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Review

Biochemistry Section

Natural Killer Cells: An Insight on their Role in Human Health and Diseases

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ABSTRACT

Natural Killer (NK) cells are crucial mediators of innate immunity, protecting human from several unwanted health hazards. They are derived from haematopoietic stem cells and exert cytotoxic effects against infections, tumours, allergic and autoimmune diseases. They also have important roles in pregnancy and organ transplantation. The immune functions exhibited by NK-cells are under tight regulation of NK-cell receptors which may be stimulatory or inhibitory. Therefore, a better understanding of NK-cell biology and its receptors may aid in the discovery of novel therapeutic strategies which could be fruitful in improving human health and diseases. However, such strategies before being used extensively in the clinical setup must be verified by further in-depth and large scale experimental studies. Thus the basic aim of this review was to harness a short update on NK-cell biology and its effect on human physiology and pathology.

Keywords: Autoimmunity, Cytolytic granules, Immunosurveillance, Immunotherapy, Infections, Innate immunity

INTRODUCTION

Living organisms are gifted with effective mechanisms which can be described as the immune system that defends them from the pathogens and toxins thereby protecting the health. Our immune system is complex and consist of number of molecules, defensive cells, tissues or organs. Overall the immune system can be categorised as an innate immune system or adaptive immune system [1], which when activated on the encounter of pathogens, protect the health by several defensive mechanisms [2].

Encounter of threats in the form of microbes or any other danger signals causes rapid activation of mediators of innate immunity and thus the innate immune system serves as the first line of defence system. The mediators of innate immunity include

- Recognition molecules (e.g., natural antibodies, CRP).
- Complement system.
- Cellular components (Phagocytes, dendritic cells, natural killer cells),
- Epithelial cells that provide a physical barrier and also release chemokines and cytokines,
- Subsets of T and B-lymphocytes having fewer antigen receptors diversity (e.g., $\Upsilon\delta$T cells, natural killer T-cells, B-1β cells [3].

Cells of innate immunity via pattern recognition receptors such as Toll-Like Receptors (TLRs) recognise the microbial or any other threats thereby mounting quick immune response. However, if the innate immunity fails to sort out the threat, adaptive immunity, which is a second line defence system takes over the role. Unlike innate immunity, the adaptive immune system is mediated by antigen-specific T-lymphocytes and B-lymphocytes which require time to get activated [4]. Though both innate and adaptive immune systems are involved in host defence mechanisms, they share quite different features [Table/Fig-1].

S.N.	Features	Innate immunity	Adaptive Immunity
1	Presence	Present in the body by birth	Developed in response to a foreign substance on exposure
2	Specificity	Non-Specific	Specific
3	Response	Fast	Slow (1-2 weeks)
4	Potency	Low	High
5	Diversity	Limited	High
6	Time	Remains throughout life once activated	Maybe short or remain life long
7	Memory	No memory	Long term memory
8	Inheritance	Inherited from parents	Not inherited
9	Allergic reaction	Not present	Immediate or delayed hypersensitivity reactions may be present
10	Complement system	Alternative and lectin pathways	Classical pathways
11	Physical barrier	Skin, mucous membrane	Spleen, lymphocytes
12	Mediators	Physical and chemical barrier, phagocytes, NK-cells, plasma proteins, dendritic cells	T-lymphocytes B-lymphocytes

[Table/Fig-1]: Comparison between innate and adaptive immunity.

Natural Killer (NK) Cells

NK-cells were first identified in 1975 in mice and humans [5]. Being the crucial part of the innate immune system, NK-cells are involved in tumour immunosurveillance and alleviating of bacterial or viral infections. As per their name, they can directly carry out their effector functions without the necessity of pre-stimulation [2]. Apart from T cells and B-cells, NK-cells are also regarded as the third lymphocytes. Though NK-cells are the mediators of innate immunity, they exhibit some features similar to that of T and B-lymphocytes [6].

