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Immune memory redefined: characterizing the longevity of natural killer cells

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Summary

Natural Killer (NK) cells respond rapidly against transformed, stressed, or virally infected cells and provide a first line immune defense against pathogen invasion and cancer. Thought to involve short-lived effector cells that are armed for battle, NK cells were not previously known to contribute in recall responses against pathogen re-encounter. Here we highlight recent discoveries demonstrating that NK cells are not limited to driving primary immune responses against foreign antigen but can mount secondary responses contributing to immune memory. We also further characterize the phenotype and function of long-lived memory NK cells generated during viral infection.

Keywords

natural killer cells; immune memory; viral infection

Introduction to immune memory

Immune memory and the ability to respond more robustly to a second encounter with a pathogen have long been associated with cells of the adaptive immune system. Decades of studies on T cells using T-cell receptor (TCR) transgenic and genetic knockout mice have unveiled many aspects of T-cell activation and differentiation in response to model pathogens (1–4). Analogous studies done on B cells have similarly revealed how B cells respond to antigen and develop into long-lived memory and plasma cells (5,6). However, even with the enormous body of work performed to elucidate the parameters that govern the differentiation of effector T and B cells into long-lived memory cells, we still do not fully understand many aspects of immune memory, including the following: (i) How are set points determined for memory cell numbers? For example, why does contraction of effector cytotoxic T lymphocytes (CTLs) consistently result in memory cells that constitute 5–10% of the total cell numbers at the peak of the response, and how are these numbers maintained so consistently throughout the lifetime of the host? (ii) How does the duration of antigen exposure control the magnitude of the effector response and memory cell numbers? For example, how does persistence of antigen during chronic infections negatively influence the memory T-cell pool? And more broadly, how does antigen concentration and availability impact T-cell priming and memory cell survival? (iii) How do growth factors, nutrients, cytokines, chemokines, and costimulation influence the quantity and quality of the effector and subsequent memory cell response? (iv) How do interactions between immune cells with

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other hematopoietic and parenchymal cells influence maintenance of the memory pool? For example, how do CD4⁺ T cells help memory B cells and CD8⁺ T cells? (v) How do effector and memory cells traffic to lymphoid and non-lymphoid sites throughout the body? There is much we have yet to learn about how our adaptive immune system functions, with the ultimate goal of providing long-term protection against numerous pathogen threats, such as malaria, tuberculosis, and human immunodeficiency virus (HIV), through effective vaccine development. Understanding the molecular mechanisms of the adaptive immune system and how different lymphocytes coordinate with one another to deliver the most potent immune response will allow us to establish the basic correlates of protection.

Newly recognized as a member of the army of lymphocytes that can respond a second time to an encounter with a previous pathogen is the NK cell. NK cells have long been compared to effector and memory CD8⁺ T cells in phenotype and function (7–9), and now several groups have recently demonstrated that NK cells become long-lived cells that can mount secondary responses against specific antigens. In mouse studies, NK cells activated specifically by a chemical hapten or virus (10,11) or non-specifically by inflammatory cytokines (12) acquire classical characteristics of immune memory that include an extended life span, the ability to self-renew, and the capacity to mount robust and protective recall responses. Because the NK cell receptor repertoire is fixed (unlike gene rearrangement leading to limitless T and B-cell receptors, NK cell receptor diversity is established at the cell population level) and certain subsets of NK cells exist at relatively high precursor frequencies, methods for tracking antigen-experienced NK cells following activation and distinguishing these cells from recent emigrants from the bone marrow were not frequently employed. Furthermore, until the recent identification of specific NK cell receptors and their cognate foreign (non-self) antigens, we lacked the proper models to study NK cells with target specificity. With these new tools available, recent experimental data generated using adoptive transfer of congenic NK cells prior to infection allowed exclusion of all host cells and specific visualization of a mature NK cell population responding to infection (11). These studies, described below, demonstrate that NK cells exhibit features of adaptive immunity that have not been fully appreciated. Now, with the ability to track and stimulate NK cells to a specific ligand, the many unanswered questions above pertaining to memory B and T-cell biology need to also be addressed in NK cells. Some of our new findings characterizing the phenotype and function of memory NK cells will be described in later sections and will begin to address the questions posed above.

Viral models to study NK cell memory

The NK cell response against cytomegalovirus (CMV) is well-characterized in mouse and humans (13–17). In mice lacking NK cells, MCMV replication occurs at a high rate. MCMV has been shown to infect conventional dendritic cells (18) as well as other cell types, leading to the expression of the viral MHC class I-like decoy molecule m157 (19–22). Resistance to MCMV in the C57BL/6 strain of mice has been mapped to the NK gene complex on chromosome 6 (23–25), which contains the *Ly49h* gene (26–28). NK cells bearing the activating Ly49H receptor are able to recognize the MCMV-encoded m157 gene product (20,21,29) (Fig. 1A) and respond to inflammatory cytokines produced by dendritic cells (30–32). Signaling primarily via DAP12 (33) (Fig. 1B) and augmented through DAP10 (34–36), Ly49H⁺ NK cells mount rapid antiviral responses during infection (11,37,38) (Fig. 1C, D). Similar to T cells, the Ly49H-bearing NK cells are able to undergo a rapid clonal-like expansion (Fig. 1E), resulting in a 3–10-fold increase in cell numbers during the first week of infection in the spleen and liver, respectively (11,37). In an adoptive transfer model, where the precursor frequency of Ly49H⁺ NK cells is reduced, 100–1000-fold expansion of these cells is readily observed in spleen and liver, respectively (11). The prolific expansion measured in transferred NK cells during acute viral infection mimics TCR transgenic

systems, where small numbers of antigen-specific T cells are similarly transferred into naive hosts and numbers can be tracked using congenic markers following infection with viruses or bacteria containing the epitope of interest. Following NK cell expansion and viral clearance, effector Ly49H⁺ NK cells undergo a contraction phase resulting in a long-lived memory pool (Fig. 1F), persisting in lymphoid and non-lymphoid organs (11). Many months later, the antigen-experienced memory NK cells can respond a second time when viral antigen is re-encountered (11) (Fig. 1A).

The Ly49H/MCMV system described above will be useful in dissecting the factors that are important in NK cell activation, proliferation, and formation of long-lived memory cells. However, having only one clearly identified receptor-viral ligand pair to work with has its limitations and is akin to possessing only one known TCR epitope for the study of all T-cell responses. As the pathogen-associated ligands for more NK cell receptors are discovered and validated, we will have multiple systems in which to test NK cell responses and the generation of NK cell memory to infectious agents. Several additional models appear to be on the way. Recently, a viral component has been identified for the activating NK cell receptor Ly49P, which like Ly49H, associates and signals via the immunoreceptor tyrosine-based activation motif (ITAM)-containing adapter molecule DAP12 (33,39). Interestingly, similar to major histocompatibility complex (MHC) restriction of T cells, Ly49P recognition of MCMV-infected cells is MHC restricted, and only the mouse strains such as Ma/My, which possess both the *Ly49p* and *H2-D^k* genes, confer resistance to MCMV infection (40,41). Although the viral m04 protein is required for recognition of infected H-2Dk-bearing cell by Ly49P⁺ NK cells (42), the precise nature of the ligand remains to be elucidated.

