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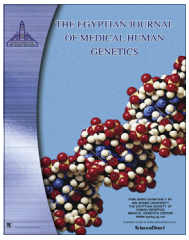

REVIEW

Natural killer cells enhance the immune surveillance of cancer

Faisal Nouroz [a,b,*], **Farzana Bibi** [a], **Shumaila Noreen** [c], **Nosheen Masood** [d]

[a] *Department of Bioinformatics, Hazara University Mansehra, Pakistan*
[b] *Department of Botany, Hazara University Mansehra, Pakistan*
[c] *Department of Zoology, University of Peshawar, Pakistan*
[d] *Department of Biotechnology, Fatima Jinnah Women University, Rawalpindi, Pakistan*

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Abstract Immune system (IS) is comprised of molecules, cells, tissues and organs involved in host defense mechanism from infectious agents or tumor cells. On crossing the cell barriers by these infectious agents, the defense mechanism is alerted by the immune system to respond against these invading microbes. Innate immune response (IIR) and acquired immune response (AIR) are working in parallel to control these invading microbes. IIR is composed of various types of phagocytes and lymphocytes, while AIR is comprised of T and B lymphocytes. All the cells of the immune system cooperatively work against infectious agents and cancerous cells but Natural killer (NK) cells are playing an important role to respond to tumor by enhancing the expression of complementary domain (CD86) on dendritic cells (DCs) and production of IL-12. NK cells demolished tumor through perforin and granzyme, which are important for immune surveillance and death of tumor cells induced by cytokines such as tumor necrosis factor (TNF), Fas ligand (CD178), interferon-γ (IFN-γ) and IL-10. These cytokines have inhibited proliferation of tumor by inducing anti-angiogenic factors and maintaining cross talk with other immune cells. Natural products like transfer factor plus, immune modulator mix, ascorbic acid, *Ganoderma lucidum*, *Agaricus blazei* teas, nitrogenated soy extract, *Andrographis paniculata* and several phytochemicals enhanced the efficiency of NK cells in controlling cancers. Further studies will unravel the impact of NK cells in cancer control and how NK efficiency can be further enhanced.

* Corresponding author at: Department of Bioinformatics, Hazara University Mansehra, Pakistan. Tel.: +92 997 414168.
E-mail address: faisalnouroz@gmail.com (F. Nouroz).

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Contents

1. Introduction

Immune system (IS) is a network of a population of molecules, cells, tissues and organs to protect the body from microbes like viruses, bacteria and other toxins. When microbes cross the natural cell barriers, the IS alerts the defense mechanism of the body and activates different types of cells to respond to these invading microbes to protect the health of the organism. The IS naturally has the ability to protect and heal the body and to prevent various diseases. A healthy IS increases the ability of the body to defend against various pathogens, microbes, decreases infection rate, allows efficient healing and improves quality of health [1]. The immune system is dependent on two types of immunities; innate immune response (IIR) and acquired immune response (AIR). The IIR is a second line of defense because it acts against invaders, who cross the physical barriers such as skin and mucosa. IIR is comprised of various phagocytes such as Natural killer cells, dendritic cells, macrophages, neutrophils, basophils and eosinophils (Fig. 1). Natural killer cells are involved in the destruction of tumor and virus-infected cells, while the dendritic cells, macrophages, basophils, eosinophils and neutrophils mediate the inflammatory response which is immediate but of short term. These cells also act as physical or chemical barriers to