1. Education and tolerance

Both NK-cells and T cells are dependent on MHC (Major Histocompatibility Complex) recognition on host cells for the functional efficiency. On chronic stimulation, these cells follow the common tactic of adaptive tolerance. Like T cells, NK-cells also undergo highly regulated selection process (positive or negative) [7].

2. Cell priming

Similar to T-lymphocytes, NK-cells on the encounter of inflammation or infections are transported from blood to the lymphoid tissue where, in presence of cytokines (such as IL-15) derived from dendritic cells, they are modulated with the enhanced ability to synthesise and secrete cytotoxic granules and IFN-α on re-stimulation. From there, these cells are then re-circulated to the peripheral tissues [8].

3. Co-stimulatory signals

The functionality of T-lymphocytes depends on T cell receptors which are activated by co-stimulatory signals from antigen MHC complex and antigen presenting cells. Likewise, NK-cells are also stimulated by modulation of receptors expressed on their surface [9].

4. Effector function

NK-cells and cytotoxic T cells on the encounter with target cells induce their lysis by releasing cytotoxic granules (perforins and granzymes), cytokines (INF-α, TNF-α), chemokines (MIP1-α, MIP-1β, RANTES) [10] and by other cytotoxic mechanism associated with Fas ligand (CD 95 ligand or Cluster of differentiation 95 ligand). Like T cells, NK-cells effector functions are triggered by IL-2, IL-15, and IL-18 [11]

5. Proliferation and immune response

NK-cells are demonstrated to show high proliferative capacity during viral infections and lymphopenic states. They can also mount a secondary immune response with more effective effector functions than primary response similar to T and B-lymphocytes [12].

Like B-cells, NK-cells are suggested to be developed from bone marrow. However, unlike T cells, they are not dependent on thymus for development. But in a recent study, $CD127^+$ NK-cells are reported to develop from thymus via GATA-3 dependent mechanisms [13].

Development of NK-Cell

Initially, NK-cells were assumed to be developed chiefly from bone marrow, but recent studies have indicated that they can also foster in liver and lymph nodes [14]. From bone marrow, Haematopoietic Stem Cells (HSC) give rise to the precursors of NK-cells which undergo phenotypic and functional maturation. NK-cells then undergo homeostasis in the presence of several factors such as transcription factors (ETS 1), soluble factors and membrane factors [15]. Maturation of NK-cells occurs in the presence of GATA-3 and IRF-2 while functional differentiation after maturation requires the involvement of MEF (Myeloid Elf like Factor), MITF (Micropthalmia Associated Transcription Factor), and C/EBP-γ (CCAAT/Enhancer binding protein gamma). IL-15 is essential for development and survival of NK-cells [16] while IL-2 derived from T cells is important for cytolytic functional maturity [17].

Morphology and types of NK-Cells

Phenotypically, human NK-cells are categorised into two major subsets based upon the expression of CD56 on their cell surface. These include $CD56^{dim}$ and $CD56^{bright}$ subsets. About 90% of NK-cell in peripheral circulation is of $CD56^{dim}$ type while 10% belong to $CD56^{bright}$ type. $CD56^{dim}$ NK-cells possess high cytolytic abilities and produce cytokines at lower concentrations. On the other hand, $CD56^{bright}$ NK-cells secrete a number of cytokines in higher concentrations and acquire cytolytic property only after activation for a prolonged period of time [18]. $CD56^{dim}$ NK-cells have low-affinity receptors for the Fc region of immunoglobulin (Ig) G (i.e., FcγRIIIa, also called CD16) [19]. The $CD56^{bright}CD16^-$ NK-cells are present in secondary lymphoid tissues like tonsils and lymph nodes [20]. The $CD56^{bright}$ type NK-cells migrate towards the region of chronic inflammation while $CD56^{dim}$ type cells are destined to the acute inflammatory sites [21]. In-vitro studies have shown $CD56^{bright}$ cells to be the precursor of $CD56^{dim}$ cells [22].