For many decades, data have existed that human NK cells produce IFN- γ and mediate cytotoxicity in response to influenza A virus infection (43). More recently, the activating Nkp46 receptor was shown to bind the hemagglutinin of influenza virus, and recognition led to NK cell-mediated destruction of target cells expressing the viral glycoprotein (44). In mouse studies, genetic ablation of Nkp46 rendered the mice susceptible to influenza infection and resulted in poor disease outcome and mortality (45). These studies in NK cells during influenza infection, along with the newly generated tools that accompany the findings, create a novel system to study the generation and survival of memory NK cells following viral infection. Furthermore, NK cells have been implicated in the control of poxviruses [ectromelia virus in mice (46–49)], and when the activating receptors and their counterpart viral ligands have been fully characterized, there will be many models to allow for the discovery of NK cell requirements during priming, effector function, and memory maintenance.

In the documented cases where humans are deficient in NK cells, these patients have been shown to be susceptible to HCMV and varicella zoster virus (VZV) infection, among other viral infections (50–53). Similar to the Ly49H⁺ NK cell response that occurs during MCMV infection, CD94/NKG2C-bearing NK cells in humans have been found to exist in higher frequency in HCMV-seropositive individuals compared to those who are CMV-seronegative (54,55). Unlike the Ly49H-bearing NK cell subset in mice, NKG2C⁺ NK cells exist in uninfected humans at low precursor frequencies (0.1%–1%). There are documented cases where this subset of NK cells greatly expands during infection *in vivo* and *in vitro* (56,57). Recently, a unique report described the preferential expansion of NKG2C⁺ NK cells (greater than 80% of total NK cells) in an immunodeficient infant at the peak of viremia during an acute HCMV infection (57). Interestingly, the magnitude and kinetics of NK cell expansion and contraction largely resembles our measurements in the mouse Ly49H⁺ NK cell response following MCMV infection (11). The viral ligand that NKG2C-bearing NK cells recognizes remains to be characterized. Although these studies indicate an important role for direct and

specific recognition of infected cells by NK cells during the immune response against viral infection, a method is required to distinguish memory NK cells from recent naive emigrants from the bone marrow that have not previously encountered virus. Discovery of a marker (or set of markers) unique to memory NK cells will allow characterization of long-lived NK cells that reside in human tissues. Also complicating immune studies in humans is the difficulty in determining with great certainty when viral infection initially occurred; thus, the frequencies and phenotype of virus-specific NK cells obtained at a given time point represents only a snapshot along the continuum of NK cell expansion, contraction, and memory.

Further characterization of memory NK cells

Using the NK cell adoptive transfer model described above, we have been further able to characterize memory NK cells following MCMV infection. Memory T cells are self-renewing, and the 'basal homeostasis' of these cells is dependent on IL-7 and IL-15 (58–73). Basal homeostasis refers to the tight regulation in number of cells of a particular lymphocyte population during steady state in an animal (i.e. in the absence of infection, chemotherapy, radiation, or disease). Survival cytokines that signal via receptors of the IL-2 receptor common γ chain family along with helper CD4⁺ T cells (74–82) have been attributed to the longevity of memory CD8⁺ T cells following infection. Although developing and mature NK cells require IL-15 signals for basal homeostasis and survival (83–88), IL-15 and signals from other common γ chain family members are not required for NK cell activation, proliferation, or function during acute viral infection (89). Although in the absence of IL-15 the primary NK cell response was driven and sustained by IL-12 and other inflammatory cytokines (89), once inflammation resolved, the remaining memory NK cells once again became dependent on IL-15 (J.C.S. and L.L.L., unpublished observations). Future studies will examine additional cytokine requirements of memory NK cells, and the influence of other immune cells on the longevity of NK cells.

Using bromodeoxyuridine (BrdU) incorporation, early reports suggested that the turnover rate of endogenous memory-phenotype T cells measured was increased compared with naive T cells (64,90,91); however, these studies did not measure antigen-experienced memory T cells. More recent studies using TCR transgenic mice have confirmed that basal homeostatic turnover over a given period of time was higher in memory T cells compared to naive T cells in a non-lymphopenic setting (59,92–95). In unirradiated mice, adoptively transferred naive T cells did not readily incorporate BrdU or dilute much CFSE when labeled prior to transfer. We compared the basal turnover rate of naive NK cells with antigen-experienced memory NK cells during the course of one week. Naive mice or mice containing memory NK cells (at day 50 post-infection) were given BrdU, and the percentage of Ly49H⁺ NK cells that incorporated BrdU was measured (Fig. 2). Roughly 4% of memory NK cells incorporated BrdU compared with 16% of the NK cells in naive mice (Fig. 2), suggesting that memory NK cells undergo basal turnover at a lower rate than NK cells that have not previously encountered cognate antigen. To measure only mature naive NK cells incorporating BrdU (and exclude recent emigrants from the bone marrow that have taken up BrdU), naive splenic NK cells were transferred at the same time BrdU treatment was initiated. In this set of control mice, 10.3% of naive NK cells incorporated BrdU, still a larger percentage compared with the memory NK cells (Fig. 2). Why do memory NK cells turn over at a slower rate than naive cells? Perhaps the decreased basal homeostatic turnover of memory NK cells allows them to remain in a more quiescent state, thus permitting memory NK cells a far greater lifespan than naive NK cells that have not been previously activated. More precise studies using CFSE-labeled memory and naive NK cells will allow us measure the overall number of divisions these cells undergo over a specified period of time and allow direct comparison with basal homeostasis of T-cell subsets. Unlike naive T

cells, naive NK cells are found in abundance in peripheral non-lymphoid organs. Future studies will examine the rate of naive and memory NK cell turnover in lymphoid compared with non-lymphoid tissues. Furthermore, because NK cells placed in a lymphopenic environment will undergo homeostatic proliferation (96–98), it will be of interest to see if such a non-antigen-specific driving force will result in the development of long-lived memory NK cells.

