ARTICLES

Adaptive immune features of natural killer cells

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In an adaptive immune response, naive T cells proliferate during infection and generate long-lived memory cells that undergo secondary expansion after a repeat encounter with the same pathogen. Although natural killer (NK) cells have traditionally been classified as cells of the innate immune system, they share many similarities with cytotoxic T lymphocytes. We use a mouse model of cytomegalovirus infection to show that, like T cells, NK cells bearing the virus-specific Ly49H receptor proliferate 100-fold in the spleen and 1,000-fold in the liver after infection. After a contraction phase, Ly49H-positive NK cells reside in lymphoid and non-lymphoid organs for several months. These self-renewing 'memory' NK cells rapidly degranulate and produce cytokines on reactivation. Adoptive transfer of these NK cells into naive animals followed by viral challenge results in a robust secondary expansion and protective immunity. These findings reveal properties of NK cells that were previously attributed only to cells of the adaptive immune system.

During an infection, naive T cells proliferate in response to encounter with cognate ligand and mediate effector functions[1–4]. This first phase of the adaptive immune response is known as the expansion phase. In many infectious models of T-cell priming *in vivo*, pathogen-specific T cells become activated and expand in number over the course of one week, undergoing more than ten divisions, and give rise to thousands of daughter cells capable of effector functions[5–7]. In the second phase, known as 'contraction', the activated T cells undergo apoptosis; a precipitous drop in cell numbers (90–95%) is observed in all tissues[6,8]. The third, or 'memory maintenance', phase[3,9–11] is when stable populations of long-lived memory T cells reside in lymphoid and non-lymphoid tissues[12,13], patrolling against previously encountered pathogens. The fourth and last phase, the secondary or recall response, occurs when memory T cells re-encounter their cognate antigen and again robustly expand in numbers to fight the pathogen challenge[1,4]. These four phases are ascribed to cells of the adaptive immune system, and the latter two phases have not been previously documented in NK cells.

NK cells have many traits in common with $CD8^+$ T cells[14–16]. The existence of immunological memory in NK cells has recently been suggested in a model of chemical hapten-induced contact hypersensitivity[17]; however, the precise mechanism and identity of the antigen-specific receptors responsible for mediating the recall responses were not defined. Here, using the well-established model of mouse cytomegalovirus (MCMV) infection in which NK cells provide host protection, we show that NK cells undergo all four phases of an immune response against a pathogen.

NK-cell expansion and contraction phases

The early virus-specific immune response against MCMV in C57BL/6 (B6) mice is dominated by NK cells expressing the Ly49H receptor, which recognizes the virally encoded m157 protein on the surface of infected cells, and these NK cells confer protection against infection[18–22]. Over the first week of MCMV infection, $Ly49H^+$ NK cells undergo a twofold to threefold expansion in the spleen and a roughly tenfold increase in the liver, as described previously[23,24] (Supplementary Fig. 1). Because $Ly49H^+$ cells constitute about 50% of total NK cells in a naive B6 mouse, we proposed that a 'ceiling' for NK-cell expansion (measured at 80–90% of total NK cells) is rapidly achieved during infection and inhibits further proliferation in a normal host. We therefore sought to investigate the proliferation potential of NK cells by experimentally decreasing the initial precursor frequency of the $Ly49H^+$-cell population. We reconstituted lethally irradiated mice with 1:1 mixed bone marrow from wild-type ($CD45.1^+$) and DAP12-deficient ($Tyrobp^{-/-}$) ($CD45.2^+$) B6 mice (which are defective in Ly49H receptor expression and function[25]), thus decreasing the initial frequency of fully functional, wild-type $Ly49H^+$ cells in the host to about 25% of the total NK cells (Fig. 1a). After infection of mixed chimaeric mice, wild-type NK cells preferentially expanded over 7 days and became the predominant NK-cell subset in the spleen (Fig. 1a). Although the chimaeric mice contained a lower initial frequency of $Ly49H^+$ NK cells than the wild-type mice, by day 7 after infection the chimaeric mice had absolute numbers of $Ly49H^+$ NK cells that were comparable to those in infected wild-type mice (data not shown). The $Ly49H^+$ cells within the $CD45.1^+$ NK-cell fraction upregulated the expression of the maturation marker KLRG1 (Fig. 1a) and preferentially incorporated bromodeoxyuridine over 7 days after infection (Fig. 1b), confirming the proliferation of this specific NK-cell subset and excluding the possibility that DAP12-deficient NK-cell populations were diminishing. Indeed, the absolute number of DAP12-deficient NK cells increased only slightly over the course of the infection (data not shown). The percentage of $Ly49H^+$ NK cells began to decline beyond day 7 after infection but continued to be elevated, compared with uninfected animals, at 15 and 28 days after infection; frequencies returned to those measured in uninfected animals by 37 days after infection (Fig. 1c). Similar expansion and contraction of $Ly49H^+$ NK cells were measured in the liver and lymph nodes of chimaeric mice during infection (Supplementary Fig. 2). The $Ly49H^+$ NK-cell response was specific for the m157 viral ligand because we did not observe preferential expansion of the Ly49H subset during infection with MCMV lacking m157 (Fig. 1d). In 1:5 wild-type:DAP12-deficient bone marrow chimaeric mice, in which the starting percentage of wild-type $Ly49H^+$ cells was about 10% of total NK cells, the $Ly49H^+$ NK cells also preferentially expanded over 7 days after infection, allowing the wild-type ($CD45.1^+$) NK cells to constitute the dominant population observed at the peak of expansion (Supplementary Fig. 3a). This expansion of $Ly49H^+$ NK cells after

