

Application of Memory Natural Killer Cells in HIV Vaccine

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Abstract. Human immunodeficiency virus (HIV) is a formidable infection targeting the intricate defense mechanisms of the human body, primarily focusing its assault on the vital white blood cells known as CD4 cells. HIV impairs these CD4 cells, reducing resistance to opportunistic infections. In 2022, 630,000 people died from HIV-related causes globally due to the lack of preventative vaccines and effective treatment. The pursuit of an effective HIV vaccine remains a critical endeavor in global health, necessitating innovative strategies that can elicit robust and durable immune responses. Natural killer (NK) cells, renowned for their swift and inherent cytotoxic prowess, constitute a critical element of innate immunity, typically thought to be deficient in memory abilities. Fascinatingly, NK cells have now become the focal point of scientific research due to emerging evidence suggesting the presence of memory-like characteristics within their functional repertoire. Through a comprehensive exploration of NK cell subpopulations and the strategic application of cytokines or other stimuli to induce the development of memory-generating NK cell subpopulations, the potential for enhancing the efficacy of HIV vaccines emerges. This approach holds promise in bolstering the creation of adaptive immunity and advancing the quest for effective HIV immunization strategies. The study offers valuable insights into the potential of NK memory cells as a viable component of future HIV vaccine regimens, suggesting avenues for further research and development in the quest to mitigate the global burden of HIV.

Keywords: HIV; vaccine; NK cells; NK memory cells.

1. Introduction

According to data from WHO, approximately 39.0 million individuals were living with HIV in 2022, with 1.3 million new HIV infections reported during the same year. The ongoing quest for an effective vaccine against human immunodeficiency virus (HIV) has proven to be one of the most challenging endeavors in the field of infectious diseases. Despite decades of dedicated research, the development of a preventative vaccine against HIV remains an elusive goal.

Existing vaccines primarily target adaptive immunity and trigger B, and T cells to have antigen-specific memory against pathogens. These vaccines have faced substantial hurdles due to the remarkable variability of HIV's envelope glycoprotein and the virus's ability to rapidly mutate. Additionally, these vaccines are also limited by the intensity of adaptive immune responses and the duration for which memory cells persist within the immune repertoire. These limitations have underscored the urgency of exploring alternative avenues that harness the full potential of the immune system.

In recent years, the scientific community has increasingly emphasized the innate aspect of the immune system, with particular emphasis on natural killer (NK) cells. NK cells provide immediate, non-specific/specific responses to infections (Fig 1) [1]. Traditionally viewed as key players in innate immunity, NK cells have now garnered attention for their potential to exhibit memory-like properties, thereby holding significant promise for enhancing the efficacy of HIV vaccine strategies. Remarkably, recent research has illuminated the memory properties in NK cells specifically in the context of exposure to HIV contraction.

By examining mechanisms underlying the differentiation of NK cell subpopulations capable of generating immunological memory, this study aims to shed light on how these cells could be harnessed to create more potent and sustained immune responses against HIV. These findings have raised the exciting prospect of leveraging NK cell memory to revolutionize the landscape of HIV vaccine development.