Using TCR transgenic models for bacterial or viral infections, several groups have shown that memory CD4⁺ and CD8⁺ T cells reside in all organs (lymphoid and non-lymphoid) in the body (99–101). It is thought that the preferential localization of a subset of memory T cells to peripheral non-lymphoid tissues allows these previously antigen-experienced cells to fight infection at the sites of microbial entry. Like NK cells, these ‘effector memory’ T cells are poised to respond rapidly and robustly during infection. To similarly examine how activated NK cells traffic during infection, we examined the localization of effector and memory NK cells in various tissues (Fig. 3). Previously, we measured effector and memory NK cell numbers in blood and spleen (11). Here, we determined the percentages of transferred NK cells at 7 and 50 days after MCMV infection in spleen, liver, lung, kidney, and mesenteric and inguinal lymph nodes (Fig. 3). Similar to our previous measurements in peripheral blood, we observed a prolific expansion of Ly49H-bearing NK cells in spleen and non-lymphoid organs (greater than 60% of total NK cells in tissue) at 7 days after infection (Fig. 3A). Perhaps not surprisingly, the mesenteric lymph nodes that drain the site of infection (intraperitoneal injection of virus) contained a higher percentage of transferred NK cells than the non-draining inguinal lymph node (Fig. 3A). Although a significant increase in the transferred Ly49H⁺ NK cells was measured in the lymph nodes at day 7 post-infection, the percentages were much lower than in other tissues mentioned (Fig. 3A). When memory NK cells were analyzed 50 days after MCMV infection, the highest percentages of Ly49H⁺ NK cells were found in spleen and non-lymphoid tissues, with very few transferred NK cells residing in either lymph node site (Fig. 3B). Thus, like effector memory T cells (94,102), memory NK cells preferentially reside in peripheral tissues. Future studies will examine whether memory NK cells found in non-lymphoid tissues have a distinct phenotype and function compared to the small pool of memory NK cells that take residence in secondary lymphoid tissues. Moreover, it will be of interest to characterize whether the remnant of memory NK cells that resides in lymph nodes is functionally similar to the ‘central memory’ T cells generated during viral infection (94,102).

Avidity maturation during the NK cell response?

One tenet of adaptive immunity is that an activated and proliferating antigen-specific cell and its daughter cells should be able to respond multiple times to repeated pathogen exposure. This has been experimentally tested using TCR transgenic T cells, and it has been observed that the same cells can undergo primary, secondary, and tertiary responses when re-exposed to the same antigenic epitope (103–106). To be able to mount a tertiary immune response, primary memory T cells that have become effectors a second time have been shown to survive and generate a pool of ‘secondary memory’ T cells. Previously, when we transferred memory NK cells into Ly49H-deficient mice and infected with MCMV, we observed a robust secondary response (11). We now demonstrate that following the recall response of memory NK cells, we are able to recover secondary memory NK cells several months later (Fig. 4A, B). Secondary memory cells were recovered at a lower frequency than primary memory NK cells because lower numbers of memory cells ($\sim 10^4$) were adoptively transferred during the secondary response compared to naive cells ($\sim 10^5$) during the primary response (Fig. 4B). Previously, we documented that memory Ly49H⁺ NK cells had a higher surface expression of the activating Ly49H receptor and the inhibitory KLRG1 receptor compared with naive NK cells (11). The more mature state of the individual

memory NK cell compared to a naive NK cell (107), along with the higher expression of Ly49H, were thought to account for the greater ability of memory NK cells to protect against viral challenge (11). Interestingly, surface levels of Ly49H and KLRG1 continued to increase on secondary memory NK cells (Fig. 4C). Whether this represents higher transcript levels or more stable surface protein on secondary memory NK cells compared with naive and primary memory NK cells remains to be determined. Again, this finding suggests that memory NK cells are more similar to effector memory CD8⁺ T cells, which express KLRG1, than central memory CD8⁺ T cells (KLRG1^{lo}) (94,108,109).

During affinity maturation of T cells with each successive round of infection, T cells containing the highest affinity TCRs for a given peptide/MHC will constitute the immunodominant response leading to a higher frequency of effector and memory cells (104,110–113). The affinity of the TCR for a given epitope along with the overall number of TCRs on the cell surface will together contribute to the overall avidity. Perhaps in a similar manner, the memory NK cell compartment could be selecting for cells with highest avidity (i.e. highest amounts of the Ly49H receptor) for the specific viral antigen. (There is no evidence for receptor editing and changes in Ly49H affinity for m157 during MCMV infection, thus overall avidity is presumably determined solely by number of receptors on a given NK cell.) Thus, although the naive NK cell pool contains cells with differing levels of the Ly49H receptor (which presumably have the same affinity for m157 on a per receptor basis), each successive round of antigen exposure selects only for memory NK cells with the highest levels of receptor (Fig. 4B, C). Interestingly, the selection of cells expressing high levels of the Ly49H receptor is specific only for Ly49H, because surface expression of activating receptors NK1.1 and Ly49D do not differ between naive, memory, and secondary memory NK cells (Fig. 4C), suggesting that engagement of viral ligand drives ‘avidity maturation’ during the NK cell response resulting in the highest avidity memory Ly49H⁺ NK cells. A potential mechanism could exist where enhanced activating signals through Ly49H^{hi} NK cells (as opposed to Ly49H^{int} NK cells) promote improved memory cell formation. The selective expansion and maintenance of the highest avidity T and NK cells might constitute an *in vivo* mechanism for optimizing the early immune detection of virally infected cells.

Concluding remarks

As discussed in the introduction, there is still much to be learned about B and T-cell memory. By comparison, our knowledge of NK cell memory is in its infancy. If NK cells are thought to be an evolutionary bridge between innate and adaptive immunity, it is not surprising that they will exhibit features of both (114). Like most innate responders, NK cells possess receptors that do not rearrange and are fixed at the germline level. They also can respond at peripheral sites of infection, trafficking rapidly and mediating effector mechanisms (cytotoxicity and cytokine release) without previous priming. As adaptive responders, NK cells possess the features of clonal expansion, longevity, and the ability to respond more robustly during subsequent encounters with the same pathogen.

With the identification of numerous NK cell receptors and downstream signaling molecules, the focus now turns to characterizing the factors and cues that govern the adaptive immune functions of NK cells. For instance, the limit of NK cell expansion appears fixed (at roughly 1000-fold) and is 1–2 logs lower than for T cells (at greater than 50,000-fold for certain epitopes) (11,115–117). How are these ‘ceilings’ established, and what are the homeostatic restraints or growth factor limitations? Perhaps competition for limiting endogenous resources and cytokines (such as IL-15) between T cells and NK cells may be regulating NK cell expansion, and experimentally supplementing IL-15 signals would maximize expansion (118,119). Does the degree of inflammation influence the kinetics or magnitude of the NK

cell response? The amount of inflammatory cytokines (such as IL-12 and type I interferon) present during T-cell priming has been shown to dictate proliferation potential and even influence cell fate decisions (i.e. generation of effector T cells that are short-lived or possess memory potential) (108,120,121). Do cell-cell interactions play an important role in regulating the expansion of NK cells during viral infection? We have new evidence that interactions with MHC class I during the NK cell response to MCMV greatly influences the ability of certain subsets to proliferate and mediate effector function (manuscript in press). With T cells, it has been shown that CD4⁺ T-cell help is required for APC maturation during the priming of CD8⁺ T cells (122–126). Is there a similar role for T-cell help in NK cell priming that has not been previously recognized? These and other questions regarding the initiation of the NK cell response require addressing.

