Impairment of NK Cell Mediated Immune Surveillance Against Acute Myeloid Leukemia

Mohammed TAHA

Department of Pharmacology and Medical Sciences, Al-Azhar University of Gaza, Faculty of Pharmacy, Gaza-Palestine

SUMMARY
Natural killer (NK) cells are cytotoxic lymphocytes contributing in innate immune responses that recognize and kill virus-infected and tumor cells without prior stimulation. Several clinical trials have indicated that NK cell-based immunotherapy represents a promising antitumor immunotherapeutic approach due to their key role in mediating graft versus leukemic effect against hematological malignancies, particularly acute myeloid leukemia. However, the antitumor activity of NK cells is inhibited as a result to immune-evasion mechanisms developed by malignant cells through alterations in the expression of activating and inhibitory receptors and their ligands, as well as secretion of soluble NK-inhibitory mediators. Until now, the exact molecular mechanisms involved in these alterations are still not defined.

Keywords: Immune evasion of acute myeloid leukemia; natural killer cells; natural killer cytotoxicity; natural killer cell immunosurveillance.

Copyright © 2022, Turkish Society for Radiation Oncology

Introduction
Natural killer (NK) cells are lymphocytes of the innate immune system which have the ability to recognize tumors and virus-infected cells without prior specific sensitization.[1,2] NK cell functions are regulated by the expression of numerous inhibitory and activating receptors which bind to ligands on healthy or transformed cells.[1-3] The antitumor activity of NK cells is mediated through direct cytotoxic function as well as regulation of other immune cells by cytokine-secreting function. NK cells play a key role in mediating graft versus leukemic (GvL) effect against hematological malignancies, particularly acute myeloid leukemia (AML).[4,5] However, tumor cells can develop immunosuppressive mechanisms to escape NK cell-mediated immunity. Hence, maintaining or improving NK cell performance is considered a major challenge. In this review, we focus on the different mechanisms involved in the evasion of hematological malignancies from NK cells surveillance. Furthermore, we will mention the different approaches used to restore and improve the efficacy of anti-tumor function of NK cells against hematological malignancies.

NK Cell Biology
NK cells are lymphocytes arising from the lymphoid origin which are considered as the third largest population of lymphocytes following T and B cells encompassing approximately 10-15% of all peripheral blood lymphocytes.[6] However, NK cells are considered as critical cells of the innate immune system due to their ability to kill the target cells directly without specific immunization.[3,7] Based on the expression of CD56 molecule, NK cells are defined as CD56+ lymphocytes. [6] Phenotypically, NK cells can be divided into many subsets based on the surface expression of CD56,
CD16, inhibitory receptors and/or activating receptors. In general, the major subpopulations of NK cells are CD56bright CD16−/+ (5-10% of NK cells) and CD56dim CD16+ NK cells (90-95% of NK cells).[6,8]

**NK Cell Cytotoxicity**

NK cells play a key role in immuno-surveillance and host defense against certain virus-infected or transformed cells mediated by direct cytolysis as well as regulation of the effector functions of other cytotoxic immune cells.[9-11] NK cell functions are controlled by a balance between activating and inhibitory signals provided by a varied group of activating receptors as (NKG2D, DNAM-1, Nkp30, Nkp44, and Nkp46) and inhibitory receptors as NKG2A.[12] In general, NK cells can recognize abnormal cells through two models: Missing-self recognition and stress-induced recognition because abnormal cells as tumor cells can change their surface phenotype by losing the expression of human leukocyte antigen (HLA) class I molecules and/or upregulating damage-associated proteins. Numerous damage-associated proteins have been expressed by tumor cells such as MICA and MICB binding with NKG2D, ligands of Nkp30 as B7-H6, a mixed-lineage leukemia protein which is a ligand of Nkp44, and CD155 and CD112 which interact with DNAM-1.[14] Consequently, NK cell activating receptors bind with their specific ligands expressed on the target cells resulting in lysis of their target cells. Alongside their activation by tumor cells and pathogens, NK cells can be directly or indirectly regulated through signals from other immune cells such as dendritic cells (DCs), macrophages, CD4+ T cells during the immune response.[14] Then, activated NK cells have the ability to kill their target cells though a variety of mechanisms, including cytolysis, signaling through the tumor necrosis factor (TNF) death receptor family members such as FAS (CD95) and TNF-related apoptosis-inducing ligand, the release of cytokines IFN-γ and TNF-α, and antibody dependent cell cytotoxicity (ADCC) via CD16. Besides the cytolysis mediated by NK cells, immune response can be regulated by NK cells through the recruitment of other immune cells.

