Published in final edited form as:

Semin Immunopathol. 2018 July ; 40(4): 343–355. doi:10.1007/s00281-018-0686-9.

Memory responses of innate lymphocytes and parallels with T cells

Moritz Rapp^{1,2,*}, Gabriela M. Wiedemann^{2,*}, and Joseph C. Sun^{2,3,+}

¹Roche Pharmaceutical Research and Early Development, Roche Innovation Center Zurich, Zurich, Switzerland ²Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY, 10065 ³Department of Immunology and Microbial Pathogenesis, Weill Cornell Medical College, New York, NY 10065

Abstract

Natural killer (NK) cells are classified as innate immune cells, given their ability to rapidly respond and kill transformed or virally infected cells without prior sensitization. Recently, accumulating evidence suggests that NK cells also exhibit many characteristics similar to cells of the adaptive immune system. Analogous to T cells, NK cells acquire self-tolerance during development, express antigen-specific receptors, undergo clonal-like expansion and can become long-lived, self-renewing memory cells with potent effector function providing potent protection against reappearing pathogens. In this review, we discuss the requirements for memory NK cell generation and highlight the similarities with the formation of memory T cells.

Keywords

Natural killer cells; immunological memory; T cells; viral infection

1. Introduction

In recent years, the classic hallmarks that distinguish the immune system into two arms, innate and adaptive, have been challenged. Natural killer (NK) cells have traditionally been classified as innate immune cells because of their ability to respond rapidly without prior sensitization and their inability to undergo somatic gene recombination processes that create diversity in T and B cell antigen receptors [1]. However, in recent days, NK cells have become appreciated to possess several features of adaptive immunity. Previous studies had shown that NK cells, like T and B cells of the adaptive immune system, originate from a common lymphoid progenitor in the bone marrow [2] and require common γ -chain-dependent cytokines for development, homeostasis, and survival [3]. More recent studies have demonstrated that expression of the recombination-activating genes (RAGs) is required for robust NK cell ontogeny, function, and fitness, although individual NK cells lack a

*Corresponding author: Joseph C. Sun, PhD, Memorial Sloan Kettering Cancer Center, 408 East 69th Street, ZRC-1462, New York, NY 10065, Phone: 646-888-3228, Fax: 646-422-0453, sunj@mskcc.org.

+These authors contributed equally to this work.

unique antigen recognition receptor generated through RAG-mediated receptor gene rearrangement like with B and T cells [4]. Moreover, NK cells undergo processes during their development that result in self-tolerance and in some respects are analogous to T cell development in the thymus [5–7]. Interestingly, similar to antigen-specific T and B cell receptors, NK cells express germline-encoded activating receptors, which recognize specific antigens of virally infected or transformed cells [8,9]. In addition, these activating NK cell receptors share many components of the signaling machinery and transcription factors downstream of T cell and B cell receptors [10]. Most notably, over the last decade several studies have demonstrated that NK cells can undergo clonal-like expansion followed by longevity, self-renewal, and recall responses, all characteristics originally associated with immunological memory in adaptive lymphocytes [9,11–13].

Immunological memory is defined as a process by which the formation of specialized long-lived cell subsets allows the host to mount a quantitatively or qualitatively greater immune response when re-exposed to a given pathogen. T cells are the best characterized example of a cell type that undergoes memory formation, and their response can be defined by three distinct phases: clonal expansion, contraction, and long-lived memory [14]. Naïve T cells encountering a cognate MHC-bound antigen through their TCR induces the clonal expansion and differentiation into effector cells. This robust proliferation is followed by the apoptosis of most of the effector population resulting in a rapid contraction of the expanded T cells. The remaining T cells can form a pool of long-lived memory cells, which upon re-encounter with their cognate antigen, induce enhanced effector function resulting in greater protection of the host. Memory T cell longevity is maintained through self-renewal, and memory cells persist in lymphoid and non-lymphoid organs [15]. This review will discuss the key findings in support of memory NK cell generation and maintenance, and will highlight the parallels with memory T cells.

2. NK cell memory

2.1 Antigen-induced NK cell memory

2.1.1 Hapten-specific memory NK cells—Although classified as innate immune cells, both murine and human NK cells can become long-lived memory cells. To our knowledge, the first evidence of antigen-driven NK cell memory comes from studies performed using models of hapten-induced contact hypersensitivity (CHS), thought to only evoke adaptive immune responses [16,17]. RAG2-deficient mice, which lack B and T cells, were sensitized with 2,4-dinitro-1-fluorobenzene (DNFB), and four weeks later NK cells could mount a memory response upon re-challenge with DNFB, resulting in CHS. Importantly, no CHS was induced by heterologous haptens (e.g. oxazolone) to which mice were not previously sensitized [16]. More recently, it was demonstrated that monobenzene, a pro-hapten metabolized by melanocytes, induces a CHS response driven by memory NK cells with a specific cytotoxic activity against melanocytes and melanoma cells highlighting the potential of memory NK cells as an effective tool in cell-based cancer immunotherapy [18]. In immunodeficient *Rag2*^{-/-}*Il2rg*^{-/-} mice lacking T, B, and NK cells, no hapten-induced CHS was observed. However, CHS could be acquired in *Rag2*^{-/-}*Il2rg*^{-/-} mice by adoptively transferring NK cells from hapten-sensitized immunocompetent donors. These transfer

experiments, together with NK cell depletion studies, suggested that NK cells are both essential and sufficient for hapten-specific secondary immune responses (Figure 1A).

Although the hapten-specific antigen receptors are unknown and the specific mechanisms behind NK cell-mediated CHS remain elusive, blocking of NKG2D, a well-characterized activation receptor, in hapten-sensitized *Rag2*^{-/-} mice partially suppressed CHS recall responses, indicating a contribution of this receptor in hapten-induced NK cell activation. However, NKG2D is expressed on essentially all NK cells and only a small subset of NK cells is presumed to be hapten-specific, suggesting that NKG2D is not a hapten-specific antigen receptor, but likely serves as an activating co-receptor that may be detecting CHS-induced stress ligands [16]. Hapten challenge-induced CHS was also abrogated in mice treated with blocking antibodies specific for P- and E-selectin or CD18, presumably by inhibition of memory NK cell migration to the site of challenge. In addition, it was found that CXCR6-expressing CD49a⁺CD49b⁻ liver-resident NK cells, a subset of NK cells similar or equivalent to the recently described small intestine group I innate lymphoid cells (ILC1) [11], mediate hapten-specific CHS responses upon hapten challenge [17,19]. Further studies showed that the function and maintenance of these hapten-specific hepatic memory NK cells depends on pro-inflammatory cytokines (IL-12, type I and II interferons) and the transcription factor aryl hydrocarbon receptor (AHR) [20,21]. Future studies will increase our understanding of the generation and maintenance of hapten-specific memory NK cells. It will be of great interest, for example, to identify the hapten-specific antigen receptors mediating CHS, and to determine why hapten-specific memory NK cells preferentially reside in the liver.

2.1.2 MCMV-specific memory NK cells—Shortly after the initial identification of memory NK cells in experimental models of hapten-induced contact hypersensitivity, NK cell memory was demonstrated during viral infection. In MCMV-infected mice, NK cell responses result in the activation, proliferation and memory formation of a subset of NK cells that express the germline-encoded activation receptor Ly49H [8,9,22], which uniquely recognizes the MCMV-encoded glycoprotein m157 expressed by infected cells (Figure 1B) [22–25]. Ligation of Ly49H with m157 leads to a signal cascade mediated by the association of the Ly49H intracellular domain with the ITAM-containing adapter molecules DAP10 and DAP12 to induce activation [26,27].

To drive optimal antigen-specific NK cell proliferation in response to viral infection, the interplay of different cytokine signaling modules is also required. In particular, proinflammatory cytokines have been demonstrated to mediate multiple cellular programs in anti-viral NK cells. Following MCMV infection, IL-18 and IL-33 are essential for MyD88-driven NK cell expansion [28,29], and IL-12 through STAT4 induces the expression of the BTB-ZF transcription factor Zbtb32, antagonizing the anti-proliferative factor Blimp-1 in NK cells [30]. IL-12 and IL-18 also up-regulate the expression of microRNA-155, which promotes NK cell survival and proliferation by inhibiting Noxa and Socs1 expression [31]. More recently, we have demonstrated that IL-12 induces STAT4-mediated epigenetic control of the transcription factors Runx1 and Runx3, which are essential for clonal expansion and memory formation suggesting yet another mechanism in the multi-faceted role of IL-12 [32].

Furthermore, a recent study demonstrated that type I IFNs and downstream STAT1 signaling are crucial for protecting activated NK cells from being killed by neighboring NK cells during the expansion phase following MCMV infection (i.e. fratricide) [33]. In addition, pro-inflammatory cytokines produced by MCMV-infected dendritic cells such as IL-12 enable NK cells to induce granzyme- and perforin-dependent cytotoxicity, and produce pro-inflammatory cytokines such as IFN- γ [34,35]. Over the course of 6-7 days after viral antigen encounter, proinflammatory cytokines along with activating receptors induce a 3- to 10-fold expansion in absolute numbers of splenic and hepatic Ly49H⁺ NK cells in C57BL/6 mice [8,9]. In Ly49H-deficient mice, adoptively transferred Ly49H⁺ NK cells can proliferate up to 100-fold in the spleen and 1000-fold in the liver during MCMV infection, demonstrating a proliferative capability nearly as high as the proliferation of antigen-specific T cells induced by various pathogens [9,14].