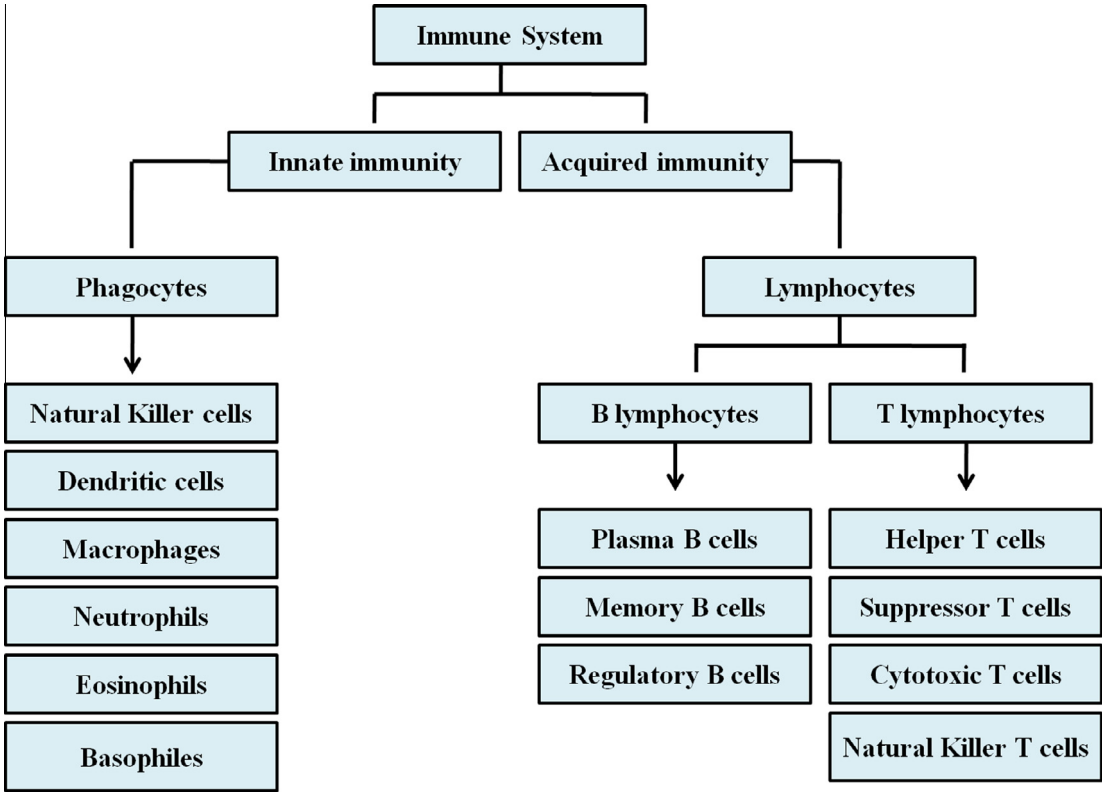

Figure 1 Type of immune system and various immune cells involved in defense system.

the invading microbes or infectious agents and control the disease. These cells phagocytose the pathogens or foreign invading microbes and help in their elimination. IIR requires no special preparation to act against pathogens and consists of chemical, mechanical, microbiological and cellular defense networks. IIR's function is to stop and return the foreign particles at the entry sites. AIR is an adaptive immune response, activated by IIR through specific signals. AIR is made up of T and B lymphocytes (Fig. 1), where B lymphocytes are involved in humoral while T lymphocytes are involved in cell mediated cell response. The T lymphocytes act through receptors (TCR) and B lymphocytes respond through antibodies. These specialized cells are involved in the elimination and prevention of the growth of pathogens. IIR and AIR responses are given through blood and physical barriers and both immunities correspond with each other through cytokines (Interleukins, Interferon's and growth factors), chemicals and hormones [2].

2. Immune system and cancer

Cancer is a disease initiated by a series of collective genetic and epigenetic changes in a normal cell. In addition to the malignant cell itself, cancer is a disease of micro environment and immunity [3]. The immune system (IS) and tumor cells are often concurrent in a dynamic equilibrium and both have a complex interaction and are interlinked [4]. Tumor has the capacity to resist the IS in order to proliferate but in response, the IS has the ability to control the growth of proliferating tumor cells. According to the theory of immune surveillance, the IS works for cancers on the basis of three stages such as exclusion, equilibrium and escape [5]. In exclusion (elimination) stage, cancer is recognized and destroyed by the IS. In equilibrium stage, IS wipes out the cancer cells completely. In escape stage, the IS remains unable to control the growth of proliferating cancerous cells and cannot demolish the tumor. In cancer, the IS is mostly pathetic due to the use of radiotherapy and chemotherapy, so research has been conducted in recent years to boost immunity and immunotherapy is carried out to treat cancer [6].

3. Role of Natural killer cells

Natural killer (NK) cells were first discovered in humans and mice in 1975 and are large granular population of leukocytes, that can directly kill the virus infected or tumor cells [4]. NK cells of the immune system specially lyse the tumor cells and virus infected cells [7]. A recent analysis suggested the response of NK cells based on the expression of major histocompatibility complex class-1 (MHC-1) molecules and inducible ligand of NK cell receptor [8]. NK cells play an important role in the immune surveillance of cancer and are accomplished to prevent the growth of tumor [9,10]. They have the ability to recognize targets directly through inhibitory or activating receptors expressed on the cell surface of tumor or other target cells [11]. The ligand on the surface of target cells (infected or tumor cells) triggers NK cell cytotoxicity and activates the receptors of NK cells. These ligands are absent on the surface of normal cells. NK cells modulate activity of other leukocytes such as dendritic cells (DCs) and T cells through cytokine. A recent study reported that NK cell derived Interferon-γ (IFN-γ) promotes macrophages toward tumor and defends the organism from carcinogen induction [12].

Table 1 Function of natural killer cells in humans triggered by cytokines, receptor and ligands. TLR: tool like receptor, TGF: tumor growth factor, Ly49A: type II transmembrane molecule, KIR: immunoglobulin like receptors, MICA: MHC-1 related ligand, HLA: human leukocyte antigen.

