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Natural Killer Cell Responses Redefine Immunological Memory

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Abstract

Immunological memory has traditionally been attributed as a unique trait of the adaptive immune system. Nevertheless, there is evidence of immunological memory in lower organisms and invertebrates, which lack an adaptive immune system. Despite their innate ability to rapidly produce effector cytokines and kill virally infected or transformed cells, NK cells also exhibit adaptive characteristics such as clonal expansion, longevity, self-renewal, and robust recall responses to antigenic or non-antigenic stimuli. This review highlights the intracellular and extracellular requirements for memory NK cell generation, and describes the emerging evidence for “memory precursor” NK cells, and their derivation.

Introduction

The immune system has classically been divided into two arms, innate and adaptive immunity. Innate immunity is poised for swift, short-lived effector responses mediated through recognition of evolutionarily conserved signals via germline-encoded receptors (1). Although initially slow in onset, adaptive immunity is considered highly “specialized” based on the ability to somatically rearrange antigen receptor genes to generate a diverse repertoire of T and B cells that can amplify antigen-specific responses through prolific clonal expansion (2-4). Because adaptive immune cells can persist long-term following recognition of cognate antigen and execute a quantitatively and qualitatively more robust response following re-challenge with the same antigen, T and B cells were thought to be the only immune population capable of generating “memory”.

The emergence of immunological memory in the adaptive immune system can be traced to lower vertebrates, including the jawless fish such as lamprey and hagfish. Early studies demonstrated that lampreys immunized with the killed bacterium *Brucella abortus* produce long-lived “antibody” capable of agglutinating *Brucella* cells upon re-challenge but not capable of agglutinating typhoid-paratyphoid cells, underscoring the antigen-specific nature of these antibody titers (5). The lamprey can also mediate delayed-type hypersensitivity

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(DTH) reactions following stimulation with *Mycobacterium tuberculosis*-fortified complete Freund's adjuvant (5). The basis for these phenomena may be attributable to the recently discovered lymphocyte-like populations and variable lymphocyte receptors, akin to primordial T and B lymphocytes and their antigen receptors, respectively (6, 7).

However, evidence also exists for immunological memory in invertebrates devoid of an adaptive immune system. Protection of the American cockroach *Periplaneta americana* against the bacterium *Pseudomonas aeruginosa* was enhanced by prior immunization with killed *P. aeruginosa* but not with saline or immunization with an array of other gram-negative organisms (8). Interestingly, this protection against *P. aeruginosa* re-challenge persisted for 14 days post-immunization (8), demonstrating both specificity and memory. Similar findings were demonstrated in the copepod *Macrocyclops albidus*, in which exposure to larvae of their natural parasite, the tapeworm *Schistocephalus solidus*, resulted in fewer infections from sibling but not unrelated parasites, the first evidence of innate immune memory in crustaceans (9). Similar protective memory responses against bacteria, parasites and fungi in other invertebrates add to the mounting evidence for innate immunity's capacity for memory responses in lower organisms (10-16), suggesting that the ability to “remember” may be evolutionarily conserved across both innate and adaptive immunity.

Natural killer cells: innate lymphocytes with adaptive features

NK cells were first described in 1975, when several groups identified a lymphocyte population in athymic (nude) mice capable of mediating “natural” (i.e. without the requirement of prior target exposure) cytotoxicity against both syngeneic and allogeneic tumor cell lines (17-20). Cytotoxic activity was maintained despite filtration of splenocytes through an anti-immunoglobulin column or treatment with carbonyl iron/magnet, excluding a contribution from T and B lymphocytes or macrophages (18, 20). Because of their capacity to rapidly secrete lytic molecules and the proinflammatory cytokine IFN-γ upon sensing pathogen-derived or host stress ligands through a repertoire of germline-encoded receptors, and because they lack RAG-mediated rearrangement of receptor genes, NK cells were characterized as a component of innate immunity (21-25).

However, current evidence has revealed striking similarities between NK cells and adaptive immune cells. NK cells develop from the common lymphoid progenitor (CLP), from which T and B cells and the newly described lineage of innate lymphoid cells (ILCs) are also derived (26). Similar to T and B cells, NK cell development, homeostasis, survival, and function require common-γ-chain-dependent cytokine signaling, particularly IL-15 (27-29). Although NK cells do not require expression of the RAG recombinase for development or generation of their receptor repertoire, nearly a third of peripheral NK cells have a history of RAG expression during ontogeny (30, 31). Furthermore, analogous to thymic T cell education, NK cells acquire functional competence through a “licensing” or “arming” process; during development, NK cells become self-tolerant (and thus gain effector function) due to engagement of self-MHC by inhibitory receptors (32-34).