The clonal-like expansion is followed by a contraction phase that is regulated by the proapoptotic factor Bim, which induces apoptosis in the majority of effector NK cells 1–2 weeks after MCMV infection [36]. However, some effector NK cells protected by autophagy-related gene 3 (Atg3)-mediated autophagic removal of damaged mitochondria, which accumulate in proliferating NK cells [37,38], survive the contraction phase and form a long-lived, self-renewing pool of memory cells in lymphoid and non-lymphoid tissues such as the liver, spleen, lung and kidney [9,39]. In mice infected with an MCMV strain lacking m157 expression, the frequency of MCMV-specific memory NK cells is highly reduced; whereas in mice infected with an m157-expressing vesicular stomatitis virus (VSV), memory cell formation is enhanced compared to mice infected with VSV expressing a control antigen, illustrating the antigen specificity of Ly49H⁺ NK cells for their cognate viral ligand. [9,28,40]. In addition, memory Ly49H⁺ NK cells can be characterized by an altered expression of several cell surface antigens. The expression of Ly49H, killer cell lectin-like receptor G1 (KLRG1), CD43, CD11b and Ly6C is increased and the expression of CD27 is decreased on Ly49H⁺ memory NK cells, indicating that memory NK cells have a mature and differentiated phenotype compared with naïve NK cells [9], although it is currently difficult to distinguish memory from effector NK cells, as activated NK cells express a similar surface receptor profile [12].

A recent study found that memory NK cells arise from a small subset of naïve Ly49H⁺ NK cells with low KLRG1 expression [41]. In contrast, NK cells expressing high levels of KLRG1 have a limited capacity to proliferate and form memory cells in response to MCMV infection. Interestingly, heterogeneity within memory NK cell progenitor populations was found to be influenced by the host microbiota and by an intact T cell compartment [41]. In RAG deficient mice, the excessive bioavailability of IL-15, due to a lack of competition by IL-15-consuming T cells, increases KLRG1 expression on naïve NK cells and drives terminal differentiation of NK cells, resulting in a reduced frequency of Ly49H⁺KLRG1^{low} memory NK cell progenitors. In addition to environmental impact of RAG-deficiency, cell intrinsic factors were also shown to be crucial for memory NK cell formation. Using RAG fate-mapping and RAG-deficient mice, it was demonstrated that NK cells with a history of RAG expression form a pool of Ly49H⁺KLRG1^{low} memory NK cell progenitors that can become long-lived memory NK cells following antigen-specific proliferation [4].

Upon MCMV re-encounter, memory NK cells undergo a secondary expansion. Although comparable expansion kinetics between memory and naïve Ly49H⁺ NK cells was observed, memory NK cells were shown to be significantly more protective to the host than naïve NK cells on a per cell basis during MCMV challenge [9]. Importantly, MCMV-experienced memory NK cells demonstrated heightened IFN- γ production and degranulation compared to naïve NK cells [9]. These data are consistent with a similar observation that was made for naïve versus memory CD8⁺ T cell expansion, indicating that a higher functional capacity and not necessarily enhanced proliferation rate of memory cells is most essential for protective recall responses [42–46].

2.1.3 HCMV-specific memory NK cells—Analogous to NK cell proliferation in MCMV-infected mice, a NK cell expansion has been observed in humans following human cytomegalovirus (HCMV) infection. Several studies have demonstrated that the frequency of NK cells expressing CD94/NKG2C, a heterodimeric activating receptor, is highly increased in HCMV-seropositive humans compared with HCMV-seronegative individuals [47–49]. In HCMV-seronegative donors typically less than 1% of all blood NK cells are NKG2C positive, whereas in seropositive donors a large spectrum is observed, but as much as 60% of the NK cell population in the peripheral blood can express NKG2C. These HCMV-induced memory-like NKG2C⁺ NK cells typically co-express the maturation marker CD57 and lack the inhibitory receptor NKG2A [49–52]. In immunosuppressed patients receiving solid organ transplants or hematopoietic stem cell transplantation, an expansion of NKG2C⁺ NK cells was observed following HCMV reactivation or acute infection [49,53–55].

During acute HCMV infection, IFN- γ production and degranulation of NKG2C⁺ NK cells is enhanced in response to HCMV-infected cells compared to NKG2C[−] NK cells from the same donor, suggesting an antigen specificity of NKG2C⁺ NK cells for HCMV-derived or induced ligands [54,56]. After the virus has been controlled, elevated frequencies of NKG2C⁺ NK cells can persist for months to years in these patients, suggesting the generation of a long-lived memory NK cell pool in humans [49,54,57,58]. Further studies showed that in HCMV-infected recipients, NKG2C⁺ NK cells from HCMV-seropositive donor grafts produce significantly more target cell-induced IFN- γ than NKG2C⁺ NK cells from HCMV-seronegative donor grafts [50]. Together, these studies demonstrate that HCMV infection induces the generation of a long-lived memory NK cell pool that is transplantable. Although *in vitro* studies have shown that HLA-E expression on HCMV-infected cells is essential for virus-induced NKG2C⁺ NK cell expansion [59], future studies are necessary to determine the precise nature of the HCMV-derived or induced antigen driving proliferation and memory formation of NKG2C⁺ NK cells *in vivo*.

2.2 Cytokine-induced NK cell memory

Besides being essential for the clonal expansion and maintenance of antigen-specific memory NK cells, proinflammatory cytokines alone have been proposed to generate NK cells with memory-like properties in the absence of antigen receptor triggering (Figure 1C). Splenic mouse NK cells stimulated *in vitro* with a combination of IL-12 and IL-18 plus low-dose IL-15 and adoptively transferred into *Rag1*^{−/−} recipient mice secrete more IFN- γ upon cytokine restimulation (IL-12 plus IL-15) or activating receptor ligation (Ly49H and NK1.1)

compared with adoptively transferred control NK cells [60]. Enhanced IFN- γ secretion was observed up to three weeks after adoptive transfer, suggesting NK cells can acquire a long-lasting antigen-independent memory of prior activation [60,61]. In addition, cytokine-activated NK cells expressed high levels of IL-2R α (CD25) and require IL-2 produced by CD4 $^{+}$ T cells to proliferate *in vivo* [62]. In contrast to the increased IFN- γ secretion, granzyme B expression and cytotoxicity from cytokine-induced memory-like NK cells was comparable with control NK cells [60].

Consistent with the results obtained in mice, IL-12/15/18-preactivated human NK cells expressed CD25, proliferated rapidly *in vitro*, and produced high levels of IFN- γ upon restimulation, suggesting a conserved program for sustained memory-like effector functions between mouse and human NK cells [62]. Furthermore, enhanced NKG2D- and DNAM-1-dependent *in vitro* cytotoxicity was observed against leukemia target cells, compared with control NK cells from the same donor. Although cytokine-experienced human NK cells adoptively transferred into NSG mice were found in similar numbers in hematopoietic tissues compared to control NK cells, cytokine-activated memory-like NK cells produced more IFN- γ in response to *ex vivo* restimulation, and were more effective in tumor growth inhibition [63]. Together, these results demonstrate a potent anti-leukemia function of memory-like human NK cells. Using a xenograft melanoma mouse model, the antitumor activity of memory-like NK cells was confirmed. In these adoptive transfer studies, IL-12/15/18-preactivated human NK cells rejected tumors more efficiently than NK cells stimulated with IL-15 alone [64]. Although these studies demonstrate the existence of NK cells with antigen-independent memory-like functionality *in vitro* and the potential use of these cells in the treatment of cancer, future studies are needed to further understand the generation and function of endogenous memory NK cells that were induced by inflammatory cytokines alone.

Using a mouse model with an inducible reporter system to track the fate of NK cells after MCMV infection, it was shown that during viral infection both antigen-specific and cytokine-activated memory NK cells are generated [65]. Further it was demonstrated that MCMV-specific NK cells possess enhanced effector function, whereas cytokine-activated NK cells survive longer in an MCMV-free environment. This study illustrates that a single infection can induce different subsets of memory NK cells, both antigen-dependent and -independent, a phenomenon that may represent an important strategy to facilitate host protection against homologous and heterologous infections [66]. Lastly, adoptive transfer of NK cells into lymphopenic mice (*Rag2* $^{-/-}$ *Il2rg* $^{-/-}$) lacking T, B, and NK cells, results in a rapid, IL-15-dependent, antigen-independent homeostatic expansion, followed by a contraction phase and the formation of a pool of long-lived NK cells [67–70]. Interestingly, NK cells undergoing homeostatic proliferation share several characteristics with MCMV-specific memory NK cells, including the ability for self-renewal and enhanced effector function [70]. The survival of these long-lived NK cells depended on Atg3- and Atg5-mediated autophagy, suggesting that this represents an essential mechanism for NK cell survival and acquisition of memory-like properties following homeostatic proliferation [71]. Future studies are needed to determine additional molecular mechanisms involved in the generation of these long-lived NK cells in lymphopenic mice, and to further investigate the memory-like functions of these cells in disease settings.