Activator	Inhibitor	Receptors	Ligands	Cytotoxicity
TLR ligand	IL-10	NKG2A	HLA	IL-2
IFN-α/β	TGF-β	NKG2D	MICA	IL-12
IL-2	MHC-1	CD16	IgG	IL-15
IL-12	KIR	NKp30	Tumor	IL-18
IL-15	Ly49A	NKp44	Tumor	IL-25
IL-18	Tumor	NKp46	Tumor	IFN-γ

4. Mechanism involved in the lysis of tumor by NK cells

Generally the NK cells are activated by different stimuli such as contact to the dendritic cells, MHC-1 negative cells, products derived by the infectious agents, direct involvement of NKR by stress induced tumor molecules, various cytokines such as IL-1, IL-2, IL-12, IL-15, IL-18, IL-21, and type I IFNs. On the stimulation of cytokine, the NK cells are converted into lymphokine-activated killer (LAK) cells. These LAK cells propagate, produce cytokines and up-regulate the effectors or adhesion molecules like perforin, NKp44, granzymes, Fas ligand (FasL), and TRAIL. These LAK cells adhere to the target cells and recognize them and increase tumor lysis activity [13–15]. Tumor necrosis factor (TNF) family ligands express themselves on the surface of NK cells. NK cells control the tumor by continued T cell antitumor response through cross talk with dendritic cells [16]. Evidence showed that cytokines such as IL-2, IL-15, IL-12, IL-18, (see Table 1) and CD40 enhance NK cell cytotoxicity against tumor target cells and the production of IFN-γ by NK cells [17]. IFN-γ and CD40 play an important role in the stimulation of dendritic cells and provoke IL-12 from dendritic cells [18].

5. Granzyme B

Previous studies reported that Granzyme B (GrB) is generated by NK cells and cytotoxic T lymphocytes (CTL) in an inactive form and contains N-terminal signal dipeptide (Glycine-Glucan). The cysteine protease creates activity of the GrB by removing the dipeptide. Perforin is released and binds to the cell membrane of tumor cells and causes the conformational changes to form the pores in the cell membrane. GrB derivatives are interacted with tumor cell surface antigen (EGFR or ErbB2), and by these pores GrB cross the threshold and enter into the cytosol of the target cells and induce apoptosis [19]. Research revealed that cancer increases expression of major histocompatibility complex class-1 (MHC-1) [20] in order to inhibit NK cell cytotoxicity and suppress the expression of NKG2D, which spoils the recognition of NK cells [21]. Previous studies reported that treatment with GrB-scFv proteins sensitized tumor to radiation and chemotherapy [22], so in future the mechanism of GrB requires further analysis. A recent study in humans reported that administration of IL-15

enhanced the activity of NK cells [23]. Another analysis showed that clinically mutual administration of IL-2, IL-15 in mice increased the efficiency of antitumor immunity in carcinoma [24].

6. Cancer stem cells

Cancer stem cells (CSCs) retain the growth of tumor and resist chemotherapy [25]. NK cells can target CSCs and are talented targets for immunotherapy in future [26]. Previous studies suggested that tumors resist immunity through dominant inhibitory effects of the immune system. The prospect of immunotherapy for cancer is now becoming a clinical reality. Besides a new approach of databases predicting the character peptide epitopes to definite MHC molecules (e.g. human leukocyte antigen-A2; HLA-A2) has been recognized. In the course of bioinformatics, the background of mutatopes for entity cancers is becoming a reality [27].

7. NK cells increase the cytotoxicity of tumor cells by natural products

Previous studies suggested that natural products increase the cytotoxic activities of NK cells and increased the level of tumor necrosis factor alpha (TNF-α) while decreasing the DNA damage in patients with late stage cancer. Studies confirmed that clinical results can be improved by a combination of nutraceuticals. Twenty patients of stage IV (bladder, breast, prostate, lung, neuroblastoma, mesothelioma, lymphoma, ovarian, gastric and osteosarcoma) cancer were treated with natural products such as transfer factor plus, immune modulator mix, ascorbic acid, IMU plus, *Agaricus blazei* teas, nitrogenated soy extract and *Andrographis paniculata*. After nutrients application, the function of NK cells and TNF-α and receptor levels were measured by phytohemagglutinin (PHA) and ELISA. Complete blood count and chemistry panels were daily counted. After 6 months, 16 of the 20 patients were alive, which showed maximum efficiency of NK cell function and TNF-α level in all four cell population. It was observed that hemoglobin, hematocrit and glutathione levels were prominent in investigated patients. It concluded that an aggressive combination of immuno-active nutraceuticals was effective in late stage cancer; while the clinical outcome evaluations are ongoing [28].

