REVIEW ARTICLE

Utilizing the Anti-leukemic Potential of Natural Killer Cells

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ABSTRACT

Acute myeloid leukemia (AML) is a rapidly growing, life-threatening subtype of blood cancer that mostly affects adults and requires hematopoietic stem cell transplantation (HSCT) as a treatment modality. Strategies such as natural killer (NK) cell-based therapy have been developed to maximize the AML and HSCT immunotherapeutic approach, harnessing the anti-leukemic immune response and graft versus leukemia (GvL) effect. A previous study has demonstrated that human cytomegalovirus (HCMV) reactivation in allogeneic HSCT patients caused a reduction in AML relapse or recurrence of disease, attributed to the killing activity of the adaptive NK cells that express $CD56^{dim}$NKG-$2C^{+}CD57^{+}$. Nonetheless, the positive influence of these adaptive NK cells in individuals with high HCMV burden, particularly in AML and HSCT patients, remains uncertain due to insufficient grasp of the receptors and ligands responsible for their anti-tumor actions. In this review, we explore potential utilization of the adaptive or 'memory-like' NK cells in acute leukemia which could potentially be harnessed for anti-leukemia purposes. The understanding will be valuable for the formulation of adoptive immunotherapy strategies, including the development of chimeric antigen receptor (CAR) NK cells possessing the adaptive or 'memory-like' characteristic.
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INTRODUCTION

Leukemia is a type of blood cancer that accounts for a significant burden of morbidity and mortality worldwide. Leukemia is classified into four different types: either acute or chronic, lymphoid, or myeloid (1,2). Among the subtypes of leukemia, acute myeloid leukemia (AML) is a particularly aggressive form that rapidly grows and affects the myeloid progenitor cells, with ability to migrate and invade the surrounding environment (3,4). The incidences of acute leukemias are reported to be about 20% in children and 33% in adolescents (5). AML is also a blood malignancy characterized by the high abnormalities of bone marrow myeloid cells, which fail to mature properly and leading to decrease average blood cell production and the onset of debilitating symptoms. AML can be a highly aggressive cancer with a poor prognosis if left untreated even though this condition is relatively rare (6).

A key therapy for AML involves hematopoietic stem cell transplantation (HSCT), commonly known as a bone marrow transplant (BMT). This procedure necessitates the use of immunosuppressive medications to prevent rejection of the transplanted cells by the patient's immune system. However, this immunosuppression leaves the patient vulnerable to infections, which can pose significant risks, potentially even threatening life (7). In the transplant setting, innate lymphoid cells can be used to harness the anti-leukemic immune response, by promoting graft versus leukemia (GvL) activity, and maximizing the treatment of AML. The administration of stem cells to the patient is also planned as a method to replace damaged or unhealthy cells, serving as one of the strategies within immunotherapy (8).

Over four decades ago, researchers discovered a population of naturally non-T lymphoid cells that can quickly destroy virally infected and tumor cells. This group of innate cells are the natural killer (NK) cells, which can be utilized to recognize a distinct mechanism of tumor blasts, primarily through a complex network of stimulatory activating and inhibitory receptors

(9,10). Remarkably, NK cell receptors also can detect a particular profile of ligands associated with oncogenic transformation expressed in nearby the tumor environment. If a tumor-associated profile is identified, NK cells will be activated, leading to the targeted destruction of the affected and targeted cancer blasts. This unique capacity to recognize and eliminate tumor and limited reactivity towards healthy tissues suggest that NK cell may be an attractive candidate for use as promising 'living drugs' in anti-cancer therapy (11,12).

