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The Memory Activation of NK Cells: New Methods in Cancer Immunotherapy

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Abstract

Cancer remains a main cause of mortality, despite the research efforts to unravel molecular mechanisms and for developing personalized targeted therapies with acceptable side effects. In cancer, both players, the aggressor (tumor cells) and the endogenous defenders (immune cells), are key therapeutic targets. Immunotherapy is nowadays considered the fourth therapeutical approach in cancer, complementing and sometimes replacing surgery and chemo- and radiotherapy. Natural killer (NK) cells, generally considered part of the innate immune system, play a critical role in defense against pathogens and tumors. Immunological memory is a hallmark of the adaptive immune system. However, NK cells have been shown to mediate Ag-specific recall responses and acquire immunological memory in a manner similar to that of T and B cells. This chapter summarizes evidence for NK cell immunotherapy, evidence and characteristics of NK cell memory and mechanisms involved in the generation and survival of these cells. There is no doubt that NK cells have major role in cancer treatments and viral infections, and in the future, NK cell immunotherapy from "a new hope" may become "a reality" for malignant diseases.

Keywords: immunotherapy, natural killer (NK) cells, innate memory, adoptive cell transfer, cancer

1. Introduction

Cancer represents one of the major causes of mortality, despite huge research efforts for deciphering the molecular mechanisms of disease and for developing new targeted and personalized therapeutic approaches with acceptable side effects. There are still problems to overcome, which are linked to the following: (a) heterogeneity of cancer cells within tumors, which is mirrored in their response to a specific therapy; for example, cancer stem cells possess inherent mechanisms for self-repair and renewal and are therefore often responsible of tumor

recurrence and metastasis; (b) complex mechanisms by which tumor cells avoid the innate and adaptive immune responses (adaptability, versatility, mimicry), including by immune suppression induction [1].

In addition to conventional therapeutic approaches (chemo- and radiotherapy), we face the shifting of the attention to the immune antitumor defense mechanisms which might offer a steady improvement to conventional therapies for tumor cells eradication. Cancer defiantly induces immune suppression and this is further deepened by anticancer therapies, which weaken the immune response, apart from killing tumor cells [2, 3].

In the last decade, immunotherapy gained a leading position in cancer research and management, due to promising results recently reported in clinical studies for (a) vaccines (sipuleucel-T) and monoclonal antibodies (ipilimumab), (b) recombinant cytokines and hematopoietic growth factors, and (c) cellular and gene therapies.

The adoptive transfer of lymphocytes with high antitumor reactivity can trigger tumor regression in metastatic melanoma [4–6]. Nowadays, autologous T lymphocytes are clinically used. *Ex vivo* multiplied tumor infiltrating lymphocytes (TILs) are adoptively transferred at the very patient from which they have been harvested and IL-2 is concomitantly administered in order to enhance T lymphocyte activation. This kind of immunotherapy is not fitted for all cancer patients, as TILs with antitumor reactivity are only seldom found in patients with other types of cancer than melanoma. Even in melanoma, TILs harvested from certain patients cannot be sufficiently expanded *ex vivo*, and this undermines the odds for their adoptive transfer. Moreover, there are some cases when tumor cells do not express class I major histocompatibility complex (MHC) molecules and therefore cannot be recognized by T lymphocytes. Natural killer (NK) cells might be an alternative to T lymphocytes, as they are able to kill tumor cells independently of MHC.

Natural killer cells are a component of the innate immune response against viruses and malignant cells. Individuals having low NK cells activity are at risk to develop cancer [7]. The presence of high NK cells number within the tumor confers a good prognostic for cancer patients [8]. NK cells represent a relatively poor population, about 1–32% from the peripheral lymphocytes in healthy individuals [9]. As opposed to T cells, which hold MHC-restricted antigen specificity, NK cells are able to directly and quickly lyse target cells without the need of an initial sensitization. It was proven that many types of tumor cells express high levels of ligands for NK cells receptors [10], which leads to their recognition and killing by NK cells [11]. The role of NK cells is regulated by (a) cytokines and chemokines which interact with inhibiting or activating receptors on NK cells [12, 13]; (b) communication with other immune cells, such as dendritic cells [14], effector TCD4+ lymphocytes [15] and regulatory T lymphocytes [16].

