The Journal of Immunology

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

Joseph C. Sun, Averil Ma and Lewis L. Lanier

J Immunol 2009; 183:2911-2914; Prepublished online 31 July 2009;
doi: 10.4049/jimmunol.0901872
http://www.jimmunol.org/content/183/5/2911

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Cutting Edge

THE JOURNAL OF IMMUNOLOGY

Cutting Edge: IL-15-Independent NK Cell Response to Mouse Cytomegalovirus Infection[1]

Joseph C. Sun,* Averil Ma,† and Lewis L. Lanier[2]*

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[3]; Smith strain) or MCMV-Δm157 was injected i.p. at 5 × 10^4 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, NKG2D, 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-dioleoyloxyl)propyl)-*N*,*N*,*N*-trimethylammonium methylsulfate (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 ^{51}Cr-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. 1*A*). 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. 1*A*). A similar expansion was not observed in the Ly49D$^+$Ly49H$^-$ NK cell subset (Fig. 1*A*). With precursor numbers of ~2 × 10^4 total Ly49H$^+$ NK cells in the spleen, the absolute number of

*Department of Microbiology and Immunology and the Cancer Research Institute and †Department of Medicine, University of California, San Francisco, CA 94143

Received for publication June 15, 2009. Accepted for publication July 13, 2009.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This study was supported by National Institutes of Health Grant AI068129. L.L.L. is an American Cancer Society Professor. J.C.S. is an Irvington Postdoctoral Fellow of the Cancer Research Institute.

[2] Address correspondence and reprint requests to Dr. Lewis L. Lanier, University of California, San Francisco, Department of Microbiology and Immunology, 513 Parnassus Avenue, Room HSE-1001G, Box 0414, San Francisco, CA 94143-0414. E-mail address: Lewis.Lanier@ucsf.edu

[3] Abbreviations used in this paper MCMV, mouse cytomegalovirus; γ_C, IL-2R common γ-chain; LAMP, lysosome-associated membrane protein; PI, postinfection; WT, wild type.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0901872