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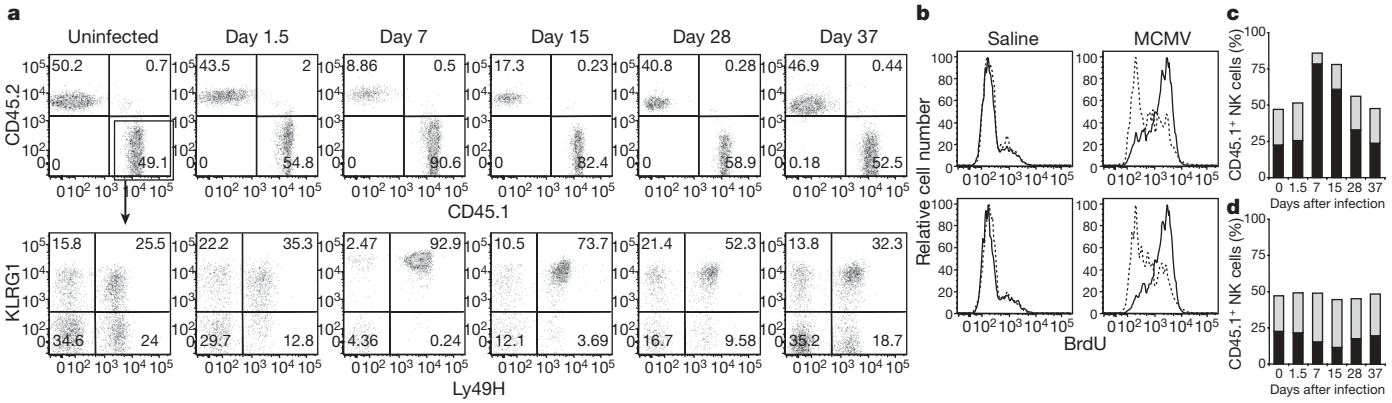

Figure 1 | Preferential expansion of wild-type, but not DAP12-deficient, NK cells during MCMV infection. Mixed-bone-marrow chimaeric mice (1:1 mixture of wild-type ($CD45.1^+$) and DAP12-deficient ($CD45.2^+$) cells) were infected with MCMV. **a**, Top: percentages of wild-type and DAP12-deficient NK cells (gated on $CD3^-$, $NK1.1^+$). Bottom: Ly49H and KLRG1 on wild-type NK cells. **b**, Top: incorporation of bromodeoxyuridine (BrdU) (day 7 after infection) of wild-type (solid lines) and DAP12-deficient (dotted lines) NK cells, gated on total NK cells. Bottom: incorporation of bromodeoxyuridine by $Ly49H^+$ (solid lines) and $Ly49H^-$ (dotted lines) wild-type NK cells, gated on $CD45.1^+$ NK cells. **c**, **d**, Percentages of $Ly49H^+$ cells within the wild-type NK-cell population after infection with MCMV (**c**) or MCMV-Δm157 (**d**). Grey bars, total wild-type cells; black bars, $Ly49H^+$ cells. Data are representative of three experiments with three to five mice per time point.

infection observed in the spleen and liver was even greater than the proliferation measured in the 1:1 chimaeras (Supplementary Fig. 3b). Together, these findings highlight the ability of a small number of precursor NK cells to expand robustly into effector cells and mount a primary response against viral infection.

We next sought to address whether maximal proliferation of $Ly49H^+$ NK cells could be achieved by adoptively transferring mature wild-type NK cells ($CD45.1^+$) into DAP12-deficient mice ($CD45.2^+$) and infecting with MCMV (Fig. 2a). Although DAP12-deficient mice have defective $Ly49H^+$ NK cells, $CD8^+$ T-cell responses to MCMV are normal, and mice clear infection within two weeks (data not shown). We were able to recover the transferred NK cells from recipient spleen (Fig. 2b) and thereby showed that early after infection (day 1.5 after infection), they were capable of becoming activated ($CD69^+$ and $NKG2D^{high}$) and producing interferon (IFN)-γ (Fig. 2b). Seven days after infection, we observed a marked increase in transferred NK cell numbers, with preferential expansion in the $Ly49H^+$ NK-cell subset (Fig. 2c). Adoptive transfer of carboxyfluorescein succinimidyl ester (CFSE)-labelled NK cells confirmed that only the $Ly49H^+$ NK cells were dividing, fully diluting their CFSE during the 7 days after infection, whereas the $Ly49H^-$ NK-cell subset remained $CFSE^{high}$ (Fig. 2d). NK cells transferred into DAP12-deficient mice expanded as much as 100-fold in the spleen after infection (Fig. 2e). This expansion was not seen when NK cells were adoptively transferred into wild-type B6 mice, which contain competing endogenous $Ly49H^+$ NK cells and thus restrict proliferation of the transferred cells (Fig. 2e). The expansion of $Ly49H^+$ NK cells was not unique to DAP12-deficient recipients because we also observed robust expansion of $Ly49H^+$ NK cells when they were adoptively transferred into C57BL/6 mice that do not possess the *Ly49h* gene (data not shown).