7.1. Mushrooms enhance NK cell mediated cytotoxicity

Study confirmed that beta glucans derived from solid cultures of *Ganoderma lucidum* (Lingzhi or Reishi) enhances NK cell mediated cytotoxicity in lung carcinoma in mice with radiation therapy only or in conjunction with radio therapy. It is more effective in controlling the growth of tumor and metastasis, prevents hair loss and healing of the wounds in tumor infected mice. The experiment conducted on 40 mice indicated that the mice treated only with radiation showed the highest number of metastatic tumor nodules (nodules = 17), the mice treated with mushroom beta glucans showed less number of metastatic tumor nodules (nodules = 2) and the mice treated with both radiation therapy and mushroom beta glucans showed only 1 nodule. The experiments on mice having cancer suggested that cytotoxicity of NK cells might be increased by natural products [29]. *G. lucidum* stimulates immunity through NK cell cytotoxicity. The results of the clinical trial suggested that it displayed potential immune-modulating effect in patients with advanced colorectal cancer. Although, these results showed its anti-cancerous activities but further investigations are required to explore the benefits of *G. lucidum* [30]. The *A. blazei* and its extracts are also reported to be used as cancer alternative medicines (CAMs). It increased the cytotoxicity of NK cells in wild type (WT) C57BL/6, C3H/HeJ, BALB/c and RAG-2 deficient mice but not from IFN-deficient mice. NK cell activation and IFN-γ production were also observed in vitro when DC rich splenocytes of wild type mice were co-incubated with an extract of *A. blazei*. These observations demonstrated that *A. blazei* increased NK cell activation through IL-12 mediated IFN-γ production [30].

7.2. Alternative phytochemicals used against cancer

Study on various plant constituents revealed that most of the phytochemicals are widely used as immune modulators against cancer or tumors. The important anti-cancerous natural plant products (phytochemicals) are Apigenin from parsley, crocetin from saffron, curcumin from turmeric, cyanidins from grapes, cranberries, raspberries, etc, Epigallocatechin gallate from green tea, fisetic from strawberries, apples, diindolylmethane (DIM) from *Brassica* vegetables, genistein from soybean, gingerol from gingers, kaempferol from grapefruit, tea, broccoli, Lycopene from tomato, resveratrol from grapes, sulforaphane from cruciferous vegetables, rosmarinic acid from rosemary, vitamin D from mushrooms and vitamin E from various plant oils [31].

8. NK cells and tumor immunotherapy

In previous studies, where NK cells were used in tumor therapy, NK cells were incubated in the presence of human and mouse tumor cell lines. The expanded NK cells were identified by physical parameters. Human NK cells expand in response to stimulation with varied tumor cell lines. Tumor derived expansion of NK cells is accompanied by rapid changes in CD16 expression levels, MHC class-l negative tumor cell line and to the EBV transformed cell line 721.221. CD16 expression was maintained on the cell surface of the expanded NK cells due to antibody dependent mechanism. If the antibodies are present against the tumor cells in the patients, it might be beneficial to expand the NK cells in the presence of 1106 mel cells. The selective expansion of NK cells was used for tumor immunotherapy [32]. A recent study used whole genome microarray data sets of the Immunological Genome Project and demonstrated that there was a close relationship between NK cells and T cells than other leukocytes because these cells shared expression of gene encoding molecules with similar signaling functions. Resting NK cells are known to share expression of genes with cytotoxic $CD8^+$ T cells, which allow NK cells to respond more rapidly to viral infection [33]. The role of T_{reg} cells in tumor immunological response is investigated in several previous studies. These regulatory T cells (T_{reg} cells) are involved in promoting various types of tumors. The tumor cells recruit and generate the T_{reg} cells to create a suppressive tumor microenvironment. The antitumor activity of T cells and NK cells is inhibited by T_{reg} cells. Experimental and clin-

ical studies on various animals and humans revealed that deletion strategies of T_{reg} cells yielded highly efficient results in enhancing anti-tumor immunity [34]. Recently the therapeutic approaches to enhance the cytotoxicity of the NK cells against cancer have been investigated. The study revealed that advancements in scientific discoveries for pathways of activating NK cells efficiency against tumor have led to the development of genetic and pharmacological techniques, which enhanced the antitumor properties of NK cells. It also allowed the investigators with several new strategies to connect the potential of NK cell based immunotherapy in the clinical studies [35].

9. Conclusion

The NK cells not only exhibit cytotoxic activities against tumor cells but also activate other immune cells against cancer cells. It takes part in adaptive immunity through cytokines and is a brilliant target for immunotherapy in future treatment due to its direct or indirect target to tumor cells. Natural products (nutraceuticals), alternative medicines, immune modulators and active hexose correlated compounds enhance the activity of NK cells. Further investigations are required to study the effect of NK cells on various cancers and how the affect of these NK cells can be enhanced.

Conflict of interest

We declared that no competing interest or conflict exist among any of the authors. All the authors agreed to submit manuscript in this journal and all ethical standards are fulfilled before submission.

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