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Anti-Tumour Treatment

Translating the anti-myeloma activity of Natural Killer cells into clinical application

Cinzia Fionda*, Helena Stabile, Rosa Molfetta, Alessandra Soriani, Giovanni Bernardini[1], Alessandra Zingoni, Angela Gismondi, Rossella Paolini, Marco Cippitelli, Angela Santoni[1]

Department of Molecular Medicine, Sapienza University of Rome, Laboratory affiliated to Istituto Pasteur Italia – Fondazione Cenci Bolognetti, Rome, Italy

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ABSTRACT

Natural Killer cells (NK) are innate effector cells with a critical role in immunosurveillance against different kinds of cancer cells, including Multiple Myeloma (MM). However, the number and/or function of these lymphocytes are strongly reduced during MM progression and in advanced clinical stages. A better understanding of the mechanisms controlling both MM and NK cell biology have greatly contributed to develop novel and combined therapeutic strategies in the treatment of this incurable hematologic malignancy. These include approaches to reverse the immunosuppressive MM microenvironment or potentiate the natural or antibody-dependent cellular cytotoxicity (ADCC) of NK cells. Moreover, chemotherapeutic drugs or specific monoclonal antibodies (mAbs) can render cancer cells more susceptible to NK cell-mediated recognition and lysis; direct enhancement of NK cell function can be obtained by means of immunomodulatory drugs, cytokines and blocking mAbs targeting NK cell inhibitory receptors. Finally, adoptive transfer of *ex-vivo* expanded and genetically manipulated NK cells is also a promising therapeutic tool for MM.

Here, we review current knowledge on complex mechanisms affecting NK cell activity during MM progression. We also discuss recent advances on innovative approaches aimed at boosting the functions of these cytotoxic innate lymphocytes. In particular, we focus our attention on recent preclinical and clinical studies addressing the therapeutic potential of different NK cell-based strategies for the management of MM.

Introduction

Multiple myeloma (MM) is a hematologic neoplasia caused by the overgrowth of malignant plasma cells (PCs) in the bone marrow (BM) [1]. MM is a multistage disease, often rising from a premalignant condition, named monoclonal gammopathy of undetermined significance (MGUS), after a transition through a phase of asymptomatic (smouldering, SMM) myeloma. Although the molecular mechanisms driving the progression from MGUS to MM remain largely undefined [2], failure of immune surveillance plays major roles in this process. Indeed, MM development is often associated with a progressive impairment of both innate and adaptive anti-tumor immune responses [3].

Current treatment options for MM include standard and high dose cytotoxic chemotherapies, haematopoietic stem cell transplantation (HSCT), Proteasome Inhibitors (PIs) and Immunomodulatory Drugs (IMiDs). These approaches significantly improved the quality of life and the outcome of myeloma patients in the last decades, but the median survival remains around 4–5 years. Therefore, novel and alternative anti-MM therapeutic strategies are needed. In this context, particular attention has been given to the therapeutic potential of Natural Killer (NK) cells as important effectors of anti-myeloma immune response.

Natural Killer cells are innate lymphoid cells, important effectors and regulators of host immune response against cancer and virus-infected cells [4]. Two phenotypically and functionally distinct major subsets of human NK cells have been described: $CD56^{bright}$ and $CD56^{dim}$ [5]. NK cells express inhibitory and activating receptors which upon interaction with their specific ligands on target cells trigger integrated signals able to induce or inhibit their activity.

Inhibitory receptors include the Killer-cell immunoglobulin-like receptors (KIR), CD94/NKG2A and ILT2/LIR-1/CD85j, all able to render NK cells tolerant versus self healthy cells after binding to MHC-class I molecules. Moreover, a relevant inhibitory receptor expressed by NK cells in cancer is Programmed cell death protein 1 (PD-1) [6].

Activating receptors include the natural cytotoxicity receptors NCRs: (NKp30, NKp44, NKp46), the C-type lectin-like receptors [NKG2D (natural-killer group 2, member D) and CD94/NKG2C],

* Corresponding author.
E-mail address: cinzia.fionda@uniroma1.it (C. Fionda).
[1] IRCCS NEUROMED, Pozzilli (IS), Italy.

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activating forms of KIR, the adhesion/co-activating receptor DNAM-1(DNAX accessory molecule-1) and the co-receptors 2B4, CS1 (cell-surface glycoprotein CD2 subset 1) and NTBA (NK, T, B-cell Antigen) [7,8]. These receptors interact with ligands highly expressed on target cells upon tumor transformation, viral infection and cell stress and trigger natural NK cell cytotoxicity. Moreover, NK cells express the receptor CD16 which binds the Fc fragment of IgG and mediates antibody dependent cellular cytotoxicity (ADCC) [9].

Several lines of evidence describe the capability of NK cells to recognize and attack malignant PCs indicating a role for these lymphocytes in the control of MM development. NK cell number and activity correlates with the disease stage and results significantly reduced in advanced clinical stages, suggesting that restoration of NK cell deficiencies could be a suitable therapeutic opportunity for MM.

In this review, we highlight findings supporting a role for NK cells as effectors of anti-myeloma immune response. Moreover, we cover current insight into complex mechanisms responsible for the NK cell dysregulation associated with the progression of this hematological disease. Finally, we summarize and discuss current and novel NK cell based-treatment options for MM.