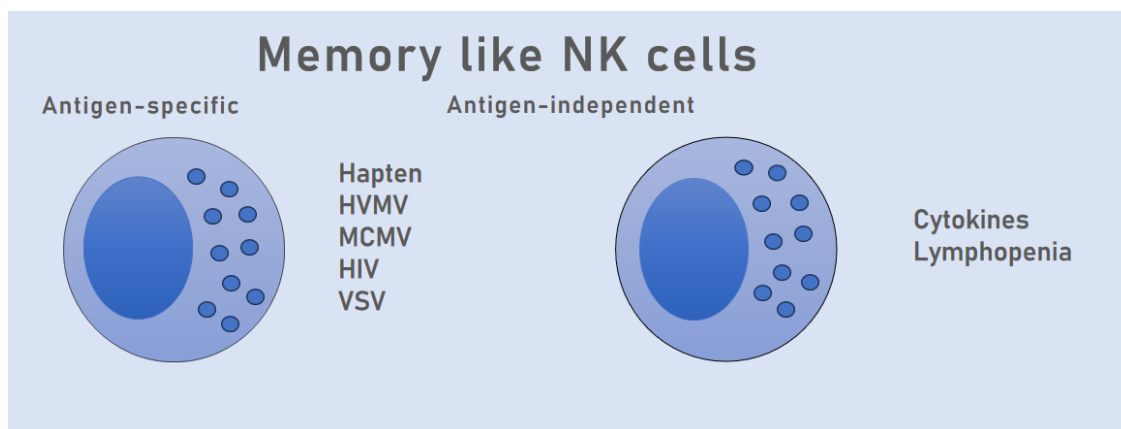


Fig 1. Categories of Memory like NK cells.

2. Rationale for the memory property of NK cells

NK cells, not relying on recombination activation genes (*RAG*), are considered without memory property for generating antigen receptors independently. However, recent cancer immunotherapy research reveals NK cells exhibiting memory-like traits in response to specific stimuli, sparking increased researcher interest.

2.1. NK memory like cells in mice and macaques

The earliest research found that transient cytokine stimulation of murine cells using interleukin IL-12, IL-15, and IL-18, leads to intensified IFN- γ production and generation during subsequent reactivation after several weeks. The augmented functional capabilities also showed in their progeny [2]. Later other researchers found NK cells pre-activated with cytokines have a notable and impactful antitumor impact when transferred into mice afflicted with lymphoma, melanoma, or ovarian cancer [3, 4]. Researchers found NK cells pre-activated beyond a span of 48 hours have down-regulated expression of inhibitory Killer-cell Immunoglobulin-like Receptors (*KIR*) [5].

This could elucidate the strong activation of NK cells upon re-stimulation.

In mouse experiments, Ly49H-bearing NK cells, when infected with MCMV, displayed CD8⁺ T cell-like traits [6]. They share antigen specificity, clonal expansion, subsequent contraction, memory formation, and the ability to trigger recall responses. The NK cell memory-like quality stems from the interaction of the Ly49H receptor with the virus-encoded m157 protein [7]. Furthermore, a study conducted on macaques revealed that splenic and hepatic NK cells from Ad26-vaccinated subjects proficiently initiated lysis of targets with congruent antigens, while demonstrating limited efficacy against mismatched antigens even five years following vaccination [8]. Collectively, these empirical instances substantiate the notion that memory properties are true in NK cells.

2.2. Physiologic mechanisms underlying the memory traits of NK cells.

The human *KIR* demonstrates expression within specific factions of NK cells and certain T cell subtypes, predominantly encompassing CD8⁺ T cells and a subset of CD4⁺ T cells. *KIR* governs NK cell activity, distinguishing healthy from infected/cancerous cells via MHC class I. Altered MHC class I triggers NK cell activation if the activation signal dominates inhibition. *KIR* gene expression, once established in a T cell or an NK cell, persists through successive generations of these cellular progenies. NK cell *KIR* expression varies in distinct subsets and memory T cells via stochastic mechanisms. An individual's receptor repertoire is molded by subtle factors tied to their MHC class I haplotype, potentially granting *KIR*-expressing NK cells the capability for specific antigen recognition and memory-like attributes.

Moreover, NK cells share similarities with B and CD8⁺ T cells (Table 1). They originate from the common lymphoid progenitor in the bone marrow. NK cells and CD8⁺ T cells both rely on cytokine

signals via the common receptor gamma-chain (IL-2R γ) family for viability and balance. In infections, both NK cells and CD8⁺ T cells activate via antigen-specific receptors, spurred by inflammatory cytokines like IL-12 and type I IFNs, leading to substantial IFN- γ production. These findings provide evidence for the notion that NK cells exhibit developmental and homeostatic features akin to adaptive lymphocytes.

Table 1. Competencies of Natural Killer Memory Cell.

