Generation of Natural Killer Cell Memory during Viral Infection

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Key Words

Immunological memory · Natural killer cells · Cytomegalovirus

Abstract

Immunological memory is classically regarded as an attribute of antigen-specific T and B lymphocytes of the adaptive immune system. Cells of the innate immune system, including natural killer (NK) cells, have been considered short-lived cytolytic cells that can rapidly respond against pathogens in an antigen-independent manner and then die off. However, NK cells have recently been described to possess traits of adaptive immunity, such as clonal expansion after viral antigen exposure to generate long-lived memory cells. In this review, we will discuss the current evidence for viral-induced NK cell memory in both mice and humans.

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Introduction

The ability of the immune system to rapidly respond and enhance the protection of the host against a previously encountered pathogen is defined as immunological

memory. Long-lived memory cells are generated after an initial infection and display heightened responses upon a secondary challenge with the same pathogen. The process of memory formation in T cells has been well studied and is divided into 3 distinct phases [1]. Upon exposure to cognate antigen, naive T cells clonally expand and differentiate into effector T cells during the 'expansion' phase. This first phase is followed by a second 'contraction' phase where the vast majority of effector T cells undergo apoptosis to form a small stable pool of surviving T cells that enter the third 'memory' phase. Memory T cells then persist throughout the host and maintain their longevity through self-renewal until a secondary encounter with their cognate antigen, when they exhibit enhanced proliferative and effector functions. Because cells of the innate immune system lack the ability to undergo somatic rearrangement of their receptor genes, it was hypothesized that these cells, including natural killer (NK) cells, lack antigen specificity and therefore could not form immunological memory. Work by our group and others has directly challenged this concept by establishing a view of NK cell memory that is based on viral antigen-driven proliferation through specific ligand/receptor interaction, which then generates a long-lived memory population with an enhanced ability to respond and protect against secondary viral challenges.

NK Cell Memory Formation in Response to MCMV Infection

In recent years, NK cells have been appreciated to possess a number of developmental and functional features in common with cells of the adaptive immune system. These similarities include the development from a common lymphoid progenitor cell [2], a requirement for common γ -chain-dependent cytokines (e.g. IL-2, IL-7, and IL-15) during development and homeostasis [3], expression of the recombination-activating genes (RAGs) during ontogeny [4], and an education process in the bone marrow that is analogous to T-cell development in the thymus [5, 6]. Moreover, much like their T-cell counterparts, which use the T-cell antigen receptor (TCR) to recognize antigens, NK cells also express activating receptors capable of directly binding virally derived antigens. For example, in C57BL/6 mice, the activating receptor Ly49H is expressed on ~50% of NK cells and binds the mouse cytomegalovirus (MCMV)-encoded glycoprotein m157 expressed on infected cells to drive the expansion of virus-specific NK cells during the acute phase of MCMV infection [7–11]. Using an experimental system in which Ly49H⁺ NK cells were adoptively transferred into mice lacking this receptor, our group observed a robust antigen-driven expansion of these Ly49H⁺ cells after MCMV infection [12]. Following viral clearance, expanded effector NK cells underwent a rapid contraction phase to establish a long-lived memory pool of antigen-specific cells that could be recovered many months following infection in a variety of peripheral tissues [12]. These memory NK cells displayed functional attributes commonly associated with memory T cells such as secondary expansion, enhanced effector function *ex vivo*, and increased protection against virus challenges compared to naive NK cells from uninfected mice [12]. Most importantly, the expansion and memory formation of virus-specific NK cells was dependent on the interaction with the viral antigen, as MCMV lacking m157 glycoprotein did not induce Ly49H⁺ NK cell expansion or the development of memory after infection [12]. Indeed, Lanier and Min-Oo recently demonstrated that MCMV-primed memory NK cells display a reduced ‘bystander’ functionality after heterologous infections and cytokine-induced activation in the absence of m157 antigen, suggesting that memory Ly49H⁺ NK cells become specialized for the purpose of controlling MCMV upon reexposure [13]. Additional models of NK cell memory have been described in settings where mice have been primed with haptens or proinflammatory cytokines alone [14, 15]. Together these

results demonstrated that NK cells, like CD8⁺ T cells, undergo activation, expansion, and contraction in an antigen-specific manner to generate long-lived memory cells in response to viral infection.