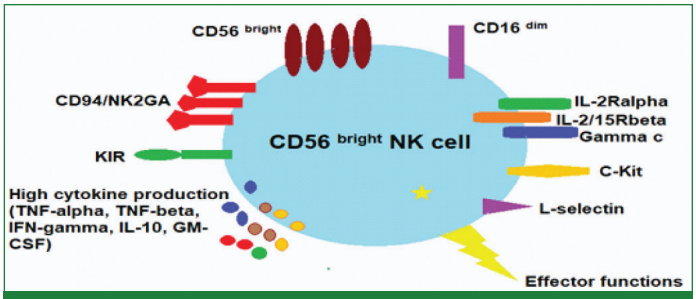
$CD56^{bright}$ cells also exhibit

- CD117 (ckit) that binds with stem cell factor SCF (A cytokine derived from stromal cells)
- IL-2 receptor a (IL-2Ra or CD25)

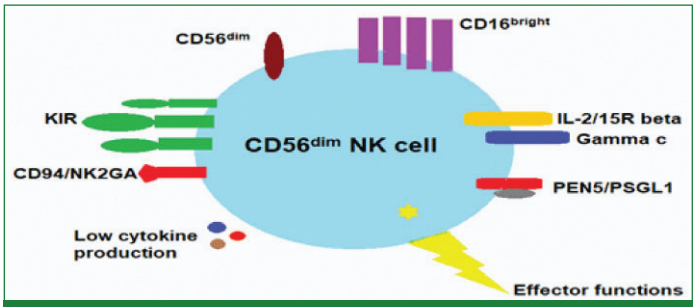
While $CD56^{dim}$ cells only demonstrate IL-2Rb/IL-2Rc (CD122/CD132) that can bind with IL-2 and IL-15 [23].

[Table/Fig-2,3] shows $CD56^{bright}$ NK-cell and $CD56^{dim}$ NK-cell [24]. Depending upon their cytokine profiles, NK-cells can also be categorised into 4 subsets namely NK1 cells, NK2 cells, NK17 cells and NK 22 cells [25].

Human NK-cell differentiates into two distinct subpopulations when they are cultured in medium supplemented with IL-2 or IL-4. These cells demonstrate the cytokine profile similar to that of Th1 and Th2 cells. The NK-cells cultured in medium with IL-12 produce NK1 cell type that secrete, INF-γ and IL-6 whereas those developed in presence of IL-4 develop into NK2 subtypes that secrete IL-13 and IL-15. Both the cell subsets though do not have significantly different cytolytic action, they share some dissimilarities such as:



[Table/Fig-2]: $CD56^{bright}$ NK-cell [24].



[Table/Fig-3]: $CD56^{dim}$ NK-cell [24].

- Cell surface antigen CD95 or Fas is comparatively high in NK1 cell than NK2 cells.
- NK1 cells are more sensitive to apoptosis induced both by antibodies or chemicals.
- NK1 cells express a high amount of IL-12Rβ2 chain mRNA upon STAT4 activation and respond more to IL-12 [26].

Human NK17 cells are characterised as $CD56^+CCR4^+ROR\gamma t^+Tbet^+IL23R^-$ cells that secrete interleukin 17 (IL-17) and interferon (IFN) γ [13]. NK22 cells secrete IL-22 and B-cell Activating factor of tumour necrosis Factor Family (BAFF) which suggests the supportive role of NK22 in enhancing mucosal immunity in conjunction with B-cells [27].

NK-22 cells are the subsets of NK-cells that express CCR6 (a chemokine receptor), Retinoic acid-related Orphan Receptor γt (RORγt) transcription factor and aryl hydrocarbon receptor on their surface [28].