Until now, therapies with NK cells have been successful mainly for patients with leukemia [17–22], but adoptive transfer of interleukin (IL)-2-activated NK cells in patients with solid tumors (melanoma or renal carcinoma) did not show clear clinical benefits [23]. Based on the fact that NK cells possess the memory of being previously activated [23–29], new strategies can be developed for enhancing *ex vivo* the antitumor activity of NK cells intended to be transferred in patients with solid tumors. The use and clinical efficiency of immune therapy with

NK cells have been limited by the difficulty to obtain sufficient cells for adoptive transfer. NK cells represent only a small fraction of blood leukocytes, have a low *ex vivo* proliferation rate and have a limited lifetime in vivo. Identifying the optimal activator for expanding NK cells in vitro is difficult, due to the high number of activating and inhibiting receptors, pairs of cooperative receptors, overlapping of the signaling transduction pathways involved in their maturation, activation and proliferation.

It was shown that the multiplication of NK cells can be achieved by modulation with cytokines [30–38]. The development of the methods for growing human NK cells in vitro has incited a special interest for immunotherapy [32, 36, 39–41]. With the development of methods for multiplication of human NK cells in vitro, these cells have incited a special interest for immunotherapy.

2. NK cells biology

NK cells are large granular cells that play a major role in the innate immune response against viruses, bacteria, as well as malignant cells [12, 24, 42]. They were first identified in 1975 by their ability to kill tumor cells without MHC restriction or prior sensitization to tumor antigens [43–45]. The name "natural killer" refers to their natural occurrence and spontaneous ability to kill malignant cells in non-immunized animals. NK cells are found in a variety of lymphoid and nonlymphoid tissues, including bone marrow, lymph nodes, spleen, peripheral blood, liver and lung. NK cells develop in the bone marrow from a common lymphoid progenitor cells.

2.1. Phenotypes

NK cells are characterized by the expression of CD16 and CD56 surface antigens and the lack of CD3/T-cell receptor molecules. In humans, there are two subsets of NK cells based on CD56 expression levels: CD56^{dim} and CD56^{bright}. Morphologically, the CD56^{dim} NK cells are large granular lymphocytes, while CD56^{bright} NK cells are small lymphocytes. The CD56^{dim} subset represents the majority of NK cell population in the peripheral blood and spleen (90–95%), exhibit a high cytotoxic potential after interaction with target cells [46, 47]. They produce negligible amounts of cytokines and bear the Fc receptors (CD16) to mediate antibody-dependent cell-mediated cytotoxicity (ADCC). In contrast, the CD56^{bright} subset predominates (approximately 90%) in lymph nodes and tonsils, have poor cytotoxic activity and produce very significant amounts of cytokines and chemokines [48]. The CD56^{bright} NK cells produce chemokines and cytokines in response to cytokine stimulation, while the CD56^{dim} population chemokines and cytokines production is stimulated by target cell recognition.

These subsets also differ in the expression of interleukin (IL)-2 receptor α chain (IL-2Rα/CD25). CD56^{bright} subset exclusively expresses CD25, while the CD56^{dim} subset lacks CD25 expression. There are differences in receptor expression between the two subsets of NK cells. As opposed to the CD56^{bright} NK cells, CD56^{dim} NK cells express high levels of killer-cell immunoglobulin-like receptors (KIRs) and low levels of CD94/natural killer group 2 (NKG2) receptors. These differences have been correlated with the specific alloreactive properties of CD56^{dim} NK cells due to their high KIR levels and their ability to kill various tumor cells [49].

The murine NK cells do not express CD56 marker, but they can be divided in four subsets according to the expression of CD11b and CD27 markers: CD11b^{low}CD27^{low}, CD11b^{low}CD27^{high}, CD11b^{high}CD27^{high}, CD11b^{high}CD27^{low} [50]. Mouse CD27^{high} NK cells predominate in lymph nodes and produce large amounts of cytokines, but in contrast with CD56^{bright} NK cells, they have cytotoxic potential. In humans, expression of CD11b and CD27 markers have also revealed four subsets of NK cells with distinct maturation stages, tissue distribution patterns and functional properties [51, 52].

The NK cell activating receptor NKp46 (Ncr1), in contrast to other human NK cell markers (CD56, CD16) or murine NK cell markers (NK1.1, DX5), is almost exclusively expressed by NK cells, and it can be used as an additional marker for identify NK cells [53, 54].

2.2. Receptors

NK cells do not express rearranged, antigen-specific receptors, but they express a variety of germ-line encoded receptors that can recognize ligands on their cellular targets.