The amplitude and kinetics of the MCMV-specific NK-cell response measured in our system mirror analogous responses in primary T cells[7,26], as well as with adoptive transfer of T-cell antigen receptor-transgenic T cells[5,27]. Furthermore, adoptive transfer of NK cells permits us to track the congenic $CD45.1^+$ cells late after infection, allowing us to distinguish between antigen-experienced NK cells and naive NK cells that have recently left the bone marrow. Using this experimental approach, we were able to recover a long-lived 'memory' pool of NK cells that can persist in lymphoid tissues as well as non-lymphoid tissues such as the liver (Fig. 3a, b). We examined whether lowering the precursor frequency permitted a greater expansion of NK cells by measuring the amplitude of the $Ly49H^+$ NK-cell response in mice after the transfer of 10^5 or 10^4 cells, and determined that both the kinetics and fold expansion were comparable in the spleen (about 100-fold) and liver (about 1,000-fold) of MCMV-infected mice, irrespective of initial transfer numbers (Fig. 3a, b). A lower threshold therefore exists at which small numbers of precursor NK cells no longer lead to enhanced overall responses. A summary of $Ly49H^+$ NK-cell fold expansion in B6 mice, the different mixed chimaeric mice and the adoptive transfer system is shown in Fig. 3c, highlighting the previously underappreciated ability of antigen-specific NK cells to undergo a profound expansion and persist after viral infection.

NK-cell memory phase

In our adoptive transfer model, the contraction phase observed during the NK-cell response to MCMV infection (Fig. 3a, b) seems to emulate the prolonged decline in effector-cell numbers observed with antigen-specific CD4 T-cell responses[26], rather than the rapid and more precipitous decline observed with most $CD8^+$ T-cell responses[8]. After the contraction phase, we were able to detect 'memory' NK cells at 70 days after infection (Fig. 4a), and we sought to determine whether these cells were still functional. These 'memory' NK cells were indeed functional, because they produced IFN-γ *ex vivo* in response to plate-bound antibody against NK1.1 or Ly49H (Fig. 4a). In fact, their response was heightened in comparison with the response by naive NK cells, in that a greater percentage of 'memory' NK cells responded to plate-bound antibodies as well as m157-expressing target cells, and the amounts of IFN-γ produced by individual cells were higher (Fig. 4b and Supplementary Fig. 4). Degranulation by 'memory' NK cells was also enhanced, as assessed by LAMP-1 (CD107a) expression after *ex vivo* stimulation with anti-NK1.1 (Fig. 4c).

'Memory' $Ly49H^+$ NK cells could be distinguished from naive $Ly49H^+$ NK on the basis of several surface antigens. In particular, 'memory' NK cells expressed higher levels of the Ly49H receptor, but not other activating receptors, than naive NK cells (Fig. 4d), perhaps permitting 'memory' NK cells to have a lower threshold for activation. 'Memory' NK cells also showed a greater expression of KLRG1, CD43 and Ly6C, and a decreased expression of CD27, suggesting that they were more mature than naive NK cells (Fig. 4d). A similar phenotype was also observed on long-lived NK cells isolated from non-lymphoid tissues such as the liver (Supplementary Fig. 5). 'Memory' NK cells also constitutively expressed higher levels of *Ifng* transcripts than naive NK cells (Fig. 4e), as assessed by studies with the IFN-γ reporter Yeti mice[28]. Taken together, these data indicate that 'memory' NK cells possess characteristics similar to those of memory T cells in both their phenotype and their ability to produce effector cytokines robustly.