NK cells were first discovered in 1970s (13,14) and since then there are numerous interests from the immunologists to understand their roles in immunity towards cancer and viruses (15,16). NK cells are the lymphocytes from similar progenitor as T- and B-cells, classified into group I innate lymphocytes (ILC) and named after their 'natural' killing activity (17). Unlike T-lymphocytes, NK cells lack surface T-cell receptors (TCRs) and do not depend on recognition by human leukocyte antigen (HLA) molecules. Consequently, they do not pose a risk of inducing graft versus host disease (GvHD). Therefore, they present a hopeful 'off-the-shelf' cell therapy product that can be pre-prepared, refined, and administered as required to numerous patients (18,19). Traditionally, NK cells are known to be part of innate immunity but there are numerous evidences showing that these cells may possess adaptive or 'memory-like' properties, by mechanism which ruled by genetic and functional characteristics, in relation to NKG2C$^+$ marker expression and human cytomegalovirus or HCMV (20,21).

Several clinical applications have been widely explored to expand the anti-tumor cytotoxic activity of NKG2C$^+$ NK cells for immunotherapeutic strategies, associated with HCMV infection or reactivation (22–25). This group of NK cells also can be induced by cytokines such as IL-12, IL-15 and IL-18 (26,27). Nonetheless, the significance of these adaptive or 'memory-like' NK cell, expressing high NKG2C+ marker as novel therapeutic approach particularly in a population with high HCMV burden and prevalence are not totally explored. It is estimated that HCMV prevalence is higher in Eastern countries as compared to the western population, with less advanced therapeutic modalities in low socioeconomic countries (28).

In recent years, NK cell-based therapies have shown great potential in treating various cancers either blood or solid, particularly in AML (29). The mechanisms by which NK cells recognize and eliminate cancer cells are not fully understood, hampers the development of more effective therapies with the patient's compromised immune system (30,31). The potential of NK cells as a therapeutic agent has been further strengthened by the recent advancements in clinical-grade methods that enable the production of large quantities of NK cells from various sources, including peripheral blood, umbilical cord blood, and induced pluripotent stem cell (iPSC) (32,33). These innovative platforms have paved the way for developing multiple strategies to enhance the activation of NK cells, increase their proliferation *in vivo* and *ex vivo*, and augment their ability to recognize and target cancer cells. As a result, a wide range of NK cell-based therapy approaches can now be explored for clinical use (34–36).

In this review, we explore a series of overviews on the HCMV-driven adaptive or 'memory-like' NK cells that express a unique set of surface protein receptors and ligands contributing to their anti-leukemic activity in the potential treatment specifically on AML. We aim to analyze the mechanism involved in the anti-tumor activities of the adaptive or 'memory-like' NK cells in adoptive NK cell therapy, chimeric antigen receptor (CAR) NK cell therapy, cytokine enhancement, combination therapies and reduced intensity conditioning.

Gaining insights into acute myeloid leukemia

AML is, indeed, a form of cancer that impacts bone marrow and blood (37), characterized by the abnormal development of myeloid cells, resulting in the accumulation of non-functional cells called blasts (Figure 1) (38). These blasts can accumulate in the bone marrow and quickly spread to the bloodstream, as well as other organs such as the spleen, lymph nodes, liver, and central nervous system (39). AML is diagnosed through various tests, including immunophenotyping, biopsy, cytochemistry, and imaging and is primarily caused by genetic mutations, chromosome translocations, and deletions (40,41). The bone marrow microenvironment (BMM) also plays a crucial role in AML pathogenesis and in the development of resistance to chemotherapy (42). Research findings have indicated that the aberrant microenvironment observed in leukemia is, to some extent, a result of the leukemic cell proliferation that interferes with the normal niches of hematopoietic progenitor cells in the bone marrow (43,44).

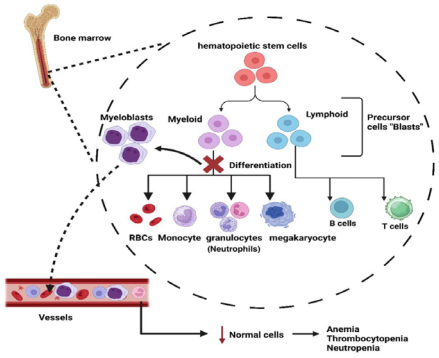

Figure 1: AML arises due to a cascade of genetic alterations occurring in a hematopoietic precursor cell, leading to a disruption in the cell differentiation process. These genetic changes interfere with the average growth and maturation of hematopoietic cells, leading to an excessive accumulation of abnormal, immature myeloid cells in both the bone marrow and the peripheral blood.