Cupedo T et al., identified $CD56^+CD127^+$LTi like cells which expressed RORC (RORγt in human), CD127, NKp46, CD56 and secreted IL-17 and IL-22 on activation. Unlike conventional NK-cells, these cells were non-toxic and did not express perforins and granzymes [29]. Cella M et al., described a similar type of cells to $CD56^+CD127^+$LTi cells and named it NK-22 cells since those cells also expressed NK-cell markers like CD56 and NKp44, and produced IL-22 on activation [30].

Functions

NK-cells exhibit functional versatility ranging from identification and lysis of target cells to the release of a vast array of cytokines and immune-homeostasis mediated through NK-cell-derived exosomes. Cytotoxicity mediated by NK-cells can be achieved via the release of perforins and granzymes (A and B) which are potential cytolytic molecules. Cytotoxicity may be natural cytotoxicity that acts against tumour cells and virus-infected cells or Antibody-Dependent Cellular Cytotoxicity (ADCC) which acts against target cells coated with IgG [31].

Various stimuli such as infectious agents or infected cells, tumours, cytokines like IL-1, IL-2, IL-15 etc activate NK-cells. Upon activation, they are converted to Lymphokine-Activated Killer cells (LAK) [31]. These activated NK-cells mediate cytolytic activity via the following pathways:

- The release of lytic granules by exocytosis and cause target cell apoptosis.
- Apoptosis mediated by caspase proteins. NK-cell surface expresses ligands like FasL and Tumour necrosis factor Related Apoptosis-Inducing Ligand (TRAIL) which bind with death receptors such as Fas/CD95 present on the target cell surface thereby leading to the apoptosis of target cells [32].
- NK-cells via the presence of low-affinity receptors for IgG (CD16) act on target cell leading to cytotoxicity [33].

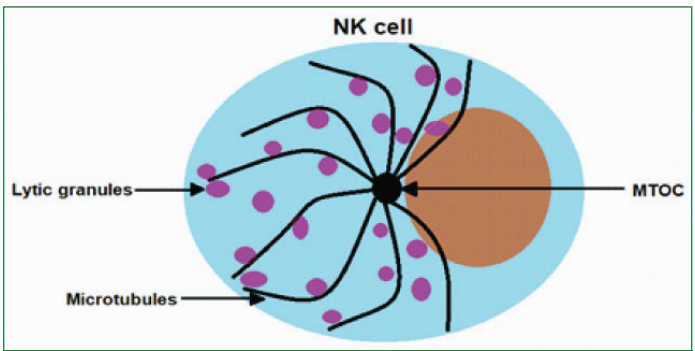
Mechanism of release of cytolytic granules

NK-cells are activated when they come across the target cells. In these cells, the cytolytic granules via mechanism associated with dynein-dynactin complex, migrate towards MTOC (Microtubule Organising centre) through the microtubules [34]. The MTOC and lytic granules are polarised towards the contact area between the NK-cells and the target cells. Under the stimulation of myosin II (actin motor proteins), the vesicles containing lytic granules switch from microtubules and navigate towards the plasma membrane through filamentous actin networks [35]. The membrane of vesicles with lytic granules contains Rab27a, Munc13-4 and R-SNARE proteins. These proteins allow docking of lytic granule membrane with the plasma membrane in association with syntaxin II (STXII) and Munc 8-12. Munc13-4 then causes priming of docked granules thereby forming a bridge between the plasma membrane and granule membrane. R-SNARE (VAMP7) protein present on granule membrane complex with Q-SNARE proteins (SNAP23) on the plasma membrane resulting in the fusion of plasma membrane with granule membrane and release of the lytic granules at immunological synapse (between NK-cells and target cell) via exocytosis [36].

After the release into synaptic clefts, these granules enter the target cells by two mechanisms:

- The lytic granules fuse with the plasma membrane of the target cell and get internalised as enlargosome (via endocytosis). The perforins then stimulate the pore formation in the membrane of enlargosome and allow leaking of granzymes into the cytoplasm of target cell [37].
- Perforins can directly form a pore in the target cell membrane by oligomerisation thereby providing access for granzymes into the cytoplasm of target cell [38].