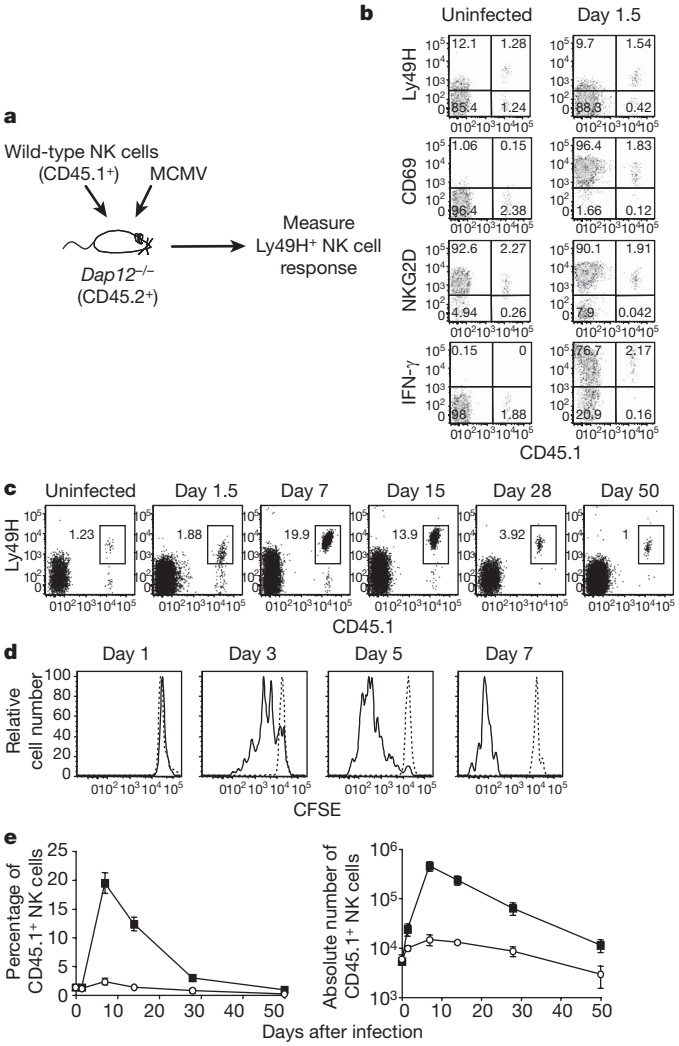

Figure 2 | Robust proliferation of adoptively transferred wild-type NK cells in DAP12-deficient mice after MCMV infection. **a**, A total of 10^5 wild-type NK cells ($CD45.1^+$) were transferred into DAP12-deficient mice ($CD45.2^+$) and infected with MCMV. **b**, Transferred NK cells ($CD45.1^+$) within the total $CD3^-$ $NK1.1^+$ gated population analysed for Ly49H, CD69, NKG2D and intracellular IFN-γ. **c**, Percentages of transferred $CD45.1^+$ $Ly49H^+$ NK cells within the total $CD3^-$ $NK1.1^+$ population after infection. **d**, CFSE-labelled wild-type NK cells (5×10^5) were transferred into DAP12-deficient hosts. $Ly49H^+$ (solid lines) and $Ly49H^-$ (dotted lines) NK cells were analysed after infection. **e**, Percentages (left graph) and absolute numbers (right graph) of transferred $CD45.1^+$ NK cells in DAP12-deficient (filled squares) or wild-type B6 (open circles) recipients after infection. Error bars show s.e.m. ($n = 3$–5). Data are representative of five experiments.

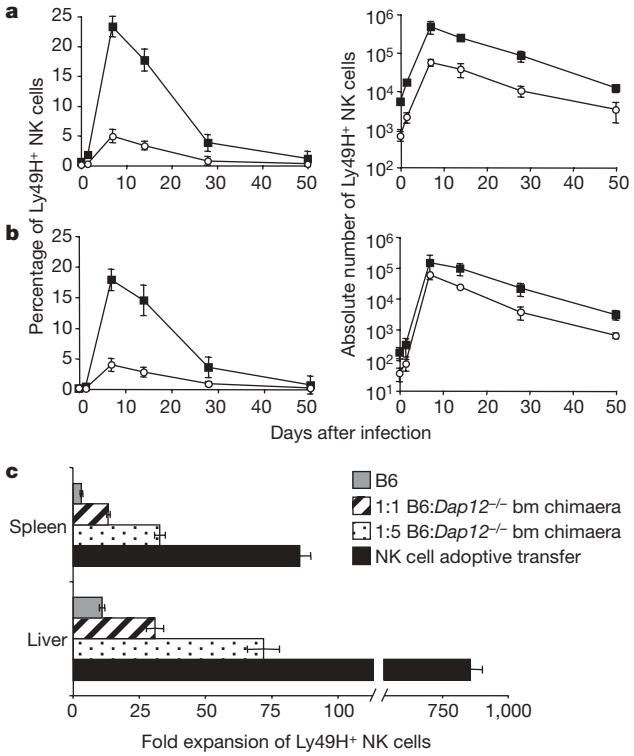

Figure 3 | Expansion and contraction of NK cells in lymphoid and non-lymphoid tissues results in 'memory' NK cells. **a**, **b**, After adoptive transfer of 10^5 (filled squares) or 10^4 (open circles) wild-type NK cells ($CD45.1^+$) into DAP12-deficient mice and MCMV infection, graphs show percentages (left) and absolute numbers (right) of $Ly49H^+$ NK cells in spleen (**a**) and liver (**b**). Error bars show s.e.m. ($n = 3$–5). Data are representative of three experiments. **c**, Fold expansions of $Ly49H^+$ NK cells over 7 days of infection were calculated in B6 mice, in 1:1 and 1:5 wild-type:DAP12-deficient bone marrow (bm) chimaeric mice, and after adoptive transfer of wild-type NK cells into DAP12-deficient mice. Error bars show s.e.m. from three experiments in each group of mice indicated.