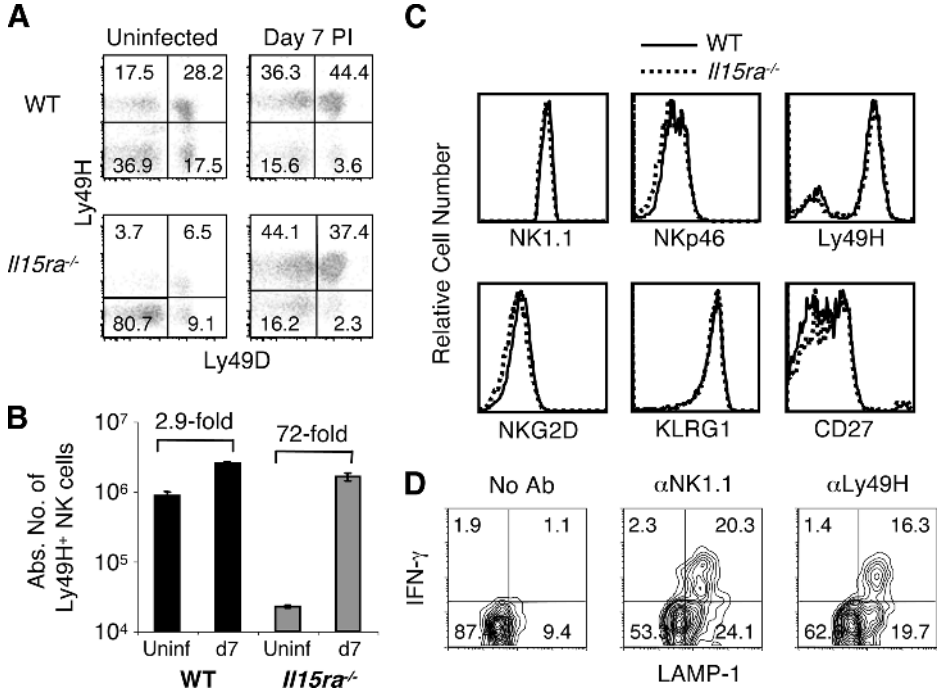

FIGURE 1. Expansion of NK cells in WT and *Il15ra*$^{-/-}$ mice. *A*, WT and *Il15ra*$^{-/-}$ mice were infected with MCMV and NK cells (CD3$^-$, NK1.1$^+$) analyzed 7 days PI (compared with uninfected mice) for expression of Ly49H and Ly49D. *B*, Graph shows the absolute numbers of Ly49H$^+$ NK cells in the spleens of uninfected (Uninf) and day 7 (d7) PI WT and *Il15ra*$^{-/-}$ mice. Error bars on graph display SEM ($n = 3$–4). Fold expansion of Ly49H$^+$ NK cells over the first 7 days of infection was calculated in 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. αLy49H, 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 (>10^6) (Fig. 1*B*). 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. 1*C*). When NK cells at day 7 PI were stimulated ex vivo with anti-NK1.1 or anti-Ly49H, these cells up-regulated LAMP-1 and produced IFN-γ (Fig. 1*D*), demonstrating that NK cells do not require IL-15 signals to mediate effector functions during MCMV infection.

Il15$^{-/-}$ mice are also deficient in NK cells (3). On day 7 PI, we observed robust expansion of Ly49H$^+$ NK cells in the spleens of MCMV-infected *Il15*$^{-/-}$ mice (Fig. 2*A*). Expression of KLRG1, a NK cell activation marker (27), was comparable in WT and *Il15*$^{-/-}$ mice (supplemental Fig. 1).[4] With <10^4 total Ly49H$^+$ NK cells in the spleen before infection, the absolute number of Ly49H$^+$ NK cells in *Il15*$^{-/-}$ mice at day 7 PI was >10^5, representing a 50-fold increase in absolute numbers (Fig. 2*A*). We tested the ability of Ly49H$^+$ NK cells from *Il15*$^{-/-}$ mice to kill m157-bearing target cells. Ly49H$^+$ NK cells isolated at day 7 PI from MCMV-infected *Il15*$^{-/-}$ mice were able to efficiently lyse m157-bearing target cells (Fig. 2*B*).

To test whether a specific viral ligand (and not inflammation alone) is required to drive NK cell proliferation, we infected *Il15*$^{-/-}$ mice with MCMV or a mutant strain lacking m157 (MCMV-Δm157). Unlike MCMV-infected *Il15*$^{-/-}$ mice, which contained a large percentage and absolute number of Ly49H$^+$ NK cells at day 7 PI (45.3-fold expansion), infection of *Il15*$^{-/-}$ mice with MCMV-Δm157 did not generate many NK cells (1.7-fold expansion) compared with uninfected controls (Fig. 2, *C* and *D*). The diminished proliferation of NK cells during infection with MCMV-Δm157 is not due to defective replication, as this mutant virus is equally or more virulent than WT MCMV (28). Adoptive transfer of WT NK cells into *Il15*$^{-/-}$ recipient mice results in the rapid loss of the transferred NK cells (1–10); however, during infection with MCMV we measured large numbers of transferred NK cells (CD45.1$^+$) at day 7 PI in spleen and liver of the *Il15*$^{-/-}$ recipients (supplemental Fig. 2, *A* and *B*). At later time points after MCMV infection (days 15 and 30 PI) transferred NK cells were difficult to recover (data not shown), suggesting that following the resolution of infection, NK cells again require IL-15 for survival. Expansion and survival of adoptively transferred WT NK cells were not observed in *Il15*$^{-/-}$ mice infected with MCMV-Δm157 (supplemental Fig. 2, *A* and *B*). Altogether, these experiments demonstrate that both viral infection and m157 are required for robust NK cell proliferation in the setting of IL-15 deficiency.