Lastly, NK cells can undergo clonal expansion and initiate antigen-specific recall responses. Perhaps the earliest evidence suggesting the possibility of NK cell memory was reported in 1964 in a model of F1 hybrid resistance. Adult F1 hybrid mice (B10 × B10.D2) reject

parental (B10 or B10.D2) bone marrow grafts (35). When primed with a bone marrow graft from one parent (B10), F1 hybrid mice more rapidly rejected a second graft from that same parent (B10) compared with a graft derived from the other parent (B10.D2) (35). Conversely, priming the F1 hybrids with a B10.D2 or allogeneic graft did not accelerate B10 graft rejection (35). Together, these findings suggested that a non-T or -B cell responded in a qualitatively different manner upon re-exposure to previously encountered antigens. The later discovery of the NK cell and the "missing-self" hypothesis (that NK cells selectively kill cells lacking self-MHC class I) implicated NK cells as the cell type mediating F1 hybrid resistance (36). Since then, NK cells have been found to possess a number of adaptive features that have redefined their role in immunity.

Antigen-dependent NK cell memory

The first evidence of anamnestic NK cell responses was in the setting of contact hypersensitivity responses to chemical haptens. *Rag2*$^{-/-}$ mice lacking both T and B lymphocytes exhibited a severe inflammatory reaction when sensitized and re-challenged with the same hapten (either DNFB or oxazolone) (37). Depletion of NK cells abrogated the contact hypersensitivity, suggesting that NK cells either directly or indirectly were responsible for the observed recall response (37). Adoptive transfer of DNFB-sensitized NK cells into *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ mice was also sufficient to drive contact hypersensitivity upon recipient challenge with DNFB, although transferable hapten-specific recall was retained only in hepatic, but not splenic NK cells (37, 38). Specifically, contact hypersensitivity depended on a subset of hepatic NK cells expressing the chemokine receptor CXCR6, which was required for the homeostasis but not antigen-recognition of these cells (38). Thus, hepatic NK cells can generate antigen-specific recall responses to haptens, although whether these cells are truly mature NK cells or a distinct subpopulation of the type 1 ILC family is unresolved (39).

NK cells can also undergo recall responses against viral pathogens (40). During the "expansion" phase of the NK cell response to MCMV infection that peaks at day 7 post-infection, the antigen-specific NK cell compartment has been measured to undergo ~100-1000-fold growth in size (40). This proliferative burst is driven by antigen-specific interactions between the activating receptor Ly49H on NK cells and the MHC-I-like viral glycoprotein m157 on the surface of infected cells (41-43). Following robust expansion of Ly49H^{+} NK cells following MCMV infection, these effector cells contract and form a long-lived pool of memory NK cells in both lymphoid and non-lymphoid tissues that is detectable at least 70 days after MCMV infection (40). These memory NK cells exhibit enhanced IFN-γ production and degranulation compared to naïve NK cells (40). The response of memory NK cells re-challenged with MCMV was found to be comparable in both kinetics and magnitude to that of naïve NK cells, yet memory NK cells conferred greater protection to susceptible neonate mice against MCMV challenge (40). Thus, MCMV-experienced NK cells are capable of recall responses, enhanced functionality, and protection against repeated MCMV exposure.

Evidence for secondary NK cell responses against different viral pathogens continues to build. Analogous to hapten-specific memory NK cell-mediated contact hypersensitivity

responses, adoptively transferred hepatic, but not splenic NK cells, from *Rag1*$^{-/-}$ mice immunized with virus-like particles expressing influenza A virus, vesicular stomatitis virus (VSV) or HIV-1 antigens afforded protection to *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ hosts challenged with the immunizing, but not unrelated, virus (38). Similar immunization-dependent and virus-specific NK cell protective responses in the absence of adaptive immunity have been described for HSV-2, vaccinia virus and influenza virus (44-46).