NK cell function, including proliferation, production of cytokines and chemokines, natural killing, lymphokine-activated killing and ADCC, depends on an intricate balance between signals from inhibitory and activating receptors. The activating receptors interact with ligands expressed on stressed, infected, or transformed cells, or antibody-opsonized targets (CD16/FcγRIIIa), while inhibitory receptors recognize MHC class I or class I-like molecules [11].

Inhibitory receptors include killer immunoglobulin-like receptors (KIRs), the c-type lectin, NKG2A/CD94 and leukocyte immunoglobulin-like receptors (LILRs) [55]. The ligands for these inhibitory receptors are mostly the major histocompatibility complex class-I (MHC-I) molecules. Inhibitory signals prevent NK cells from becoming activated, blocking degranulation and cytokine production. Ligation of MHC-I molecules to the inhibitory receptors acts as a form a NK cell tolerance. By this mechanism, NK cells save healthy cells from killing as long as they express normal levels of MHC class I molecules and low amounts of stress-induced self-molecules. During the NK cell development, signals coming from inhibitory receptors help NK cells to be "educated" to respond to MHC-I deficient cells [56].

Activating receptors include the natural cytotoxicity receptors or NCRs, the c-type lectins, NKG2D and NKG2C/CD94, the SLAM family receptors and others. NK cells express the low-affinity Fc receptor or CD16 and the death ligands FasL and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) that after interaction with death receptors induce apoptosis of the target cell. Although some of the ligands to activation receptors are already present on healthy cells, the expressions of many of them are induced upon cell stress [57].

In case of cellular transformation or viral infection, surface MHC class I expression on the cell surface is downregulated or lost to escape from recognition by T cells. When mature NK cells encounter that cell, their inhibitory receptors are not engaged, and the unsuppressed activating signals can trigger cytokine secretion and attack of the targeted cell. In parallel, cellular stress and DNA damage upregulate "stress ligands" that can be recognized by activating NK receptors.

NK cells kill tumor targets through a variety of mechanisms, including release of cytoplasmic granules containing perforin and granzyme, expression of tumor necrosis factor (TNF) family members FasL or TNF-related apoptosis-inducing ligand (TRAIL), that induce tumor cell apoptosis by interacting with their respective receptors Fas and TRAIL receptor (TRAILR) as well as ADCC [58].

During tumor progression, tumor cells develop the ability through which they escape from NK cell response. These mechanisms include downregulation of adhesion molecules, costimulatory ligands or ligands for activating receptors, upregulation of MHC-I molecules, soluble MIC, FasL or NO expression [59, 60], secretion of immunosuppressive factors such as IL-10, transforming growth factor (TGF)-β or indoleamine 2,3-dioxygenase (IDO) and resisting Fas- or perforin-mediated apoptosis [61]. In cancer patients, there are observed some NK cell defects, including decreased numbers in peripheral blood and in tumor infiltrate, reduced expression of activation receptor or intracellular signaling molecules and overexpression of inhibitory receptors, decreased cytotoxicity, defective proliferation and cytokine production.

2.3. Cytokines

Cytokines are the main regulators of growth, proliferation, survival and differentiation for various cell types involved in the innate and adaptive immunity. The exhibition of NK cells to cytokines induces enhanced proliferation, augmented cytokine production, higher cytotoxicity against target cells and upregulation of cytotoxic and adhesion molecules. The common γ-chain interleukins (ILs) such as IL-2, IL-15, IL-21 can activate NK cells, and the combination of IL-12 and IL-18 is especially potent to trigger interferon (IFN)-γ.