Capabilities	Innate immune response	Adaptive immune response	Antigen-specific NK memory cell	Antigen-independent NK memory cell
rapid	Yes	No	Yes	Yes
Indiscriminate reaction to cytokines	Yes	No	Yes	Yes
Antigen-specific response	No	No	Yes	No
Clonal expansion	No	Yes	Yes	Yes
Long-lived progeny	No	Yes	Yes	Yes
Immune reaction upon encountering the same pathogen again	No	Yes	No	Yes

3. Feasibility of NK cells combined with T cells in HIV vaccine manufacturing.

3.1. Problems with vaccines targeting T cells.

Developing an HIV vaccine is tough due to the virus's rapid mutation and evasion of the immune system, with efforts focusing on stimulating T-cell responses. However, this approach faces challenges, including HIV's genetic variability, weak T cell responses, difficulty targeting key viral sites, immune activation control, and limited protection and durability. Since T cell is antigen-specific, HIV is known for its high genetic variability and rapid mutation rate. This diversity allows the virus to constantly change its surface proteins (especially the envelope glycoprotein, gp120/gp41), making it difficult for the immune system to generate a lasting T-cell response.

HIV has developed a lot of methods to avoid detection by the immune system, including generating mutations in its genetic material. T-cell responses that target one part of the virus may lead to the emergence of escape mutants that are not detected by immunity. Additionally, HIV could establish hidden latent infections in CD4⁺ T cells, creating enduring HIV reservoirs. These latent reservoirs often elude T-cell responses, enabling the virus to persist even if other immune responses succeed. Research consensus shows that treated individuals primarily have latently infected memory T cells [9], challenging traditional secondary immunity approaches. Consequently, NK cells are explored for HIV vaccine feasibility due to these hurdles.

3.2. The effectiveness of NK cell vaccines

Compared with adaptive immune cells, NK cells have numerous advantages. During the early stage of an infection caused by a virus, the proliferative rate of cytotoxic CD56^{low} NK cells surpasses that of CD8⁺ T cells. Through activation triggered by IL-2 and IL-15, NK cells can significantly hinder the entry of a virus into CD4⁺ T cells by stimulating the production of β -chemokines, thus effectively curtailing the dissemination of HIV-1. Additionally, Research demonstrates that NK cells that have been prepped through CMV exhibit distinctive epigenetic signatures [10]. This phenomenon exemplifies the capacity for NK cells to undergo programming when encountering pathogens (or conceivably, vaccines). Infections, according to these findings, have the capacity to affect the variety of NK cells and influence their subsequent responses, thus implying that vaccines could be used to enhance NK cell cytotoxicity against pathogens.

In the realm of HIV vaccine research, the literature underscores the vital significance of antigen-dependent cell-mediated cytotoxicity (ADCC) against pathogens. By involving non-neutralizing

antibodies, NK cells are primarily responsible for this cytotoxicity. This pathway indicates the critical function that NK cells have in immunology for HIV, and it also emerges as a pivotal route toward eliciting safeguarding vaccine reactions. Furthermore, levels of plasma HIV RNA and the advancement of AIDS can be largely impacted by NK cells along with specific receptors known as KIR according to compelling genetic evidence. This experimental evidence suggests vaccines targeted at NK cells are promising in HIV immunology. A study assessed the capacity of NK cells to react in a manner reliant on antibodies when they encounter T cells from different donors bearing HIV-1 antigens. The findings demonstrated a strong activation of NK cells through an antibody-mediated response [11]. Additionally, the tested target cells exhibited susceptibility to antibody-dependent cytotoxicity, underscoring the potency of this immune mechanism.

A study conducted a comparative analysis of NK cell-mediated ADCC between individuals who were infected with SARS-CoV-2 and those who had been vaccinated. The research revealed that both antibodies generated by natural SARS-CoV-2 infection and those induced by anti-SARS-CoV-2 vaccines have the potential to provoke noteworthy levels of NK cell-mediated ADCC activity. Furthermore, the administration of the BNT162b2 vaccine was observed to elicit the production of exceptionally potent antibodies, which, in turn, facilitated robust activation of NK cells [12]. The finding suggests that a similar approach could be explored for an HIV vaccine. In the context of HIV, activating NK cells through vaccine-induced antibodies might contribute to better control of the virus and infected cells.