Mechanisms of Memory NK Cell Formation

Activation and Expansion

Recent studies have also shed light on the molecular mechanisms controlling the formation of NK cell memory in response to MCMV infection. NK cells deficient in the IL-12 receptor or STAT4 signaling do not undergo clonal proliferation and are defective in memory NK cell generation following MCMV infection [16]. In addition, signals from proinflammatory cytokines (including IL-12, IL-18, and type I IFN) are necessary and sufficient to drive the expression of the transcription factor Zbtb32, which is essential for the proliferation and protective function of antigen-specific NK cells during MCMV infection [17]. Zbtb32 acts as an important molecular checkpoint to promote a proproliferative state in activated NK cells by antagonizing the tumor suppressor factor Blimp-1 [17]. Although the precise mechanisms of how Zbtb32 antagonizes Blimp-1 function in antigen-specific NK cells remain to be elucidated, the finding that proinflammatory cytokines are essential for maximal Zbtb32 expression provides a mechanistic explanation for how and why inflammatory signals are required for the robust proliferation of antigen-specific NK cells during MCMV infection, even when high amounts of viral antigen are present [16]. This pathway in NK cells may be analogous to ‘signal 3’ in the widely accepted model of T cell activation, which hypothesizes that 3 independent and coordinated signals from the T-cell antigen receptor (signal 1), costimulatory receptors such as CD28 (signal 2), and cytokine receptors such as IFNAR and IL-12R (signal 3) are required for maximal effector function [1]. Indeed, costimulatory activating signals are also required for the proliferation of antigen-specific NK cells in the presence of antigen and proinflammatory signals because Ly49H⁺ NK cells lacking the activating receptor DNAM-1 fail to expand and form long-lived memory cells following MCMV infection [18]. Thus, the signaling requirements to drive the optimal activation and proliferation of antigen-specific NK cells are analogous to their T-cell counterparts: receptor engagement with antigen (Ly49H-m157, signal 1), costimulatory signaling (DNAM-1, signal 2), and proinflammatory cytokine signaling (IL-12, STAT4, Zbtb32, signal 3) (fig. 1). Whether antigen-spe-

cific NK cells require additional cytokine or costimulatory signals for clonal proliferation and memory formation will be interesting topics for future research.

Contraction

Induction of apoptosis in effector CD8⁺ T cells following viral clearance is an essential mechanism to prevent immune-mediated pathology by regulating the numbers of cytolytic lymphocytes [19], and therefore the contraction phase may also be a critical determinant in the development of NK cell memory in response to viral infection. During T-cell memory formation, Bcl-2 family proteins such as Bcl-2 and Bim play contrasting roles in the survival of antigen-specific effector T cells [20–22]. Similarly, effector NK cells require Bim-mediated proapoptotic signaling during the contraction phase to form a stable pool of memory cells [23]. Although it has been shown that prosurvival cytokines, such as IL-15, are required for the survival of adoptively transferred Ly49H⁺ effector NK cells [24], the protective pathways that antigen-specific NK cells use to combat apoptosis and mediate survival to form memory cells remain to be fully addressed (fig. 1). Our group has demonstrated that expression of microRNA-155 in virus-specific NK cells functions to suppress Noxa and suppressor of cytokine signaling 1 (SOCS1) during NK cell activation and expansion to enhance survival in response to MCMV infection (fig. 1) [25]. Recent studies have also demonstrated that the self-catabolic process of autophagy is required for the survival of antigen specific CD8⁺ T cells in the effector-memory transition phase in response to viral infections [26, 27]. Therefore, given the functional similarities between NK cells and CD8⁺ T cells, it will be of interest to investigate whether autophagy plays a critical role in the survival of virus-specific NK cells during the contraction phase, and to further understand the molecular mechanisms behind how autophagy mediates the formation of long-lived innate and adaptive lymphocytes.

NK Cell Memory Formation in Response to Other Viral Infections

In addition to evidence supporting NK cell memory formation during MCMV infection, several recent studies have suggested that NK cells are essential for secondary responses in other viral infections. Adoptive transfer experiments in mice have demonstrated that CXCR6⁺ hepatic NK cells primed with virus-like particles are sufficient and required for protective recall responses against

Fig. 1. Mechanisms of NK cell memory formation. During MCMV infection, naive NK cells that express the activating receptor Ly49H recognize the virally encoded glycoprotein m157 on the surface of MCMV-infected host cells, resulting in the robust activation and proliferation of antigen-specific NK cells. This process is critically dependent on proinflammatory IL-12 signaling through STAT4 and Zbtb32, and costimulatory signaling through the activating receptor DNAM-1. During clonal proliferation, antigen-specific NK cells maintain viability by increasing the expression of miR155 to antagonize the prodeath factors Noxa and SOCS1; however, at the peak of virus-driven expansion, effector NK cells undergo BIM-mediated cell death to form a stable pool of long-lived memory NK cells in a process that depends on endogenous IL-15. MCMV-elicited memory NK cells can respond robustly to a secondary challenge with MCMV but are less responsive to heterologous infection.