3. Parallels between NK cell and T cell memory

Immunological memory has long been considered a hallmark of adaptive immunity. Yet, in the past years, the emerging evidence of NK cell memory has blurred the line segregating innate from adaptive immunity [72]. Surprisingly, the more insights we gain into the mechanisms of NK cell development, activation, and memory formation, the more we uncover clear similarities between T cells and NK cells.

3.1 Development

Among the differences between T cells and NK cells is the dependence of T cells on RAG enzymes for V(D)J recombination of antigen receptor genes, thus enabling the abundant diversity of T cell receptors. RAG-mediated generation of receptor diversity has long been considered to be the prerequisite for antigen-specific primary immunological responses, as well as memory formation and recall responses. RAG-deficient mice have no functional B or T cells, yet they have normal numbers of NK cells [4,73]. Interestingly, a sizable percentage of NK cells express RAG proteins during ontogeny, raising the question of whether a functional role exists for RAG enzymes in NK cell development [4,73]. Furthermore, it was recently shown that both RAG1 and RAG2 bind at thousands of sites throughout the genome and outside of the antigen receptor loci [74]. In another recent study, RAG (via the DNA damage/repair pathway) was shown to commonly mediate cellular fitness and longevity of NK cells and T cells, revealed during clonal expansion [4].

Both NK cells and T cells derive from a common lymphoid progenitor (CLP). T cell development critically takes place in the thymus, where T cells undergo positive and negative selection. By contrast, NK cell development is thymus-independent, as athymic nude mice display normal numbers of NK cells [75]. Instead, bone marrow defects resulting in reduced NK cell activity and responsiveness suggested that the bone marrow is the primary site of NK cell development [76]. Despite these differences, NK cells and T cells share many features of development, including dependency on similar cytokines, shared transcription factors, and analogous education processes [72].

Thymocytes are educated and selected through their interaction with MHC class I and II, where self-reactive T cells undergo apoptosis and are eliminated [77]. Similarly, developing NK cells are dependent on MHC class I interactions for functional maturation (i.e. “licensing” or education) [6]. NK cells are endowed with a large repertoire of germline-encoded inhibitory receptors specific for MHC class I, the most prominent of which are Ly49 receptors in mice and KIR in humans. Binding of these receptors to self-MHC class I during development is a crucial step towards acquisition of functional competence, and NK cells that either do not possess inhibitory MHC class I receptors or do not encounter cognate MHC class I ligands during maturation (i.e. “unlicensed” NK cells) remain hyporesponsive [78–80]. Another shared feature of NK cells and T cells is their dependency on cytokines of the common gamma chain family (e.g. IL-2, IL-7 and IL-15) during maturation, homeostasis and activation, although the precise role of these cytokines may differ between different T cell and NK cell subsets *in vivo* [81].

3.2 Three signals of activation

Immunological memory is defined by a more rapid and robust secondary immune response to a previously encountered stimulus. In T cells, memory formation begins with the primary recognition of an antigen, which, under the right conditions, results in clonal expansion of an antigen-specific T cell. This process is eventually followed by a contraction phase in which most of the T cell clones undergo apoptosis and results in the survival of a small number of long-lived memory T cells [14,82,83]. Many of these steps show a distinct similarity to NK cell memory formation during viral infection.

The first step towards clonal expansion is lymphocyte activation, and classic T cell activation requires three signals. Signal 1 is the ligation of the T cell receptor (TCR) to cognate antigen presented on MHC, Signal 2 involves the binding of co-stimulatory receptors such as CD28 to their B7 ligands on antigen-presenting cells (APC), and Signal 3 is mediated by pro-inflammatory cytokines [84–86]. Similarly, NK cells also require 3 signals for efficient primary activation for clonal expansion and memory formation. As with Signal 1 in T cells, the binding of an activating NK cell receptor to its cognate ligand is an absolute requirement for the robust formation of immunological memory following viral infection. To date, relatively little is known about the ligands for most activating NK cell receptors in mouse and human. Arguably the best characterized receptor-ligand encounter occurs during MCMV infection, where ligation of the NK cell receptor Ly49H to the viral glycoprotein m157 leads to NK cell activation crucial to NK cell clonal expansion and memory generation. MCMV strains that are engineered to be m157-deficient do not induce clonal expansion or memory, whereas recombinant vesicular stomatitis virus (VSV) or vaccinia virus (VacV) expressing m157 potentially induce NK cell memory formation [40]. Notably, Ly49H receptor signaling through the ITAM-containing adaptor protein DAP12 is closely related to signaling downstream of the TCR [87]. In humans, the interaction of CD94/NGK2C with HLA-E is involved in adaptive NK cell responses during HCMV infection, yet the exact nature of this interaction is not well defined, as CD94/NGK2C-independent HCMV memory has also been suggested [57,59]. With the recent discovery of a novel viral ligand for mouse NK1.1 [88], it will be great interest to investigate whether this receptor-ligand interaction can drive clonal expansion of NK cells and produce NK cell memory in analogous manner to the Ly49H-m157 interaction. Additional NK cell receptors and their cognate ligands, which may be involved in the recognition of other viral and bacterial infections, remain to be elucidated.

Comparable to T cells, NK cells need a co-stimulatory Signal 2 in order to expand after viral infection. During MCMV infection, activation of the co-stimulatory molecule DNAX-accessory molecule 1 (DNAM-1) through binding of its ligands CD155 and CD112, was crucial for NK cell expansion and memory formation. NK cells deficient in DNAM-1 or its downstream signaling kinases Fyn and PKC η showed impaired expansion and differentiation into long-lived memory cells during MCMV infection [89]. In human NK cell responses to HCMV infection, costimulatory signals might be provided by the interaction of CD2 with its ligand CD58, although a detailed role in NK cell expansion and memory formation has yet to be described [90,91]. In addition, although NK cells express CD28, and

also CTLA-4 upon activation, it is still unclear whether this costimulatory receptor serves as Signal 2 *in vivo*, as it does for T cell activation and clonal expansion.

Beyond antigen receptor ligation and co-stimulation, in certain settings T cells require a Signal 3, namely the presence of pro-inflammatory cytokines such as IL-12 or type I IFNs, for full activation, expansion, and memory formation [84,86]. Several studies have found that NK cells require proinflammatory cytokine signals for their activation, function, and adaptive responses. IL-12 and downstream STAT4 signaling are critical, as defective memory generation was observed in IL-12 receptor-deficient and STAT4-deficient mice infected with MCMV [34]. Furthermore, IL-18, IL-33, and the adapter molecule MyD88 are required for optimal expansion of NK cells in viral infections, yet they are dispensable for the recall response [28,29]. IFN- α and STAT1 have been shown to be crucial for NK cell expansion upon MCMV infection due to prevention of fratricide [33], and IL-12 and type I IFNs both drive the expression of transcription factor Zbtb32, which promotes NK cell expansion by suppression of Blimp-1, an anti-proliferative transcription factor and key transcriptional regulator of T cell proliferation and differentiation [30,92]. Thus, Zbtb32 in NK cells may be analogous to Bcl6 in T cells, as both BTB-ZF transcription factor family members target Blimp-1 activity [92]. Moreover, Runx1 and Runx3, two central transcription factors in T cell development and function, have very recently been shown to be downstream targets of STAT4 in NK cells, critically promoting expansion and survival during MCMV infection [32,93,94]. Taken together, these findings highlight the similarities between NK cells and T cells during primary activation (Figure 2).

3.3 Contraction and memory formation

As described above, clonal expansion is followed by a contraction phase that generates both NK and T cell memory. The kinetics of these phases after initial pathogen challenge is strikingly comparable in T cell and NK cell responses, with maximum effector cell frequencies occurring 7 – 10 days after antigen encounter [36,95]. The subsequent contraction phase is critical to memory cell numbers for both T cells and NK cells, as it determines how many effector cells avoid death to become long-lived. The contraction phase for both T and NK cell responses is typically characterized by the apoptosis of greater than 90% of the effector pool. In T cells, apoptosis is mediated by interactions of pro-apoptotic Bim with pro-survival Bcl-2 [96,97]. Similarly, Bcl-2 downregulation and Bim-mediated apoptosis are critical to the contraction phase of the NK cell response, as shown by an impaired contraction and insufficient protection to secondary viral challenge in *Bcl2l1*^{-/-} mice [30,36]. Moreover, IL-15 has been described to play a role in the survival of activated T cells during contraction phase by upregulation of Bcl-2 [98–100]. In NK cells, IL-15 limits expression of Bim and drives the expression of Mcl-1, which is critical to the survival of memory NK cells [40,101].

Another shared mechanism enabling selective survival during the contraction phase in T cells and NK cells is autophagy, process by which defective cellular components (e.g. misfolded proteins) and damaged organelles (e.g. mitochondria) are removed from the cell. Atg5- or Atg7-deficient mice lacking essential components of the autophagy machinery showed impaired memory T cell differentiation due to reduced T cell survival during the

contraction phase, whereas the primary immune response is unaffected [102]. Similarly, Atg3-mediated autophagy of mitochondria (i.e. mitophagy) was critical for the survival of memory NK cells during the contraction phase following MCMV infection, whereas the initial expansion was not affected by NK cell-specific deletion of Atg3 [38]. This process of mitophagy in NK cells was shown to be controlled by two mitochondria-associated proteins BNIP3 and BNIP3L and ultimately led to the formation of a stable memory NK cell pool after infection, as removal of damaged mitochondria and reactive oxygen species promoted NK cell survival [38]. These studies highlight yet another conserved pathway for the generation of memory in both innate and adaptive lymphocytes.