Similar to the expansion of NK cells in MCMV-infected mice, human NK cells expressing the activating heterodimeric CD94/NKG2C receptor are preferentially found in the peripheral blood of healthy individuals seropositive for HCMV, compared to donors who were HCMV-seronegative or seropositive for other herpesvirus infections (47-49). This CD94/NKG2C^{+} subset commonly co-expresses the maturation marker CD57 and lacks expression of the inhibitory NKG2A receptor (49-52). Although the ligand driving expansion of CD94/NKG2C^{+} NK cells *in vivo* has yet to be elucidated, *in vitro* studies demonstrated that shRNA-mediated knockdown of HLA-E on HCMV-infected fibroblasts abrogated this expansion (53). HCMV reactivation in patients receiving allogeneic hematopoietic stem cell grafts, umbilical cord blood grafts or solid-organ transplants also precipitates expansion of CD94/NKG2C^{+} NK cells, followed by persistence of these cells for months to years (49, 54-57). Furthermore, the transfer of NKG2C^{+} NK cells in grafts from HCMV-seropositive donors resulted in enhanced target cell-induced IFN-γ production upon secondary CMV exposure in the recipient compared to grafts from HCMV-seronegative donors (50). In combination, these studies highlight the antigen-specificity, longevity and transplantability of human memory NK cell responses to HCMV. A recent study also identified a subset of CD16^{+}CD56^{+} FcεRIγ^{-} NK cells associated with prior HCMV and HSV-1 infection that persisted upwards of 9 months and was capable of mediating enhanced antibody-dependent cellular cytotoxicity (ADCC) in the presence of HCMV and HSV-1-infected cells coated by virus-specific antibodies (58, 59). Thus, human NK cells appear to have evolved redundant mechanisms to generate immunological memory.

Several studies have also demonstrated expansion of long-lived CD94/NKG2C^{+} human NK cells in response to HIV-1, hantavirus, chikungunya virus, hepatitis B virus (HBV) or hepatitis C virus (HCV), although it should be noted that this expansion occurred only in individuals previously infected with HCMV (60-64), suggesting that superinfection with these viruses may trigger HCMV reactivity. Interestingly, a recent study in rhesus macaques established that primate NK cells can achieve pathogen-specific memory independent of HCMV exposure (65). Infection of rhesus macaques with simian-human immunodeficiency virus (SHIV) or SIV elicited splenic and hepatic memory NK cells capable of lysing Gag- and Env-pulsed dendritic cells in an NKG2A- and NKG2C-dependent fashion for as long as 5 years post-infection (65). Vaccinating the macaques with recombinant adenovirus expressing HIV-1 *Env* or SIV *Gag* likewise produced robust, stable, and antigen-specific NK cell memory (65).

The benefit of pathogen-specific NK cell memory is clear in the case of persistent or repeated encounter with the same virus. However, there is some evidence that suggests there may be a costly trade-off associated with NK cell memory. A recent study highlighted that human NK cell receptor diversity increases with age (66). CD57^{+}NKG2C^{+} NK cells that

expand during HCMV infection are far from clonal, displaying substantial heterogeneity for other receptors (67). However, high NK cell receptor diversity was associated with greater risk of HIV-1 acquisition in Kenyan women (66). Given that viral challenge may enhance NK cell receptor diversity, human NK cells appear to risk unresponsiveness to novel antigens in order to better protect against previously encountered pathogens (66). The consequence of NK cell receptor diversity for human health and disease thus requires further exploration.

Mechanisms of NK cell memory generation during viral infection

Analogous to the generation of T cell memory against pathogens (4), NK cells progress through three phases during their response to MCMV: expansion, contraction, and memory maintenance (68). During each stage, both intracellular and extracellular cues are necessary for establishing a long-lived memory NK cell pool (Figure 1). In addition to antigen engagement by activating receptors (analogous to TCR engagement for T cell activation), NK cells require proinflammatory cytokine signaling for robust expansion (4). The proinflammatory cytokine IL-12, through a STAT4-dependent, but IFN-γ-independent mechanism, is indispensable for optimal MCMV-specific NK cell clonal expansion as well as memory NK cell formation (69). IL-12 and STAT4 may be responsible for programming activated NK cells early during MCMV infection for memory formation (69). Members of the IL-1 family of cytokines, particularly IL-33 and IL-18, are similarly necessary for amplifying NK cell proliferation in response to MCMV, but are dispensable for recall responses (70, 71). A recent study also identified a role for type I interferon and downstream STAT1 signaling in shielding proliferating NK cells from fratricide (killing by other NK cells) by modulating their cell surface expression of NK group 2 member D ligands (NKG2DL) (72).

