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Review

Memory responses of natural killer cells

Clair D. Geary[a,b], Joseph C. Sun[a,b,*]

[a] Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY 10065, United States
[b] Department of Immunology and Microbial Pathogenesis, Weill Cornell Medical College, New York, NY 10065, United States

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ABSTRACT

Natural killer (NK) cells have traditionally been classified as a cellular component of the innate immune system, given their ability to rapidly produce effector cytokines and kill infected or transformed cells without prior exposure. More recently, NK cells have been shown to possess features of adaptive immunity such as clonal expansion, longevity, and robust recall responses. NK cell memory can be broadly divided into two categories: antigen-specific and antigen-independent. In the first case, exposure to certain viral or hapten stimuli endows NK cells with antigen-specific immunological memory, similar to T and B cells. In the second case, exposure of NK cells to specific cytokine milieus can imprint long-lasting changes on effector functions, resulting in antigen-independent memory-like NK cells. In this review, we discuss the various conditions that promote generation of these two categories of memory NK cells, and the mechanistic requirements underlying these processes.

1. Introduction

Natural killer (NK) cells were first identified in the 1970s as a novel lymphocyte population capable of mediating cytotoxicity against tumor targets without the need for prior sensitization [1–4]. They express a combination of germline-encoded inhibitory receptors, which recognize self molecules such as MHC class I, and activating receptors that bind stress-induced host molecules, pathogen-encoded ligands, and the Fc portion of antibodies [5]. When activating signals outweigh inhibitory signals, the NK cell's effector functions are triggered, leading to production of pro-inflammatory cytokines such as IFN-γ and TNF, and to target cell cytotoxicity mediated by perforin and granzymes.

NK cells are critical for control of viral infections in both humans and mice. Patients with rare genetic deficiencies in NK cell numbers or functionality are highly susceptible to viral infections, most notably herpesviruses, including human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), and varicella zoster virus (VZV), and human papillomavirus (HPV) [6]. Similarly, mice that are genetically deficient in NK cells or have been depleted of NK cells using antibodies have increased susceptibility to infection with mouse cytomegalovirus (MCMV), HSV-1, and vaccinia virus, among others (reviewed in [7]).

Immunological memory, defined as possessing a quantitatively or qualitatively greater response upon secondary immune challenge, is best characterized in mammalian adaptive lymphocytes, but has recently been demonstrated in invertebrates [8], and in innate immune cells within mammals [9]. For T cells, the well-studied process of memory generation can be delineated into three distinct phases: expansion, contraction, and memory [10]. In the expansion phase, naïve T cells that encounter their cognate antigen (foreign peptides presented on MHC molecules) undergo clonal expansion and differentiation into effector cells. This is followed by a rapid contraction phase, during which the majority of effector cells undergo apoptosis to generate a small pool of surviving "memory" cells. These memory T cells maintain their longevity through self-renewal and persist in both lymphoid and non-lymphoid organs. Upon re-encounter with cognate antigen, they display enhanced effector function and host protection.

Although NK cells have traditionally been considered part of the innate immune system, it is increasingly appreciated that they share many features with adaptive lymphocytes. NK cells develop from the same common lymphoid progenitor that gives rise to T and B cells [11]. Like T and B cells, they require common gamma chain-dependent cytokines for their development and homeostasis [12–14], and their responsiveness is tuned through an "education" or "licensing" process analogous to T cell development in the thymus [15–17]. Most notably, studies over the last decade have demonstrated that mature NK cells possess features of immunological memory. This review will discuss the key findings in support of NK cell memory, and the mechanisms involved in the generation and survival of memory NK cells.

* Corresponding author at: Memorial Sloan Kettering Cancer Center, 408 East 69th Street, ZRC-1462, New York, NY 10065, United States.
E-mail address: sunj@mskcc.org (J.C. Sun).

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2. Antigen-dependent generation of NK cell memory

2.1. Hapten-specific NK cell memory

The first demonstration of antigen-specific recall responses by NK cells was in the setting of hapten-induced contact hypersensitivity (CHS) [18]. Although previously thought to be T cell-mediated, robust CHS was observed following immunization and challenge of RAG2-deficient mice, which lack B and T cells, with a single chemical hapten such as 2, 4-dinitro-1-fluorobenzene (DNFB) [18,19]. Specificity during challenge was demonstrated when delayed hypersensitivity occurred only when the same hapten (DNFB) was used for challenge, and not a different hapten (e.g. oxazolone). NK cells were shown to accumulate at the site of hapten administration in the skin, and depletion and transfer studies showed that NK cells were both necessary and sufficient for the hapten-specific secondary response. Interestingly, the recall response was found to be mediated by a subset of hepatic NK cells expressing the chemokine receptor CXCR6, but not by splenic NK cells. Further studies showed the hapten response is mediated by $CD49a^{+}DX5^{-}$ tissue-resident cells [20], and required pro-inflammatory cytokines including IL-12, type I and II IFNs [21] and expression of the transcription factor AHR [22]. Antibody-mediated blockade of NKG2D, or molecules involved in trafficking of NK cells, such as CD18 and P- or E-selectin, prevented development of CHS responses at secondary sites [18]. Thus, hepatic NK cells are capable of generating an antigen-specific recall response to haptens (Fig. 1**A**). However, many questions remain to be addressed, including the identity of the antigen receptor, the precise nature of the ligand, and whether these cells are best classified as mature NK cells or a distinct subset of ILC1s [23].