**Mechanisms of AML Escape from NK Cell Immunity**

Although the cytotoxic activity of NK cells against leukemia cells and their beneficial role in immunotherapy, many tumors including AML can evade the immunosurveillance of NK cell by destroying the precise balance between inhibitory and activating signals.[28-32] Commonly, AML cells are able to escape NK cell immunosurveillance through various mechanisms: i) alteration of NK cells, ii) immunosuppressive properties of AML cells, and iii) interactions with other immune cells (Fig. 1).

**Alterations of NK Cell By AML**

AML cells are capable to alter the expression of NK cell receptors and their ligands, resulting in a significant impairment of NK cell functions, however, the molecular mechanisms responsible for these alterations are still unknown.

**Alterations of the Expression of NK Cell Receptors**

Several reports have shown a clear decrease in the expression of NK activating receptors on circulating NK cells of AML patients such as natural cytotoxic receptors, NCRs (Nkp30, Nkp44 and Nkp46), NKG2D, and DNAM-1.[28,30,31,33-35] These alterations are associated with impaired anti-leukemic activity of NK cells, a decreased cytokine production, and risk of tumor relapse. Notably, Fauriat et al.,[30] (2007) showed that NCRs downregulation on NK cells was associated...
with poor prognosis for AML patients, and significantly lower 5-year survival rates than their NCRbright counterparts. Interestingly, the phenotypic and functional abnormalities of NK cells are partially or totally restored in patients achieving remission, which suggest that the presence of AML cells is responsible for NK cells abnormalities.[30] Moreover, Olive’s team in 2017 showed a strong correlation between NKp46 expression on NK cells of AML patients at diagnosis and the clinical outcomes after allogeneic stem cell transplantation. They found that patients with high expression of NKp46 at diagnosis had better progression-free survival and overall survival (OS) than patients with low expression of NKp46.[36] Regarding the expression of inhibitory receptors, it is clear that failure to achieve remission in AML patients is strongly associated with overexpression of NKG2A and inhibitory KIRs.[31,33]

Alterations of the Expression of NK Cell Receptors Ligands
Another strategy by which AML can escape from NK cell immunosurveillance is decreasing the expression and shedding of surface ligands for various NK cell activating receptors on AML cells themselves.[34,37-40] For example, leukemic blasts are characterized by a sharp decrease in the expression of MICA/B and ULBPs (ligands of NKG2D), CD48 (a ligand for 2B4), NCR-specific ligands, and DNAM-1 ligands (CD112/CD155).[34,38,39,41,42] These alterations were associated with reduction of the effector functions of NK cells and OS among those patients. On other hand, DNAM-1 ligands (CD112 and CD155) are highly expressed on AML blasts of patients younger than 65 years. However, NK recognition and killing of leukemic blasts is reduced due to downregulation of DNAM-1 on NK cells of AML patients, hypothesizing a converse relationship between DNAM-1 ligands expression on leukemic blasts and DNAM-1 expression on NK cells. [43] Besides the classical alterations of the expression of NK receptors and their ligands in AML, Olive’s team reported alteration in the maturation profile of NK cells in AML patients. They found three different groups of AML patients based on NK maturation profile: hypomutation, intermediate maturation, and hypermaturation. Interestingly, the findings revealed that patients with hypomutation profile have decreased OS and relapse-free survival compared to patients with intermediate and hypermaturation.[44]
Alterations of NK Cell at Genetic Level

Some attempts were performed at gene level to try to identify the molecular mechanisms of NK functions defect in hematological malignancies. In this context, Costello's team aimed to realize the mechanisms underlying NCRs down-regulation in NK cells from AML patients.[32] They found that AML-NK cells showed a specific transcriptomic signature compared to NK cells from healthy volunteers, disappeared by NK cells expansion. Although the gene expression of E26 transformation-specific 1 (ETS-1) transcription factor (a potential regulatory element of NCRs expression) was decreased in presence of AML blasts, the expression of ETS-1 and NCRs was restored following AML-NK cells expansion. This proposes that ETS-1 may regulate NCRs expression.[32] In addition, miRNAs, which play an important role in fundamental NK cell biology,[45] can be well accepted to participate in many aspects of AML, including proliferation, differentiation, survival, apoptosis and invasion by targeting oncogenes or tumor suppressors.[46,47] A study showed a selective loss of immature NK cells subset and a clear reduction in the cytolytic granules containing perforin and granzyme B among NK cells in leukemic mice and the NK cells in cytolytic granules containing perforin and granzyme B of immature NK cells subset and a clear reduction in the mor suppressors.[46,47] A study showed a selective loss of apoptosis and invasion by targeting oncogenes or tumor suppressors.[46,47]