The whole mechanism of lytic granules in NK-cells is shown in [Table/Fig-4,5] [39].



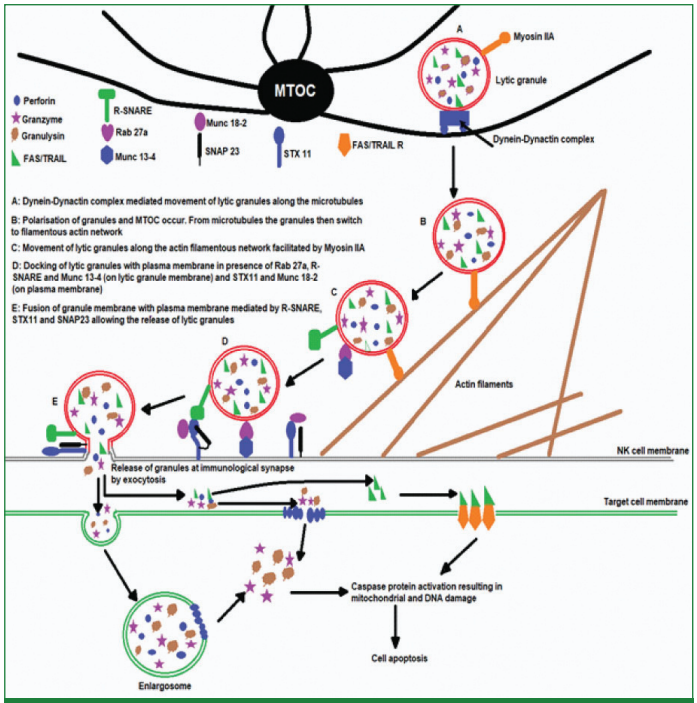
[Table/Fig-4]: Lytic granules in NK-cell [39].

Cytolytic granules once inside the cytoplasm induce apoptosis of target cells by mechanisms such as:-

- Activation of caspases
- Mitochondrial and DNA damage
- Activation of Fas Ligand and TRAIL

Cytolytic Granules of NK-Cells

The cytolytic granules in NK-cells have the properties similar to that of lysosomes, hence they are also described as secretory lysosomes. The lytic granules of NK-cells are as follows:-



[Table/Fig-5]: Release of lytic granules and target cell apoptosis [39].

Granzyme Clusters	Chromosome	Granzyme coded	MOA	Site of cleavage
A	5	A and K (tryptases)	Caspase-dependent	After lysine or arginine residues
B	14	B (Aspase) and H (Chymase)	Caspase-dependent and independent	B: After the aspartic acid residue H: Hydrophobic residues
M	19	M	Caspase-dependent and independent	Leucine or methionine residue

[Table/Fig-6]: Types of granzymes with site and mode of action.

1. Granzymes

Granzymes are serine proteases coded by five genes arranged in three clusters. Five different granzymes have been identified viz., granzymes A, B, H, K and M [Table/Fig-6]. Granzymes are first synthesised in an inactive form and then signalled to lytic granules via a mannose-6-phosphate-dependent pathway. In the granules, they are activated by cathepsin C and tightly packed with serglycine [40,41].

2. Perforins

Perforin is a pore-forming protein with a molecular weight of 65 kDa and consists of 555 amino acids. Initially, it is synthesised as an inactive precursor that contains:-

- Membrane attack complex/perforin (MACPF) domain and a signal peptide at N-terminus end
- C2 domain and EGF like domain at the C-terminus end [42].

Once the peforins reach lytic granules, they are activated by cathepsin L which causes proteolytic cleavage of last 20 amino acid residues at the C-terminal end of perforin molecule [43]. To prevent the disintegration of granule membrane perforins in lytic granule are inhibited by several means such as acidic environment, calreticulin (increases the resistance of membrane of granule to osmotic lysis) and serglycin [44].

