cancer Institute (Frederick, MD) and Taconic, respectively. Il15−/−, Il15ra−/−, Il15−/− × Il15ra−/−, and Rag1−/− × Il2rb−/− B6 mice were bred at the University of California, San Francisco, CA (UCSF). Experiments were done according to the UCSF Institutional Animal Care and Use Committee guidelines. A salivary gland stock of mouse CMV (MCMV; Smith strain) or MCMV-Δm157 was injected i.p. at 5 × 10^6 PFU (21). Neutralizing anti-IL-12 p70 (clone C17.8; 750 µg) was injected i.p. 24 h before infection.

Flow cytometry and functional assays

Cells were stained with Abs against NK1.1, CD3, Ly49H, Ly49D, KLRG1, NKp46, NKp46, CD27, and DX5 (CD49b) (eBioscience or BD Pharmingen). Flow cytometry was performed using a LSRII apparatus with CellQuest software (BD Biosciences).

Splenocytes were enriched for NK cells by using a NK cell isolation kit (Miltenyi Biotec) followed by autoMACS magnetic bead separation. NK cells were incubated in tissue culture plates treated with N-1-(2,3-dioleoyloxy)proply-N,N,N-trimethylammonium methysulfate (Sigma-Aldrich) and coated with anti-NK1.1, anti-Ly49H, or PBS for 5 h at 37°C in the presence of GolgiPlug (BD Pharmingen), followed by staining for lysosome-associated membrane protein (LAMP)-1 and intracellular IFN-γ (BD Pharmingen) (22). NK cells were used as effector cells in a 4-h 3Cr-release assay (23) against Ba/F3 cells and m157-transfected Ba/F3 cells (22).

Results and Discussion

Functional NK cell responses in IL-15Rα- and IL-15 deficient mice

The spleens of Il15ra−/− mice contain <0.1% CD3−NK1.1+ NK cells compared with 2–5% in wild-type (WT) B6 mice (5). The absolute number of NK cells is decreased and the percentage of NK cells bearing the Ly49H receptor is lower in Il15ra−/− (~10%) mice compared with WT mice (~50%) (Fig. 1A). During the NK cell response against MCMV in WT mice, the Ly49H+ NK cells preferentially proliferate during the first several days of infection (21, 24, 25), a response specific for the m157 gene product of MCMV (22, 26). When we infected WT and Il15ra−/− mice with MCMV, both mice showed an increase in Ly49H+ NK cell numbers and comprised >80% of total NK cells at day 7 postinfection (PI) (Fig. 1A). A similar expansion was not observed in the Ly49D+Ly49H- NK cell subset (Fig. 1A). With precursor numbers of ~2 × 10^4 total Ly49H+ NK cells in the spleen, the absolute number of
Ly49H+ NK cells in Il15ra−/− mice at day 7 PI expanded ~72-fold to become comparable to the numbers found in uninfected WT B6 mice (>10⁶) (Fig. 1B). NK cells from MCMV-infected Il15ra−/− mice expressed comparable levels of activating receptors (NK1.1, NKp46, Ly49H, and NKG2D) and activation markers (KLRG1 and CD27) as WT mice (Fig. 1C). When NK cells at day 7 PI were incubated with plate-bound Abs against NK1.1 and Ly49H (or PBS as a control), plots are gated on NK cells (CD3−, DX5−) expressing LAMP-1 and intracellular IFN-γ. All data presented are representative of at least two independent experiments. α-Ly49H. Anti-Ly49H αNK1.1, anti-NK1.1.

**FIGURE 1.** Expansion of NK cells in WT and Il15ra−/− mice. A, WT and Il15ra−/− mice were infected with MCMV and NK cells (CD3+, NK1.1+) analyzed 7 days PI (compared with uninfected mice) for expression of Ly49H and Ly49D. B, Graph shows the absolute numbers of Ly49H+ NK cells in the spleens of uninfected (Uninf) and day 7 (d7) WT and Il15ra−/− mice. C, NK cells from WT (solid lines) and Il15ra−/− (dotted lines) mice at day 7 PI were analyzed for surface expression levels of NK1.1, NKp46, Ly49H, NKG2D, KLRG1, and CD27. D, Enriched NK cells from Il15ra−/− mice at day 7 PI were incubated with plate-bound Abs against NK1.1 and Ly49H (or PBS as a control). Plots are gated on NK cells (CD3−, DX5−) expressing LAMP-1 and intracellular IFN-γ. All data presented are representative of at least two independent experiments. α-Ly49H. Anti-Ly49H; αNK1.1, anti-NK1.1.