Interestingly, IL-12, IL-18, and type I interferon signaling act synergistically to drive maximal expression of the BTB-ZF family transcription factor Zbtb32, which controls the proliferative burst of virus-specific NK cells by antagonizing the anti-proliferative factor Blimp-1 (73). In parallel, IL-12- and IL-18-mediated induction of miR-155 regulates effector and memory NK cell numbers during MCMV infection by regulating targets such as Noxa and SOCS1 (74). Although the mechanism by which SOCS1 impairs the development of effector NK cells is unclear, the potent restraint that constitutive SOCS1 activity places on STAT signaling may have some influence. Lastly, akin to the necessity of costimulation for T cell activation (“signal 2”), NK cells require the costimulatory molecule DNAX accessory molecule 1 (DNAM-1) and downstream signaling through the Src-family tyrosine kinase Fyn and the serine-threonine protein kinase C isoform eta (PKCη) for optimal expansion of effector NK cells and their differentiation into memory NK cells (75).

Following viral infection, contraction of effector lymphocytes serves to eliminate activated $CD8^+$ T and NK cells to stave off immunopathology (76, 77). Mitochondrial apoptosis mediated by the proapoptotic factor Bim shapes the size, maturity, and functionality of the memory NK cell pool in response to MCMV (78), similar to $CD8^+$ T cells (79). The accumulation of depolarized mitochondria and mitochondrial-released reactive oxygen species (ROS) in effector NK cells after virus-driven expansion results in either cell death, or

clearance of damaged mitochondria, resulting in NK cell survival (80). Analogous to the autophagy-dependent survival and memory formation of virus-specific effector $CD8^+$ T cells (81-83), surviving NK cells undergo the self-catabolic process of mitophagy during the contraction-to-memory phase transition, requiring the autophagosome machinery component *Atg3* and the mitophagy-specific receptors BCL2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3) and BNIP3-like (BNIP3L or Nix) to promote their survival (80). Mitophagy in contracting NK cells is induced by mechanistic target of rapamycin (mTOR) inhibition or AMP-activated protein kinase (AMPK) activation (80), suggesting that mitophagy may also link other cellular metabolic processes, similar to the catabolic processes memory T cells employ to fuel oxidative phosphorylation during non-proliferative states (84).

In addition to intracellular mechanisms, extracellular cues can also promote the maintenance of NK cell memory. Adoptive transfer of $Ly49H^+$ NK cells isolated from MCMV-infected hosts at day 7 or day 21 post-infection into IL-15-deficient recipients led to decreased persistence of the transferred cells, supporting a role for IL-15-dependent maintenance of NK cells during contraction (85). IL-15 was previously shown to promote NK cell survival via Mcl-1 (86). miR-155-mediated suppression of Noxa may also aid long-term survival of memory NK cells (74). Thus, generating long-lived, MCMV-specific memory NK cells requires a complex combination of intracellular and extracellular signals during both the early and late phases of the antiviral response.

Identifying memory NK cell precursors

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 die after infection, and $KLRG1^{lo}$ memory precursor effector cells (MPECs) that are long-lived and participate in secondary responses (87). 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 (88). KLRG1 is associated with NK cell maturation, as indicated by the greater percentage of $KLRG1^{hi}$ NK cells that are also of the most mature $CD11b^+$ $CD27^-$ phenotype, suggesting that NK cell maturation is antagonistic to their memory potential (88). However, because memory NK cells are themselves $KLRG1^{hi}$ $CD11b^+$ $CD27^-$, yet still competent to undergo robust secondary expansion following MCMV challenge, KLRG1 alone does not dictate proliferative potential (40, 88).