3. FasL and TRAIL

FasL and TRAIL are pro-apoptotic molecules [45].

4. Granulysin

Granulysin is synthesised in two forms viz., 15KDa and 9KDa isoforms which differ both in functions and cellular locations. The

15KDa isoforms are found in vesicles that lack perforin or granzymes while 9KDa isoforms are obtained from 15KDa isoform by proteolytic cleavage and are present in lytic granules. Granulysin is effective against bacteria, fungi, parasites and tumours. It induces the synthesis of various cytokines (IFN-α, IL-6, IL-10) and chemokines (MLP-1α) [46].

Others

Small peptides such as peptide LL-37 (Cathelicidin), defensins 1-3 lysosomes associated proteins etc are also present in lytic granules. They have a role in NK-cell activation and show direct anti-microbial functions [47].

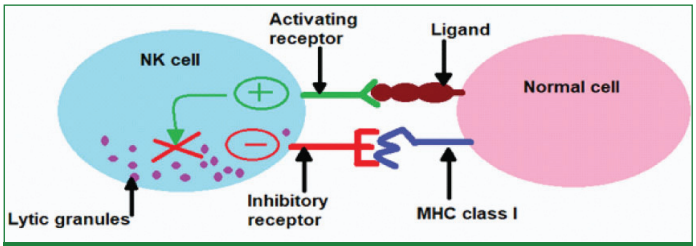
NK-cells also produce a number of cytokines and chemokines. Examples of cytokines include IL-3, IL-5, IL-10, IL-13, TNF-α, GM-CSF whereas examples of chemokines are monocyte chemo-attractant protein -1, lymphotacin, IL-8 etc., [48]. NK-cells can cause co-stimulation of T and B-cells via expression of co-stimulatory ligands such as OX40 and CD40. Activated NK-cells also express MHC II molecules and ligand against co-stimulatory receptors on T cells (CD80, CD86, CD70, OX40), thereby enhancing proliferation of T cells specific to the antigens [49]. Activated NK-cells also release exosome that has cytotoxic activity due to the presence of NK-cell-related functional proteins such as perforins and $CD56^{bright}$ [50].

Receptors of NK-Cells

The functions of NK-cells are mediated by the receptors expressed on their surface. Broadly these receptors may be activating receptors or inhibitory receptors. Examples of activating receptors are C type leptin receptors (NKG2C, CD94, NKG2D), immunoglobulin-like receptor (2B4) and cytotoxicity receptors (NKp44, NKp46). Similarly, inhibitory receptors include leucocyte inhibitory receptors (LIR-1, LIR-2), Killer Immunoglobulin-like Receptors (KIR) and Ig like receptors (CD158) [51,52].

The cytotoxic functions of NK-cells are governed by the balance between the signals transmitted by activating and inhibiting receptors [53]. The inhibitory receptors of NK-cell, on their cytoplasmic domain, consists of immunoreceptor tyrosine-based inhibitory motifs. These receptors identify self-MHC I molecules thereby preventing the activation of NK-cells and host cell lysis [54]. However, tumour cells and cells infected with virus down-regulate the expression of MHC I proteins, making such cells vulnerable to lysis by NK-cells. In this case, there is no suppression of activation receptors causing transmission of potent stimulatory signals [55].