Ly49H+ NK cells in Il15ra−/− mice at day 7 PI expanded ~72-fold to become comparable to the numbers found in uninfected WT B6 mice (>10⁶) (Fig. 1B). NK cells from MCMV-infected Il15ra−/− mice expressed comparable levels of activating receptors (NK1.1, NKp46, Ly49H, and NKG2D) and activation markers (KLRG1 and CD27) as WT mice (Fig. 1C). When NK cells at day 7 PI were incubated with plate-bound Abs against NK1.1 and Ly49H (or PBS as a control), plots are gated on NK cells (CD3−, DX5−) expressing LAMP-1 and intracellular IFN-γ. All data presented are representative of at least two independent experiments. α-Ly49H. Anti-Ly49H; αNK1.1, anti-NK1.1.

*The online version of this article contains supplemental material.*
NK cell response in Il15−/− × Il15ra−/− mice dependent on IL-12

IL-12 is produced by dendritic cells and granulocytes in response to viral and bacterial infection and is required for the generation of Th1 cells, as well as for inducing proliferation and IFN-γ in activated CD8+ T cells and NK cells (reviewed in Ref. 29). Additionally, IL-12 plays an important role in NK cell production of IFN-γ and NK cell blastogenesis during MCMV infection (30, 31), and NK cell proliferation in response to MCMV infection is somewhat impaired in Il12−/− mice (32, 33). To address whether IL-12 contributes to NK cell expansion in the setting of IL-15 deficiency, we injected Il15−/− × Il15ra−/− mice with a neutralizing anti-IL-12 Ab before infection. Uninfected Il15−/− × Il15ra−/− mice have very few peripheral Ly49H+ NK cells, but 7 days following infection, significant numbers and percentages of Ly49H+ NK cells were detected in the spleen (78%) and liver (91%) (Fig. 4A). However, absolute numbers of Ly49H+ NK cells at day 7 PI were ~30-fold less in anti-IL-12 treated mice compared with control mice (Fig. 4B). The overall expansion of Ly49H+ NK cells in Il15−/− × Il15ra−/− mice was ~70-fold vs a 2-fold increase in anti-IL-12-treated Il15−/− × Il15ra−/− mice (Fig. 4B). Thus, IL-12 contributes greatly to the overall NK cell response following MCMV infection in mice lacking the ability to produce or respond to IL-15.

Future studies are required to determine whether the small number of NK cells that do proliferate during MCMV infection represent a unique IL-15-independent subset or new bone marrow emigrants that are rescued from death by IL-12 and

FIGURE 3. Ly49H+ NK cell expansion in Rag2−/− × Il2rg−/− mice. A. Rag2−/− × Il2rg−/− mice were infected with MCMV and the percentages of NK cells (CD3−, NK1.1+) in the spleen were determined 7 days PI (compared with uninfected mice). NK cells were analyzed for expression of Ly49H, Ly49D, and KLRG1. B. Graph shows the absolute numbers (Abs. No.) of Ly49H+ NK cells in the spleens of WT and Il15−/− mice at day 7 PI. Error bars on graph display SEM (n = 3–4). Fold expansion of Ly49H+ NK cells was calculated. All data presented are representative of at least two independent experiments.

FIGURE 2. NK cell expansion in WT and Il15−/− mice. A. Graph shows the absolute numbers (Abs. No.) of Ly49H+ NK cells in the spleens of WT and Il15−/− mice before and 7 days (d7) after MCMV infection. Error bars on graph display SEM (n = 3–4). Fold expansion of Ly49H+ NK cells was calculated. Uninf, Uninfected. B. Enriched NK cells from Il15−/− mice at day 7 PI were incubated with Ba/F3 cells or m157-expressing Ba/F3 cells at different ratios. C, Il15−/− mice were infected with MCMV or MCMV-Δm157 and NK cells (CD3−, NK1.1+) from the spleen were analyzed 7 days PI (compared with uninfected mice) for expression of Ly49H, Ly49D, and KLRG1. D, Graph shows the absolute numbers (Abs. No.) of Ly49H+ NK cells in the spleens of uninfected Il15−/− mice and infected Il15−/− mice at day 7 PI. Error bars on graph display SEM (n = 3–4). Fold expansion of Ly49H+ NK cells in Il15−/− mice infected with MCMV or MCMV-Δm157 was calculated. All data presented are representative of at least two independent experiments.

(Fig. 3B). Similar results were obtained analyzing Rag1−/− × Il2rb−/− mice (supplemental Fig. 4). Collectively, these data demonstrate that during MCMV infection NK cells do not require cytokines of the γc family for their activation and proliferation.
inflammatory cytokine signaling. Moreover, although we have shown that IL-12 is involved in NK cell expansion in the absence of IL-15, other factors might also contribute to their proliferation and survival. In conclusion, our surprising findings show that IL-12 is involved in NK cell expansion in the absence of IL-15, other factors might also contribute to their proliferation and survival. In conclusion, our surprising findings

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**Disclosures**

The authors have no financial conflict of interest.

**References**