Nonetheless, a study has provided evidence suggesting that diminishing numbers of hematopoietic stem cells

may indicate the persistence of leukemic stem cells and the subsequent occurrence of leukemic relapse (45). Recently, another research investigation demonstrated that exposure to supernatants derived from AML inhibited the cell proliferation, cell cycling, colony formation, and differentiation of healthy $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) by expression of Transforming growth factor beta 1 or TGF-β1 (46).

According to the American Cancer Society, projections for leukemia in the United States in 2023 is estimated to be approximately 59,610 new cases, and 23,710 leukemia-related deaths. Of these cases, around 20,380 are expected to be new cases, with the majority occurring in adults. Additionally, it is anticipated that there will be about 11,310 deaths attributed explicitly to AML, almost exclusively affecting adults (47). In Malaysia, leukemia caused the 6^{th} most common cancers with 4,273 cases as reported by National Cancer Registry (2019) (48). When it comes to the management of AML, it is imperative to tackle this substantial healthcare issue as such using effective strategies that typically involve a combination of chemotherapy, targeted therapy, and stem cell transplantation (49). It is worth noting that ongoing research aims to develop new therapies and improve outcomes for patients with AML. However, the treatment of AML entails two separate stages; the initial phase being remission induction therapy, which is designed to eradicate leukemia cells from the bloodstream and bone marrow (50). The primary objective of this phase is to achieve remission, wherein the leukemia is no longer detectable. Following the successful remission induction, the second phase, known as post remission therapy or remission continuation therapy, begins (51). The purpose of post-remission therapy is to target remaining aberrant cells that could be dormant but possess the capacity to cause a relapse and progress the disease. (52). This comprehensive approach aims to eradicate residual leukemia cells and minimize the risk of recurrence.

NK cell characterization and function

Natural Killer (NK) cells constitute a subset of lymphocytes that play a critical role in the immune system's defense against infected or transformed cells (53), characterized by their ability to identify and eradicate target cells without prior sensitization making them part of the innate immune response (54). NK cells are a type of cytotoxic lymphocyte that can directly kill target cells through the release of cytotoxic granules or by inducing apoptosis (55). They are often recognized from other lymphocytes, such as T-cells and B-cells, by the presence of specific surface markers, including neural adhesion molecule (NCAM) or CD56, and CD16 (FcγRIIIa), with the lack of TCRs or immunoglobulins (Igs) (56,57). NK cells also characterized by their $CD56^{bright}$ or $CD56^{dim}$ expressions (58) and in some cases, they lack of CD56 (or $CD56^{negative}$/$CD56^-$) expression (59,60).

NK cells have the capability to distinguish healthy cells from abnormal or stressed cells, including virus-infected cells and tumors (61). This recognition is intermediated through a balance on the surface NK cells activating and inhibitory receptors (62). These receptors, such as NKG2D (natural killer group 2, member D) and DNAM-1 (DNAX accessory molecule-1, CD226), recognize ligands expressed on target cells, often induced during cellular stress or infection (63) and activation of these receptors' triggers NK cell activation and cytotoxicity. NK cells also express inhibitory receptors, including killer cell immunoglobulin-like receptors (KIRs) and CD94-NKG2A, which recognize major histocompatibility complex class I (MHC-I) molecules on target cells (64). When these inhibitory receptors engage their ligands, they suppress NK cell activation and prevent the killing of healthy cells (65). Once activated, NK cells exert their