NK-cell recall phase

Antigen-specific memory T cells undergo secondary expansion, and we investigated whether 'memory' NK cells also possessed this adaptive immune characteristic. Because mice previously immunized with MCMV contain neutralizing antibodies and memory cytotoxic T lymphocytes, we could not challenge directly with a second dose of virus to examine recall responses. Therefore, after the primary expansion and contraction phase in our adoptive transfer model, we enriched for 'memory' $Ly49H^+$ NK cells ($CD45.1^+$) at 40–50 days after infection, adoptively transferred these cells into a second DAP12-deficient mouse ($CD45.2^+$) and infected it with MCMV (Fig. 5a). Although the transferred 'memory' NK cells were present in small numbers in uninfected recipients and at day 1.5 after infection, an expanded population of transferred $Ly49H^+$ NK cells could be readily detected at 7 and 14 days after infection (Fig. 5b). Thus, like memory T cells, 'memory' NK cells are self-renewing, long-lived, and can mount a secondary response after viral challenge, with expansion measured at greater than 100-fold in the spleen (Fig. 5c). When CFSE-labelled naive and 'memory' NK cells were transferred into separate DAP12-deficient recipient mice and proliferation kinetics were compared after MCMV infection, we observed a similar rate of CFSE dilution at day 3 after infection and found that both groups of NK cells were CFSE-negative (indicating at least ten divisions) by day 6 after infection (Fig. 5d). Although large numbers of naive NK cells were easier to obtain and thus more cells were transferred into recipient animals than 'memory' NK cells, the kinetics and magnitude of expansion between naive and 'memory' NK-cell populations were comparable (Fig. 5d). Overall, MCMV-specific NK-cell responses after adoptive transfer of normalized naive and 'memory' NK cell numbers showed no significant differences in the expansion and contraction phases (Fig. 5e). Several careful studies of $CD8^+$ T-cell responses have also revealed similarities in naive and memory T-cell proliferation rates[29–33], indicating that the increased memory T-cell responses could be attributed to a higher precursor frequency rather than a differential rate of proliferation. Although phenotypic and functional differences exist between naive and 'memory' NK cells, it is not surprising that the kinetics of expansion between these populations do not differ, given the property of naive NK cells as 'ready-to-respond' effectors[14]. In fact, memory $CD8^+$ T cells have often been compared to NK cells for their ability to mediate effector functions rapidly[14].

Last, we wished to determine whether 'memory' NK cells are more protective than naive NK cells. We adoptively transferred equal numbers of naive and 'memory' $Ly49H^+$ NK cells into newborn mice,

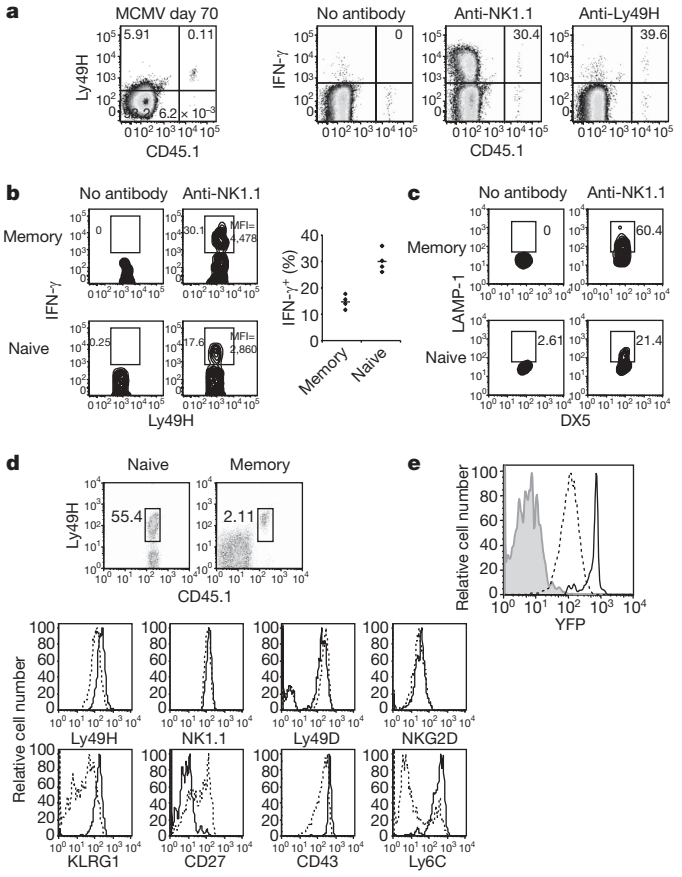

Figure 4 | Function and phenotype of 'memory' NK cells. **a**, A total of 10^5 wild-type NK cells (CD45.1$^+$) transferred into DAP12-deficient mice (CD45.2$^+$) were analysed 70 days after MCMV infection. Left: percentage of CD45.1$^+$ Ly49H$^+$ cells within the total NK-cell population. Right: percentages of CD45.1$^+$ NK cells producing IFN-γ after stimulation. **b**, 'Memory' NK cells compared with naive NK cells from uninfected mice after stimulation with anti-NK1.1. Left: percentages of Ly49H$^+$ NK cells (gated on total NK cells) producing IFN-γ. Right: percentages of Ly49H$^+$ NK cells producing IFN-γ (four mice per group; horizontal bar is mean; $P = 0.0009$). MFI, mean fluorescence intensity. **c**, LAMP-1 on 'memory' NK cells (day 55 after infection) versus naive NK cells (gated on Ly49H$^+$ NK cells) after stimulation with anti-NK1.1. **d**, Surface markers on 'memory' NK cells (day 45 after infection; solid line) versus naive NK cells (dotted lines). **e**, NK cells from day 7 MCMV-infected Yeti mice were transferred into DAP12-deficient *Rag2*$^{-/-}$ mice. The graph shows yellow fluorescent protein (YFP) in 'memory' Ly49H$^+$ NK cells (thick solid line) after 28 days compared with Ly49H$^+$ NK cells (dotted line) and T cells (thin solid line) in uninfected Yeti mouse.