Furthermore, we do not understand how the contraction phase of the NK cell response is regulated. Like with most T-cell responses, the peak of the effector NK cell expansion occurs at around 7–8 days after infection, regardless of the precursor frequency of antigen-specific NK cells (11). Why does the contraction phase begin at a set time point? How does availability of resources and antigen play a role in the NK cell response? With T cells, it has been shown that a short period of TCR stimulation can lead to ‘programmed’ durations of expansion and contraction (127–132), and it remains to be seen whether NK cells behave in similar manner or require a longer duration of receptor ligation and signaling. We have also observed that the prolonged contraction phase following Ly49H⁺ NK cell expansion mimics CD4⁺ T-cell contraction rather than the more rapid contraction phase documented for CD8⁺ T-cell responses (11,127,133). Why this happens is still unclear, and using genetic deletions of cytokine receptors and transcription factors known to play a role in cell differentiation and survival will help to determine how memory cell ‘set points’ are established during the contraction phase.

We need to investigate the requirements for the maintenance of memory NK cells. What cytokines and stromal elements are involved in maintaining memory NK cells in secondary lymphoid and peripheral non-lymphoid tissues? How do the initial signals received by NK cells during priming influence the quantity, quality, and localization of memory NK cells? Our findings would premise that specific antigen receptor ligation is essential for the generation of memory NK cells (11), as would data from von Andrian and colleagues suggesting that a specific subset of NK cells (expressing a cluster of receptors) is responsible for the contact sensitivity reactions observed in T- and B-cell-deficient mice (10). The secondary response of NK cells measured during viral or chemical hapten challenge has been shown to be receptor and antigen dependent, as both blocking the responsible activating receptor or altering the specific antigen completely ablated the recall response (10,11). In contrast, Yokoyama and colleagues (12) stimulated NK cells with inflammatory cytokines rather than foreign antigen, and in a ‘non-specific’ manner drove NK cells to become effector and ‘memory-like’ cells. It remains to be determined whether the longevity of the memory NK cells introduced to inflammation alone compares with the lifespan of memory cells driven by both specific receptor ligation and exposure to inflammatory cytokines (produced by viruses or chemicals). Furthermore, it will be of interest to assess the magnitude and quality of the secondary response of memory NK cells primed by cytokines alone.

Because NK cells can respond more rapidly than naive T cells and as memory NK cells are found in abundance in peripheral tissues, understanding the factors that promote survival of long-lived memory NK cells will aid in design of vaccines against pathogens where traditional B-cell and T-cell-focused strategies have failed. Unlike with memory T cells, our data suggest that memory NK cells may not persist for the lifespan of the host (11). In our mouse model of MCMV infection, memory NK cells are difficult to detect after 4–5 months

but are still present, because they can be found after re-challenge with MCMV (authors' unpublished observations). However, when 4–5 months in the lifespan of a mouse is extrapolated to a comparable duration of time in humans, this constitutes the equivalent of a priming strategy that could provide NK cell immunity for greater than two decades (when one considers that a mouse in captivity can live for 2 to 2.5 years, and far fewer in the wild). If a vaccine could be designed to specifically induce protective memory NK cells for a period of 15–20 years in humans, this would provide a new strategy against many viral pathogens such as herpes, hepatitis, and HIV that are contracted during the sexually reproductive years. Future studies will need to address the presence and longevity of memory NK cells at common portals of virus entry, such as mucosal tissues, which will help control the initial burst of viral infection and replication.

Although NK cells have been proven to exhibit many of the canonical features of immune memory in mouse models, identification and characterization of long-lived memory NK cells in humans that can respond robustly to repeat pathogen exposure needs to be done. In the meantime, we will use the powerful experimental mouse tools to carefully define the molecular mechanisms and cell-cell interactions that promote and sustain memory T cells, memory B cells, and now memory NK cells. Although the boundaries segregating innate and adaptive immunity may be blurring, we importantly need to establish how to harness the immune system at the cellular and molecular level to generate and sustain protective immunity. Once the correlates of immune protection are determined, we can better design vaccines that elicit the most productive and long-lived responses against unconquered pathogens.

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References

1. Goldrath AW, Bevan MJ. Selecting and maintaining a diverse T-cell repertoire. *Nature*. 1999; 402:255–w62. [PubMed: 10580495]
2. Harty JT, Badovinac VP. Shaping and reshaping CD8+ T-cell memory. *Nat Rev Immunol*. 2008; 8:107–119. [PubMed: 18219309]
3. Kaeche SM, Wherry EJ. Heterogeneity and cell-fate decisions in effector and memory CD8+ T cell differentiation during viral infection. *Immunity*. 2007; 27:393–405. [PubMed: 17892848]
4. Williams MA, Bevan MJ. Effector and memory CTL differentiation. *Annu Rev Immunol*. 2007; 25:171–192. [PubMed: 17129182]
5. Dorner T, Radbruch A. Antibodies and B cell memory in viral immunity. *Immunity*. 2007; 27:384–392. [PubMed: 17892847]
6. McHeyzer-Williams LJ, McHeyzer-Williams MG. Antigen-specific memory B cell development. *Annu Rev Immunol*. 2005; 23:487–513. [PubMed: 15771579]
7. Lanier LL. NK cell recognition. *Annu Rev Immunol*. 2005; 23:225–274. [PubMed: 15771571]
8. Lanier LL. Back to the future--defining NK cells and T cells. *Eur J Immunol*. 2007; 37:1424–1426. [PubMed: 17523211]
9. Raulet DH. Interplay of natural killer cells and their receptors with the adaptive immune response. *Nat Immunol*. 2004; 5:996–1002. [PubMed: 15454923]
10. O'Leary JG, Goodarzi M, Drayton DL, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat Immunol*. 2006; 7:507–16. [PubMed: 16617337]

11. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature*. 2009; 457:557–561. [PubMed: 19136945]
12. Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci USA*. 2009; 106:1915–1919. [PubMed: 19181844]
13. Guma M, Angulo A, Lopez-Botet M. NK cell receptors involved in the response to human cytomegalovirus infection. *Curr Top Microbiol Immunol*. 2006; 298:207–223. [PubMed: 16323417]
14. Sun JC, Lanier LL. The natural selection of herpesviruses and virus-specific NK receptors. *Viruses*. 2009; 1:362–382. [PubMed: 20151027]
15. Vidal SM, Lanier LL. NK cell recognition of mouse cytomegalovirus-infected cells. *Curr Top Microbiol Immunol*. 2006; 298:183–206. [PubMed: 16329187]
16. Wilkinson GW, et al. Modulation of natural killer cells by human cytomegalovirus. *J Clin Virol*. 2008; 41:206–212. [PubMed: 18069056]
17. Yokoyama WM, Kim S, French AR. The dynamic life of natural killer cells. *Annu Rev Immunol*. 2004; 22:405–429. [PubMed: 15032583]
18. Andrews DM, Andoniou CE, Granucci F, Ricciardi-Castagnoli P, Degli-Esposti MA. Infection of dendritic cells by murine cytomegalovirus induces functional paralysis. *Nat Immunol*. 2001; 2:1077–1084. [PubMed: 11668340]
19. Adams EJ, et al. Structural elucidation of the m157 mouse cytomegalovirus ligand for Ly49 natural killer cell receptors. *Proc Natl Acad Sci USA*. 2007; 104:10128–10133. [PubMed: 17537914]
20. Arase H, Mocarski ES, Campbell AE, Hill AB, Lanier LL. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science*. 2002; 296:1323–1326. [PubMed: 11950999]
21. Smith HR, et al. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc Natl Acad Sci USA*. 2002; 99:8826–8831. [PubMed: 12060703]
22. Tripathy SK, Smith HR, Holroyd EA, Pingel JT, Yokoyama WM. Expression of m157, a murine cytomegalovirus-encoded putative major histocompatibility class I (MHC-I)-like protein, is independent of viral regulation of host MHC-I. *J Virol*. 2006; 80:545–550. [PubMed: 16352579]
23. Scalzo AA, Fitzgerald NA, Simmons A, La Vista AB, Shellam GR. *Cmv-1*, a genetic locus that controls murine cytomegalovirus replication in the spleen. *J Exp Med*. 1990; 171:1469–1483. [PubMed: 2159050]
24. Scalzo AA, et al. The effect of the *Cmv-1* resistance gene, which is linked to the natural killer cell gene complex, is mediated by natural killer cells. *J Immunol*. 1992; 149:581–589. [PubMed: 1378069]
25. Yokoyama WM, Plougastel BF. Immune functions encoded by the natural killer gene complex. *Nat Rev Immunol*. 2003; 3:304–316. [PubMed: 12669021]
26. Brown MG, et al. Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science*. 2001; 292:934–937. [PubMed: 11340207]
27. Daniels KA, Devora G, Lai WC, O'Donnell CL, Bennett M, Welsh RM. Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H. *J Exp Med*. 2001; 194:29–44. [PubMed: 11435470]
28. Lee SH, et al. Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C-type lectin superfamily. *Nat Genet*. 2001; 28:42–45. [PubMed: 11326273]
29. Davis AH, Guseva NV, Ball BL, Heusel JW. Characterization of murine cytomegalovirus m157 from infected cells and identification of critical residues mediating recognition by the NK cell receptor Ly49H. *J Immunol*. 2008; 181:265–275. [PubMed: 18566392]
30. Andoniou CE, et al. Interaction between conventional dendritic cells and natural killer cells is integral to the activation of effective antiviral immunity. *Nat Immunol*. 2005; 6:1011–1019. [PubMed: 16142239]
31. Dalod M, et al. Dendritic cell responses to early murine cytomegalovirus infection: subset functional specialization and differential regulation by interferon alpha/beta. *J Exp Med*. 2003; 197:885–898. [PubMed: 12682109]

32. Krug A, et al. TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. *Immunity*. 2004; 21:107–119. [PubMed: 15345224]
33. Smith KM, Wu J, Bakker AB, Phillips JH, Lanier LL. Ly-49D and Ly-49H associate with mouse DAP12 and form activating receptors. *J Immunol*. 1998; 161:7–10. [PubMed: 9647200]
34. Coudert JD, Scarpellino L, Gros F, Vivier E, Held W. Sustained NKG2D engagement induces cross-tolerance of multiple distinct NK cell activation pathways. *Blood*. 2008; 111:3571–3578. [PubMed: 18198346]
35. Orr MT, et al. Ly49H signaling through DAP10 is essential for optimal natural killer cell responses to mouse cytomegalovirus infection. *J Exp Med*. 2009; 206:807–817. [PubMed: 19332875]
36. Tassi I, Le Friec G, Gilfillan S, Takai T, Yokoyama WM, Colonna M. DAP10 associates with Ly49 receptors but contributes minimally to their expression and function in vivo. *Eur J Immunol*. 2009; 39:1129–1135. [PubMed: 19247984]
37. Dokun AO, Kim S, Smith HR, Kang HS, Chu DT, Yokoyama WM. Specific and nonspecific NK cell activation during virus infection. *Nat Immunol*. 2001; 2:951–956. [PubMed: 11550009]
38. Dörner BG, et al. Coordinate expression of cytokines and chemokines by NK cells during murine cytomegalovirus infection. *J Immunol*. 2004; 172:3119–3131. [PubMed: 14978118]
39. Makrigiannis AP, et al. Cloning and characterization of a novel activating Ly49 closely related to Ly49A. *J Immunol*. 1999; 163:4931–4938. [PubMed: 10528196]
40. Desrosiers MP, et al. Epistasis between mouse Klra and major histocompatibility complex class I loci is associated with a new mechanism of natural killer cell-mediated innate resistance to cytomegalovirus infection. *Nat Genet*. 2005; 37:593–599. [PubMed: 15895081]
41. Dighe A, Rodriguez M, Sebastian P, Xie X, McVoy M, Brown MG. Requisite H2k role in NK cell-mediated resistance in acute murine cytomegalovirus-infected MA/My mice. *J Immunol*. 2005; 175:6820–6828. [PubMed: 16272339]
42. Kielczewska A, et al. Ly49P recognition of cytomegalovirus-infected cells expressing H2-Dk and CMV-encoded m04 correlates with the NK cell antiviral response. *J Exp Med*. 2009; 206:515–523. [PubMed: 19255146]
43. Santoli D, Trinchieri G, Koprowski H. Cell-mediated cytotoxicity against virus-infected target cells in humans. II. Interferon induction and activation of natural killer cells. *J Immunol*. 1978; 121:532–538. [PubMed: 150448]
44. Mandelboim O, et al. Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature*. 2001; 409:1055–1060. [PubMed: 11234016]
45. Gazit R, et al. Lethal influenza infection in the absence of the natural killer cell receptor gene *Ncr1*. *Nat Immunol*. 2006; 7:517–523. [PubMed: 16565719]
46. Delano ML, Brownstein DG. Innate resistance to lethal mousepox is genetically linked to the NK gene complex on chromosome 6 and correlates with early restriction of virus replication by cells with an NK phenotype. *J Virol*. 1995; 69:5875–5877. [PubMed: 7637035]
47. Fang M, Lanier LL, Sigal LJ. A role for NKG2D in NK cell-mediated resistance to poxvirus disease. *PLoS Pathog*. 2008; 4:e30. [PubMed: 18266471]
48. Jacoby RO, Bhatt PN, Brownstein DG. Evidence that NK cells and interferon are required for genetic resistance to lethal infection with ectromelia virus. *Arch Virol*. 1989; 108:49–58. [PubMed: 2480764]
49. Parker AK, Parker S, Yokoyama WM, Corbett JA, Buller RM. Induction of natural killer cell responses by ectromelia virus controls infection. *J Virol*. 2007; 81:4070–4079. [PubMed: 17287257]
50. Biron CA, Byron KS, Sullivan JL. Severe herpesvirus infections in an adolescent without natural killer cells. *N Engl J Med*. 1989; 320:1731–1735. [PubMed: 2543925]
51. Etzioni A, Eidenschenk C, Katz R, Beck R, Casanova JL, Pollack S. Fatal varicella associated with selective natural killer cell deficiency. *J Pediatr*. 2005; 146:423–425. [PubMed: 15756234]
52. Orange JS. Human natural killer cell deficiencies and susceptibility to infection. *Microbes Infect*. 2002; 4:1545–1558. [PubMed: 12505527]
53. Orange JS. Human natural killer cell deficiencies. *Curr Opin Allergy Clin Immunol*. 2006; 6:399–409. [PubMed: 17088643]