During viral infection, two subsets of effector CD8⁺ T cells have been described to develop: terminally differentiated KLRG1^{hi} short-lived effector cells (SLECs) that are thought to die after infection, and KLRG1^{lo} memory precursor effector cells (MPECs) that are long-lived and participate in secondary responses [103]. Analogous to CD8⁺ T cells, recent evidence supports the idea of heterogeneity within antiviral NK cell populations that can dictate memory potential. KLRG1^{lo}Ly49H⁺ NK cells preferentially generate memory NK cells compared to KLRG1^{hi} cells, which have a limited capacity for MCMV-driven expansion. However, because memory NK cells express high levels of the terminal differentiation marker KLRG1 and yet can be robustly recalled, it is likely that KLRG1 may serve a different function in NK cells as it does in T cells.

3.4 Phenotype and location of memory T and NK cells

Memory T cells have been divided into central memory T cells (T_{cm}) and effector memory T cells (T_{em}) in the literature. T_{cm} home to lymphoid organs, whereas T_{em} circulate in the periphery. Upon antigen re-encounter, T_{cm} show greater expansion, whereas T_{em} only poorly expand but have rapid and robust effector potential [104,105]. Functionally, memory NK cells after viral infection appear to resemble T_{em} cells more than T_{cm}, as they circulate throughout the host and respond to secondary challenge with robust production of IFN- γ [9]. By contrast, hapten-specific memory NK cells home to the liver in a CXCR6-dependent manner and respond to challenge with an increase in cytotoxicity [17], although some have speculated that these hapten-specific cells may actually be ILC1 rather than conventional NK cells [11,106].

Phenotypically, memory NK cells share features with T_{em}. T_{em} are characterized by surface expression of CD44, CD27, CD127, KLRG1 and Ly6C and low expression of CCR7 and CD62L, whereas T_{cm} show high expression of CD44, CD27, CD127, CD62L and CCR7 and low expression of KLRG1 [107]. Memory NK cells display a mature phenotype, with high expression of KLRG1, CD11b, CD43 and Ly6C and low expression of CD27 and CD62L [9]. Because a subset of effector T cells can become tissue resident during the memory phase (T_{rm}), future studies are required to determine whether a subset within the memory Ly49H⁺ NK cell pool following MCMV infection can reside in the tissues without circulating.

On the transcriptional level, memory T cells and memory NK cells share many common features. In a transcriptome analysis, memory NK cells and T cells commonly regulated 47 genes including those coding for effector functions (Gzmb, Fasl), migration (CD62L,

CD49a) and apoptosis (Casp1) [108]. Furthermore, epigenetic modifications have been described to play a role both in NK cell and in T cell memory formation [58,109,110]. Several epigenetic modifications during memory T cell formation have been described, including histone hyperacetylation, changes in histone methylation with high H3K4me3 levels, and DNA methylation of the IFN- γ locus [111]. Likewise, STAT4 signaling in NK cells facilitates a permissive epigenetic landscape, shown by increases of H3K4me3 at gene promoter regions throughout the genome [32]. Interestingly, the methylation profile of HCMV-specific NKG2C⁺ memory NK cells in humans contains epigenetic marks at the IFN- γ locus shared with memory CD8⁺ T cells and CD4⁺ Th1 cells [58,112,113]. Future studies using techniques such as ATAC-seq of T cells and NK cells at different time points of infection will reveal whether these innate and adaptive lymphocytes possess a shared epigenetic signature as they transition from naïve to effector to memory cells.

3.5 The question of antigen-specificity

Antigen-specificity is considered the hallmark of immunological memory. Yet, as described above, NK cell memory or memory-like status can also be induced by cytokine stimulation or under homeostatic conditions in lymphopenia [11]. As these cytokine-induced memory-like NK cells are long-living and show enhanced IFN- γ production upon secondary stimulation, but do not display antigen-specificity, the question of whether this phenotype may be considered “memory” in the classic definition remains to be resolved [60,70]. Interestingly, a corresponding mouse T cell population has been described, and termed “innate memory T cell” [114]. The formation of these innate memory T cells occurs under the same conditions as memory-like NK cells: either during lymphopenia or following cytokine stimulation (with IL-4 in this case) [114]. Thus, they are generated in the absence of antigen, yet are characterized by rapid secretion of IFN- γ upon encounter of pathogens or proinflammatory cytokines, with kinetics comparable to classic memory T cells [114]. The exact role of these innate or virtual memory T cells remains to be elucidated, and whether they represent the T cell parallel to cytokine-induced memory-like NK cells.

4. Conclusion

The rapidly growing evidence of NK cell memory in mice and humans has provided a new understanding and perception of the features of innate immune cells, and is currently challenging the classic understanding of immunological memory as a trait restricted to B and T cells. The fact that NK cells are able to mount an antigen-specific immune response which resembles a T cell response in all phases of activation, expansion, contraction, and memory formation has challenged the restrictions we have previously placed upon adaptive and innate immunity.

In recent days, NK cells have been assigned to a subset of innate lymphoid cells (ILC), and rebranded as cytotoxic “ILC1s” [115]. Because NK cell memory-like phenotypes can be induced by cytokines or under homeostatic conditions during lymphopenia, future studies will determine whether other RAG-independent ILC subtypes are also capable of memory formation. Indeed, a recent study described a memory-like phenotype in lung ILC2s, where the cells rapidly expanded following injection of papain or IL-33 and subsequently

contracted to persist over several months [116]. Moreover, lymphoid tissue inducer (LTi) cells, which belong to the ILC3 subset, are known to be crucial for CD4⁺ T cell memory maintenance by promoting survival of memory CD4 T cells [117]. Notably, liver ILC1s are able to undergo homeostatic proliferation after transfer into lymphopenic *Rag2*^{-/-}*Il2rg*^{-/-} mice and they survive for at least 28 days after transfer, suggesting their capacity to acquire longevity [71].

Evidence for adaptive immune responses in RAG-dependent innate lymphocytes has been described in recent studies, which showed clonal expansion of a gamma delta ($\gamma\delta$) T cell clone in response to CMV reactivation after allogeneic hematopoietic stem cell transplantation (HSCT), and memory $\gamma\delta$ T cell responses in the intestines against bacterial pathogens such as *Listeria* and *Salmonella* [118,119]. Future studies will determine whether, and under which circumstances, ILCs and innate lymphocytes acquire memory-like properties.

Although our understanding of the molecular mechanisms behind NK cell memory is quickly expanding, many questions remain to be addressed. Because NK cell memory has been best characterized in the setting of cytomegalovirus infection in mouse and humans, the molecular mechanisms underlying memory formation with additional pathogens and stimuli are still unclear. Moreover, as antigen receptors and their respective antigens involved in NK cell memory generation are identified, we will begin to address whether NK cell memory may play a role in other pathological conditions such as cancer, autoimmunity, or metabolic diseases. Furthermore, we can begin to test whether NK cell memory can be harnessed for vaccination against various pathogens that evade anamnestic T and B cell responses. More extensive studies on the adaptive features of innate lymphocytes will help us to better understand and therapeutically benefit patients suffering from infectious diseases.

Acknowledgments

We thank Clair Geary and Nicholas Adams for insightful discussions and the critical review of the manuscript. M.R. was supported by a fellowship from the German Academic Exchange Service (DAAD; Germany). G.M.W. was supported by Deutsche Forschungsgemeinschaft DFG (Forschungsspendium GZ: WI 4927/1-1). J.C.S. was supported by the Ludwig Center for Cancer Immunotherapy, the Burroughs Wellcome Fund, the American Cancer Society, and grants from the NIH (AI100874, AI130043, AI123658, and P30CA008748).

References

- 1Lanier LL. NK cell recognition. *Annu Rev Immunol.* 2005; 23:225–274. DOI: 10.1146/annurev.immunol.23.021704.115526 [PubMed: 15771571]
- 2Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell.* 1997; 91(5):661–672. [PubMed: 9393859]
- 3Di Santo JP. Natural killer cell developmental pathways: a question of balance. *Annu Rev Immunol.* 2006; 24:257–286. DOI: 10.1146/annurev.immunol.24.021605.090700 [PubMed: 16551250]
- 4Karo JM, Schatz DG, Sun JC. The RAG recombinase dictates functional heterogeneity and cellular fitness in natural killer cells. *Cell.* 2014; 159(1):94–107. DOI: 10.1016/j.cell.2014.08.026 [PubMed: 25259923]
- 5Fernandez NC, Treiner E, Vance RE, Jamieson AM, Lemieux S, Raulet DH. A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. *Blood.* 2005; 105(11):4416–4423. DOI: 10.1182/blood-2004-08-3156 [PubMed: 15728129]