2.2. MCMV-specific NK cell memory

Antigen-specific responses to pathogens were long thought to be unique to B and T cells that are able to undergo somatic rearrangement of their antigen receptor gene loci. However, antigen specificity has also been demonstrated for the germline-encoded activating receptor Ly49H, which is expressed on a subset of NK cells in C57BL/6 mice, and uniquely recognizes the MCMV-encoded glycoprotein m157 [24–27]. This receptor-ligand interaction results in the activation and proliferation of $Ly49H^{+}$ NK cells [25,28]. Following adoptive transfer of $Ly49H^{+}$ NK cells into mice lacking the receptor, this virus-specific NK cell compartment expanded up to 1000-fold within 7 days following MCMV infection, before then undergoing a contraction phase to generate a pool of memory cells [29]. These long-lived memory NK cells were found to persist in both lymphoid and non-lymphoid tissues, and were detectable several months after infection.

2.2.1. Properties of MCMV-specific memory NK cells

Compared to naïve NK cells, memory NK cells possess a unique transcriptional profile [30], and show enhanced effector functions, including heightened production of IFN-γ and degranulation [29]. Thus, although memory NK cells proliferate at a similar rate to naïve cells upon secondary MCMV infection, they provide greater protection against lethal challenge [29]. These studies demonstrate that NK cells, like T cells, differentiate through expansion and contraction phases to generate long-lived memory cells in response to viral infection (Fig. 1**B**).

MCMV-specific memory NK cells have a more mature phenotype than resting cells, including higher expression of KLRG1, CD11b, Ly6C, CD43 and lower expression of CD27. Within the naïve NK cell pool, it is the $KLRG1^{lo}$ subset that have the greatest capacity to expand and form memory [31]. KLRG1 expression on NK cells has been shown to depend on the host microbiota and the availability of IL-15, since acute antibiotic treatment or the presence of an intact T cell compartment to compete for IL-15 leads to a reduced frequency of $KLRG1^{+}$ NK cells. Additionally, KLRG1 expression is regulated in a cell-intrinsic matter by the activity of the RAG recombinase during ontogeny [32]. However, because all memory NK cells express high levels of KLRG1 and yet are able to expand at a comparable level to naïve cells, this suggests that while KLRG1 may be a marker of heterogeneity within the naïve NK cell pool, its expression alone does not determine the proliferative capability of NK cells. Notably, memory NK cells express higher levels of Ly49H, but not of other activating receptors relative to naïve cells, and the expression of Ly49H is even greater on secondary memory NK cells [33]. This suggests there may be a selection process in which NK cells with the highest avidity for MCMV-infected cells are more likely to form memory cells.

2.2.2. Mechanisms of MCMV-specific memory formation

2.2.2.1. Signal 1. Memory T cell generation requires a trio of independent signals to promote activation, clonal proliferation, and maximal effector function [10]. Signal 1 is the antigen-specific interaction of the TCR with peptide presented on MHC, signal 2 is co-stimulation through receptors such as CD28, and signal 3 is provided by pro-inflammatory cytokines. As discussed above, the Ly49H-m157 interaction plays a critical role in the generation of MCMV-specific memory NK cells, as infection with an MCMV strain lacking m157 (MCMVΔm157) does not induce expansion or development of memory NK cells [29], while vesicular stomatitis virus (VSV) expressing m157 promotes greater expansion and memory cell formation than VSV expressing an irrelevant antigen (e.g. OVA) [34,35]. Thus, similar to T cells, NK cells require this 'Signal 1' via Ly49H for full activation of effector function (Fig. 2). Notably, infection with MCMVΔm157 can drive expansion and memory cell generation when an alternative Signal 1 is provided; this was demonstrated for $Ly49D^{+}$ NK cells following infection with MCMVΔm157 and injection of splenocytes from BALB/c mice that express $H\text{-}2D^{d}$ which is recognized by Ly49D [36]. Future studies are necessary to determine whether other activating receptors, such as NKp46, can promote memory cell formation.

Interestingly, a recent study identified another MCMV-encoded ligand that is recognized by an activating NK receptor. MCMV m12 directly interacts with both activating and inhibitory NKR-P1 family receptors with differing affinities [37]. It will be of great interest to determine if the interaction of m12 with the activating NKR-P1C receptor (more commonly known as NK1.1) can contribute towards the formation of MCMV-specific NK cell memory, either on its own or in combination with the m157-Ly49H interaction.

2.2.2.2. Signal 2. NK cells require the co-stimulatory molecule DNAX accessory molecule-1 (DNAM-1) and the downstream signaling molecules PKCη and Fyn for optimal expansion and differentiation into memory cells [38]. Notably, the DNAM1 ligands CD155 and CD122 are upregulated on dendritic cells and macrophages early after MCMV infection [38]. Recently, it was shown that co-expression of Ly49D promotes the survival and differentiation of $Ly49H^{+}$ memory NK cells [39]. Thus, in this context, it is unclear whether Ly49D behaves as a true 'Signal 2′, or a second 'Signal 1′ along with Ly49H. Similarly, NKG2D was found to amplify the proliferation of $Ly49H^{+}$ NK cells, but was unable to drive expansion of NK cells in the absence of Ly49H [40]. Conversely, $Ly49H^{+}$ NK cells that co-express the inhibitory receptors Ly49A or NKR-P1 B do not expand as well as MCMV-specific NK cells that lack expression of these inhibitory receptors [39,41], suggesting that inhibitory receptors may function as brakes to control the extent of NK cell activation and expansion. Together these studies show the importance of a co-stimulatory 'Signal 2′ for the optimal response of NK cells to MCMV infection (Fig. 2). Future studies are needed to address whether traditional co-stimulatory molecules, such as CD28 and GITR, or checkpoint molecules, such as CTLA-4 and PD-1, that are known to modulate T cell responses, also play important roles in the formation of MCMV-specific memory NK cells.

2.2.2.3. Signal 3. In addition to providing the viral antigen m157,