Anti-myeloma activity of NK cells

A number of studies provided compelling evidence for an important role of NK cells in the control of MM. Initial *in vitro* studies demonstrated that resting NK cells from healthy donors (HDs) have the capability to recognize and lyse MM cell lines as well as cancer cells from MM patients [10]. These observations were confirmed by findings showing that allogeneic and autologous NK cells can kill primary malignant PCs [11–13]. More recently, Wagner and colleagues have demonstrated that IL-15 is able to prime *in vitro* $CD56^{bright}$ NK cells from newly diagnosed MM patients thus enhancing responses against autologous MM cells. Moreover, upon treatment of relapsed/refractory MM patients with an IL-15 super-agonist, a significant increase of $CD56^{bright}$ NK cell degranulation and cytokine production in response to autologous malignant PCs or MM cell lines have been documented [14].

Of note, clinical observations also highlighted a role for NK cells in anti-myeloma response. The number, the activation and the cytotoxic functions of NK cells can increase during and after HSCT, and this increase is often associated with a better overall survival in MM patients [15]. Moreover, the efficacy of IMiDs in MM patients is largely dependent on their strong stimulatory effects on NK cell effector functions [16].

In this context, several studies shed light on the mechanisms involved in MM cell recognition by NK cells. In particular, NKG2D, DNAM-1 and NCR activating receptors were shown to play an important role in recognition and killing of MM cells expressing specific ligands [e.g. MHC class I related chain A and B (MICA/MICB), different UL16-binding proteins (ULBP1-6) and the DNAM1 ligands poliovirus receptor (PVR/CD155) and Nectin-2/CD112] [17–19].

Overall, these findings demonstrate that NK cells are important effectors of anti-myeloma immune response and might represent relevant therapeutic tools.

Defective NK cell recruitment in MM

Multiple myeloma cells contribute to create a strong immunosuppressive microenvironment not only able to negatively affect NK cell activity but also to prevent BM infiltration by NK cells.

Several studies monitored the number, phenotype and functional properties of NK cells in myeloma patients at different clinical stages highlighting a strict correlation between MM progression and alteration of NK cell distribution and function.

Peripheral blood (PB) NK cell number remains unchanged or increases in MGUS or MM patients in earlier stages but often decreases in more advanced disease [3,20–22]. Interesting results were obtained using MM mouse models where the number and frequency of BM NK cells inversely correlate with malignant PC number [23]. Notably, NK cells were already reduced in number at early asymptomatic phase of the disease, and this was attributed to impaired trafficking into BM due to changes of selected chemokine expression [23]. In the BM, changes of inflammatory chemokine levels during pathological conditions dictates NK cell persistence versus mobilization into circulation by modulating expression and/or function of the chemokine receptor CXCR4 involved in BM positioning of NK cells [24]. The peculiar MM BM microenvironment, characterized by up-regulated levels of several inflammatory chemokines, including the CXCR3 receptor ligands CXCL9 and CXCL10, and by reduced levels of the CXCR4 ligand, CXCL12, is thus poorly suitable for NK cell persistence in the MM niche [23,25]. Indeed, CXCR3 deficiency on NK cells improves their persistence and activation state in the BM of MM-bearing mice. Of note, increased serum levels of CXCR3 ligands negatively correlate with MM patient survival. The levels of CXCL10 also associate with remarkable changes of NK cell subset distribution in BM [26]. Indeed, in MM patients with high CXCL10 serum levels the frequency of $CD56^{bright}$ NK cells increases, whereas the frequency of the more cytotoxic subset $CD56^{low}CD16^{low}$ [27] decreases. The clinical significance of the latter observation is still unclear but suggests that a deeper understanding of BM NK cell functional state in relation to CXCR3 ligand expression is required in MM patients.

Defective NK cell activity in MM

NK cells remain functional in MGUS patients, but during MM progression, NK cell functions can be significantly altered, and ultimately compromised in advanced disease. Moreover, a positive correlation exists between NK cell activity and disease-free survival of MM patients [28,29].

Different mechanisms may contribute to the decline of NK cell surveillance during MM evolution (Fig. 1), including a peculiar tumor-induced cytokine microenvironment. Several factors exerting strong NK cell inhibitory effects, such as TGF-ß, IL-6, IL-10, PGE2 and IDO, are produced in MM-BM microenvironment, either by malignant PCs or immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) [30]. The altered production and function of cytokines, such as IL-2 [31] and IL-15 [32] that mediate NK cell development, survival, proliferation and activation may have also a prominent role.

Modulation of the receptor repertoire on NK cells from MM patients can account for defective NK cell functions. As reported by several studies [29,33–37], PB NK cells from MGUS patients express levels of NKG2D equivalent to HDs, while a reduction was observed in MM patients. Accordingly, lytic activity of NK cells from MGUS patients results comparable to that of HDs, whereas it is significantly lower in MM patients [29,33]. Furthermore, a lower expression of NKG2D and of other two relevant activating receptors, NKp30 and 2B4, was appreciated in BM with respect to PB NK cells of the same MM patients [34], thus suggesting an alteration of NK cell phenotype at the tumor site. Accordingly, DNAM-1 expression was reduced on the $CD56^{dim}$ NK cell subset of patients with active disease as compared to that from patients with complete response to therapy or compared to HDs [19]. Surface CD16 levels were also lower on NK cells from MM patients [38], thus suggesting a defective ADCC. Among inhibitory receptors, Konjević et al. reported a significant increase in the expression of KIR receptor, CD158a [36]. Moreover, particular attention has been given to PD-1, due to its peculiar expression on NK cells isolated from MM but not from HDs. The concomitant increase of its ligand PD-L1 on myeloma cells or MDSCs during MM progression may account for the strong PD-1-mediated inhibition of NK cell effector functions [6,37].

Relevant to this, malignant PCs can also use different mechanisms to alter their surface expression of NK cell activating and inhibitory ligands, thus rendering NK cells progressively unable to efficiently