- 6Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L, French AR, Sunwoo JB, Lemieux S, Hansen TH, Yokoyama WM. Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature*. 2005; 436(7051):709–713. DOI: 10.1038/nature03847 [PubMed: 16079848]
- 7Orr MT, Lanier LL. Natural killer cell education and tolerance. *Cell*. 2010; 142(6):847–856. DOI: 10.1016/j.cell.2010.08.031 [PubMed: 20850008]
- 8Dokun AO, Kim S, Smith HR, Kang HS, Chu DT, Yokoyama WM. Specific and nonspecific NK cell activation during virus infection. *Nat Immunol*. 2001; 2(10):951–956. DOI: 10.1038/ni714 [PubMed: 11550009]
- 9Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature*. 2009; 457(7229):557–561. DOI: 10.1038/nature07665 [PubMed: 19136945]
- 10Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. *Nat Immunol*. 2008; 9(5):495–502. DOI: 10.1038/ni1581 [PubMed: 18425106]
- 11O'Sullivan TE, Sun JC, Lanier LL. Natural Killer Cell Memory. *Immunity*. 2015; 43(4):634–645. DOI: 10.1016/j.immuni.2015.09.013 [PubMed: 26488815]
- 12Sun JC, Lopez-Verges S, Kim CC, DeRisi JL, Lanier LL. NK cells and immune “memory”. *J Immunol*. 2011; 186(4):1891–1897. DOI: 10.4049/jimmunol.1003035 [PubMed: 21289313]
- 13Sun JC, Ugolini S, Vivier E. Immunological memory within the innate immune system. *EMBO J*. 2014; 33(12):1295–1303. DOI: 10.1002/embj.201387651 [PubMed: 24674969]
- 14Williams MA, Bevan MJ. Effector and memory CTL differentiation. *Annu Rev Immunol*. 2007; 25:171–192. DOI: 10.1146/annurev.immunol.25.022106.141548 [PubMed: 17129182]
- 15Carbone FR. Tissue-Resident Memory T Cells and Fixed Immune Surveillance in Nonlymphoid Organs. *J Immunol*. 2015; 195(1):17–22. DOI: 10.4049/jimmunol.1500515 [PubMed: 26092813]
- 16O'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(5):507–516. DOI: 10.1038/ni1332 [PubMed: 16617337]
- 17Paust S, Gill HS, Wang BZ, Flynn MP, Moseman EA, Senman B, Szczepanik M, Telenti A, Askenase PW, Compans RW, von Andrian UH. Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. *Nat Immunol*. 2010; 11(12):1127–1135. DOI: 10.1038/ni.1953 [PubMed: 20972432]
- 18van den Boorn JG, Jakobs C, Hagen C, Renn M, Luiten RM, Melief CJ, Tuting T, Garbi N, Hartmann G, Hornung V. Inflammasome-Dependent Induction of Adaptive NK Cell Memory. *Immunity*. 2016; 44(6):1406–1421. DOI: 10.1016/j.immuni.2016.05.008 [PubMed: 27287410]
- 19Peng H, Jiang X, Chen Y, Sojka DK, Wei H, Gao X, Sun R, Yokoyama WM, Tian Z. Liver-resident NK cells confer adaptive immunity in skin-contact inflammation. *J Clin Invest*. 2013; 123(4):1444–1456. DOI: 10.1172/JCI66381 [PubMed: 23524967]
- 20Majewska-Szczepanik M, Paust S, von Andrian UH, Askenase PW, Szczepanik M. Natural killer cell-mediated contact sensitivity develops rapidly and depends on interferon-alpha, interferon-gamma and interleukin-12. *Immunology*. 2013; 140(1):98–110. DOI: 10.1111/imm.12120 [PubMed: 23659714]
- 21Zhang LH, Shin JH, Haggadone MD, Sunwoo JB. The aryl hydrocarbon receptor is required for the maintenance of liver-resident natural killer cells. *J Exp Med*. 2016; 213(11):2249–2257. DOI: 10.1084/jem.20151998 [PubMed: 27670593]
- 22Smith HR, Heusel JW, Mehta IK, Kim S, Dorner BG, Naidenko OV, Iizuka K, Furukawa H, Beckman DL, Pingel JT, Scalzo AA, Fremont DH, Yokoyama WM. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc Natl Acad Sci U S A*. 2002; 99(13):8826–8831. DOI: 10.1073/pnas.092258599 [PubMed: 12060703]
- 23Arase H, Mocarski ES, Campbell AE, Hill AB, Lanier LL. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science*. 2002; 296(5571):1323–1326. DOI: 10.1126/science.1070884 [PubMed: 11950999]
- 24Brown MG, Dokun AO, Heusel JW, Smith HR, Beckman DL, Blattenberger EA, Dubbelde CE, Stone LR, Scalzo AA, Yokoyama WM. Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science*. 2001; 292(5518):934–937. DOI: 10.1126/science.1060042 [PubMed: 11340207]

- 25Daniels 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(1):29–44. [PubMed: 11435470]
- 26Lanier LL. DAP10- and DAP12-associated receptors in innate immunity. *Immunol Rev.* 2009; 227(1):150–160. DOI: 10.1111/j.1600-065X.2008.00720.x [PubMed: 19120482]
- 27Orr MT, Sun JC, Hesslein DG, Arase H, Phillips JH, Takai T, Lanier LL. Ly49H signaling through DAP10 is essential for optimal natural killer cell responses to mouse cytomegalovirus infection. *J Exp Med.* 2009; 206(4):807–817. DOI: 10.1084/jem.20090168 [PubMed: 19332875]
- 28Madera S, Sun JC. Cutting edge: stage-specific requirement of IL-18 for antiviral NK cell expansion. *J Immunol.* 2015; 194(4):1408–1412. DOI: 10.4049/jimmunol.1402001 [PubMed: 25589075]
- 29Nabekura T, Girard JP, Lanier LL. IL-33 receptor ST2 amplifies the expansion of NK cells and enhances host defense during mouse cytomegalovirus infection. *J Immunol.* 2015; 194(12):5948–5952. DOI: 10.4049/jimmunol.1500424 [PubMed: 25926677]
- 30Beaulieu AM, Zawislak CL, Nakayama T, Sun JC. The transcription factor Zbtb32 controls the proliferative burst of virus-specific natural killer cells responding to infection. *Nat Immunol.* 2014; 15(6):546–553. DOI: 10.1038/ni.2876 [PubMed: 24747678]
- 31Zawislak CL, Beaulieu AM, Loeb GB, Karo J, Canner D, Bezman NA, Lanier LL, Rudensky AY, Sun JC. Stage-specific regulation of natural killer cell homeostasis and response against viral infection by microRNA-155. *Proc Natl Acad Sci U S A.* 2013; 110(17):6967–6972. DOI: 10.1073/pnas.1304410110 [PubMed: 23572582]
- 32Rapp M, Lau CM, Adams NM, Weizman OE, O'Sullivan TE, Geary CD, Sun JC. Core-binding factor beta and Runx transcription factors promote adaptive natural killer cell responses. *Sci Immunol.* 2017; 2(18)doi: 10.1126/sciimmunol.aan3796
- 33Madera S, Rapp M, Firth MA, Beilke JN, Lanier LL, Sun JC. Type I IFN promotes NK cell expansion during viral infection by protecting NK cells against fratricide. *J Exp Med.* 2016; 213(2):225–233. DOI: 10.1084/jem.20150712 [PubMed: 26755706]
- 34Sun JC, Madera S, Bezman NA, Beilke JN, Kaplan MH, Lanier LL. Proinflammatory cytokine signaling required for the generation of natural killer cell memory. *J Exp Med.* 2012; 209(5):947–954. DOI: 10.1084/jem.20111760 [PubMed: 22493516]
- 35Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol.* 2003; 3(2):133–146. DOI: 10.1038/nri1001 [PubMed: 12563297]
- 36Min-Oo G, Bezman NA, Madera S, Sun JC, Lanier LL. Proapoptotic Bim regulates antigen-specific NK cell contraction and the generation of the memory NK cell pool after cytomegalovirus infection. *J Exp Med.* 2014; 211(7):1289–1296. DOI: 10.1084/jem.20132459 [PubMed: 24958849]
- 37Green DR, Galluzzi L, Kroemer G. Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science.* 2011; 333(6046):1109–1112. DOI: 10.1126/science.1201940 [PubMed: 21868666]
- 38O'Sullivan TE, Johnson LR, Kang HH, Sun JC. BNIP3- and BNIP3L-Mediated Mitophagy Promotes the Generation of Natural Killer Cell Memory. *Immunity.* 2015; 43(2):331–342. DOI: 10.1016/j.immuni.2015.07.012 [PubMed: 26253785]
- 39Sun JC, Beilke JN, Lanier LL. Immune memory redefined: characterizing the longevity of natural killer cells. *Immunol Rev.* 2010; 236:83–94. DOI: 10.1111/j.1600-065X.2010.00900.x [PubMed: 20636810]
- 40Firth MA, Madera S, Beaulieu AM, Gasteiger G, Castillo EF, Schluns KS, Kubo M, Rothman PB, Vivier E, Sun JC. Nfil3-independent lineage maintenance and antiviral response of natural killer cells. *J Exp Med.* 2013; 210(13):2981–2990. DOI: 10.1084/jem.20130417 [PubMed: 24277151]
- 41Kamimura Y, Lanier LL. Homeostatic control of memory cell progenitors in the natural killer cell lineage. *Cell Rep.* 2015; 10(2):280–291. DOI: 10.1016/j.celrep.2014.12.025 [PubMed: 25578733]
- 42Badovinac 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(10):4933–4942. [PubMed: 12734336]