54. Guma M, Angulo A, Vilches C, Gomez-Lozano N, Malats N, Lopez-Botet M. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. *Blood*. 2004; 104:3664–3671. [PubMed: 15304389]
55. Guma M, et al. Human cytomegalovirus infection is associated with increased proportions of NK cells that express the CD94/NKG2C receptor in aviremic HIV-1-positive patients. *J Infect Dis*. 2006; 194:38–41. [PubMed: 16741880]
56. Guma M, et al. Expansion of CD94/NKG2C+ NK cells in response to human cytomegalovirus-infected fibroblasts. *Blood*. 2006; 107:3624–3631. [PubMed: 16384928]
57. Kuijpers TW, Baars PA, Dantin C, van den Burg M, van Lier RA, Roosnek E. Human NK cells can control CMV infection in the absence of T cells. *Blood*. 2008; 112:914–915. [PubMed: 18650467]
58. Becker TC, et al. Interleukin 15 is required for proliferative renewal of virus-specific memory CD8 T cells. *J Exp Med*. 2002; 195:1541–1548. [PubMed: 12070282]
59. Goldrath AW, et al. Cytokine requirements for acute and Basal homeostatic proliferation of naive and memory CD8+ T cells. *J Exp Med*. 2002; 195:1515–1522. [PubMed: 12070279]
60. Jameson SC. Maintaining the norm: T-cell homeostasis. *Nat Rev Immunol*. 2002; 2:547–556. [PubMed: 12154374]
61. Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nat Immunol*. 2003; 4:1191–1198. [PubMed: 14625547]
62. Kieper WC, et al. Overexpression of interleukin (IL)-7 leads to IL-15-independent generation of memory phenotype CD8+ T cells. *J Exp Med*. 2002; 195:1533–1539. [PubMed: 12070281]
63. Kondrack RM, Harbertson J, Tan JT, McBreen ME, Surh CD, Bradley LM. Interleukin 7 regulates the survival and generation of memory CD4 cells. *J Exp Med*. 2003; 198:1797–1806. [PubMed: 14662907]
64. Ku CC, Murakami M, Sakamoto A, Kappler J, Marrack P. Control of homeostasis of CD8+ memory T cells by opposing cytokines. *Science*. 2000; 288:675–678. [PubMed: 10784451]
65. Li J, Huston G, Swain SL. IL-7 promotes the transition of CD4 effectors to persistent memory cells. *J Exp Med*. 2003; 198:1807–1815. [PubMed: 14676295]
66. Ma A, Koka R, Burkett P. Diverse functions of IL-2, IL-15, and IL-7 in lymphoid homeostasis. *Annu Rev Immunol*. 2006; 24:657–679. [PubMed: 16551262]
67. Marrack P, Kappler J. Control of T cell viability. *Annu Rev Immunol*. 2004; 22:765–787. [PubMed: 15032596]
68. Mortier E, et al. Macrophage-and dendritic-cell-derived interleukin-15 receptor alpha supports homeostasis of distinct CD8+ T cell subsets. *Immunity*. 2009; 31:811–822. [PubMed: 19913445]
69. Schluns KS, Kieper WC, Jameson SC, Lefrancois L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. *Nat Immunol*. 2000; 1:426–432. [PubMed: 11062503]
70. Schluns KS, Lefrancois L. Cytokine control of memory T-cell development and survival. *Nat Rev Immunol*. 2003; 3:269–279. [PubMed: 12669018]
71. Surh CD, Sprent J. Regulation of mature T cell homeostasis. *Semin Immunol*. 2005; 17:183–191. [PubMed: 15826823]
72. Tan JT, Ernst B, Kieper WC, LeRoy E, Sprent J, Surh CD. Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype CD8+ cells but are not required for memory phenotype CD4+ cells. *J Exp Med*. 2002; 195:1523–1532. [PubMed: 12070280]
73. Zhang X, Sun S, Hwang I, Tough DF, Sprent J. Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15. *Immunity*. 1998; 8:591–599. [PubMed: 9620680]
74. Belz GT, Wodarz D, Diaz G, Nowak MA, Doherty PC. Compromised influenza virus-specific CD8(+)-T-cell memory in CD4(+)-T-cell-deficient mice. *J Virol*. 2002; 76:12388–12393. [PubMed: 12414983]
75. Bevan MJ. Helping the CD8(+) T-cell response. *Nat Rev Immunol*. 2004; 4:595–602. [PubMed: 15286726]