which are susceptible to MCMV because they lack mature NK cells[34], challenged them with MCMV, and monitored survival. Newborn mice receiving 'memory' NK cells showed significant protection against viral infection compared with an equivalent number of naive NK cells ($P = 0.0024$; Fig. 5f). At least tenfold more naive NK cells than 'memory' NK cells were required to mediate protection against MCMV infection. When newborn recipients were treated with an antibody that blocks the Ly49H receptor, protection was abrogated ($P = 0.03$; Fig. 5f). Thus, like T cells, NK cells have the ability to 'remember' previously encountered pathogens and are able to mediate more efficient protective immunity against subsequent infection.

Conclusion

Because the innate immune system is considered to be evolutionarily older than the adaptive immune system, NK cells bearing receptors that recognize major histocompatibility complex (MHC) and MHC-like molecules may have arisen as a predecessor to T cells, which express clonally selected receptors for the recognition of a limitless

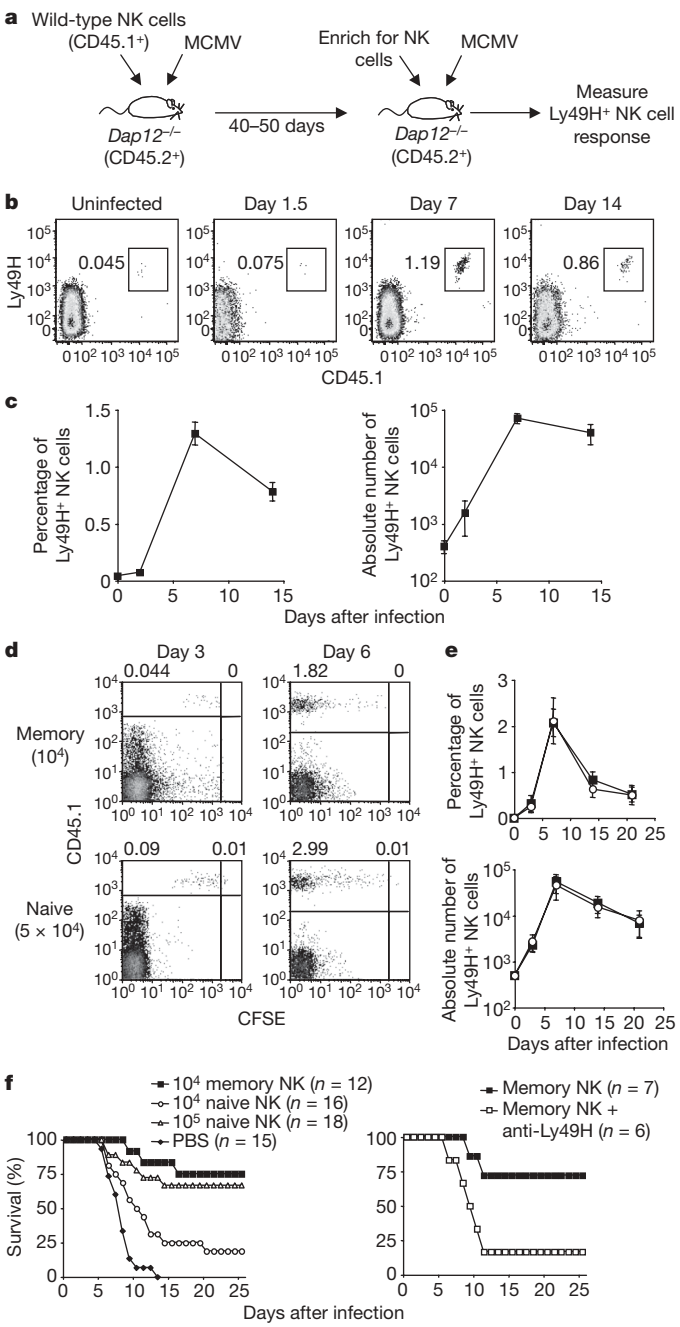

Figure 5 | Secondary expansion and protective immunity in 'memory' NK cells. **a**, A total of 10^5 wild-type NK cells (CD45.1$^+$) transferred to DAP12-deficient mice (CD45.2$^+$) were isolated 40–50 days after primary infection with MCMV and transferred into DAP12-deficient mice (CD45.2$^+$). After infection of the second host, Ly49H$^+$ NK cells were analysed. **b**, Percentages of transferred 'memory' Ly49H$^+$ NK cells in the second host. **c**, Percentages (left) and absolute numbers (right) of Ly49H$^+$ NK cells within the total NK-cell population in the second host. Error bars show s.e.m. ($n = 3$–5). Data are representative of five experiments. **d**, Analysis of CSFE-labelled 'memory' and naive NK cells transferred into DAP12-deficient recipient mice (days 3 and 6 after infection). **e**, Expansion and contraction of 'memory' (filled squares) and naive (open circles) NK cells (10^4 input) shown as a percentages (top) and absolute numbers (bottom) of Ly49H$^+$ NK cells within the total NK-cell population. Error bars show s.e.m. ($n = 3$–5). Data are representative of three experiments. **f**, Survival of DAP12-deficient neonatal mice receiving 10^4 or 10^5 naive NK cells, or 10^4 'memory' NK cells (or PBS as control) followed by MCMV infection, with or without anti-Ly49H blocking. Data were pooled from three experiments.