- 43Belz GT, Bedoui S, Kupresanin F, Carbone FR, Heath WR. Minimal activation of memory CD8+ T cell by tissue-derived dendritic cells favors the stimulation of naive CD8+ T cells. *Nat Immunol.* 2007; 8(10):1060–1066. DOI: 10.1038/ni1505 [PubMed: 17767161]
- 44Grayson JM, Harrington LE, Lanier JG, Wherry EJ, Ahmed R. Differential sensitivity of naive and memory CD8+ T cells to apoptosis in vivo. *J Immunol.* 2002; 169(7):3760–3770. [PubMed: 12244170]
- 45Jabbari A, Harty JT. Secondary memory CD8+ T cells are more protective but slower to acquire a central-memory phenotype. *J Exp Med.* 2006; 203(4):919–932. DOI: 10.1084/jem.20052237 [PubMed: 16567385]
- 46Zimmermann 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.* 1999; 29(1):284–290. [PubMed: 9933110]
- 47Guma 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(12):3664–3671. DOI: 10.1182/blood-2004-05-2058 [PubMed: 15304389]
- 48Guma M, Cabrera C, Erkizia I, Bofill M, Clotet B, Ruiz L, Lopez-Botet M. 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(1):38–41. DOI: 10.1086/504719 [PubMed: 16741880]
- 49Lopez-Verges S, Milush JM, Schwartz BS, Pando MJ, Jarjoura J, York VA, Houchins JP, Miller S, Kang SM, Norris PJ, Nixon DF, Lanier LL. Expansion of a unique CD57(+)NKG2Chi natural killer cell subset during acute human cytomegalovirus infection. *Proc Natl Acad Sci U S A.* 2011; 108(36):14725–14732. DOI: 10.1073/pnas.1110900108 [PubMed: 21825173]
- 50Foley B, Cooley S, Verneris MR, Curtsinger J, Luo X, Waller EK, Anasetti C, Weisdorf D, Miller JS. Human cytomegalovirus (CMV)-induced memory-like NKG2C(+) NK cells are transplantable and expand in vivo in response to recipient CMV antigen. *J Immunol.* 2012; 189(10):5082–5088. DOI: 10.4049/jimmunol.1201964 [PubMed: 23077239]
- 51Hendricks DW, Balfour HH Jr, Dunmire SK, Schmeling DO, Hogquist KA, Lanier LL. Cutting edge: NKG2C(hi)CD57+ NK cells respond specifically to acute infection with cytomegalovirus and not Epstein-Barr virus. *J Immunol.* 2014; 192(10):4492–4496. DOI: 10.4049/jimmunol.1303211 [PubMed: 24740502]
- 52Lopez-Verges S, Milush JM, Pandey S, York VA, Arakawa-Hoyt J, Pircher H, Norris PJ, Nixon DF, Lanier LL. CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset. *Blood.* 2010; 116(19):3865–3874. DOI: 10.1182/blood-2010-04-282301 [PubMed: 20733159]
- 53Della Chiesa M, Falco M, Podesta M, Locatelli F, Moretta L, Frasson F, Moretta A. Phenotypic and functional heterogeneity of human NK cells developing after umbilical cord blood transplantation: a role for human cytomegalovirus? *Blood.* 2012; 119(2):399–410. DOI: 10.1182/blood-2011-08-372003 [PubMed: 22096237]
- 54Foley B, Cooley S, Verneris MR, Pitt M, Curtsinger J, Luo X, Lopez-Verges S, Lanier LL, Weisdorf D, Miller JS. Cytomegalovirus reactivation after allogeneic transplantation promotes a lasting increase in educated NKG2C+ natural killer cells with potent function. *Blood.* 2012; 119(11):2665–2674. DOI: 10.1182/blood-2011-10-386995 [PubMed: 22180440]
- 55Muccio L, Bertaina A, Falco M, Pende D, Meazza R, Lopez-Botet M, Moretta L, Locatelli F, Moretta A, Della Chiesa M. Analysis of memory-like natural killer cells in human cytomegalovirus-infected children undergoing alpha-beta+T and B cell-depleted hematopoietic stem cell transplantation for hematological malignancies. *Haematologica.* 2016; 101(3):371–381. DOI: 10.3324/haematol.2015.134155 [PubMed: 26659918]
- 56Wu Z, Sinzger C, Frascaroli G, Reichel J, Bayer C, Wang L, Schirmbeck R, Mertens T. Human cytomegalovirus-induced NKG2C(hi) CD57(hi) natural killer cells are effectors dependent on humoral antiviral immunity. *J Virol.* 2013; 87(13):7717–7725. DOI: 10.1128/JVI.01096-13 [PubMed: 23637420]
- 57Beziat V, Liu LL, Malmberg JA, Ivarsson MA, Sohlberg E, Bjorklund AT, Retiere C, Sverremark-Ekstrom E, Traherne J, Ljungman P, Schaffer M, Price DA, Trowsdale J, Michaelsson J, Ljunggren HG, Malmberg KJ. NK cell responses to cytomegalovirus infection lead to stable imprints in the

- human KIR repertoire and involve activating KIRs. *Blood*. 2013; 121(14):2678–2688. DOI: 10.1182/blood-2012-10-459545 [PubMed: 23325834]
- 58Luetke-Eversloh M, Hammer Q, Durek P, Nordstrom K, Gasparoni G, Pink M, Hamann A, Walter J, Chang HD, Dong J, Romagnani C. Human cytomegalovirus drives epigenetic imprinting of the IFNG locus in NKG2Chi natural killer cells. *PLoS Pathog*. 2014; 10(10):e1004441.doi: 10.1371/journal.ppat.1004441 [PubMed: 25329659]
- 59Rolle A, Pollmann J, Ewen EM, Le VT, Halenius A, Hengel H, Cerwenka A. IL-12-producing monocytes and HLA-E control HCMV-driven NKG2C+ NK cell expansion. *J Clin Invest*. 2014; 124(12):5305–5316. DOI: 10.1172/jci77440 [PubMed: 25384219]
- 60Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci U S A*. 2009; 106(6):1915–1919. DOI: 10.1073/pnas.0813192106 [PubMed: 19181844]
- 61Keppel MP, Yang L, Cooper MA. Murine NK cell intrinsic cytokine-induced memory-like responses are maintained following homeostatic proliferation. *J Immunol*. 2013; 190(9):4754–4762. DOI: 10.4049/jimmunol.1201742 [PubMed: 23530145]
- 62Ni J, Miller M, Stojanovic A, Garbi N, Cerwenka A. Sustained effector function of IL-12/15/18-preactivated NK cells against established tumors. *J Exp Med*. 2012; 209(13):2351–2365. DOI: 10.1084/jem.20120944 [PubMed: 23209317]
- 63Romee R, Rosario M, Berrien-Elliott MM, Wagner JA, Jewell BA, Schappe T, Leong JW, Abdel-Latif S, Schneider SE, Willey S, Neal CC, Yu L, Oh ST, Lee YS, Mulder A, Claas F, Cooper MA, Fehniger TA. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. *Sci Transl Med*. 2016; 8(357):357ra123.doi: 10.1126/scitranslmed.aaf2341
- 64Ni J, Holsken O, Miller M, Hammer Q, Luetke-Eversloh M, Romagnani C, Cerwenka A. Adoptively transferred natural killer cells maintain long-term antitumor activity by epigenetic imprinting and CD4(+) T cell help. *Oncoimmunology*. 2016; 5(9):e1219009.doi: 10.1080/2162402X.2016.1219009 [PubMed: 27757318]
- 65Nabekura T, Lanier LL. Tracking the fate of antigen-specific versus cytokine-activated natural killer cells after cytomegalovirus infection. *J Exp Med*. 2016; 213(12):2745–2758. DOI: 10.1084/jem.20160726 [PubMed: 27810928]
- 66Min-Oo G, Lanier LL. Cytomegalovirus generates long-lived antigen-specific NK cells with diminished bystander activation to heterologous infection. *J Exp Med*. 2014; 211(13):2669–2680. DOI: 10.1084/jem.20141172 [PubMed: 25422494]
- 67Jamieson 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(2):864–870. [PubMed: 14707057]
- 68Prlic M, Bazar BR, Farrar MA, Jameson SC. In vivo survival and homeostatic proliferation of natural killer cells. *J Exp Med*. 2003; 197(8):967–976. DOI: 10.1084/jem.20021847 [PubMed: 12695488]
- 69Ranson T, Vosshenrich CA, Corcuff E, Richard O, Muller W, Di Santo JP. IL-15 is an essential mediator of peripheral NK-cell homeostasis. *Blood*. 2003; 101(12):4887–4893. DOI: 10.1182/blood-2002-11-3392 [PubMed: 12586624]
- 70Sun JC, Beilke JN, Bezman NA, Lanier LL. Homeostatic proliferation generates long-lived natural killer cells that respond against viral infection. *J Exp Med*. 2011; 208(2):357–368. DOI: 10.1084/jem.20100479 [PubMed: 21262959]
- 71O'Sullivan TE, Geary CD, Weizman OE, Geiger TL, Rapp M, Dorn GW 2nd, Overholtzer M, Sun JC. Atg5 Is Essential for the Development and Survival of Innate Lymphocytes. *Cell Rep*. 2016; 15(9):1910–1919. DOI: 10.1016/j.celrep.2016.04.082 [PubMed: 27210760]
- 72Sun JC, Lanier LL. NK cell development, homeostasis and function: parallels with CD8(+) T cells. *Nat Rev Immunol*. 2011; 11(10):645–657. DOI: 10.1038/nri3044 [PubMed: 21869816]
- 73Mombaerts P, Iacomini J, Johnson RS, Herrup K, Tonegawa S, Papaioannou VE. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell*. 1992; 68(5):869–877. [PubMed: 1547488]
- 74Teng G, Maman Y, Resch W, Kim M, Yamane A, Qian J, Kieffer-Kwon KR, Mandal M, Ji Y, Meffre E, Clark MR, Cowell LG, Casellas R, Schatz DG. RAG Represents a Widespread Threat to the