76. Janssen EM, Lemmens EE, Wolfe T, Christen U, von Herrath MG, Schoenberger SP. CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. *Nature*. 2003; 421:852–856. [PubMed: 12594515]
77. Khanolkar A, Fuller MJ, Zajac AJ. CD4 T cell-dependent CD8 T cell maturation. *J Immunol*. 2004; 172:2834–2844. [PubMed: 14978084]
78. Matloubian M, Concepcion RJ, Ahmed R. CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. *J Virol*. 1994; 68:8056–8063. [PubMed: 7966595]
79. Shedlock DJ, Shen H. Requirement for CD4 T cell help in generating functional CD8 T cell memory. *Science*. 2003; 300:337–339. [PubMed: 12690201]
80. Sun JC, Bevan MJ. Defective CD8 T cell memory following acute infection without CD4 T cell help. *Science*. 2003; 300:339–342. [PubMed: 12690202]
81. Sun JC, Williams MA, Bevan MJ. CD4+ T cells are required for the maintenance, not programming, of memory CD8+ T cells after acute infection. *Nat Immunol*. 2004; 5:927–933. [PubMed: 15300249]
82. von Herrath MG, Yokoyama M, Dockter J, Oldstone MB, Whitton JL. CD4-deficient mice have reduced levels of memory cytotoxic T lymphocytes after immunization and show diminished resistance to subsequent virus challenge. *J Virol*. 1996; 70:1072–1079. [PubMed: 8551565]
83. Burkett PR, Koka R, Chien M, Chai S, Boone DL, Ma A. Coordinate expression and trans presentation of interleukin (IL)-15 α and IL-15 supports natural killer cell and memory CD8+ T cell homeostasis. *J Exp Med*. 2004; 200:825–834. [PubMed: 15452177]
84. Cooper MA, et al. In vivo evidence for a dependence on interleukin 15 for survival of natural killer cells. *Blood*. 2002; 100:3633–3638. [PubMed: 12393617]
85. Huntington ND, et al. Interleukin 15-mediated survival of natural killer cells is determined by interactions among Bim, Noxa and Mcl-1. *Nat Immunol*. 2007; 8:856–863. [PubMed: 17618288]
86. Kennedy MK, et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J Exp Med*. 2000; 191:771–780. [PubMed: 10704459]
87. Lodolce JP, et al. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity*. 1998; 9:669–676. [PubMed: 9846488]
88. Lucas M, Schachterle W, Oberle K, Aichele P, Diefenbach A. Dendritic cells prime natural killer cells by trans-presenting interleukin 15. *Immunity*. 2007; 26:503–517. [PubMed: 17398124]
89. Sun JC, Ma A, Lanier LL. Cutting edge: IL-15-independent NK cell response to mouse cytomegalovirus infection. *J Immunol*. 2009; 183:2911–2914. [PubMed: 19648279]
90. Sprent J, Tough DF. Lymphocyte life-span and memory. *Science*. 1994; 265:1395–1400. [PubMed: 8073282]
91. Tough DF, Sprent J. Turnover of naive-and memory-phenotype T cells. *J Exp Med*. 1994; 179:1127–1135. [PubMed: 8145034]
92. Blattman JN, Grayson JM, Wherry EJ, Kaech SM, Smith KA, Ahmed R. Therapeutic use of IL-2 to enhance antiviral T-cell responses in vivo. *Nat Med*. 2003; 9:540–547. [PubMed: 12692546]
93. Murali-Krishna K, Lau LL, Sambhara S, Lemmonier F, Altman J, Ahmed R. Persistence of memory CD8 T cells in MHC class I-deficient mice. *Science*. 1999; 286:1377–1381. [PubMed: 10558996]
94. Wherry EJ, et al. Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat Immunol*. 2003; 4:225–234. [PubMed: 12563257]
95. Williams MA, Tyznik AJ, Bevan MJ. Interleukin-2 signals during priming are required for secondary expansion of CD8+ memory T cells. *Nature*. 2006; 441:890–893. [PubMed: 16778891]
96. Jamieson AM, Isnard P, Dorfman JR, Coles MC, Raulet DH. Turnover and proliferation of NK cells in steady state and lymphopenic conditions. *J Immunol*. 2004; 172:864–870. [PubMed: 14707057]
97. Prlic M, Blazar BR, Farrar MA, Jameson SC. In vivo survival and homeostatic proliferation of natural killer cells. *J Exp Med*. 2003; 197:967–976. [PubMed: 12695488]
98. Ranson T, Voshchenrich CA, Corcuff E, Richard O, Muller W, Di Santo JP. IL-15 is an essential mediator of peripheral NK-cell homeostasis. *Blood*. 2003; 101:4887–4893. [PubMed: 12586624]

99. Lefrancois L, Masopust D. T cell immunity in lymphoid and non-lymphoid tissues. *Curr Opin Immunol.* 2002; 14:503–508. [PubMed: 12088686]
100. Masopust D, Vezys V, Marzo AL, Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science.* 2001; 291:2413–2417. [PubMed: 11264538]
101. Reinhardt RL, Khoruts A, Merica R, Zell T, Jenkins MK. Visualizing the generation of memory CD4 T cells in the whole body. *Nature.* 2001; 410:101–105. [PubMed: 11242050]
102. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol.* 2004; 22:745–763. [PubMed: 15032595]
103. Badovinac VP, Messingham KA, Hamilton SE, Harty JT. Regulation of CD8+ T cells undergoing primary and secondary responses to infection in the same host. *J Immunol.* 2003; 170:4933–4942. [PubMed: 12734336]
104. Busch DH, Pilip I, Pamer EG. Evolution of a complex T cell receptor repertoire during primary and recall bacterial infection. *J Exp Med.* 1998; 188:61–70. [PubMed: 9653084]
105. Jabbari A, Harty JT. Secondary memory CD8+ T cells are more protective but slower to acquire a central-memory phenotype. *J Exp Med.* 2006; 203:919–932. [PubMed: 16567385]
106. Woodland DL. Jump-starting the immune system: prime-boosting comes of age. *Trends Immunol.* 2004; 25:98–104. [PubMed: 15102369]
107. Huntington ND, et al. NK cell maturation and peripheral homeostasis is associated with KLRG1 up-regulation. *J Immunol.* 2007; 178:4764–4770. [PubMed: 17404256]
108. Joshi NS, et al. Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. *Immunity.* 2007; 27:281–295. [PubMed: 17723218]
109. Sarkar S, Kalia V, Haining WN, Konieczny BT, Subramaniam S, Ahmed R. Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. *J Exp Med.* 2008; 205:625–640. [PubMed: 18316415]
110. Bachmann MF, Speiser DE, Ohashi PS. Functional management of an antiviral cytotoxic T-cell response. *J Virol.* 1997; 71:5764–5768. [PubMed: 9223463]
111. Busch DH, Pamer EG. T cell affinity maturation by selective expansion during infection. *J Exp Med.* 1999; 189:701–710. [PubMed: 9989985]
112. McHeyzer-Williams MG, Davis MM. Antigen-specific development of primary and memory T cells in vivo. *Science.* 1995; 268:106–111. [PubMed: 7535476]
113. Turner SJ, La Gruta NL, Kedzierska K, Thomas PG, Doherty PC. Functional implications of T cell receptor diversity. *Curr Opin Immunol.* 2009; 21:286–290. [PubMed: 19524428]
114. Sun JC, Lanier LL. Natural killer cells remember: an evolutionary bridge between innate and adaptive immunity? *Eur J Immunol.* 2009; 39:2059–2064. [PubMed: 19637199]
115. Butz EA, Bevan MJ. Massive expansion of antigen-specific CD8+ T cells during an acute virus infection. *Immunity.* 1998; 8:167–175. [PubMed: 9491998]
116. Masopust D, Murali-Krishna K, Ahmed R. Quantitating the magnitude of the lymphocytic choriomeningitis virus-specific CD8 T-cell response: it is even bigger than we thought. *J Virol.* 2007; 81:2002–2011. [PubMed: 17151096]
117. Murali-Krishna K, et al. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity.* 1998; 8:177–187. [PubMed: 9491999]
118. Fehniger TA, et al. Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8+ T cells. *J Exp Med.* 2001; 193:219–231. [PubMed: 11208862]
119. Stoklasek TA, Schluns KS, Lefrancois L. Combined IL-15/IL-15Ralpha immunotherapy maximizes IL-15 activity in vivo. *J Immunol.* 2006; 177:6072–6080. [PubMed: 17056533]
120. Kolumam GA, Thomas S, Thompson LJ, Sprent J, Murali-Krishna K. Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. *J Exp Med.* 2005; 202:637–650. [PubMed: 16129706]
121. Mescher MF, et al. Signals required for programming effector and memory development by CD8+ T cells. *Immunol Rev.* 2006; 211:81–92. [PubMed: 16824119]