3.3. Utilizing NK cells to boost HIV vaccines.

3.3.1. The subtypes of NK cells

The extensive variety of NK cell receptors suggests a broad spectrum of potential subgroups within the NK cell population. NK cells can be categorized into distinct subtypes based on their surface markers and functional characteristics. There are two main subtypes of NK cells: CD56^{bright} and CD56^{dim} NK cells. CD56^{bright} NK cells are characterized by high expression of CD56 and low or absent expression of CD16. This subtype participates in the generation of cytokines, particularly IFN- γ and other immune regulatory molecules. They are important for promoting immune responses, modulating inflammation, and supporting adaptive immunity. They also interact with dendritic cells and T cells to enhance the overall immune response. When compared to CD56^{bright} NK cells, CD56^{dim} NK cells produce more CD16 and less CD56. The primary role of CD56^{dim} NK cells lies in their cytotoxic capabilities, encompassing their capacity to directly eliminate target cells.

3.3.2. Possible directions for improving vaccines: selecting NK cell subsets.

Given the diverse categorization of NK cells, with certain subsets exhibiting the potential to enhance adaptive immunity and certain subsets having the property of memory like B cell or T cell, a prospective approach for enhancing vaccine efficacy involves the deliberate identification and selection of distinct NK cell subsets that wield favorable influence on adaptive immunity.

The CD56^{bright} subset of NK cells holds significant clinical significance due to its unique response to minimal IL-2 doses. Selective expansion of this effective lymphocyte group in HIV-infected individuals could be induced by a minimal amount of IL-2 cytokine. Notably, the expanded CD56^{bright} NK-cell population demonstrates the favorable expression of CD16. CD16 on NK cells binds to exposed Fc regions, bridging the NK cell and another cell. This engagement results in the production of cytotoxic granules encompassing perforin and granzymes, which induce apoptosis (programmed cell death) in the target cell [13]. Thus, IL-2 cytokine-induced expansion enhances their ability to mediate ADCC effectively.

The experiment indicates that the educated KIR3DL1+ NK cell subset holds promises for use in an HIV vaccine [14]. Their ability to respond in an antibody-dependent manner to HIV antigens, robust activation, and selective targeting of HIV-infected cells make them a potentially valuable component of an integrated HIV vaccine strategy. However, further research, including clinical trials,

is needed to fully evaluate and exploit this specific NK cell subset within the realm of HIV vaccine development.

One subtype of NK cells known as single-self-KIR+NKG2C+ adaptive NK cells (ADAPT-NK) cells were shown to be effective at targeting the "missing self". Studies showed that ADAPT-NK cells exhibit potent cytotoxicity without signs of depletion against tumor cell lines. When ADAPT-NK cells were combined with a particular molecule (anti-CD16/IL-15/anti-CD33 tri-specific engager), they almost completely wiped out the drug resistance of CD45dim blast subtypes [15].

3.3.3. Optimization of NK memory cell line in HIV vaccines

Optimizing NK memory cells in HIV vaccines would be a method worth trying. For pathogens like HIV that can diminish the quantity and responsiveness of T cells, generating a substantial population of memory NK cells through vaccination could potentially curb the proliferation and dissemination of HIV, thereby effectively managing the virus. Utilizing NK memory cells combined with conventional methods could enhance vaccines in many aspects: enhanced long-term immunity, broadened immune response, and improved response in immunocompromised individuals.

There are several reasonable strategies to optimize the memory capabilities of NK cells in HIV vaccines: induction memory-like NK cells. Investigate strategies to induce or enhance adaptive capabilities in NK cells, such as long-lasting functional changes that allow them to respond more effectively upon re-exposure to HIV antigens. Researchers have now found several cytokines combinations could improve the function of memory-like NK cells [2].

Formulation of the vaccine and strategy to present the antigen. Develop vaccine formulations and antigen presentation strategies that favor the generation of NK memory cells. This could involve optimizing the presentation of HIV antigens to NK cells and utilizing adjuvants that promote memory cell formation.