vesicular stomatitis virus, influenza A, and human immunodeficiency virus (HIV) [28]. Furthermore, NK cells previously exposed to herpes simplex virus 2 (HSV-2) or vaccinia virus infection display enhanced IFN- γ production and protection upon rechallenge in a process that is specific to the priming virus but independent of the adaptive immune system [29, 30]. These studies collectively support recall responses of memory NK cells in several models but are still limited by unknown interactions between NK cell receptors and cognate pathogen-encoded antigens that may be mediating these responses. It has been reported that the activating receptor Nkp46 can specifically recognize influenza hemagglutinin [31, 32], but this interaction was not reported to induce clonal expansion of NK cells in the lungs of influenza-infected mice [33]. The identification of viral antigens and their corresponding activating NK cell receptor pairs that may mediate enhanced recall responses in these models will further strengthen the concept of antigen-specific NK cell memory.

Human NK Cell Memory in Response to Viral Infections

In humans, NK cells are essential for controlling viral infections. Patients with rare genetic deficiencies resulting in diminished NK cell numbers or function have an increased susceptibility to infection with the herpesvirus family [Epstein-Barr virus (EBV), herpes simplex virus, human cytomegalovirus (HCMV), varicella zoster virus, and human papillomavirus] [34]. Interestingly, NK cells expressing the activating CD94-NKG2C receptor are found at a higher frequency in HCMV-seropositive healthy individuals compared to HCMV-seronegative individuals [35, 36]. Although the viral (or host) ligand induced by HCMV remains unknown, virally induced HLA-E has been shown to be critical for triggering expansion of NKG2C+ NK cells in response to HCMV [37]. Furthermore, NKG2C is likely important in the recognition of HCMV because human NKG2C+ NK cells robustly expand in allogeneic transplant patients during acute HCMV infection [35, 38–40]. NKG2C and the maturation marker CD57 are also expressed at high levels in a unique subset of NK cells that remain at an increased percentage in HCMV-seropositive individuals and further increase after HCMV reactivation [35, 38, 41]. In addition, evidence of epigenetic ‘imprinting’ was found at the IFN- γ loci in NKG2C+ NK cells following HCMV exposure that resembles CD8+ memory T cells and Th1 cells [42], suggesting a mechanism behind the higher IFN- γ production by memory NK cells. In humans with a deletion in NKG2C, NK cell differentiation was compromised during HCMV infection, resulting in an altered adaptive immunity and defective control of the virus [43, 44], demonstrating the importance of this receptor in defense against HCMV.

NKG2C+ NK cells have also been observed to expand in HCMV-seropositive patients with hepatitis C virus (HCV), hepatitis B virus (HBV), or HIV [36, 45, 46], and can rapidly proliferate following hantavirus infection to persist for over a year [47], resembling the kinetics of Ly49H+ NK cells following MCMV infection. Similar to the Ly49H+ NK cell response against MCMV infection, IL-12 (produced by inflammatory CD14+ monocytes) was implicated as a critical factor in driving the differentiation and prolific expansion of NKG2C+ NK cells through induction of CD25 in response to HCMV infection [37]. Furthermore, increased frequencies of NKG2C+ NK cells have been reported in the peripheral blood of HCMV+ children coinfecting with EBV compared to HCMV+ children [48]. However, a recent longi-

tudinal analysis of EBV students showed that acute EBV infection did not cause an expansion of peripheral NKG2C+ NK cells regardless of the previous infection status with HCMV, suggesting that the expansion of NKG2C+ NK cells may be specific to HCMV infection rather than a generalized response to acute herpesvirus family infections [49]. Understanding the precise mechanisms underlying the expansion and persistence of these human memory NK cells could enhance the efficacy of vaccine design against HCMV, hepatitis virus, and HIV.

Concluding Remarks

We have reviewed the current evidence for virally induced memory NK cells in both mice and humans that has recently been described in the literature. These studies collectively support a model in which NK cells display virus-specific expansion to form long-lived memory cells that exhibit specific functional recall responses. Although our group and others have identified several important pathways driving the clonal proliferation of virus-specific NK cells during MCMV infection, the molecular mechanisms that govern the survival of effector NK cells during the effector-memory transition remain largely unknown. It will also be of interest to investigate whether NK cells, like CD8+ T cells, utilize self-catabolic processes such as autophagy to mediate cell survival during the contraction phase and to understand the metabolic processes utilized by NK cells during distinct phases of the response to viral infection. Future studies are needed to address other types of activating receptor-ligand driven memory NK cell formation during viral infections and whether memory NK cells can be identified by unique cell surface markers to eliminate the need for adoptive transfer model systems. Finally, both mouse and human studies are required to determine whether the NK cell compartment can be harnessed in immunization strategies against viral pathogens where no vaccine or cure currently exists.

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Disclosure Statement

The authors declare no financial conflicts of interest.

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