antigen repertoire in the context of MHC. Alternatively, NK cells may have co-evolved with T cells. As an evolutionary bridge, NK cells might conceivably possess attributes of both innate and adaptive immunity, and we are only beginning to unearth characteristics of the latter. The categorization of NK cells within the immune system is beginning to undergo a paradigm shift, which will have major implications for our approach to vaccination strategies for the generation of immunological memory against pathogens.

METHODS SUMMARY

Mice and infections. C57BL/6 and congenic CD45.1$^+$ mice were purchased from the National Cancer Institute. DAP12-deficient mice[25], backcrossed 13 generations onto C57BL/6, were bred and maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee. R. Locksley provided Yeti mice[28]. Mixed-bone-marrow chimaeric mice were generated as described[35]. Mice were infected by intraperitoneal injections of Smith strain MCMV (5×10^4 plaque-forming units (p.f.u.)) and MCMV-Δm157 (10^5 p.f.u.) (provided by U. Koszinowski)[36]. Newborn mice were infected with 2×10^3 p.f.u. of MCMV.

NK-cell enrichment and adoptive transfer. NK cells were isolated with an NK-cell Isolation Kit (Miltenyi Biotec) and injected intravenously into adult recipients or intraperitonally into neonatal recipients one day before viral infection. Newborn mice were given 50 μg of anti-Ly49H monoclonal antibody (mAb) 3D10 one day before infection.

***Ex vivo* stimulation of NK cells.** Tissue culture plates treated with *N*-(1-(2,3-dioleoyloxyl)propyl)-*N*,*N*,*N*-trimethylammonium methylsulphate (Sigma) were coated with anti-NK1.1 or anti-Ly49H (provided by W. Yokoyama) and cells were incubated for 5 h at 37 °C in the presence of Golgiplug (BD Pharmingen), followed by staining for intracellular cytokines. NK cells were co-cultured with Ba/F3 cells or m157-transduced Ba/F3 (ref. 18) before intracellular cytokine staining.

Flow cytometry. Fc receptors were blocked with 2.4G2 mAb before surface staining with indicated antibodies or isotype-matched control immunoglobulin (BD or eBiosciences). Staining with bromodeoxyuridine was performed with a BrdU Flow Kit (BD). Labelling of cells with CFSE was performed in accordance with the manufacturer's instructions (Invitrogen). Samples were acquired on an LSRII (BD) and analysed with FlowJo software (TreeStar).

Statistical methods. The Mann–Whitney nonparametric *U*-test was used to compare survival between groups of mice. A value of 25 days was assigned to survivors living more than 25 days after MCMV infection. Student's *t*-test was used to compare groups in *ex vivo* stimulation experiments.

Received 23 October; accepted 24 November 2008.
Published online 11 January 2009.