Alterations of Interaction Between NK Cells and Other Immune Cells

Cellular interactions between NK cells and other immune cells are also altered in AML patients resulting in more possibilities of immune escape. NK cells play a key role in regulation of DCs by killing immature DCs to limit inflammation and inappropriate T cell tolerization. Fauriat and his colleagues (2005) have noticed the inability of NK cells from AML patients to kill immature DCs which might result in an abnormal interaction between T cells and immature DCs and induction of tolerogenic T cells.[54] Further, the number of Treg cells, which are the predominant immune suppressor cells, are increased among AML patients compared to healthy donors, while their numbers are reduced in patients with complete remission (CR).[55]

Restoration of NK Cell Cytotoxic Functions

Whereas impaired NK cells are associated with the progression of AML, recovery and boosting the effector functions of NK cells are essential for the control and eradication of AML. In general, there are different approaches used to restore and enhance the effectiveness of anti-tumor function of NK cells, including cytokines, monoclonal antibodies (mAbs), and adoptive transfer of NK cells.[14,26,56-59]

Cytokines

Several cytokines have been confirmed to enhance NK cell proliferation and/or cytotoxicity against several types of tumors. Cytokines are used for this purpose either by direct infusion of cytokines in vivo to boost the autologous NK cell numbers and functions or by in vitro incubation of allogeneic NK cells with cytokines before adoptive NK cell immunotherapy. IL-2 is the first cytokine approved for use in patients to improve NK cells activity, where it restored NK cell receptor expression and increased NK cell activity against autologous AML cells in vitro.[60] However, infusion of IL-2 into patients was accompanied by limited clinical outcomes because...
that monoclonal anti-CD123 antibody (CD123 is over-
ADCC. Consistent with the meaning, studies showed
ceptor expressed by NK cells to induce NK-mediated
cytotoxicity on NK cells, which can be presented to NK
cells in vivo through several cell types, including mono-
cytes, macrophages and DCs.[63] Infusion of IL-15 into
patients increased the cell numbers of circulating NK
cells and upregulated the expression of the activating
NK cell receptor NKp30, which augmented NK cell cy-
totoxicity in AML patients.[35,64]

mAbs and Checkpoint Inhibitors
mAbs can realize anti-tumor effects by modifying the
activity of their target proteins and by redirecting the
effector immune cells to cancer cells. mAbs treatment
based on NK cells includes mAbs which target tumor-
associated antigens to induce NK cell, and mAbs which
target and block immune checkpoint proteins to en-
hance NK cell cytotoxicity.[14,26,57] By targeting tu-
morelated antigens, a specific immune response
can be achieved against the tumor cells. In the case
of NK cells, these antibodies directly target tumor-assoc-
ated antigens and also bind to FcγRIIIA (CD16a) re-
ceptor expressed by NK cells to induce NK-mediated
ADCC. Consistent with the meaning, studies showed
that monoclonal anti-CD123 antibody (CD123 is over-
expressed on AML stem cells showing anti-leukemic
activity) improved the binding to CD16a and enhanced
the anti-leukemic activity of NK cells against AML
xenograft models.[65,66] Further, Koerner et al.[67]
(2017) found that an Fc-optimized CD133 antibody had
a greater affinity to NK cells and more cytotoxic activity
for NK cells without relevant toxicity to hematopoietic
progenitors in a human AML xenograft model.

The second strategy based on mAbs to recover NK
cell effector functions is using targeted mAbs to block
specific immune checkpoint proteins to enhance the
cytotoxic activity of NK cells. One of these inhibitory
proteins is NKG2A, an inhibitory receptor expressed
in NK cells and binds with HLA-E ligand. The expres-
sion of HLA-E is often upregulated in some cancer
cells to escape from NK cell cytotoxicity. In addition,
NKG2A expression on tumor-infiltrating NK cells has
increased in cancer cells.[68,69] As a result, blocking
NKG2A by a humanized antibody called monalizumab
improves the cytotoxic activity of NK cells in mice en-
grafted with primary leukemia cells as well as against
HLA-E+ target cells.[70,71] Another checkpoint affect-
ing the functional activity of NK cells is programmed
cell death protein 1 (PD-1) which was recently discov-
ered in a mature CD56dim NK cells where its expression
significantly suppresses NK function against PD-1 lig-
and expressing tumor targets.[72,73] In addition, the
expression of programmed cell death ligand-1 (PD-L1)
is observed in AML blasts.[74] PD-1 antibodies such as
pembrolizumab and nivolumab, preventing PD-1/
PD-L1 interaction, have been developed and their ef-
fect to enhance endogenous NK cell cytotoxic function
remains attractive.[75] Interestingly, blocking PD-1
or PD-L1 increases the cytotoxic activity of NK cells,
and decreases the growth of some tumors in xenograft
models.[76,77] In AML, nivolumab in combination
with idarubicin and cytarabine decreased the progres-
sion of AML in patients with newly diagnosed AML
and also increased the OS.[78]

