Natural Killer Cells: A Review on Their Role in Cancer Immunotherapy

Shailaza Shrestha*, S P Saxena2, Rahul Rai3

1PhD Scholar, Department of Biochemistry, Heritage Institute of Medical Sciences, Varanasi, Uttar Pradesh, India. 
2Professor, Department of Biochemistry, Hind Institute of Medical Sciences, Lucknow, Uttar Pradesh, India. 
3PhD Scholar, Department of Anatomy, Heritage Institute of Medical Sciences, Varanasi, Uttar Pradesh, India.

ABSTRACT
NK cells are major immunosurveillance cell comprising about 15% of cells in the circulation. Highly controlled regulation between activatory and inhibitory receptors mediates the functions of NK cells. These cells target the tumorous cells that lack MHC (Major histocompatibility Complex) or have altered molecules on their surfaces whereas healthy cells are protected from lysis by NK cells due to inhibitory receptors and MHC I interactions. NK cells, chiefly allogenic variants, are used in the treatment of various cancers such as breast cancer, glioma, neuroblastoma, renal cancer, melanoma etc. NK cells act by release of perforins and granzymes or by activating ADCC (Antibody dependent cellular cytotoxicity) pathway. Though NK cells have evolved as a promising therapeutic regimen for malignancies, their limited concentration in blood has made it difficult to be used widely. Thus researches are being focused on exvivo activation and expansion of NK cells so as to increase the cell number, viability and cytotoxic functions.

Key words: Natural Killer Cells (NK Cells), Major Histocompatibility Complex (MHC), Tumors.

*Correspondence to:
Shailaza Shrestha,
PhD Scholar, Department of Biochemistry, Heritage Institute of Medical Sciences, Varanasi, Uttar Pradesh, India.

INTRODUCTION
A consensus in 2012 indicated about millions of new cases, which about 8.2 million deaths related to cancer, which indicates cancer to be one of the major health threats worldwide1. The conventional techniques used in the treatment of cancers were surgery or the use of ionising radiations or chemotherapeutics, but the major drawbacks observed in these methods were higher rates of tumor relapse, due to resistance developed by the timorous cells to the drugs and radiations2. To overcome these drawbacks, and to make the cancer treatment more reliable, safe and to prevent relapse recently researches are being focused on cancer immunotherapy’s, in which several cytokines (IL-2, IL-12 etc.), immune cells (T cells, dendritic cells, natural killer cells and NK cells, cytokine induced killer cells or (IK), monoclonal antibodies are used3. It was in 1971, natural killer cells were first described when Cudkowicz and Benett showed, and mice undergoing lethal irradiation could reject allogenic parental allograft of bone marrow cells4. Later in 1975, cells capable of mediating such action were characterised to be radioresistant5 having identity similar to lymphoid cells with cytoplasmic granules6 and ability to spontaneously kill tumours cells in vitro, the mechanism of which was MHC (major histocompatibility complex) restricted. These cells were later recognised as NK cells8. However the therapeutic role of NK cells remained hidden till 1990s when target cell recognition phenomenon of NK cells was elucidated and in early 2000s when antileukemic effect of NK cells was demonstrated in HCT (Hematopoietic cell transplantation)10.

BIOLOGY OF NK CELL
Keissling and Herberman were the first to name and characterise NK cells11. These cells were named considering the fact that, the term natural meant the natural occurs of these cells and killer meant their ability to kill lymphoma and leukemic cells12. The primary origin of NK cells that have pivotal role in innate immunity

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NK CELL RECEPTORS

Highly sophisticated receptors network control the fate of NK cells, aiding them to differentiate to abnormal cells from normal. There is a well regulated balance between the inhibiting the activating signals derived from various receptors. When inhibiting signal is dominated by activating signal, lysis of target cells occurs.

NK cell receptors are of two types namely activating receptors and inhibitory receptors. Major inhibitory receptors include killer Ig like receptors (KIR) and C-type lectin receptors (KNG2A), whereas activating receptors are natural cytotoxic receptors (NCRs), Nkp30, Nkp44, NKGD2 etc. The first NK cell receptor named Ly-49 (MHC-1 specific) was discovered in mouse. Ly-49 receptors are integral membrane proteins type II and homologs that can recognize MHC-1 molecules such as H-2d and H-2k. Several receptors about more than 20, belonging to Ly-49 have been characterized, out of which 13 are inhibitory and 8 are stimulatory to the NK cells. The other category of receptors, that have been discovered and which are chiefly expressed in man, are the transmembrane proteins related to the Ig (immunglobulin) superfamily and can recognize the groups such as HLA-C (Human leukocyte and HLA-A alleles). Of these, HLA-C is mainly involved in the regulation of NK cell functions.