NK cell responses in Rag2$^{-/-}$ × *Il2rg*$^{-/-}$ *mice*

The *Rag2*$^{-/-}$ × *Ilr2g*$^{-/-}$ mouse is currently the best model of NK cell deficiency. Without the common γ-chain (γ_C), NK cells cannot receive signals from any cytokine of the γ_C family, including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. In naive *Rag2*$^{-/-}$ × *Il2rg*$^{-/-}$ mice, NK cells were barely detectable (0.05% in spleen and 0.2% in liver) (Fig. 3*A* and supplemental Fig. 3). When we infected WT and *Rag2*$^{-/-}$ × *Il2rg*$^{-/-}$ mice with MCMV and measured NK cell responses at day 7 PI, *Rag2*$^{-/-}$ × *Il2rg*$^{-/-}$ mice showed an increase in total NK cell numbers in spleen (comprising 1.6% of splenocytes) and liver (comprising 1% of hepatic lymphocytes) (Fig. 3*A* and supplemental Fig. 3). In *Rag2*$^{-/-}$ × *Il2rg*$^{-/-}$ mice, as with the other models of IL-15 deficiency, only the NK cells expressing Ly49H (and not Ly49D$^+$Ly49H$^-$ NK cells) expanded vigorously, up-regulating KLRG1 (Fig. 3*A*). With <1000 total Ly49H$^+$ NK cells in the spleens of uninfected *Rag2*$^{-/-}$ × *Il2rg*$^{-/-}$ mice, the absolute number of Ly49H$^+$ NK cells at day 7 PI became >10^5 (320-fold expansion)

[4] The online version of this article contains supplemental material.

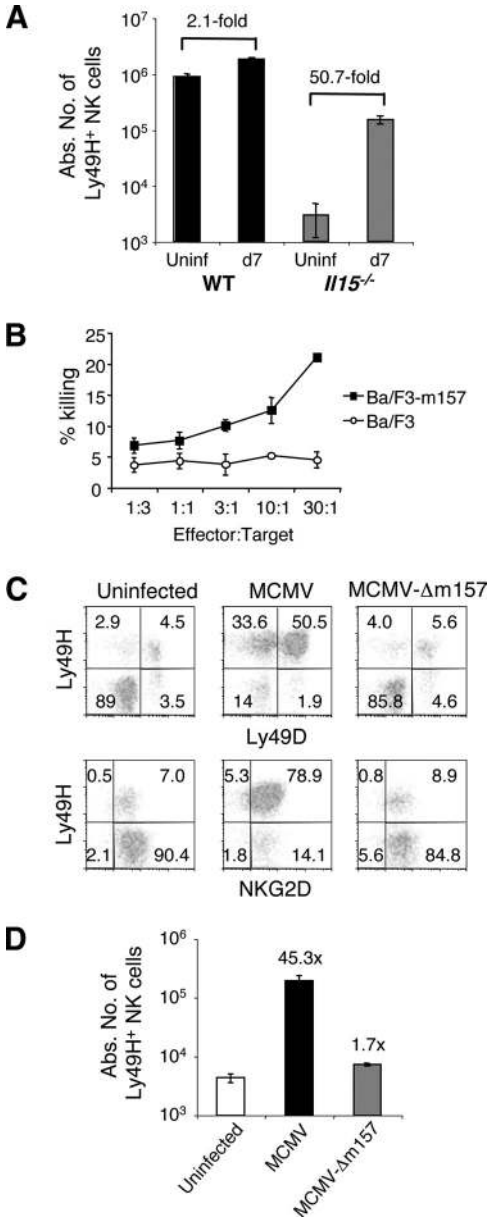

FIGURE 2. NK cell expansion in WT and $Il15^{-/-}$ mice. *A*, Graph shows the absolute numbers (Abs. No.) of Ly49H$^+$ NK cells in the spleens of WT and $Il15^{-/-}$ mice before and 7 days (d7) after MCMV infection. Error bars on graph display SEM ($n = 3–4$). Fold expansion of Ly49H$^+$ NK cells was 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 NKG2D. *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. 3*B*). Similar results were obtained analyzing $Rag1^{-/-} \times Il2rb^{-/-}$ mice (supplemental Fig. 4). Collectively, these data demonstrate that during MCMV infection NK cells do not require cytokines of the γ_C family for their activation and proliferation.