122. Bennett SR, Carbone FR, Karamalis F, Flavell RA, Miller JF, Heath WR. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature*. 1998; 393:478–480. [PubMed: 9624004]
123. Bennett SR, Carbone FR, Karamalis F, Miller JF, Heath WR. Induction of a CD8+ cytotoxic T lymphocyte response by cross-priming requires cognate CD4+ T cell help. *J Exp Med*. 1997; 186:65–70. [PubMed: 9206998]
124. Guermónprez P, Valladeau J, Zitvogel L, Thery C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol*. 2002; 20:621–667. [PubMed: 11861614]
125. Ridge JP, Di Rosa F, Matzinger P. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature*. 1998; 393:474–478. [PubMed: 9624003]
126. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature*. 1998; 393:480–483. [PubMed: 9624005]
127. Badovinac VP, Porter BB, Harty JT. Programmed contraction of CD8(+) T cells after infection. *Nat Immunol*. 2002; 3:619–626. [PubMed: 12055624]
128. Kaech SM, Ahmed R. Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. *Nat Immunol*. 2001; 2:415–422. [PubMed: 11323695]
129. Masopust D, Kaech SM, Wherry EJ, Ahmed R. The role of programming in memory T-cell development. *Curr Opin Immunol*. 2004; 16:217–225. [PubMed: 15023416]
130. Mercado R, Vijh S, Allen SE, Kerksiek K, Pilip IM, Pamer EG. Early programming of T cell populations responding to bacterial infection. *J Immunol*. 2000; 165:6833–6839. [PubMed: 11120806]
131. van Stipdonk MJ, et al. Dynamic programming of CD8+ T lymphocyte responses. *Nat Immunol*. 2003; 4:361–365. [PubMed: 12640451]
132. van Stipdonk MJ, Lemmens EE, Schoenberger SP. Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. *Nat Immunol*. 2001; 2:423–429. [PubMed: 11323696]
133. Homann D, Teyton L, Oldstone MB. Differential regulation of antiviral T-cell immunity results in stable CD8+ but declining CD4+ T-cell memory. *Nat Med*. 2001; 7:913–919. [PubMed: 11479623]

Fig. 1. Naïve and memory NK cells respond against MCMV

Following MCMV infection of a dendritic cell, the m157 glycoprotein is expressed. NK cells from C57BL/6 mice express the activating Ly49H receptor, which associates with and signals through the ITAM-containing DAP12 and the DAP10 adapter molecules.

Recognition of m157 by Ly49H-bearing NK cells (A) leads to activation via DAP12 and DAP10 (B). In addition to Ly49H ligation, IL-12 and pro-inflammatory cytokines produced by the infected dendritic cell act on NK cells allowing them to mediate cytotoxicity via perforin and granzymes (C), secrete cytokines such as IFN- γ (D), and undergo clonal-like proliferation (E). Following NK cell expansion and viral clearance, effector Ly49H⁺ NK cells undergo a contraction phase resulting in a long-lived memory pool (F). These memory NK cells can then respond again several months later when viral antigen is re-encountered (A).

Fig. 2. Homeostasis of naive and memory NK cells

Memory NK cells were generated by adoptive transfer of wildtype NK cells (CD45.1⁺) into Ly49H-deficient mice (CD45.2⁺) followed by MCMV infection. Memory NK cells were analyzed for BrdU incorporation at 50 days post-infection (top plot). BrdU was given in the drinking water for 7 days, and splenic NK cells were purified and stained for antibodies against NK1.1, CD3, CD45.1, Ly49H, and BrdU. The plot is gated on NK1.1⁺ CD3⁻ CD45.1⁺ lymphocytes, and the percentage of Ly49H⁺ NK cells that have taken up BrdU is shown. As a control, naive NK cells were transferred into Ly49H-deficient mice (without infection) concurrent with BrdU treatment (middle plot). In the bottom plot, wildtype B6 mice (not receiving any transferred cells) were given BrdU and analyzed 7 days later.

Fig. 3. Localization and maintenance of memory NK cells in lymphoid and non-lymphoid tissues
Effector and memory NK cells were generated by adoptive transfer of wildtype NK cells (CD45.1⁺) into Ly49H-deficient mice (CD45.2⁺) followed by MCMV infection. (A) Effector NK cells were analyzed 7 days post-infection in spleen, liver, lung, kidney, and mesenteric and inguinal lymph nodes. Plots are gated on NK1.1⁺CD3⁻ lymphocytes, and the percentage of transferred Ly49H⁺ NK cells (CD45.1⁺) is shown for each tissue. (B) Memory NK cells were analyzed 50 days after infection.

Fig. 4. Generation and phenotype of primary and secondary memory NK cells

(A) Schematic of experiment. Memory NK cells were generated by adoptive transfer of naïve wildtype NK cells (CD45.1⁺) into Ly49H-deficient mice (CD45.2) followed by infection with MCMV. Secondary memory NK cells were generated by adoptive transfer of memory NK cells into new Ly49H-deficient recipients (CD45.2) followed by infection with MCMV. Memory and secondary memory cells in the spleen were analyzed 30–50 days after infection. (B) Naïve Ly49H⁺ NK cells from uninfected wildtype B6 mice were compared with primary and secondary memory Ly49H⁺ NK cells. Plots are gated on total NK cells (CD3⁺ NK1.1⁻) and percentage and mean fluorescence intensity is shown for Ly49H⁺ NK cells (CD45.1⁺). (C) Surface expression of NK1.1, Ly49D, Ly49H, and KLRG1 are shown for naïve, memory, and secondary memory NK cells. Histogram plots are gated on CD45.1⁺ Ly49H⁺ cells within the total NK cell population.