- Lymphocyte Genome. *Cell*. 2015; 162(4):751–765. DOI: 10.1016/j.cell.2015.07.009 [PubMed: 26234156]
- 75Su HC, Ishikawa R, Biron CA. Transforming growth factor-beta expression and natural killer cell responses during virus infection of normal, nude, and SCID mice. *J Immunol*. 1993; 151(9):4874–4890. [PubMed: 8409446]
- 76Kumar V, Ben-Ezra J, Bennett M, Sonnenfeld G. Natural killer cells in mice treated with ⁸⁹strontium: normal target-binding cell numbers but inability to kill even after interferon administration. *J Immunol*. 1979; 123(4):1832–1838. [PubMed: 383842]
- 77Klein L, Kyewski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat Rev Immunol*. 2014; 14(6):377–391. DOI: 10.1038/nri3667 [PubMed: 24830344]
- 78Anfossi N, Andre P, Guia S, Falk CS, Roetynck S, Stewart CA, Bresó V, Frassati C, Reviron D, Middleton D, Romagne F, Ugolini S, Vivier E. Human NK cell education by inhibitory receptors for MHC class I. *Immunity*. 2006; 25(2):331–342. DOI: 10.1016/j.immuni.2006.06.013 [PubMed: 16901727]
- 79Belanger S, Tu MM, Rahim MM, Mahmoud AB, Patel R, Tai LH, Troke AD, Wilhelm BT, Landry JR, Zhu Q, Tung KS, Raulet DH, Makrigiannis AP. Impaired natural killer cell self-education and “missing-self” responses in Ly49-deficient mice. *Blood*. 2012; 120(3):592–602. DOI: 10.1182/blood-2012-02-408732 [PubMed: 22661698]
- 80Chalifour A, Scarpellino L, Back J, Brodin P, Devedre E, Gros F, Levy F, Leclercq G, Hoglund P, Beermann F, Held W. A Role for cis Interaction between the Inhibitory Ly49A receptor and MHC class I for natural killer cell education. *Immunity*. 2009; 30(3):337–347. DOI: 10.1016/j.immuni.2008.12.019 [PubMed: 19249231]
- 81Ma 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. DOI: 10.1146/annurev.immunol.24.021605.090727 [PubMed: 16551262]
- 82Badovinac VP, Porter BB, Harty JT. Programmed contraction of CD8(+) T cells after infection. *Nat Immunol*. 2002; 3(7):619–626. DOI: 10.1038/ni804 [PubMed: 12055624]
- 83Ahmed R, Gray D. Immunological memory and protective immunity: understanding their relation. *Science*. 1996; 272(5258):54–60. [PubMed: 8600537]
- 84Mescher MF, Curtsinger JM, Agarwal P, Casey KA, Gerner M, Hammerbeck CD, Popescu F, Xiao Z. Signals required for programming effector and memory development by CD8+ T cells. *Immunological reviews*. 2006; 211:81–92. DOI: 10.1111/j.0105-2896.2006.00382.x [PubMed: 16824119]
- 85Curtsinger JM, Schmidt CS, Mondino A, Lins DC, Kedl RM, Jenkins MK, Mescher MF. Inflammatory cytokines provide a third signal for activation of naive CD4+ and CD8+ T cells. *J Immunol*. 1999; 162(6):3256–3262. [PubMed: 10092777]
- 86Curtsinger JM, Valenzuela JO, Agarwal P, Lins D, Mescher MF. Type I IFNs provide a third signal to CD8 T cells to stimulate clonal expansion and differentiation. *Journal of immunology* (Baltimore, Md: 1950). 2005; 174(8):4465–4469.
- 87Wu J, Cherwinski H, Spies T, Phillips JH, Lanier LL. DAP10 and DAP12 form distinct, but functionally cooperative, receptor complexes in natural killer cells. *J Exp Med*. 2000; 192(7):1059–1068. [PubMed: 11015446]
- 88Aguilar OA, Berry R, Rahim MMA, Reichel JJ, Popovic B, Tanaka M, Fu Z, Balaji GR, Lau TNH, Tu MM, Kirkham CL, Mahmoud AB, Mesci A, Krmpotic A, Allan DSJ, Makrigiannis AP, Jonjic S, Rossjohn J, Carlyle JR. A Viral Immune-evasion Controls Innate Immunity by Targeting the Prototypical Natural Killer Cell Receptor Family. *Cell*. 2017; 169(1):58–71 e14. DOI: 10.1016/j.cell.2017.03.002 [PubMed: 28340350]
- 89Nabekura T, Kanaya M, Shibuya A, Fu G, Gascoigne NR, Lanier LL. Costimulatory molecule DNAM-1 is essential for optimal differentiation of memory natural killer cells during mouse cytomegalovirus infection. *Immunity*. 2014; 40(2):225–234. DOI: 10.1016/j.immuni.2013.12.011 [PubMed: 24440149]
- 90Rolle A, Halenius A, Ewen EM, Cerwenka A, Hengel H, Momburg F. CD2-CD58 interactions are pivotal for the activation and function of adaptive natural killer cells in human cytomegalovirus

- infection. *Eur J Immunol*. 2016; 46(10):2420–2425. DOI: 10.1002/eji.201646492 [PubMed: 27469079]
- 91Liu LL, Landskron J, Ask EH, Enqvist M, Sohlberg E, Traherne JA, Hammer Q, Goodridge JP, Larsson S, Jayaraman J, Oei VYS, Schaffer M, Tasken K, Ljunggren HG, Romagnani C, Trowsdale J, Malmberg KJ, Beziat V. Critical Role of CD2 Co-stimulation in Adaptive Natural Killer Cell Responses Revealed in NKG2C-Deficient Humans. *Cell Rep*. 2016; 15(5):1088–1099. DOI: 10.1016/j.celrep.2016.04.005 [PubMed: 27117418]
- 92Crotty S, Johnston RJ, Schoenberger SP. Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation. *Nat Immunol*. 2010; 11(2):114–120. DOI: 10.1038/ni.1837 [PubMed: 20084069]
- 93Collins A, Littman DR, Taniuchi I. RUNX proteins in transcription factor networks that regulate T-cell lineage choice. *Nat Rev Immunol*. 2009; 9(2):106–115. DOI: 10.1038/nri2489 [PubMed: 19165227]
- 94Egawa T, Tillman RE, Naoe Y, Taniuchi I, Littman DR. The role of the Runx transcription factors in thymocyte differentiation and in homeostasis of naive T cells. *J Exp Med*. 2007; 204(8):1945–1957. DOI: 10.1084/jem.20070133 [PubMed: 17646406]
- 95Busch DH, Pamer EG. T lymphocyte dynamics during *Listeria monocytogenes* infection. *Immunol Lett*. 1999; 65(1–2):93–98. [PubMed: 10065633]
- 96Grayson JM, Zajac AJ, Altman JD, Ahmed R. Cutting edge: increased expression of Bcl-2 in antigen-specific memory CD8+ T cells. *J Immunol*. 2000; 164(8):3950–3954. [PubMed: 10754284]
- 97Kurtulus S, Tripathi P, Opferman JT, Hildeman DA. Contracting the ‘mus cells’—does down-sizing suit us for diving into the memory pool? *Immunol Rev*. 2010; 236:54–67. DOI: 10.1111/j.1600-065X.2010.00920.x [PubMed: 20636808]
- 98Yajima T, Yoshihara K, Nakazato K, Kumabe S, Koyasu S, Sad S, Shen H, Kuwano H, Yoshikai Y. IL-15 regulates CD8+ T cell contraction during primary infection. *J Immunol*. 2006; 176(1):507–515. [PubMed: 16365444]
- 99Rubinstein MP, Lind NA, Purton JF, Filippou P, Best JA, McGhee PA, Surh CD, Goldrath AW. IL-7 and IL-15 differentially regulate CD8+ T-cell subsets during contraction of the immune response. *Blood*. 2008; 112(9):3704–3712. DOI: 10.1182/blood-2008-06-160945 [PubMed: 18689546]
- 100Tang C, Yamada H, Shibata K, Yoshida S, Wajjwalku W, Yoshikai Y. IL-15 protects antigen-specific CD8+ T cell contraction after *Mycobacterium bovis* bacillus Calmette-Guerin infection. *J Leukoc Biol*. 2009; 86(1):187–194. DOI: 10.1189/jlb.0608363 [PubMed: 19389797]
- 101Huntington ND, Puthalakath H, Gunn P, Naik E, Michalak EM, Smyth MJ, Tabarias H, Degli-Esposti MA, Dewson G, Willis SN, Motoyama N, Huang DC, Nutt SL, Tarlinton DM, Strasser A. Interleukin 15-mediated survival of natural killer cells is determined by interactions among Bim, Noxa and Mcl-1. *Nat Immunol*. 2007; 8(8):856–863. DOI: 10.1038/ni1487 [PubMed: 17618288]
- 102Xu X, Araki K, Li S, Han JH, Ye L, Tan WG, Konieczny BT, Bruinsma MW, Martinez J, Pearce EL, Green DR, Jones DP, Virgin HW, Ahmed R. Autophagy is essential for effector CD8(+) T cell survival and memory formation. *Nat Immunol*. 2014; 15(12):1152–1161. DOI: 10.1038/ni.3025 [PubMed: 25362489]
- 103Kaech SM, Wherry EJ. Heterogeneity and cell-fate decisions in effector and memory CD8+ T cell differentiation during viral infection. *Immunity*. 2007; 27(3):393–405. DOI: 10.1016/j.immuni.2007.08.007 [PubMed: 17892848]
- 104Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol*. 2004; 22:745–763. DOI: 10.1146/annurev.immunol.22.012703.104702 [PubMed: 15032595]
- 105Huster KM, Stemmerger C, Busch DH. Protective immunity towards intracellular pathogens. *Curr Opin Immunol*. 2006; 18(4):458–464. DOI: 10.1016/j.coi.2006.05.008 [PubMed: 16765577]
- 106Klose CSN, Flach M, Mohle L, Rogell L, Hoyler T, Ebert K, Fabiunke C, Pfeifer D, Sexl V, Fonseca-Pereira D, Domingues RG, Veiga-Fernandes H, Arnold SJ, Busslinger M, Dunay IR, Tanriver Y, Diefenbach A. Differentiation of type 1 ILCs from a common progenitor to all