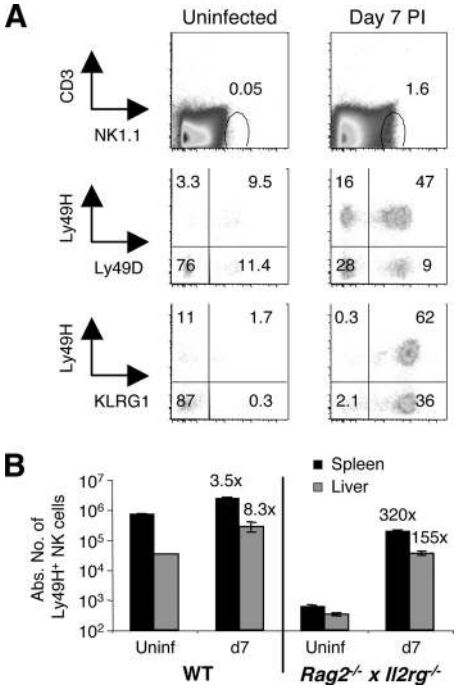

FIGURE 3. Ly49H$^+$ NK cell expansion in $Rag2^{-/-} \times Il2rg^{-/-}$ mice. *A*, $Rag2^{-/-} \times Il2rg^{-/-}$ mice were infected with MCMV and the percentages of NK cells (CD3$^-$, NK1.1$^+$) in the spleen were determined 7 days PI (compared with uninfected mice). NK cells were analyzed for expression of Ly49H, Ly49D, and KLRG1. *B*, Graph shows the absolute numbers (Abs. No.) of Ly49H$^+$ NK cells in the spleens and livers of uninfected and day 7 (d7) PI WT and $Rag2^{-/-} \times Il2rg^{-/-}$ mice. 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.

NK cell response in $Il15^{-/-} \times Il15ra^{-/-}$ 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 $Il15^{-/-} \times Il15ra^{-/-}$ mice with a neutralizing anti-IL-12 Ab before infection. Uninfected $Il15^{-/-} \times Il15ra^{-/-}$ 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. 4*A*). 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. 4*B*). The overall expansion of Ly49H$^+$ NK cells in $Il15^{-/-} \times Il15ra^{-/-}$ mice was ~70-fold vs a 2-fold increase in anti-IL12-treated $Il15^{-/-} \times Il15ra^{-/-}$ mice (Fig. 4*B*). 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

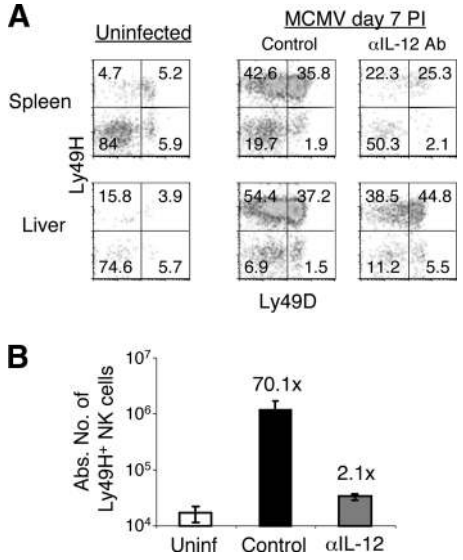

FIGURE 4. Ly49H$^+$ NK cell expansion in *IL15*$^{-/-}$ × *Il15ra*$^{-/-}$ mice is blocked with an Ab against IL-12. *Il15*$^{-/-}$ × *Il15ra*$^{-/-}$ mice were treated with a neutralizing Ab against IL-12 (or PBS as control) and infected with MCMV. *A*, Percentages of NK cells (CD3$^-$, NK1.1$^+$) in the spleen and liver 7 days PI (compared with uninfected mice) were analyzed for expression of Ly49H and Ly49D. αIL-12, Anti-IL-12. *B*, Graph shows the absolute numbers (Abs. No.) of Ly49H$^+$ NK cells in the spleen of uninfected and day 7 PI mice with and without anti-IL-12 treatment. Error bars on graph display SEM (n = 2–4). Fold expansion of Ly49H$^+$ NK cells is shown. All data presented are representative of at least two independent experiments. αIL-12, Anti-IL-12.

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 contribute added insight into the cytokines (or lack thereof) that NK cells require during an immune response against viral infection.

Acknowledgments

We thank Drs. Jody Baron, Wayne Yokoyama, Anne Hill, and Ulrich Koszinowski for generously providing reagents.

Disclosures

The authors have no financial conflict of interest.