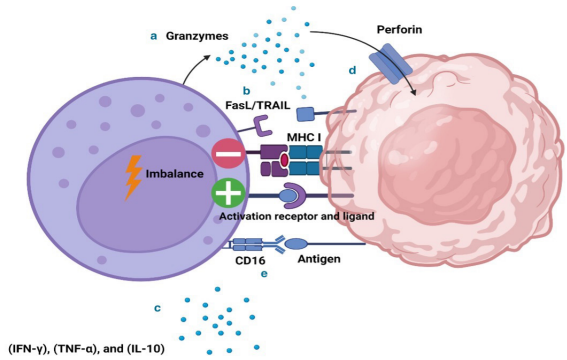

Figure 2: Explains various killing mechanisms of NK cells to eliminate target cells upon the formation of an immunological synapse.

cytotoxic function through various mechanisms such as below (Figure 2).

a) Release of cytotoxic granules: NK cells contain specialized granules called lytic granules or cytotoxic granules containing perforin and granzymes (66). Perforin forms pores in the recognized cell membrane, facilitating the access of granzymes. Granzymes then activate caspases, leading to target cell apoptosis (67).

b) Death receptor-mediated pathway: NK cells can also express death receptors, such as Fas ligand (FasL) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which can induce apoptosis in target cells by engaging their respective receptors (55).

c) Production of cytokines: NK cells are an effective source of cytokines, including interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin-10 (IL-10). These cytokines modulate the immune response by enhancing antigen presentation, activating other immune cells, and promoting inflammation (68).

d) Immunoregulation: NK cells play a fundamental role in regulating the immune response by interrelating with other immune cells, such as dendritic cells, macrophages, and T-cells (69). NK cells cause the adaptive immune response through cytokine production, direct cytotoxicity, and crosstalk with antigen-presenting cells.

e) Antibody-dependent cellular cytotoxicity (ADCC): NK cells express CD16 (FcγRIIIa), which can bind to the Fc region of antibodies bound to target cells (70). This interaction allows NK cells to recognize and kill antibody-coated target cells, enhancing the immune response against pathogens and facilitating the efficacy of antibody-based therapies (71).

Adaptive or 'memory-like' NK cells

NK cells that are capable of mounting adaptive immune responses are referred to as adaptive NK cells, and the controversial 'memory-like' NK cells, or 'memory' NK cells (21). Adaptive NK cells have been demonstrated to improve effector functions and cytotoxic response with re-exposure to particular antigens, in contrast to the conventional NK cells, which are thought of as a constituent of the innate immune system and do not exhibit 'memory-like' activity (72). Like conventional antigen recognition in the adaptive immune system (T- and B-cells), the adaptive NK cells have the ability to identify specific antigens mainly with specificity on tumor antigens or certain viral antigenic epitopes, undertake clonal expansion, and develop into long-lived NK cells (73,74). Consequently, they can 'remember' the previous interactions and respond more forcefully and quickly after being exposed to similar antigen, produce more cytokines and are also more cytotoxic (75,76).

Adaptive NK cells frequently express distinct cell surface markers or receptors. These markers allow for the identification and differentiation of these NK cells from other subsets, in which responses are observed in both humans and non-human primates (77). Stary and Stary, (2020) have identified three distinct adaptive NK cell responses: including (I) the enduring long-lasting memory of numerous distinct haptens and viral antigens in NK cells within murine liver tissues, which suggests the potential existence of a comparable phenomenon in humans, (II) infection of CMV that linked to the increase of NKG2C$^+$ NK cells, and expansion of Ly49H$^+$ NK cells in mouse, upon CMV peptide recognition and also (III) restimulation of NK cells that derived from cytokines which exhibit augmented production of IFN-γ (25). Interestingly, stimulation of adaptive NK cells with cytokines exhibits similarities to the educated immunity observed in myeloid cells (27).

Adaptive NK cell roles in cancer immunotherapy

NK cells play a significant and notable part in immune surveillance against blood cancer and leukemia (78). NK cells as part of the innate immune system, and with their ability to detect and destroy cancer cells without prior sensitization are the important players in the immune response against this hematological malignancy (11,79–81). NK cells have been comprehensively studied in the context of AML, which they play a crucial role in immune surveillance against AML by identifying and eliminating abnormal leukemic cells. However, the interaction between NK cells and AML can be complex due to various factors that influence the immune response. In terms of functionality, NK cells respond differently to leukemic targets, demonstrated a higher capacity to recognize lymphoid leukemia as compared to myeloid leukemia (82).