The receptors (both KIRs and Ly-49), that are inhibitory in nature, conduct their action via ITIMs (Immunoreceptor tyrosine-based inhibitory motifs). On binding of specific MHC molecules, the tyrosine residues are phosphorylated causing inhibition of stimulatory signals. On the other hand, stimulatory receptors lack ITIMs and mediate their action via association with DAP 12, which is a ITAM (Immunoreceptor tyrosine based activation motif) bearing adaptor molecule. Receptors associated with NKG2 family have also been identified in human and mice. Such receptors contain CD94 molecule (a C type lectin) which can exert both excitatory and inhibitory signals. Inhibitory signals occur when CD94 binds with Qa-1b (in case of mouse) and HLA-E (in case of human in target cells).

MECHANISM OF ACTION

Studies have shown that CD56 dim type NK cells are the major circulating fraction and most potent against tumor cells. Similarly, a 11 year follow up study has demonstrate that, reduced NK cell cytotoxicity is associated with the increased risk of cancer. An increased infiltration of NK cells in cancer cells have proven to be a positive prognostic marker in carcinomas of colorectum, stomach and lungs.

Direct clearance of tumor cells

The cells deficient of MCH-I or the cells with stress induced protein molecules are targeted by NK cell. Moreover, certain cells with expressed MHC-I are also targeted via activating receptor induced recognition of stress induced ligand. Several mechanisms have been developed to explain the direct killing of tumor cells by NK cells. These include:

- Release of cytoplasmic granules with perforin and granzymes causing caspase dependent or independent apoptosis of tumor cells.
- FasL (Fas ligand), TRAIL (Tumor necrosis factor related apoptosis inducing ligand) dependent mechanisms.
The document discusses the role of natural killer (NK) cells in cancer immunotherapy. It highlights the secretion of effector molecules like IFNs, IL-2, IL-12 etc which not only stop tumor angiogenesis and stimulate adoptive immunity but also induce production of nitric oxide causing the destruction of tumor cells via NO signaling mechanisms. ADCC (antibody-dependent cellular cytotoxicity) is also discussed.

The text mentions that NK cells recognize target cells coated by antibodies, enhancing the NK cell-mediated ADCC with rapid activation and degranulation of NK cells. Immune checkpoints, such as PD-1 and PD-L1, play a crucial role in regulating NK cell activity. CD3 depletion, autologous and allogenic sources of NK cells, and the potential of NK cell-based immunotherapy are also covered.

NK cells are involved in direct and indirect clearance of tumor cells. Indirect clearance involves dendritic cells that enhance the antigen-specific cytolytic responses, cross-presenting tumor-specific antigens to NK cells, and activating NK cells via antigen presentation by Ag-Pulsed DCs. These activated NK cells can then lyse target cells via ADCC.

NK cells are key players in cancer immunotherapy, and their role includes the induction of antitumor activity, especially in solid tumors and hematological malignancies. NK92 cells are used extensively for clinical trials due to their cytotoxic potential.

NK cell lines
About seven NK cell lines have been developed, namely NK92, YT, HANK-1, KHYG-1, NKL, NKYS and NKG which are used in the production of "Off the shelf" anticancer products. Of these cell lines, NK92, KHYG-1 and NKG are well known for the anti-tumor function. NK92, clinically the most studied cell line, is FDA approved safe and beneficial therapy for a wide range of tumors. In the study of Tonn et al., NK92 was used to treat both solid tumors and hematological malignancies. Use of NK92 cells showed partial efficacy in some end stage cancer like renal cancer and melanoma.

Steps of NK cell base immunotherapy
NK cell-based immunotherapy involves three major steps as follows:

1. **KIR typing**
   - Of several NK cell receptors identified, KIR family is the major determinant in NK cell response. KIR, since is strongly polymorphic, its typing is very critical. The advantage of KIR typing is that it facilitates the selection of allogenic donor and prognostication of autologous NK therapy.
   - KIR typing involves genotyping, categorisation of A/B haplotypes, phenotyping and alleotyping. Gene expression can be assessed by real-time PCR or flow cytometry. Allele polymorphism is seen in all KIR genes that are inhibitory in nature. Therefore single nucleotide polymorphism assays are being used for rapid clinical typing.
   - Patients receiving grafts with stronger KIR allele demonstrated lower relapse rate, better survival and lower mortality rates.