NK Cell-Based Adoptive Immunotherapy
The strategy of using NK cells as adoptive immunother-
apy depends on the valuable effects of NK cell allore-
activity which is induced by the mismatch between
HLA class I molecules on recipient cells and KIRs on
donor NK cells. Several clinical reports have shown
that donor NK cell alloreactivity is a key therapeutic el-
ement in the success of transplant and killing leukemia
through GvL effect without development of graft versus
host disease as well as controlling infections.[79,80] In
general, donor-derived NK cells are mainly obtained
from donor PBMCs by separating protocols; however,
attaining sufficient numbers of NK cells from PBMCs
to achieve a therapeutic effect has been a major limita-
tion.[81] Therapeutically, a phase I clinical trial showed
that infusion of IL-15 plus IL-21 stimulated NK cells
which were given after hematopoietic stem cell trans-
plantation (HSCT) reduced progression of leukemia
compared with patients who have subjected to HSCT
without NK cell infusion.[82] Moreover, infusion
of multiple doses of NK cells expanded ex vivo with
feeder cells was effective in minimizing leukemia rel-
apse. A present study showed that infusion of IL-2 ac-
tivated NK cells into patients with hematologic malig-
nancies 2 months following HSCT was associated with
increasing the expression of activating receptors on the
reconstituting NK cells as well as increasing degranu-
lation and cytokine production.[83] After follow-up,
a CR of hematologic malignancies was observed in 11
patients out of 16 treated patients.

Conclusion

NK cells are a specific group of lymphocytes playing
a key role in the innate immune responses against
virus-infected cells as well as different types of cancer.
Although NK cells play a major role in immunosurveillance against AML cells, it was demonstrated that NK cells in AML patients have impaired anti-leukemic activity due to multiple mechanisms of innate immune escape, including down-regulation of activating receptors expression, up-regulation of inhibitory NKG2A expression, down-regulation of NK-activating ligands, and secretion of soluble NK-inhibitory factors as well as other immunosuppressant mechanisms. However, the specific molecular mechanisms involved in these alterations are still not well defined. Hence, there is a persistent need for complete understanding of how AML escapes the natural defenses of immune system.

**References**

44. Chretien A-S, Fauriat C, Orlanducci F, Galseran C, Rey
NK Cell Dysfunction in AML

Taha

57. Gauthier M, Laroye C, Bensoussan D, Boura C, De-
56. Chiossone L, Vienne M, Kerdiles YM, Vivier E. Natural killer cell immunotherapies against cancer: checkpoint
55. Shenghui Z, Yixiang H, Jianbo W , Kang Y , Laixi B, Fauriat C. Defective killing of dendritic cells by autol-
54. Fauriat C. Defective killing of dendritic cells by autol-
43. 57. Gauthier M, Laroye C, Bensoussan D, Boura C, De-
42. 56. Chiossone L, Vienne M, Kerdiles YM, Vivier E. Natural killer cell immunotherapies against cancer: checkpoint

57. Gauthier M, Laroye C, Bensoussan D, Boura C, De-
56. Chiossone L, Vienne M, Kerdiles YM, Vivier E. Natural killer cell immunotherapies against cancer: checkpoint
55. Shenghui Z, Yixiang H, Jianbo W , Kang Y , Laixi B, Fauriat C. Defective killing of dendritic cells by autol-
54. Fauriat C. Defective killing of dendritic cells by autol-
43. 57. Gauthier M, Laroye C, Bensoussan D, Boura C, De-
42. 56. Chiossone L, Vienne M, Kerdiles YM, Vivier E. Natural killer cell immunotherapies against cancer: checkpoint

57. Gauthier M, Laroye C, Bensoussan D, Boura C, De-
56. Chiossone L, Vienne M, Kerdiles YM, Vivier E. Natural killer cell immunotherapies against cancer: checkpoint
55. Shenghui Z, Yixiang H, Jianbo W , Kang Y , Laixi B, Fauriat C. Defective killing of dendritic cells by autol-
54. Fauriat C. Defective killing of dendritic cells by autol-


72. Davis Z, Felices M, Lenvik TR, Badal S, Hinderlie P, Blazar BR, et al. PD-1 is expressed at low levels on all peripheral blood natural killer cells but is a significant suppressor of NK function against PD-1 ligand expressing tumor targets. Blood 2019;134(Supplement_1):621.