Interestingly, KLRG1 expression on naïve NK cells may be dictated during development by the activity of the RAG recombinase. NK cells with a history of RAG activity during ontogeny preferentially expand and persist as memory cells following MCMV infection (Figure 2A), due to an enhanced overall cellular fitness measured by the ability to repair DNA breaks which can occur during stresses such as rapid proliferation or exposure to ionizing radiation (30). In contrast, the absence of RAG expression in developing NK cells results in diminished expression of the DNA damage repair machinery and a subsequent impairment in DNA double-strand break resolution following DNA damage (30). Thus,

although the underlying mechanisms remain to be elucidated, RAG activity during ontogeny shields NK cells from global genomic instability and apoptosis. Furthermore, *Rag2*$^{-/-}$ NK cells display a transcriptional signature markedly similar to that observed in SLECs rather than MPECs (SLEC and MPEC signatures published in (89)) (Figure 2B), supporting the hypothesis that RAG dictates functional heterogeneity within the NK cell compartment. It will be of interest to determine whether the degree of genomic integrity similarly specifies SLEC versus MPEC fate.

Although RAG appears to determine KLRG1 expression levels in a cell-intrinsic manner, KLRG1 expression can also be influenced in a cell-extrinsic manner. One study demonstrated that T cells can restrain NK cell maturation by limiting the availability of IL-15, driving the preferential generation of KLRG1^{lo} memory progenitors at the expense of KLRG1^{hi} NK cells (Figure 2A) (88). Signals derived from the host commensal microbiota were also shown to control expression of KLRG1 in NK cells (Figure 2A) (88). Treatment with broad-spectrum antibiotics in the drinking water diminished KLRG1 expression and boosted the frequency of memory NK cells compared with untreated wild-type mice, suggesting that the host microbiota regulates the NK cell pool containing memory potential (88). Thus, the foundation for NK cell memory formation may be laid even prior to encountering virus.

MCMV-specific memory NK cells display greater cell surface expression of not only KLRG1 but also Ly49H compared with naïve NK cells, and expression of these receptors is further enhanced in secondary memory NK cells (90). In contrast, naïve, memory and secondary memory NK cells express comparable levels of the activating receptors NK1.1 and Ly49D (90). These data together imply that there may be selection for NK cells with the greatest avidity for ligand during successive rounds of MCMV infection (Figure 2A). T cells undergo a process of affinity maturation, whereby T cells bearing TCRs with highest affinity for peptide rise to clonal dominance during the response to a pathogen (91-94). A similar process may occur with NK cells responding against infection. There is currently no evidence to support the idea that NK cells undergo somatic mutation of their antigen receptor genes (i.e. the affinity of each Ly49H receptor for m157 is equivalent), indicating that NK cell avidity for viral antigen is determined solely by the number of Ly49H receptors on the surface of a given NK cell. However, it remains to be determined at what stage of the antiviral response activating signals through Ly49H select for memory precursors to constitute the long-lived pool.

Emerging evidence also illustrates the contribution of the activating receptor Ly49D and the inhibitory receptor Ly49A, both of which recognize the MHC class I molecule H-2D^{d}, during the NK cell memory response to MCMV (95). Adoptive transfer of B10.D2 (H-2D^{d}-sufficient background) NK cells into MCMV-infected syngeneic Ly49H-deficient recipients demonstrated that Ly49D^{+}Ly49A^{-}Ly49H^{+} NK cells preferentially differentiated into memory NK cells compared with Ly49D^{-}Ly49A^{+}Ly49H^{+} NK cells (95). Similar to how MCMV infection can break the anergic state of unlicensed NK cells in B6 mice, it appears that acute viral infection can also breach tolerance of activating receptors for self-MHC class I, a phenomenon that has functional consequences for host protection and NK cell memory (95-98).

Recent human studies have suggested that epigenetic heterogeneity is also associated with differential capacity for NK cell longevity (Figure 2A). The aforementioned $CD16^+$ $CD56^+$ $Fc\varepsilon RI\gamma^-$ NK cells are a subset of memory-like NK cells that can be isolated from HCMV-seropositive individuals, although they can be characterized by either the absence or presence of CD94/NKG2C (58, 59, 99, 100). Interestingly, this population lacks expression of the tyrosine kinase SYK, the signaling adaptors DAB2 and EAT-2, and the transcription factors promyelocytic leukemia zinc finger protein (PLZF) and Helios due to promoter hypermethylation at several of these loci (99, 100). Another study has identified epigenetic imprinting at the *Ifng* conserved non-coding sequence (CNS) 1 in $NKG2C^+$ NK cells from HCMV-seropositive individuals that is critical for *Ifng* transcriptional activity in response to stimulation through NKG2C (101). It is unclear whether this epigenetic heterogeneity is a cause or consequence of NK cell memory. Nevertheless, receptor heterogeneity, the microbiota, and history of RAG expression during ontogeny precipitate differential fitness of cells within the naïve NK pool. During viral infection, selective pressure may then be acting on the effector pool to preferentially select memory precursors to establish the memory pool.