In the setting of HSCT, adaptive NK cells are known to be produced in response to viral infection, particularly those express NKG2C$^+$, and have been linked to a protective effect against leukemia recurrence. Studies have revealed that transplant recipients who display an increase in adaptive NKG2C$^+$ NK cells experience significantly lower rates of leukemia relapse (83–85). AML is the only leukemia for which the direct function of CD94/NKG2C-HLA-E interaction in killing leukemic cells is conceivable; as in ALL, the cells often express low amounts of HLA-E (86,87). However, in donor/recipient pairings with a mismatch of KIR who undergoing haploidentical HSCT, the cytotoxicity abilities of adaptive NK cells can also be dependent on alloreactive KIR$^+$ NKG2A$^-$ NK cells stimulated by HCMV (88). Moreover, considering the efficacy of adaptive NK cells in mediating ADCC, significant benefits can be achieved in HSCT patients who have a substantial number of this subset by using immune engagers that enhance the response against leukemic blasts (89–92). This approach involves utilizing therapeutic agents that bridge NK cells to leukemic cells via specific antibodies, thereby triggering NK cell-mediated killing of the leukemic blasts (93).

NK cells with their activity and functionality have been linked with a reduced risk of incidence of cancer and improved prognosis in various cancer (94–97). NK cell cytotoxicity and their secretion of pro-inflammatory cytokines and chemokines contribute to developing an effective anti-tumor immune response (98,99). NK cell-mediated ADCC has also been connected in the therapeutic efficacy of tumor antigen-specific antibodies and checkpoint blockers (98,100). In solid tumors, NK cells infiltrating the tumor are primarily found in two key regions: the invasive boundary and areas with abundance of stromal cells (101,102).

There are limited clinical studies directly assessing the relationship between adaptive NKG2C$^+$ NK cell expansion with tumor immune infiltrates. As such, NKG2C$^+$ NK cell expression has been reported to be downregulated in tumor-infiltrating NK cells, including in non-small cell lung cancer (NSCLC), gastrointestinal stromal tumor (GIST), and breast cancer (101,103,104). Irrespective of their thorough response to anti-HER2 antibodies, patients displayed comparable proportions of circulating NKG2C$^+$ NK cells as well as their absolute numbers (105). Moreover, CD57$^+$ NK cells, typically associated with adaptive NKG2C$^+$ NK cells, which significantly limited within tumor infiltrates as in comparison to those in peripheral blood. These findings provide further evidence that adaptive NKG2C$^+$ NK cells

are not abundant in solid cancers (105).

The efficient characteristics of adaptive NKG2C⁺ NK cells make them particularly promising for cancer immunotherapy. These features include their ability to carry out effector roles including cytotoxicity activity and cytokine production when activated by antibodies (91). Additionally, adaptive NKG2C+ NK cells exhibit oligoclonal expression of inhibitory killer cell immunoglobulin-like receptors (iKIR) without NKG2A, which is noteworthy. Another advantage is their prolonged lifespan compared to other NK cell subsets. Moreover, *in vitro* studies propose that adaptive NKG2C⁺ NK cells may exhibit resistance to suppression by contact-dependent myeloid-derived suppressor cells (MDSC) and regulatory T-cells (Tregs), potentially providing them with an advantage within the tumor microenvironment (106,107). The potential of NK cell infusions has been mainly investigated as adjuvant therapy in allogeneic hematopoietic stem cell transplantation (allo-HSCT) or following immunosuppressive chemotherapy for haematological malignancies. In these settings, the effect of KIR-based NK alloreactivity on the risk of relapse also has been demonstrated (108). Previous studies have shown a favorable safety profile for allogeneic NK cell infusions, as they do not result in GvHD and are associated with minimal severe adverse reactions (109,110). Additionally, when engineered to express chimeric antigen receptor (CAR), NK cell infusions have shown promising results without significant cytokine release syndrome or neurotoxicity (111).