IL-2 and IL-15 are the best studied cytokine activators of NK cells; they have central role for NK cell development and homeostasis, induce proliferation, costimulate cytokine production and enhance cytotoxic effector mechanisms. The both cytokine share the IL-2/15Rβ and γ_c as the primary signaling subunits, but these cytokine require α receptor subunit for efficient ligation of the IL-2/15R$\beta\gamma_c$. IL-15 plays an important role in the maturation, survival and homeostatic expansion of NK cells. For generating in vivo functional NK cells, IL-15R signaling is required [62]. The first surface marker exhibited by NK cell progenitors is IL-2/15R β (CD122), which is expressed even before the cell lineage marker, NK1.1 (CD161c or NKR-P1C) [63]. The factors which further regulate differentiation and homeostasis of mature NK cells are largely unknown. Mature NK1.1+ cells continuously need IL-15 for survival [64–66] and NK cells can be stimulated in vitro by IL-15 or IL-2. Once NK cells are activated, their differentiation can be induced/increased by cytokines. It has been shown that IL-15 is a powerful *ex vivo* stimulator for immune cells. Preclinical studies have shown that NK cells, naïve/memory T CD8+ lymphocytes and dendritic cells cultivated with IL-15 develop enhanced functions when being adoptively transferred in animals [67–69]. Several studies shown that coadministration of IL-15/IL-15Ra complexes on NK cells enhanced in vivo activity, and the use of IL-15/IL-15Rα complexes remains highly promising as an IL-15 immunotherapy [70–72]. IL-2 and IL-15 are also used in *ex vivo* activation and/or expansion of NK cells for adoptive therapy and to support the expansion and function of NK cells after infusion.

IL-21 is a cytokine structurally-related to IL-2, IL-4, IL-15. IL-21Rα is expressed by lymphoid tissues, shows similarities with IL-2/IL-15R β chain and forms a complex with the common γ chain [73]. IL-21 exerts antitumor effects through its ability to induce activation and proliferation of cytotoxic cells (T CD8+ cytotoxic lymphocytes, NK cells, NKT cells). Meanwhile, IL-21 suppresses Foxp3 expression and the expansion of immunosuppressive Treg lymphocytes. Accordingly, IL-21 was associated with antitumor activity in clinical practice [74]. The discovery of IL-21 was linked to its role in NK cells proliferation and maturation. Further studies have provided however contradictory data, highlighting both the activator and the suppressor role of IL-21. Soluble IL-21 alone does not induce significant proliferation in mature mouse NK cells, and IL-21R knockout mice possess normal NK cells number. Meanwhile, IL-21 synergizes with IL-2, IL-15 and Flt-3L for NK cells generation in the bone marrow and in umbilical cord blood. IL-21 can activate the cytotoxic activity of NK cells through over expression of costimulating receptors and cytolytic molecules (perforin, granzymes). IL-21 enhances multipotent progenitor maturation from bone marrow and activates peripheral NK cells in the absence of other stimuli.

IL-12, originally identified as "NK cell stimulatory factor (NKSF)" based on its ability to enhance NK cell cytotoxicity. The primary effects of IL-12 on NK cells are IFN-γ and TNF-α production. In vitro and in vivo studies have shown that IL-12 acts on NK cells in concert with other activating cytokines, such as IL-2 and IL-18, or with receptor-based interactions from pathogenic cells.

Although the IL-18Rα is constitutively expressed on unstimulated NK cells and can induce NK cell proliferation alone, IL-18 has been described as a costimulatory cytokine that functions synergistically with IL-12 and IL-15. NK cells from IL-18 deficient mice have impaired cytotoxicity and IFN-γ production. These findings indicate the importance of IL-18 to NK-mediated host defense.

Successful adoptive cell transfer (ACT) and *ex vivo* modulation of cellular functions with cytokines has aroused the interest for immunotherapy in cancer. Currently, immunotherapy is known as the forth treatment alternative in cancer, after surgery, chemo- and radiotherapy.

3. NK cells for adoptive transfer

3.1. Autologous NK cells

Based on results from experimental animal models, adoptive transfer of autologous NK cells seems to be safe and promising for cancer therapy. Initial trials of adoptive NK cells involved infusion of CD56 bead-selected autologous NK cells from a leukapheresis product followed by administration of systemic cytokines [75]. Upon cytokine stimulation, NK cells become lymphokine-activated killer (LAK) cells and exhibit greater cytotoxicity against tumor cells. Although administration of cytokines improve the antitumor activity of NK cells in vitro, only limited antitumor activity of LAK cells was observed in cancer patients [23]. Similar results were also obtained when autologous NK cells and systemic IL-2 were administrated to patients with lymphoma. Partially effective clinical outcomes were observed in metastatic renal cell carcinoma (RCC) patients that received a combination of high-dose IL-2 and LAK cell infusions, while

treatment of non-Hodgkin's lymphoma and RCC patients with *ex vivo* IL-2-activated autologous NK cells followed by daily subcutaneous IL-2 injection shown no improvement in the disease status. In patients with recurrent malignant glioma infusion of NK cells combined with IFN-α was safe and partially effective. It is known that high IL-2 doses induced severe toxic side effects such as vascular leak syndrome and also promote expansion of regulatory T cells that directly inhibit NK-cell functions and induce activation-induced cell death of NK cells. The adoptive transfer of IL-2-activated LAK cells was more successful rather than administering IL-2 systemically. Other cytokines, such as IL-12, IL-15, IL-18 and IL-21, have been successfully tested in preclinical cancer models [76]. Adoptively transferring autologous NK cells has been evaluated clinically for cancer immunotherapy and was found to greatly improve clinical responses without any obvious adverse side effects in metastatic renal cell carcinoma (RCC), malignant glioma and breast cancer patients. However, these autologous NK cells could not yet exhibit their full cytotoxic capacity in vivo and were not consistently effective in cancer patients; this may be due to MHC class I expression in cancer patients that suppress autologous NK cells in vivo.

As was shown, adoptively transferring autologous NK cells was found to greatly improve clinical responses without any obvious adverse side effects in some cancers. However, these NK cells could not yet exhibit their full cytotoxic capacity in vivo, this may be due to many tumors expressing high levels of human leukocyte antigen (HLA) class I receptor and/or low levels of ligands for activating receptors that suppress autologous NK cells in vivo. For these reasons, they are focused on therapies using allogenic NK cells from related donors or other strategies to prevent such NK cell resistance.

3.2. Allogeneic NK cells

Using the allogeneic NK cells has the advantage of the inherent alloreactivity afforded by the "missing self" concept. When donor NK cells encounter an altered MHC environment, the NK cells can be "re-educated" by host HLA and can acquire cytotoxicity against host tumor cells without causing graft versus host disease (GVHD). Allogenic NK cells with KIR mismatch have greater tumor-killing activity and the ability to control acute myeloid leukemia (AML) relapse [77, 78]. Adoptively transferred human-mismatched (haploidentical) allogeneic NK cells have been shown to be a safe therapy with minimal toxicity and have been more successful for cancer immunotherapy, including against leukemia and solid cancers. In some clinical trials using adoptively transferred haploidentical allogeneic NK cells to treat AML patients, including pediatric AML patients and older AML patients no graft-versus-host disease (GvHD) response was observed and NK cell therapy was well tolerated. Besides hematopoietic-derived tumors, strategies using adoptively transferred haploidentical allogeneic NK cells can also expand in patients with various malignancies, including metastatic melanoma, renal cell carcinoma, Hodgkin's disease and poor-prognosis AML

3.3. Memory-like NK cells

NK cells are traditionally considered members or the innate branch of the immune system that responds rapidly but lack immunologic specificity in the form of a clonal antigen receptor and memory of prior activation. Recently several groups have challenged this paradigm of NK cells as pure innate lymphocytes and demonstrated memory-like functions in NK cells.

The immune system capacity to learn from previous encounters with pathogens, and respond more rapidly and effectively upon secondary infection has been termed adaptive immunity or immunological memory. After primary encounter with antigen, naïve antigen-specific T or B cells proliferate vigorously and some of them differentiate into memory cells [79]. This stage represents the expansion phase, when the naïve cells clonally expand. Following the primary response, in the contraction phase, the majority of effector cells die and surviving cells enter the memory phase. Upon reencounter with their cognate antigen, memory cells exert their functional responses more rapidly than do naïve cells. This response to a second antigen exposure called the "recall response."

Antigen-specific responses and memory responses, both are hallmarks of adaptive immunity. Innate responses do not require pre-sensitization and rely on germline encoded receptors and do not require clonal expansion. NK cells have long been categorized as a component of innate immunity. Although NK cells lack the ability to undergo somatic rearrangements of their receptors, these cells are developmentally and functionally more related to adaptive immune lymphocytes than innate immune cells.

Initially it was believed that NK cells act in the first days of infection, but now we know that NK cells function in parallel and complementary to the adaptive immune response over extended periods of time. Moreover, there are many evidences for adoptive-like features of NK cells. Evidence for adoptive-like features of NK cells has come from a variety of studies, and NK cell-mediated memory can be generated in response to haptens, viruses and following combined cytokine activation.

3.3.1. NK cell response to haptens

NK cell memory was first described in a mouse model of hapten-induced contact hypersensitivity (CHS), induced by chemical haptens such as 2,4-dinitro-1-fluorobenzene (DNFB) and 4-ethoxymethylene-2-phenyloxazol-5-one (oxazolone) and picryl chloride [25]. T- and B-deficient mice developed vigorous specific contact hypersensitivity responses to haptens and response persisted for at least four weeks. The mice exhibited enhanced recall responses to the same chemical, but not to a different one, demonstrating antigen specificity. Furthermore, contact hypersensitivity responses could be conferred to naive mice by adoptive transfer of natural killer cells from sensitized mice.

These responses possessed the hallmarks of adaptive immunity: they were sensitization dependent, persisted for at least four weeks and were only elicited by haptens to which mice had previously been sensitized. These observations indicate that natural killer cells can mediate long-lived, antigen-specific adaptive recall responses independent of B cells and T cells. It was shown that transfer of a subset of liver-derived NK cells, not splenic or naïve NK cells, could confer the recall response. NK cell memory to haptens depended on expression of CXCR6, a chemokine receptor on hepatic NK cells critical for intrahepatic survival and homeostasis [80]. CXCR6 is a chemo-attractant receptor which is expressed on roughly 50% of liver NK cells. The molecular mechanism leading to the generation of antigen-specific memory NK cells remained elusive.

3.3.2. NK cell response to viruses

Also, NK cells could mount recall responses to diverse viral antigens such as vesicular stomatitis virus (VSV), virus-like particles (VLP) containing influenza A-derived hemaglutinin and/or matrix protein 1, or VLP containing the HIV-1-derived Gag protein and/or Env protein [80]. In order to confer the recall response, liver NK cells had to express the CXCR6. Blocking CXCR6 with antibodies can impair the recall response. NK cells can respond more vigorously upon secondary stimulation against challenges with additional irritants (such as fluorescein isothiocyanate [FITC]), as well as the viruses vaccinia, and herpes simplex virus (HSV)-2 [81–83].

A central issue that remains unresolved is how NK cells can recognize and differentiate between all these different antigens, since there are no known VSV, vaccinia, HSV-2, or HIV-specific NK receptors. The ability of NK cells to respond to such a wide diversity of distinct antigens, including pathogens that are not endemic to mice, such as HIV1, is puzzling and suggests that a hitherto unknown recombination-activating genes (RAG)-independent receptor diversification mechanism may exist in NK cells [84].

Most of the evidence that NK cells exhibit a memory-like adaptive response has come from studies involving mouse cytomegalovirus (MCMV) infection. NK cells play a crucial role in the protective immune response to herpesvirus family members, especially to CMV infection. It have been shown that a subset of NK cells in the C57BL/6 strain of mice bearing the activating receptor Ly49H, specifically recognize the MCMV-expressed ligand m157 and proliferate in response to viral infection [85–87]. Ly49H, a germ-line encoded receptor, is expressed by a very large fraction of NK cells in naïve mice (~50% of NK cells). During viral infection, a Ly49H-expressing subset of NK cells is extended and rapidly responds upon reinfection with MCMV, similar to classic lymphocytes memory. The germ-line encoded MCMV-specific receptors exist too in mouse strains, such as Bagg Albino (BALB/c), non-obese diabetic (NOD) and others, and that clonal expansions of MCMV-specific NK cells could also be observed in those strains [88]. Similar to CD8+ T cell response, the proliferation was Ag-specific because infection of mice with a mutant MCMV lacking m157 did not cause expansion of Ly49H+ NK cells [25]. Ly49H+ NK cells adoptively transferred in mice lacking functional Ly49H receptor proliferate 100-1000-fold after MCMV infection, and after a contraction phase, persist for months. When these cells are restimulated *ex vivo* with agonistic antibodies against NK1.1 or Ly49H, they respond more robustly and offer increased protection against MCMV infection then naïve NK cells. MCMV-induced NK cell memory is critically dependent on the IL-12; NK cells that lack the IL-12 receptor do not proliferate in response to MCMV [89].