Antigen-independent NK cell memory

Although proinflammatory cytokine signaling is critical in driving the clonal expansion and maintenance of MCMV-specific memory NK cells, proinflammatory cytokines alone were found to be capable of supporting NK cell memory properties in the absence of antigen. Splenic NK cells pre-activated with a cocktail of IL-12, IL-15, and IL-18, and adoptively transferred into $Rag1^{-/-}$ mice become long-lived and can be identified in these recipients up to 3 weeks following transfer (102). These cytokine-induced “memory-like” NK cells retained a cell-intrinsic capacity for enhanced IFN-γ production, but not cytotoxicity, when re-stimulated with cytokine, plate-bound antibody or target cells (102, 103). Human NK cells pre-activated with cytokines also displayed the same properties (104). The progeny of cytokine-induced memory-like NK cells similarly exhibited enhanced effector functions (102, 104), suggesting that NK cell memory properties may be epigenetically inherited. These data may have implications for the antigen-independent self-renewal of memory NK cells following clearance of pathogens, although future studies are necessary to dissect the contribution of cytokine-induced memory-like NK cells during a recall response to a pathogen against which pathogen-specific memory NK cells already exist. Given that antigen-specific NK cells demonstrate diminished bystander activation to heterologous infection (105), cytokine-induced memory-like NK cells may represent a strategy for the host to nonspecifically respond to a new proinflammatory stimulus. Recent studies have shown that NK cells pre-activated with IL-12, IL-15, and IL-18 demonstrate enhanced persistence and antitumor activity against established, irradiated mouse tumors compared with naïve NK cells, suggesting that harnessing the sustained effector functions of cytokine-induced memory-like NK cells may represent a potential enhancement to adoptive NK cell immunotherapy (106). ILCs are thought to respond exclusively to cytokine cues (107), and it will be of interest to determine whether cytokines can similarly support longevity in other innate lymphocytes.

Lastly, in an NK cell-deficient lymphopenic host, adoptively transferred NK cells undergo a rapid, antigen-independent homeostatic proliferation to fill the empty niche, a process that

requires common-γ-chain-dependent cytokine signaling (108-110). These same cytokines, as well as TCR-mediated self-peptide/MHC interactions, are known to support the acquisition of memory-like characteristics in naïve T cells undergoing lymphopenia-induced proliferation (111). Similarly, following homeostatic proliferation in *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ or sublethally irradiated recipients, adoptively transferred NK cells contract to form a long-lived population that persists in both lymphoid and nonlymphoid organs for at least 6 months (112). These NK cells display an enhanced capacity for IFN-γ production and degranulation when stimulated *ex vivo* 10 days after transfer, but the functionality of these homeostatically-driven NK cells following contraction is unclear (112). Nevertheless, homeostatically-expanded NK cells self-renew and are capable of mounting a robust proliferative response when challenged with MCMV 6 months after transfer, thus sharing some properties with MCMV-specific memory NK cells (112). Emerging evidence demonstrates that *Atg5*-mediated autophagy is critical for the survival of NK cells during homeostatic proliferation, suggesting that this is one mechanism by which NK cells acquire memory properties following lymphopenia-driven proliferation (113).

Conclusions

Immunological memory represents just one example among the adaptive features NK cells exhibit during their dynamic life span. Mounting evidence in both mice and humans points to the remarkable capacity of NK cells to generate memory responses in both infectious and noninfectious settings. Virus infection models are allowing us to uncover the molecular mechanisms necessary for NK cell memory formation and maintenance, yet the pathways that govern cytokine- and lymphopenia-induced memory-like NK cells remain poorly understood. NK cells have expanded the classical definition of immunological memory found in textbooks. It will now be of interest to determine whether a similar capacity for longevity, self-renewal, and robust recall responses exists within the newly described ILC lineages, which generally respond to proinflammatory cytokine cues. NK cells have a diverse repertoire of cell surface activating receptors, and future studies are necessary to address whether other activating receptors are sufficient to drive NK cell memory when exposed to cognate ligands. Resolving these questions will facilitate NK cell vaccination strategies and adoptive NK cell immunotherapies for viral infection and malignancy.