References

1. Burkett, P. R., R. Koka, M. Chien, S. Chai, F. Chan, A. Ma, and D. L. Boone. 2003. IL-15Rα expression on CD8$^+$ T cells is dispensable for T cell memory. *Proc. Natl. Acad. Sci. USA* 100: 4724–4729.
2. Huntington, N. D., H. Puthalakath, P. Gunn, E. Naik, E. M. Michalak, M. J. Smyth, H. Tabarias, M. A. Degli-Esposti, G. Dewson, S. N. Willis, et al. 2007. Interleukin 15-mediated survival of natural killer cells is determined by interactions among Bim, Noxa and Mcl-1. *Nat. Immunol.* 8: 856–863.
3. Kennedy, M. K., M. Glaccum, S. N. Brown, E. A. Butz, J. L. Viney, M. Embers, N. Matsuki, K. Charrier, L. Sedger, C. R. Willis, et al. 2000. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med.* 191: 771–780.
4. Koka, R., P. R. Burkett, M. Chien, S. Chai, F. Chan, J. P. Lodolce, D. L. Boone, and A. Ma. 2003. Interleukin (IL)-15Rα-deficient natural killer cells survive in normal but not IL-15Rα-deficient mice. *J. Exp. Med.* 197: 977–984.
5. Lodolce, J. P., D. L. Boone, S. Chai, R. E. Swain, T. Dassopoulos, S. Trettin, and A. Ma. 1998. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity* 9: 669–676.
6. Lodolce, J. P., P. R. Burkett, D. L. Boone, M. Chien, and A. Ma. 2001. T cell-independent interleukin 15Rα signals are required for bystander proliferation. *J. Exp. Med.* 194: 1187–1194.
7. Ma, A., R. Koka, and P. Burkett. 2006. Diverse functions of IL-2, IL-15, and IL-7 in lymphoid homeostasis. *Annu. Rev. Immunol.* 24: 657–679.
8. Prlic, M., B. R. Blazar, M. A. Farrar, and S. C. Jameson. 2003. In vivo survival and homeostatic proliferation of natural killer cells. *J. Exp. Med.* 197: 967–976.
9. Schluns, K. S., K. D. Klonowski, and L. Lefrancois. 2004. Transregulation of memory CD8 T-cell proliferation by IL-15Rα$^+$ bone marrow-derived cells. *Blood* 103: 988–994.
10. Cooper, M. A., J. E. Bush, T. A. Fehniger, J. B. VanDeusen, R. E. Waite, Y. Liu, H. L. Aguila, and M. A. Caligiuri. 2002. In vivo evidence for a dependence on interleukin 15 for survival of natural killer cells. *Blood* 100: 3633–3638.
11. Burkett, P. R., R. Koka, M. Chien, S. Chai, D. L. Boone, and A. Ma. 2004. Coordinate expression and *trans* presentation of interleukin (IL)-15Rα and IL-15 supports natural killer cell and memory CD8$^+$ T cell homeostasis. *J. Exp. Med.* 200: 825–834.
12. Dubois, S., J. Mariner, T. A. Waldmann, and Y. Tagaya. 2002. IL-15Rα recycles and presents IL-15 In *trans* to neighboring cells. *Immunity* 17: 537–547.
13. Ferlazzo, G., M. Pack, D. Thomas, C. Paludan, D. Schmid, T. Strowig, G. Bougras, W. A. Muller, L. Moretta, and C. Munz. 2004. Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs. *Proc. Natl. Acad. Sci. USA* 101: 16606–16611.
14. Koka, R., P. Burkett, M. Chien, S. Chai, D. L. Boone, and A. Ma. 2004. Cutting edge: murine dendritic cells require IL-15Rα to prime NK cells. *J. Immunol.* 173: 3594–3598.
15. Lucas, M., W. Schachterle, K. Oberle, P. Aichele, and A. Diefenbach. 2007. Dendritic cells prime natural killer cells by *trans*-presenting interleukin 15. *Immunity* 26: 503–517.
16. Mortier, E., T. Woo, R. Advincula, S. Gozalo, and A. Ma. 2008. IL-15Rα chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via *trans* presentation. *J. Exp. Med.* 205: 1213–1225.