helper-like innate lymphoid cell lineages. *Cell*. 2014; 157(2):340–356. DOI: 10.1016/j.cell.2014.03.030 [PubMed: 24725403]

- 107Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999; 401(6754):708–712. DOI: 10.1038/44385 [PubMed: 10537110]
- 108Bezman NA, Kim CC, Sun JC, Min-Oo G, Hendricks DW, Kamimura Y, Best JA, Goldrath AW, Lanier LL. Molecular definition of the identity and activation of natural killer cells. *Nat Immunol*. 2012; 13(10):1000–1009. DOI: 10.1038/ni.2395 [PubMed: 22902830]
- 109Zediak VP, Johnnidis JB, Wherry EJ, Berger SL. Cutting edge: persistently open chromatin at effector gene loci in resting memory CD8+ T cells independent of transcriptional status. *J Immunol*. 2011; 186(5):2705–2709. DOI: 10.4049/jimmunol.1003741 [PubMed: 21278341]
- 110Dunn J, McCuaig R, Tu WJ, Hardy K, Rao S. Multi-layered epigenetic mechanisms contribute to transcriptional memory in T lymphocytes. *BMC Immunol*. 2015; 16:27.doi: 10.1186/s12865-015-0089-9 [PubMed: 25943594]
- 111Gray SM, Kaech SM, Staron MM. The interface between transcriptional and epigenetic control of effector and memory CD8(+) T-cell differentiation. *Immunol Rev*. 2014; 261(1):157–168. DOI: 10.1111/imr.12205 [PubMed: 25123283]
- 112Lee J, Zhang T, Hwang I, Kim A, Nitschke L, Kim M, Scott JM, Kamimura Y, Lanier LL, Kim S. Epigenetic modification and antibody-dependent expansion of memory-like NK cells in human cytomegalovirus-infected individuals. *Immunity*. 2015; 42(3):431–442. DOI: 10.1016/j.immuni.2015.02.013 [PubMed: 25786175]
- 113Schlums H, Cichocki F, Tesi B, Theorell J, Beziat V, Holmes TD, Han H, Chiang SC, Foley B, Mattsson K, Larsson S, Schaffer M, Malmberg KJ, Ljunggren HG, Miller JS, Bryceson YT. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. *Immunity*. 2015; 42(3):443–456. DOI: 10.1016/j.immuni.2015.02.008 [PubMed: 25786176]
- 114Jameson SC, Lee YJ, Hogquist KA. Innate memory T cells. *Adv Immunol*. 2015; 126:173–213. DOI: 10.1016/bs.ai.2014.12.001 [PubMed: 25727290]
- 115Artis D, Spits H. The biology of innate lymphoid cells. *Nature*. 2015; 517(7534):293–301. DOI: 10.1038/nature14189 [PubMed: 25592534]
- 116Martinez-Gonzalez I, Matha L, Steer CA, Ghaedi M, Poon GF, Takei F. Allergen-Experienced Group 2 Innate Lymphoid Cells Acquire Memory-like Properties and Enhance Allergic Lung Inflammation. *Immunity*. 2016; 45(1):198–208. DOI: 10.1016/j.immuni.2016.06.017 [PubMed: 27421705]
- 117Withers DR, Gaspal FM, Mackley EC, Marriott CL, Ross EA, Desanti GE, Roberts NA, White AJ, Flores-Langarica A, McConnell FM, Anderson G, Lane PJ. Cutting edge: lymphoid tissue inducer cells maintain memory CD4 T cells within secondary lymphoid tissue. *J Immunol*. 2012; 189(5):2094–2098. DOI: 10.4049/jimmunol.1201639 [PubMed: 22855716]
- 118Ravens S, Schultze-Florey C, Raha S, Sandrock I, Drenker M, Oberdorfer L, Reinhardt A, Ravens I, Beck M, Geffers R, von Kaisenberg C, Heuser M, Thol F, Ganser A, Forster R, Koenecke C, Prinz I. Human gammadelta T cells are quickly reconstituted after stem-cell transplantation and show adaptive clonal expansion in response to viral infection. *Nat Immunol*. 2017; 18(4):393–401. DOI: 10.1038/ni.3686 [PubMed: 28218745]
- 119Sheridan BS, Romagnoli PA, Pham QM, Fu HH, Alonzo F 3rd, Schubert WD, Freitag NE, Lefrancois L. gammadelta T cells exhibit multifunctional and protective memory in intestinal tissues. *Immunity*. 2013; 39(1):184–195. DOI: 10.1016/j.immuni.2013.06.015 [PubMed: 23890071]

A Generation of hapten-specific memory NK cells **B** Generation of MCMV-specific memory NK cells **C** Generation of cytokine-induced memory NK cells

Figure 1. Antigen-specific and -nonspecific mechanisms underlying NK cell memory formation
(A) Hapten-induced contact hypersensitivity initiates the formation of long-lived, hapten-specific CXCR6⁺NKG2D⁺CD49a⁺CD49b⁻ liver-resident memory NK cells. Function and maintenance of hapten-specific memory NK cells depend on the expression of the pro-inflammatory cytokines IL12, type I and II IFNs, and the transcription factor aryl hydrocarbon receptor (AHR). Hapten challenge induces CHS, which requires NKG2D, CXCR6, CD18, AHR, L-, P- and E-selectin and results in increased degranulation of hapten-sensitized NK cells. **(B)** Ligation of the NK cell activation receptor Ly49H with the MCMV-encoded glycoprotein m157 expressed on infected dendritic cells results in the activation of KLRG1^{lo}DNAM1⁺Ly49H⁺ NK cells followed by a clonal-like expansion, contraction and memory formation. Proliferation and memory generation require the expression of different cytokines and transcription factors, including IL-12, IL-18, IL-33, type I IFNs, Zbtb32, STAT1, STAT4, miR-155, Runx1 and Runx3. MCMV-specific memory NK cells express high levels of the cell surface marker Ly49H, KLRG1, CD43, CD11b and Ly6C, and lack CD27 expression. Upon MCMV challenge, virus-specific memory NK cells proliferate rapidly and produce high levels of granzymes, perforin, and IFN-γ. **(C)** NK cells stimulated with a combination of IL-12, IL-15 and IL-18 differentiate into cells with antigen-

independent, memory-like properties. Cytokine-activated memory-like NK cells are long-lived, express the cell surface receptor CD25, require IL-2 and IL-15 for survival and secrete IFN- γ upon cytokine-restimulation, demonstrating a long-lasting antigen-independent memory of prior activation.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Parallel pathways of activation and memory generation shared by NK cells and T cells during viral infection

Both NK cells (**A**) and T cells (**B**) require three signals for proper activation. Signal 1 is the ligation of antigen to its specific receptor, exemplified by viral m157 to the Ly49H receptor in NK cells, or MHC-I-bound peptides to the respective TCR in T cells. Signal 2 is the binding of a co-stimulatory receptor. In NK cells, signal 2 is mediated by binding of DNAM-1 to its cognate ligands CD112 or CD155, and in T cells co-stimulation is mediated by CD80/CD86 ligation to CD28 on the T cell surface. In both NK cells and T cells, Signal 3 represents the presence of pro-inflammatory cytokines such as IL-12 or type I IFN. Full activation results in clonal expansion, followed by a contraction phase, in which most of the activated T or NK cells undergo apoptosis. IL-15, Bcl2 interactions with pro-apoptotic Bim and autophagy are known mechanisms that protect NK and T cells from apoptosis during this stage, and lead to memory cell formation. Memory NK and effector memory T cells share common epigenetic features as well as common surface markers like KLRG1 and

Ly6C. Upon secondary antigen encounter, both memory NK cells and effector memory T cells exhibit high and rapid secretion of IFN- γ .

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript