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Improving efficacy of cancer immunotherapy by genetic modification of natural killer cells

RACHEL A. BURGA[1,2], TUONGVAN NGUYEN[3], JANE ZULOVICH[3], SARAH MADONNA[3], LOYDA YLISASTIGUI[3], ROHAN FERNANDES[1,2] & ERIC YVON[3]

[1]Institute for Biomedical Sciences, The George Washington University, Washington, DC, USA, [2]Children's National Health System, Washington, DC, USA, and [3]The University of Texas, MD Anderson Cancer Center, Houston, Texas, USA

Abstract

Natural killer (NK) cells are members of the innate immune system that recognize target cells via activating and inhibitory signals received through cell receptors. Derived from the lymphoid lineage, NK cells are able to produce cytokines and exert a cytotoxic effect on viral infected and malignant cells. It is their unique ability to lyse target cells rapidly and without prior education that renders NK cells a promising effector cell for adoptive cell therapy. However, both viruses and tumors employ evasion strategies to avoid attack by NK cells, which represent biological challenges that need to be harnessed to fully exploit the cytolytic potential of NK cells. Using genetic modification, the function of NK cells can be enhanced to improve their homing, cytolytic activity, *in vivo* persistence and safety. Examples include gene modification to express chemokine, high-affinity Fc receptor and chimeric antigen receptors, suicide genes and the forced expression of cytokines such as interleukin (IL)-2 and IL-15. Preclinical studies have clearly demonstrated that such approaches are effective in improving NK-cell function, homing and safety. In this review, we summarize the recent advances in the genetic manipulations of NK cells and their application for cellular immunotherapeutic strategies.

Key Words: *gene transfer, immunotherapy, natural killer cells*

Introduction

As part of the innate immune system, natural killer (NK) cells play an important role in host responses against viral infections and cancers, including leukemia. Although the complex biological networks at play in the anti-tumor activity of NK cells are not fully elucidated, the field of cellular immunotherapy is actively evaluating NK cells as a novel therapeutic for patients with cancer. However, we anticipate that genetic modification is necessary to fully exploit the potential of NK cells to treat patients with advanced malignancies where overcoming tumor immune evasion strategies are critical. The focus of this review is to summarize the recent progress in the gene modification of NK cells to optimize the tumor-specific NK-cell response to improve their *in vivo* persistence, homing and resistance to the immune suppressive tumor microenvironment.

NK-cell biology

Early reports of NK cells first identified a population of tumor-killing lymphocytes with fast-acting effects [1]. Later termed NK cells, these lymphocytes were found to lyse tumors in a manner entirely distinct from the antigen-specific mechanisms used by T cells [2–5]. Indeed, NK cells are members of the innate immune system that recognize target cells by activating and inhibitory receptors expressed on their surface and the balance between both signals will determine the NK-cell response against virally infected or malignant cells. NK cells are generated in the bone marrow and are derived from lymphoid progenitors which themselves develop from hematopoietic stem cells (HSC) [6]. In the early stages of differentiation, NK progenitor cells are dependent on the expression of the transcription factor inhibition of DNA binding 2 (ID2) and the interleukin (IL)-2 receptor beta chain (CD122) [7]. However, as they mature, NK cells are identified based on their variable expression of CD56, CD34, CD117, CD94 and CD16 [6]. NK-cell development is also dependent on high levels of IL-15, which interacts with components of the IL-2 receptor on the surface of the NK progenitor cell to elicit marked cell proliferation and activation of NK

Correspondence: Eric Yvon, PhD, The University of Texas, MD Anderson Cancer Center, Department of Stem Cell Transplantation and Cellular Therapy, 1515 Holcombe Blvd, Unit 065, Houston, TX 77030-4009, USA. E-mail: ESYvon@mdanderson.org

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effector function [8]. As they fully develop, mature NK cells can be located in the bone marrow, lymph nodes, spleen, thymus and tonsil and can be further subdivided into three distinct subsets on the basis of their expression of CD56 [9] $CD56^{bright}$ cells have low cytolytic activity, and instead support an immune-regulatory environment by producing cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1, chemokine (C-C) ligands, CCL3 and CCL5 [2,10–12]. Along with expressing high levels of CD56, this subset also expresses the C-C chemokine receptors CCR7 and CCR5, which contributes to their selective accumulation in secondary lymphoid organs [13]. The $CD56^{dim}$ NK subset, in contrast, expresses CD16 (FCyRIII) and has characteristically potent cytotoxic ability against target cells, as well as produces high levels of self-sustaining IFN-γ [2,14] $CD56^{dim}$ cells uniquely express chemokines and factors involved in leukocyte trafficking such as CX3CR1, CXCR1, CXCR2, chemerin and the sphingosine 1-phosphate moleculate and can be located in both peripheral blood and lymphoid organs [15,16]. Of note, both the $CD56^{dim}$ and the $CD56^{bright}$ subsets can express CXCR3, CXCR4 and CD62L, which support transport into the peripheral circulation [17]. The third subset of CD56 negative NK cells are rare and represent a dysfunctional population of NK cells found in the setting of HIV viremic patients; they have impaired function and produce low levels of immune-modulatory cytokines [18,19]. Although these three subsets represent a gradient of CD56 expression, it is mostly the $CD56^{bright}$ and $CD56^{dim}$ subsets that are important in innate immunity and play a role in tumor lysis. As discussed in subsequent sections, the rapidly growing tumor micro-environment can introduce an altered chemokine and cytokine landscape. Because of their dependence on chemokine gradients and cytokines, the repertoire and distribution of NK cells can consequently be affected [15].

As mentioned previously, unlike other cells of lymphoid origin, NK-cell recognition is not governed by high-resolution antigen specificity but rather through a balance between activating and inhibitory signals received by receptors known as killer immunoglobulin-like receptors (KIR). KIRs on NK cells can be classified according to two haplotypes: A and B. NK cells of the A haplotype characteristically have multiple inhibitory receptors and only one activating receptor. NK cells of the B haplotype express a variety of both inhibitory and activating receptors [20]. NK cells can also express (i) receptors from theNKG2 family, which can be either inhibitory or activating (NKG2A, B, C, D, E, F, and H); (ii) the activating receptor DNAX Accessory Molecule-1 (DNAM-1); (iii) the 2B4 family of receptors; and (iv) the natural cytotoxicity receptors (NCRs) NKp30, NKp44, NKp46 to recognize tumor antigens, viral ligands, and heat shock associated proteins [21]. The majority of NK cells express receptors of the NKG2 family, which uniquely bind to major histocompatability complex (MHC) class I molecules to initiate an inhibitory signal. Because MHC class I molecules, specifically histocompatibility antigen, alpha chain E (HLA-E), are expressed on all nucleated cells, the inhibitory response following engagement with an NKG2 receptor serves as a recognition of "self," which hinders lytic attack and thus prevents rampant NK-based autoimmunity [22]. NK cells recognize tumor cells by engaging their activating receptors with tumor antigens or viral proteins on the cell surface, while simultaneously not seeing ligands to engage their inhibitory receptors. The lack of MHC on the surface of these foreign cells is known as the "missing self" hypothesis, by which the non-engagement of inhibitory receptors such as NKG2A leads to NK cell activation and tumor lysis [23]. Accordingly, the mimetic upregulation of MHC molecules illustrates a method of immune evasion employed by tumor cells preventing any consequent tumor cell lysis. NK cells exert their cytotoxic function directly through granzyme B and perforin-mediated apoptosis of target cells or by promoting expression of Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL) to signal death of the target cells [24]. Additionally, the crosslinking of CD16 on NK cells to the Fc portion on a target cell ligand can trigger antibody-dependent cellular cytotoxicity (ADCC), which can in turn lead to target cell lysis [25,26].

Because of their unique biological properties, NK cells are relevant effector cells for adoptive immunotherapy. However, the use of NK cells in such therapeutic approaches comes with advantages and disadvantages, especially when compared with the better-established T-cell effector population. The main disadvantage would be the poor *in vivo* persistence of adoptively transferred NK cells compared with T cells. On the other hand, NK cells are "serial killers" and therefore can efficiently kill multiple targets. Another advantage is the possibility of using an allogeneic source of NK cells, which would help overcome the inhibitory signals provided by the MHC molecules, expressed on the tumor cells, without the risk of graft-versus-host disease (GvHD) [27]. Finally, the "cytokine storm" initiated by the pro-inflammatory cytokines release by T cells, such as TNF-α, IL-1 and IL-6, would not be observed because NK cells produce different cytokines on activation. To the best of our knowledge, no pre-clinical or clinical study directly compared NK cells and T cells as cytotoxic effector cells for the treatment of malignant diseases. Although NK cells can demonstrate successful cytotoxic activity against tumor cells, lytic function in the tumor