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Abbreviations used in this article

MCMV mouse CMV

ILC	innate lymphoid cell
DNFB	2,4-dinitro-1-fluorobenzene
HCMV	human CMV
SOCS1	suppressor of cytokine signaling 1
KLRG1	killer cell lectin-like receptor G1

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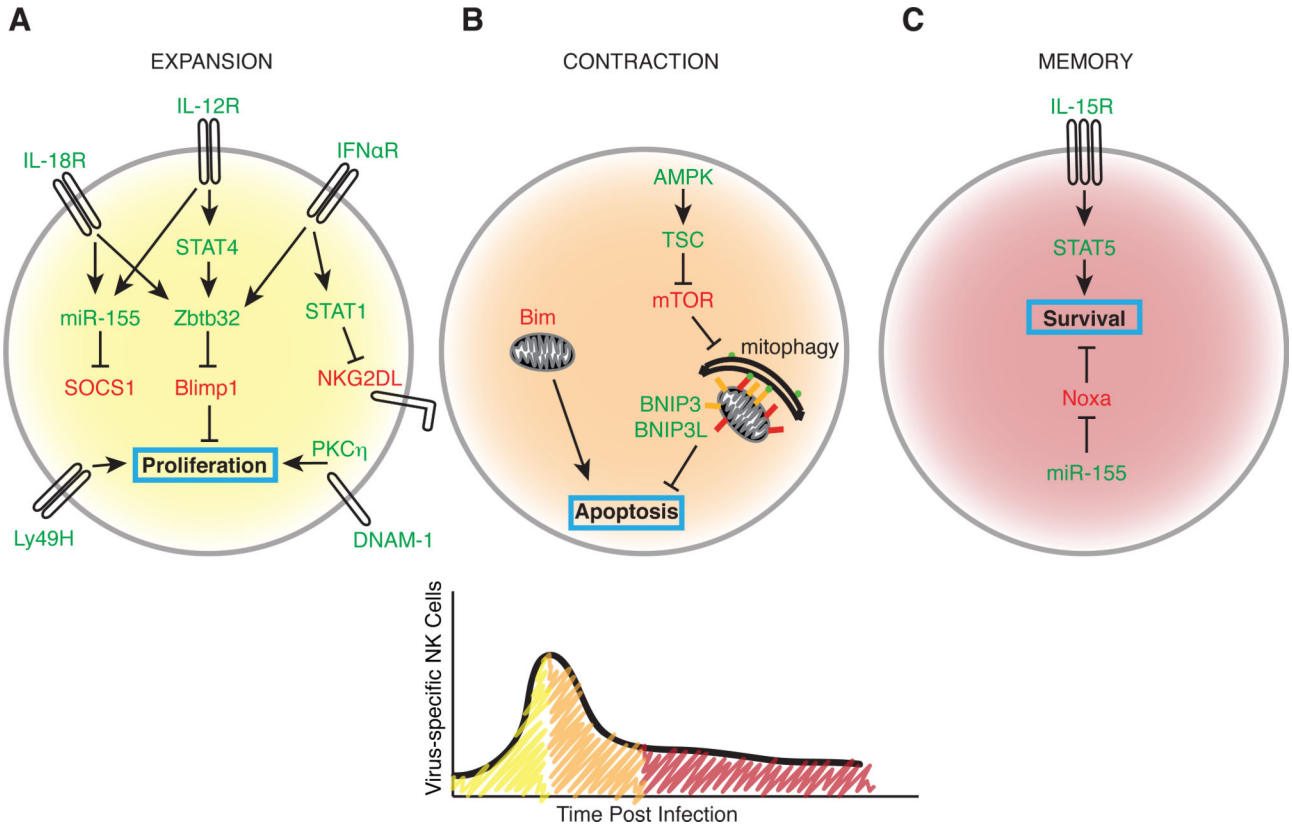

FIGURE 1.