17. Sandau, M. M., K. S. Schluns, L. Lefrancois, and S. C. Jameson. 2004. Cutting edge: transpresentation of IL-15 by bone marrow-derived cells necessitates expression of IL-15 and IL-15Rα by the same cells. *J. Immunol.* 173: 6537–6541.
18. Mattei, F., G. Schiavoni, F. Belardelli, and D. F. Tough. 2001. IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation. *J. Immunol.* 167: 1179–1187.
19. Nguyen, K. B., T. P. Salazar-Mather, M. Y. Dalod, J. B. Van Deusen, X. Q. Wei, F. Y. Liew, M. A. Caligiuri, J. E. Durbin, and C. A. Biron. 2002. Coordinated and distinct roles for IFN-αβ, IL-12, and IL-15 regulation of NK cell responses to viral infection. *J. Immunol.* 169: 4279–4287.
20. Zhang, X., S. Sun, I. Hwang, D. F. Tough, and J. Sprent. 1998. Potent and selective stimulation of memory-phenotype CD8$^+$ T cells in vivo by IL-15. *Immunity* 8: 591–599.
21. Sun, J. C., J. N. Beilke, and L. L. Lanier. 2009. Adaptive immune features of natural killer cells. *Nature* 457: 557–561.
22. Arase, H., E. S. Mocarski, A. E. Campbell, A. B. Hill, and L. L. Lanier. 2002. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* 296: 1323–1326.
23. Sun, J. C., and L. L. Lanier. 2008. Tolerance of NK cells encountering their viral ligand during development. *J. Exp. Med.* 205: 1819–1828.
24. Daniels, K. A., G. Devora, W. C. Lai, C. L. O'Donnell, M. Bennett, and R. M. Welsh. 2001. Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H. *J. Exp. Med.* 194: 29–44.
25. Dokun, A. O., S. Kim, H. R. Smith, H. S. Kang, D. T. Chu, and W. M. Yokoyama. 2001. Specific and nonspecific NK cell activation during virus infection. *Nat Immunol.* 2: 951–956.
26. Smith, H. R., J. W. Heusel, I. K. Mehta, S. Kim, B. G. Dorner, O. V. Naidenko, K. Iizuka, H. Furukawa, D. L. Beckman, J. T. Pingel, et al. 2002. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc. Natl. Acad. Sci. USA* 99: 8826–8831.
27. Huntington, N. D., H. Tabarias, K. Fairfax, J. Brady, Y. Hayakawa, M. A. Degli-Esposti, M. J. Smyth, D. M. Tarlinton, and S. L. Nutt. 2007. NK cell maturation and peripheral homeostasis is associated with KLRG1 up-regulation. *J. Immunol.* 178: 4764–4770.
28. Bubic, I., M. Wagner, A. Krmpotic, T. Saulig, S. Kim, W. M. Yokoyama, S. Jonjic, and U. H. Koszinowski. 2004. Gain of virulence caused by loss of a gene in murine cytomegalovirus. *J. Virol.* 78: 7536–7544.
29. Trinchieri, G. 2003. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat. Rev. Immunol.* 3: 133–146.
30. Orange, J. S., and C. A. Biron. 1996. Characterization of early IL-12, IFN-αβ, and TNF effects on antiviral state and NK cell responses during murine cytomegalovirus infection. *J. Immunol.* 156: 4746–4756.
31. Orange, J. S., and C. A. Biron. 1996. An absolute and restricted requirement for IL-12 in natural killer cell IFN-γ production and antiviral defense: studies of natural killer and T cell responses in contrasting viral infections. *J. Immunol.* 156: 1138–1142.
32. Andrews, D. M., A. A. Scalzo, W. M. Yokoyama, M. J. Smyth, and M. A. Degli-Esposti. 2003. Functional interactions between dendritic cells and NK cells during viral infection. *Nat. Immunol.* 4: 175–181.
33. French, A. R., H. Sjolin, S. Kim, R. Koka, L. Yang, D. A. Young, C. Cerboni, E. Tomasello, A. Ma, E. Vivier, et al. 2006. DAP12 signaling directly augments proproliferative cytokine stimulation of NK cells during viral infections. *J. Immunol.* 177: 4981–4990.