2. **Cell processing**
   - **NK cell purification**
     - After selection of suitable donor via KIR typing, the NK cells are purified and processed ex vivo. The quality of NK cells is assessed in terms of cell count, function, purity, viability and phenotype. Immunogenetic separation technique yields purified form of NK cell mixture that consists of more than 90% of NK cells with very little amount of T and B lymphocytes.

NK cell processing or activation ex vivo
Purified NK cells can be activated in several ways. One of the common methods is by using cytokines such as IL-2, IL-15 or IFN type. An alternative to this method is to activate NK cells by artificially presenting the cells with membrane bound ligands like IL-15 and IL-21.

3. **Use of chimeric antigen receptor (CAR)**
   - Use of CAR can also aid to potentiate NK cells. Genetic insertion of CAR with specificity for tumor-associated antigens can enhance the specificity and cytotoxicity of NK cells.

The text concludes with the importance of NK cells in cancer immunotherapy, highlighting the potential of NK cell-based therapies and the need for further research to improve their efficacy and safety.
Augmentation of NK cells
It can be mediated via

Combination of NK cells with therapeutic antibody/ Chimeric protein

One of the important NK cell dependent therapy for cancer is ADC⁵. Use of monoclonal antibody against CD137 or blocking antibodies for KIR or NKG2A increases NK cell induced cell lysis⁶. Another method of augmentation is incubation of NK cells with specific killer cell engager. This not only stimulates cytolytic activity of NK cells but also increases production of INFγ, TNFα and IL8⁷.

Combination with medication

NK cells that are adoptively transferred not only target cancerous cells, but also interact with immune system, therefore combination therapy should be applied to improve efficacy⁸. Most of the combined regimen includes injection of IL 2 in low dose after NK cell infusion but recent studies are being focused on newer agents. As for example, non-Hodgkin lymphoma is being treated using granzyme B and FasL along with lenalidomide as medical supplement which enhances ADC⁹. Drugs such as spironolactone upregulates NKG2D² whereas doxorubicin upregulates TRAIL R2 causing enhancement of antitumor functions of NK cells. NKG2D can also be upregulated by histone deacetylase inhibitor⁹.

Modulation of tumor cells

Tumor cells can be epigenetically modulated with histone deacetylase inhibitor, demethylating agents and proteosome inhibitors⁹. Such modulation upregulates NKG2D and prevents its loss from NK cells⁹. In a study of William et al, lentiviral shRNA was used to target almost 10000 human genes so as to silence JAK 1 and JAK 2. This increased susceptibility of tumor cells to lysis by NK cells⁹.

Genetic modifications of NK cells

Recent advances in the field of NK cell immunotherapy include genetic manipulation of NK genes via siRNA, that involves suppression of inhibitory receptors and over expression of activating receptors⁹. Further NK cells can also be engineered to express chimeric antigen receptor⁹ and cytokines (via transfer of cytokine genes)⁹, which not only activate but also improve proliferation, survival and antitumor activity of NK cells⁹.

CONCLUSION

NK cells have been recently evolved as the potential therapeutic agent in cancer, which are resistant to the contemporary therapies used. NK cells, major cells in tumor immunosurveillance, can destroy tumor cells that lack or have altered MHC-I proteins, via release of cytotoxic granules. Normal cells are protected from lytic effect due to interactions between KIRs and MHC-I protein on the cell surface.

However, NK cells, though evolved as a novel immunotherapeutic strategy, have some limitations which hinder their widespread use. NK cell therapy, both autologous and allogenic, requires about 10⁹ lymphocytes. Since the peripheral blood from donor comprises only 15% of NK cells, it possesses a challenge in acquiring NK cells in sufficient amount as well as difficulties in expanding the cell ex vivo. Besides, in spite of presence of efficient immunosurveillance system, many tumors can still develop in body creating the disease. Therefore, it is necessary to understand the mechanism by which the tumor cells escape immunosurveillance and researches are to be focused on development of more promising approaches for expansion and activation of immune cells involved in cancer therapy so as to attain higher cytotoxicity ability, survival rates and desirable clinical outcomes.

REFERENCES


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