Most NK cell products used for adoptive cell transfer in clinical settings are produced before going through *in vitro* modification. These protocols, such as in cytokine-induced memory-like (CIML) and haploidentical NK cell donor selection, aim to increase the number and improve NK cell function for therapeutic reasons. Nevertheless, the polyclonal NK cell expansions can result in varying alloreactive NK cells proportion and NKG2A co-expression, which may impact their effectiveness against tumors.

Human cytomegalovirus and its impact on NK cell activity

Human cytomegalovirus (HCMV) is a virus that infects an enormous part of the global population (112). It is estimated that up to 91% to 97.6% of the population in some developing countries, including Malaysia (92%) (113), are seropositive for HCMV, meaning they have been infected with the virus at some point in their lives and have developed antibodies against it (114). HCMV infection is generally symptomless in healthy individuals, but it can cause severe disease in people with undermined immune systems, such as organ transplant recipients, people with HIV/AIDS, and newborn infants (115). In these populations, HCMV can cause a range of complications, including pneumonia, encephalitis, hepatitis, and retinitis (116). One of the challenges with HCMV is that the virus can remain dormant in the body after primary infection, meaning that it can reactivate at a later time and cause disease

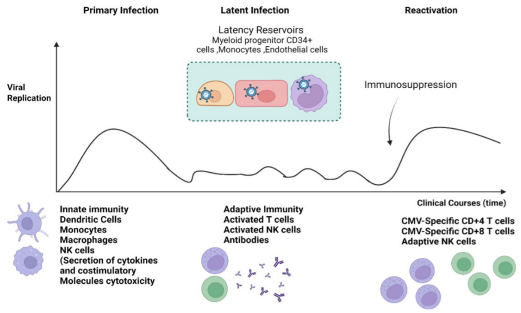

Figure 3: Illustrates the immune responses against HCMV. The stages of response depend on the phases of infection.

(Figure 3) (117). This reactivation can occur in people with weakened immune systems as well as in healthy individuals.

HCMV, a widespread β-herpesvirus, exclusively reproduces in human cells. Similar to other herpesviruses, this virus exhibits latency following initial infection, and reactivation is more likely to occur especially when the immune system is compromised (118). Upon primary infection, HCMV triggers the innate immune response such as in antigen presenting cells (APC); releasing inflammatory cytokines and co-stimulatory molecules (119). These mechanisms are crucial in impeding viral replication until the adaptive immune response can mount an effective defense. HCMV establishes a latent infection following dissemination to myeloid lineage cells such as monocytes and CD34⁺ cells (120). CMV-specific CD4 T-cells have been observed to emerge approximately 7 days after the peak of HCMV replication (121). These cells exhibit T-helper 1 type (Th1) characteristics and produce IFN-γ and TNF-α. Subsequently, CMV-specific CD8 T-cells detected in the peripheral blood, targeting against HCMV and possess the ability to lyse CMV peptide-presenting target cells (122). Over the course of several weeks to months following primary infection, CMV-specific CD8 T-cells undergo transition into effector memory T-cells. Remarkably, despite the persistence of latent infection in the host, these the virus is not completely eliminated the by the T-cells. The process of restoring adaptive immunity occurs gradually over several months to many years through the engraftment stem cells from donors (122).