In human, studies have focused on the memory NK cell response in infection with cytomegalovirus (CMV), Hanta virus or Chikungunya virus [90–92]. The results shown that NK cells that express the germ-line encoded NKG2C receptor appeared in increased frequency in response to infections with these viruses. During acute HCMV infection, NKG2C+ NK cells expanded in number, and diminished partially in numbers after the resolution of the acute phase. These memory NK cells persisted for up to a year. After expansion, the NKG2C+ NK cells produced significantly more IFN-γ in response than NKG2C- NK cells. A follow-up study demonstrated that after adoptive transfer of NKG2C+ NK cells from CMV-seropositive donors exhibited

enhanced effector function against a secondary CMV challenge [93]. In the Hanta virus infection or Chikungunya virus infection, NKG2C+ NK cells expanded three- to fourfold compared to uninfected controls. In both cases, these increases were only seen in patients who were HCMV seropositive, raising the possibility that the increase in NKG2C+ NK cells reflected reactivation of latent CMV. It has been noted that in HCMV-infected patients, after the acute infection was cleared, NKG2C+ NK cell numbers remained elevated in contrast with uninfected individuals. It is possible that these NKG2C+ NK cells present in HCMV-seropositive individuals respond to other infections. Another study has demonstrated that the combination of CMV-infected fibroblasts plus IL-12-producing monocytes induced the expansion of NKG2C+ NK cell in vitro [94].

3.3.3. Cytokine-induced NK cell responses

Memory-like NK cells could be induced in vitro by cytokines activation in both mice and humans. NK cells from Rag 1-deficient mice were pre-activated overnight in vitro with IL-12, IL-18 and IL-15, and then adoptively transferred into syngeneic Rag-1$^{-/-}$ recipients. After resting in vivo, when the NK cells had reverted to a quiescent state, the cytokine pre-activated NK cells were phenotypically similar to control NK cells. They expressed similar levels of CD69, CD11b, CD27, B220, as well as the cytokine receptors CD122, IL12Rβ1, IL-15Rα and CD127. Also, they expressed comparable levels of granzyme B and lysed target cells similar to control NK cells in vitro [27, 95]. However, up to 3 weeks following adoptive transfer, the cytokine pre-activated NK cells were found to respond more robustly compared to resting NK cells. These pre-activated NK-cells displayed enhanced IFN-γ production upon either activating receptor ligation (Ly49H or NK1.1 receptors) or cytokines (IL-12 and IL-15) restimulation. This enhanced ability to produce IFN-γ occurs in cells that have not undergone division and those that have replicated. It has been demonstrated that the enhanced functionality of memory-like NK cells is not due to alteration in IFN-γ transcription or mRNA stability.

Adoptively transferred cytokine pre-activated NK cells proliferated rapidly in an IL-2-dependent manner into recipient mice bearing MHC class I-deficient RMA-S lymphoma or B16-Rae1ε melanoma cell lines [96]. Exposure of NK cells to cytokines upregulated the IL-2Rα chain, making these cells more responsive to IL-2. It was observed a significantly reduced tumor growth and prolonged survival in recipient mice, pre-activated NK cells exhibited enhanced functionality months following adoptive transfer, and were able to mediate more effective in vivo antitumor responses.

Human NK cells also exhibit enhanced IFN-γ production after short-term pre-activation with various combinations of IL-12, IL-15 and IL-18 [97]. Both NK cell subsets, CD56^{bright} and CD56^{dim}, exhibited cytokine-induced memory-like NK cell. Cytokine pre-activation led to extensive proliferation, and memory-like NK cells maintained their capacity for enhanced recall responses. Similar to mice, IFN-γ mRNA transcript levels did not differ between control and memory-like NK cells. In contrast to murine memory-like NK cells, phenotypic differences were identified between pre-activated NK cells and controls. Human memory-like NK cells had increased CD94, NKG2A, NKp46 and CD69 surface expression and reduced KIR and CD57 expression. Human memory-like NK cells were also shown to be responsive to low concentrations of IL-2.

A pre-clinical study has shown that human memory-like NK cells also have potential as anti-leukemia cellular therapy [98]. They exhibited enhanced IFN-γ production and increased cytotoxicity when restimulated with leukemia cell lines or acute myeloid leukemia (AML) blasts in vitro. Following adoptive transfer into immunodeficient NOD-severe combined immunodeficiency (SCID)-$\gamma_c^{-/-}$ mice, human cytokine-induced memory-like NK cells exhibited increased IFN-γ production following restimulation [99].

Therefore, human NK cells can acquire memory-like properties after a brief cytokine pre-activation.

4. Conclusion

Although, the concept of NK cell memory is rather new, many studies in recent years have provided substantial evidence for adaptive features of the NK cell response. The enhanced function of memory NK cells makes them an area of interest for future use in preventing or treating inflammatory diseases, infectious diseases and cancer.

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