1. Harty, J. T. & Badovinac, V. P. Shaping and reshaping CD8$^+$ T-cell memory. *Nature Rev. Immunol.* **8,** 107–119 (2008).
2. Kaech, S. M., Wherry, E. J. & Ahmed, R. Effector and memory T-cell differentiation: implications for vaccine development. *Nature Rev. Immunol.* **2,** 251–262 (2002).
3. Sprent, J. & Surh, C. D. T cell memory. *Annu. Rev. Immunol.* **20,** 551–579 (2002).
4. Williams, M. A. & Bevan, M. J. Effector and memory CTL differentiation. *Annu. Rev. Immunol.* **25,** 171–192 (2007).
5. Butz, E. A. & Bevan, M. J. Massive expansion of antigen-specific CD8$^+$ T cells during an acute virus infection. *Immunity* **8,** 167–175 (1998).
6. Goldrath, A. W. & Bevan, M. J. Selecting and maintaining a diverse T-cell repertoire. *Nature* **402,** 255–262 (1999).
7. Murali-Krishna, K. *et al.* Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* **8,** 177–187 (1998).
8. Badovinac, V. P., Porter, B. B. & Harty, J. T. Programmed contraction of CD8$^+$ T cells after infection. *Nature Immunol.* **3,** 619–626 (2002).
9. Jameson, S. C. Maintaining the norm: T-cell homeostasis. *Nature Rev. Immunol.* **2,** 547–556 (2002).
10. Marrack, P. & Kappler, J. Control of T cell viability. *Annu. Rev. Immunol.* **22,** 765–787 (2004).
11. Schluns, K. S. & Lefrancois, L. Cytokine control of memory T-cell development and survival. *Nature Rev. Immunol.* **3,** 269–279 (2003).
12. Lefrancois, L. & Masopust, D. T cell immunity in lymphoid and non-lymphoid tissues. *Curr. Opin. Immunol.* **14,** 503–508 (2002).
13. Masopust, D., Vezys, V., Marzo, A. L. & Lefrancois, L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* **291,** 2413–2417 (2001).
14. Lanier, L. L. NK cell recognition. *Annu. Rev. Immunol.* **23,** 225–274 (2005).
15. Lanier, L. L. Back to the future—defining NK cells and T cells. *Eur. J. Immunol.* **37,** 1424–1426 (2007).
16. Glas, R. *et al.* Recruitment and activation of natural killer (NK) cells *in vivo* determined by the target cell phenotype. An adaptive component of NK cell-mediated responses. *J. Exp. Med.* **191,** 129–138 (2000).
17. O'Leary, J. G., Goodarzi, M., Drayton, D. L. & von Andrian, U. H. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nature Immunol.* **7,** 507–516 (2006).
18. Arase, H. *et al.* Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* **296,** 1323–1326 (2002).
19. Brown, M. G. *et al.* Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science* **292,** 934–937 (2001).
20. Daniels, K. A. *et al.* Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H. *J. Exp. Med.* **194,** 29–44 (2001).
21. Smith, H. R. *et al.* Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc. Natl Acad. Sci. USA* **99,** 8826–8831 (2002).
22. Vidal, S. M. & Lanier, L. L. NK cell recognition of mouse cytomegalovirus-infected cells. *Curr. Top. Microbiol. Immunol.* **298,** 183–206 (2006).
23. Dokun, A. O. *et al.* Specific and nonspecific NK cell activation during virus infection. *Nature Immunol.* **2,** 951–956 (2001).
24. Yokoyama, W. M., Kim, S. & French, A. R. The dynamic life of natural killer cells. *Annu. Rev. Immunol.* **22,** 405–429 (2004).
25. Bakker, A. B. *et al.* DAP12-deficient mice fail to develop autoimmunity due to impaired antigen priming. *Immunity* **13,** 345–353 (2000).
26. Homann, D., Teyton, L. & Oldstone, M. B. Differential regulation of antiviral T-cell immunity results in stable CD8$^+$ but declining CD4$^+$ T-cell memory. *Nature Med.* **7,** 913–919 (2001).
27. Williams, M. A., Ravkov, E. V. & Bevan, M. J. Rapid culling of the CD4$^+$ T cell repertoire in the transition from effector to memory. *Immunity* **28,** 533–545 (2008).
28. Stetson, D. B. *et al.* Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J. Exp. Med.* **198,** 1069–1076 (2003).
29. Badovinac, V. P., Messingham, K. A., Hamilton, S. E. & Harty, J. T. Regulation of CD8$^+$ T cells undergoing primary and secondary responses to infection in the same host. *J. Immunol.* **170,** 4933–4942 (2003).
30. Belz, G. T. *et al.* Minimal activation of memory CD8$^+$ T cell by tissue-derived dendritic cells favors the stimulation of naive CD8$^+$ T cells. *Nature Immunol.* **8,** 1060–1066 (2007).
31. Grayson, J. M. *et al.* Differential sensitivity of naive and memory CD8$^+$ T cells to apoptosis *in vivo*. *J. Immunol.* **169,** 3760–3770 (2002).
32. Jabbari, A. & Harty, J. T. Secondary memory CD8$^+$ T cells are more protective but slower to acquire a central-memory phenotype. *J. Exp. Med.* **203,** 919–932 (2006).
33. Zimmermann, C., Prevost-Blondel, A., Blaser, C. & Pircher, H. Kinetics of the response of naive and memory CD8 T cells to antigen: similarities and differences. *Eur. J. Immunol.* **29,** 284–290 (1999).
34. Bukowski, J. F., Warner, J. F., Dennert, G. & Welsh, R. M. Adoptive transfer studies demonstrating the antiviral effect of natural killer cells *in vivo*. *J. Exp. Med.* **161,** 40–52 (1985).
35. Sun, J. C. & Bevan, M. J. Cutting edge: long-lived CD8 memory and protective immunity in the absence of CD40 expression on CD8 T cells. *J. Immunol.* **172,** 3385–3389 (2004).
36. Bubic, I. *et al.* Gain of virulence caused by loss of a gene in murine cytomegalovirus. *J. Virol.* **78,** 7536–7544 (2004).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank the Lanier laboratory for comments and discussions, and R. Locksley and W. Seaman for critical reading of this manuscript. The work was supported by National Institutes of Health grant AI068129. J.C.S. is supported by the Irvington Institute for Immunological Research. J.N.B. is supported by the Juvenile Diabetes Research Foundation. L.L.L. is an American Cancer Society Research Professor.

Author Contributions J.C.S. and J.N.B. contributed to project planning, experimental work, data analysis and writing the manuscript. L.L.L. contributed to project planning, data analysis and writing the manuscript.

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doi:10.1038/nature07833

Adaptive immune features of natural killer cells

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Nature **457**, 557–561 (2009)

In the right panel of Fig. 4b of this Article the *x*-axis labels for 'Naive' and 'Memory' were inadvertently reversed in the histogram. The 'Naive' label should be on the left and 'Memory' on the right.