On the other side, HCMV infection is found to drive maturation of NK cells towards highly different stages of impaired T-cell immunity in HSCT patients (123). Recent studies also show HCMV reactivation rapidly develop NK cells expression following umbilical cord blood transplantation (UCBT) (124,125). In HCMV-reactivating patients, NK cells reach complete maturation quickly than in non-infected individuals (126). These NK cells exhibit a distinct phenotype

characterized by low percentages of $CD56^{bright}$ and high percentages of mature $CD56^{dim}$ NK cells, characterized by the expression of $NKG2C^+NKG2A^-KIR^+Siglec\text{-}7^-CD57^+$ phenotypes (127,128). These mature NK cells also exhibit competency in the of cytokines production as well as cytolytic activity and persist and elevate even one year after HSCT (122,126). The expansion of specific group of NK cell subset observed in HCMV-reactivating patients is similar of a population of Ly49H-positive NK cells that undergo proliferation in mice infected with murine CMV (MCMV) (129,130).

Interestingly, HCMV infection/reactivation in HSCT patients has a similar impact on adaptive NK cell growth compared to $HCMV^+$ healthy individuals (85). However, the occurrence of HCMV-driven adaptive $NKG2C^+$ NK cells can vary and is influenced by multiple factors, including the conditioning regimen received prior to transplantation. Reduced-intensity conditioning regimen has shown to favor expansion of adaptive NK cells compared to myeloablative regimens (85). Furthermore, studies have also established that adaptive $NKG2C^+$ NK cells can persist in T-cell depleted and T-cell replete HSCT patients in the long term without detectable HCMV replication (88,131–133).

The mechanisms behind the development of adaptive $NKG2C^+$ cells have been a subject of investigation. Initial investigations showed that co-culturing peripheral blood mononuclear cells (PBMC) obtained from healthy individuals infected with HCMV with HCMV-infected fibroblasts resulted in a rise in NK cell $NKG2C^+$ expression (134,135). This expansion was found to be hindered by anti-CD94 monoclonal antibody, suggesting that CD94/NKG2C receptor-specific recognition of a viral ligand promotes their expansion in response to cytokines such as IL-2 or IL-15. Other studies have suggested the involvement of IL-12 and HLA-E in this process(134). However, attempts to identify a specific viral ligand for CD94/NKG2C using HCMV deletion mutants lacking genes responsible for downregulating HLA class I expression were not successful. Additionally, the function of the NK cell with CD94/NKG2C receptor in initiating effector functions on cells that are infected with HCMV has not been definitively demonstrated through blocking experiments with specific monoclonal antibodies such as monoclonal antibodies (mAbs) (136–138).

Chimeric antigen receptor (CAR) NK cell therapy

One strategy to enhance the function and effectiveness of NK cells against tumor is the utilization of chimeric antigen receptors (CARs). CARs have been known an artificial fusion protein that combine an extracellular domain responsible for recognizing antigens with intracellular signaling components, activating the cells upon antigen binding (139). Typically, the CARs-antigen-binding domain is originated from a single-chain variable fragment of an antibody specific to the target antigen (140). However, CARs can also be constructed using the extracellular portion of native cellular receptors, leveraging their natural specificity for receptor-ligand interactions (141). These CARs can be introduced into immune effector cells to redirect their specificity towards a specific target. Traditionally, CARs have been designed for T-cells, incorporating CD3ζ and co-stimulatory molecules (142). Successful generation of CAR-NK cells using these conventional CAR designs has been demonstrated, showing effective and specific targeting of tumors while maintaining a favorable safety profile (143–146).

CONCLUSION

The review delved into the anti-tumour mechanism of adaptive NK cells activated by HCMV in healthy individuals. This investigation encompassed the examination surface protein receptors and ligands, analyzing the effectiveness of cytotoxicity on leukemia cells and evaluating the receptor binding and interaction involved in the mechanism of cytotoxicity. It is suggested that adaptive or 'memory-like' NK cells can be obtained from peripheral blood and bone marrow from healthy donors through isolation techniques, and AML and allo-HSCT patients and analyzing them using various laboratory techniques and bioinformatics tools. The identified unique surface receptors and ligands on HCMV-driven adaptive NK cells, demonstrate their higher cytotoxic activity against leukemia cells, and elucidate the crucial role of specific receptor binding and interaction in their mechanism of action. These findings may inform the development of effective anti-leukemia therapies.

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