Regulation of NK cells during each phase of the response to viral infection. *A*, Expansion. IL-12, IL-18, and type I interferon converge to drive Ly49H^{+} NK cell proliferation by inducing Zbtb32 and subsequently suppressing Blimp-1. IL-12 and IL-18 also cooperate to regulate SOCS1 in a miR-155-dependent mechanism. Signaling through IFNαR also independently contributes to memory formation by protecting against NKG2D-mediated fratricide. The costimulatory molecule DNAM-1 facilitates a PKCη-dependent proliferative burst. *B*, Contraction. Memory formation hinges on averting Bim-mediated mitochondrial apoptosis. Recycling dysfunctional mitochondria via mitophagy mediated by BNIP3 and BNIP3L promotes the contraction-to-memory phase transition. *C*, Memory maintenance. IL-15 signaling is required as NK cells contract and for the maintenance of memory cells in peripheral tissues. miR-155-mediated suppression of Noxa promotes memory NK survival. Green font represents positive regulators of memory. Red font represents negative regulators of memory. Cell colors correspond to the infection time course.

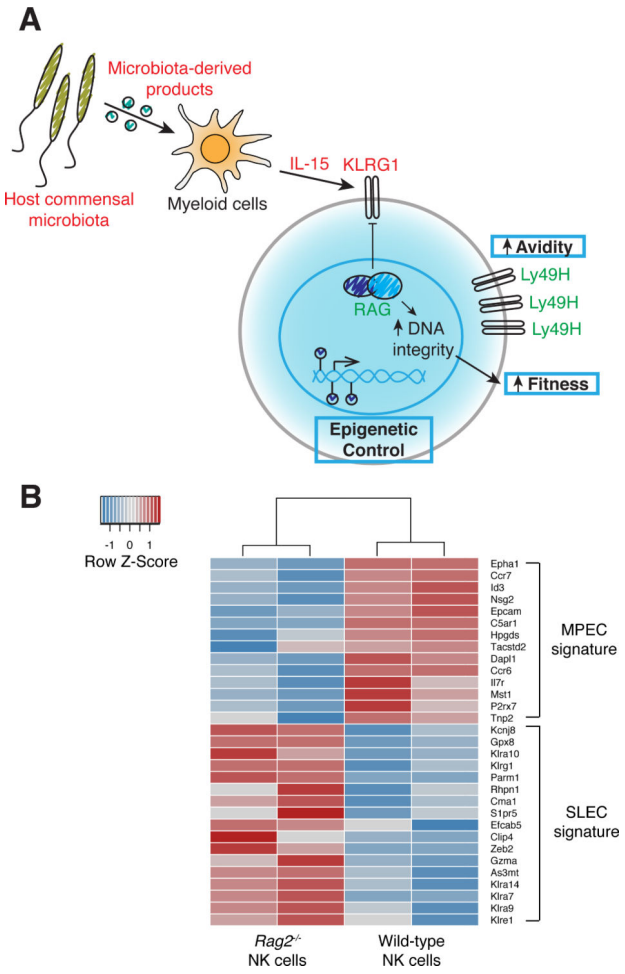

FIGURE 2.

Functional heterogeneity within the effector NK cell pool. *A*, Multiple mechanisms regulate memory precursor identity in NK cells. KLRG1 expression inversely correlates with NK cell memory potential. RAG expression during ontogeny not only negatively regulates KLRG1 expression but also promotes enhanced NK cell fitness by supporting optimal expression of DNA damage repair enzymes to maintain DNA integrity. Host commensal microbiota-derived products and IL-15 signaling, the availability of which is determined by competition with conventional T cells, drive NK cell expression of KLRG1. Greater avidity for ligand via higher cell surface concentration of Ly49H and epigenetic programs that drive a particular suite of memory genes may also converge to dictate NK cell memory precursor identity. Green font represents positive regulators of memory potential. Red font represents negative regulators of memory potential. *B*, Comparison of the gene expression profile of *Rag2*$^{-/-}$ and wild-type NK cells with that of MPECs and SLECs. *Rag2*$^{-/-}$ and wild-type NK cells were purified from mixed bone marrow chimeric mice and RNA-sequencing performed. Heat map shows the relative mRNA expression in *Rag2*$^{-/-}$ and wild-type NK cells of the top differentially expressed genes between MPEC and SLEC populations, as previously described (89). The transcriptional signature of *Rag2*$^{-/-}$ NK cells resembles that of SLECs whereas wild-type NK cells exhibit an MPEC-like signature.