Epigenetic modification of NK memory cells. Exploring epigenetic modifications or signaling pathways as potential targets to enhance the generation of NK memory cells. Epigenetic changes might play a role in establishing long-lasting functional changes in NK cells. CAR-NK cells would be a promising research realm. Endeavors have been made to direct CAR-NK cells towards HIV-infected CD4+ T cells. Findings demonstrated that under in vitro conditions, CAR-NK cells can restrain the replication of HIV within CD4+ T cells [16]. The result shows the possibility of genetically modifying NK cells to enhance cytotoxicity.

Figure out the mechanism of NK memory cell maintenance. Study the factors that contribute to the maintenance and survival of NK memory cells over time. This could involve identifying cytokine signaling pathways, metabolic requirements, or other mechanisms that sustain memory-like properties. Additionally, investigate how aging affects the development and maintenance of NK memory cells, as older individuals might have altered NK cell function and responses.

Nonetheless, it's crucial to emphasize that the notion of NK memory cells remains a subject of ongoing investigation, and there are several engineering challenges and considerations that would need to be addressed before this approach could be fully realized. As our comprehension of the physiological functions and characteristics of memory NK cells deepens and vaccine engineering advances, it is believed that memory NK cells can have an impactful impact on HIV vaccine strategy development.

4. Discussion

NK cell memory continues to be a subject of controversy within current research. Research has uncovered the following factors attributed to NK cell memory trait. Firstly, cytokine-mediated signaling emerges as a pivotal determinant in the establishment and sustenance of memory-like properties in NK cells. Secondly, the intricate network of receptors is fundamental to the memory behavior exhibited by NK cells. Inhibitory and activating receptors interact dynamically, creating a finely tuned-recognition system. Initial engagement triggers activating receptors, initiating effector functions, which boosts receptor upregulation and priming, leading to stronger effector responses

upon re-stimulation. Thirdly, epigenetic modifications, encompassing DNA methylation and histone remodeling, constitute an additional dimension implicated in NK cell memory. These modifications bestow stable alterations upon the genetic landscape of NK cells, conferring nuanced transcriptional profiles conducive to augmented responsiveness upon re-challenge, for example IRF8 gene in MCMV infections. Besides, metabolic reprogramming emerges as a contributory determinant in the memory-like attributes of NK cells. Substantial alterations in metabolic pathways, notably glycolysis and oxidative phosphorylation, are instrumental in shaping NK cell function and memory. Notably, the metabolic configuration of memory-like NK cells may be distinct, thus substantiating their heightened and sustained effector potential upon re-engagement.

5. Conclusion

In the context of HIV eradication, current antiretroviral therapy (ART) falls short due to its inability to target latent HIV residing within T cells. Moreover, preventative HIV vaccines meet obstacles like variability and mutation of HIV, weak and ineffective T cell responses, inadequate targeting of key viral sites, and limited durability. This paper provides a comprehensive analysis of experimental evidence for memory properties in NK cells, delves into the mechanisms underlying the differentiation of NK memory cells, and sheds light on their potential as a pivotal component in enhancing the effectiveness of an HIV vaccine. By leveraging cytokine stimulation, CD16 culture, or other stimuli, it may drive the differentiation of NK cells into subpopulations conducive to adaptive immunity, such as memory NK cells, and ADAPT-NK. By capitalizing on NK cells' memory-like attributes and their ability to bridge innate and adaptive immunity, researchers have advanced strategies against HIV. NK memory cells in HIV vaccine development hold promising potential, building upon breakthroughs in understanding NK cell biology and its memory-like properties. There are some valid directions to develop an HIV vaccine in a manner that targets NK cells: 1) Using specific stimuli to promote and select NK cell subsets that are more conducive to developing memory NK cells. 2) Develop vaccine formulation and antigen presentation strategy that favor the generation of NK memory cells and induce robust immune response. 3) Genetically engineered and edited CAR-NK cells have the potential to augment the efficacy of NK cells in the production of HIV vaccines.

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