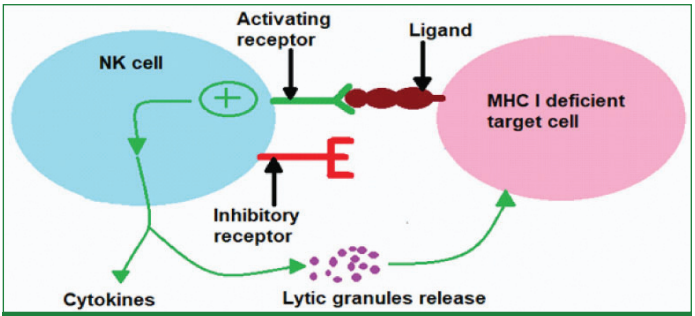
Class I MHC molecules are decreased in tumour cells or cells infected with a virus. NK-cell when circulates in the body binds with the MHC I molecules. If the cells are normal i.e., express sufficient amount of MHC I molecules, NK-cells remain in inactive form as shown in [Table/Fig-7a] [56]. If the cells express MHC I molecules in less than the normal level, NK-cells are activated as they bind with such cells. Activated NK-cells release cytokines, stored granules and lyse the target cells [Table/Fig-7b]. NK-cell also targets the cells with normal MHC I but show excess expression of induced ligands [Table/Fig-7c].



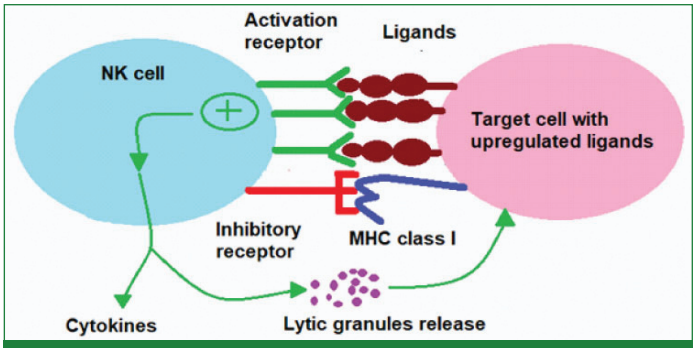
[Table/Fig-7a]: NK-cell and normal cell [56].

Sites of NK-Cells in the Body

NK-cells are present in the human body since foetal life. At the gestational age of 6th week, NK-cells appear in the foetal liver and



[Table/Fig-7b]: NK-cell and MHC I deficient cell (missing self) [56].



[Table/Fig-7c]: NK-cell and target cell with stress induced ligands [56].

they are present in the spleen at the gestational age of 15th week [57]. Foetal NK-cells are immature during the first trimester. The dynamic phase of development is followed by a steady phase in the second trimester. In the third trimester, foetal NK-cells develop into an adult like NK-cells. However, the activity is comparatively lower than that present in adult human beings. This fact may be correlated with the view that newborns are more susceptible to viral infections (like herpes virus) as NK-cells are considered the first line defence against these viruses [58].

Besides peripheral circulation, NK-cells are also present in the uterus and are commonly called uterine natural killer (uNK) cells. Unlike peripheral NK-cells, uNK-cells lack CD16 but express CD94. uNK-cells secrete cytokines like IFN-γ, MIP 1α, CSF 1 and GM-CSF [59]. uNK-cells are predominantly present at the implantation site where they regulate trophoblast invasion [60], vascular remodelling of the spiral artery and maintain decidual integrity [61]. These cells also facilitate immune tolerance and maintain pregnancy by regulating Th17 (T helper 17) cells at the feto-maternal interface [62]. They down-regulate expression of ST2L, a soluble receptor on the endometrial surface, which binds with IL-33. Imbalance in IL-33/ST2L regulation may cause Recurrent Pregnancy Loss (RPL) [63].

NK-Cells in Various Diseases

Being the indispensable part of immune system, NK-cells play a crucial role in various diseases to protect the body from adverse health effects.

1. Infections

NK-cells are most active against viral infections such as flavivirus (Japanese encephalitis), yellow fever, dengue, tick-borne encephalitis, influenza, HIV infection and hepatitis [64]. They are also effective against bacteria causing respiratory infections. NK-cells are also found to be associated with virus-mediated asthma [65]. Target cells infected by micro-organisms down-regulate expression of MHC I proteins thereby making them unsuitable for elimination by T cells. However, these cells become more susceptible to lysis by NK-cells [66]. Cytokines such as IFN-γ from NK-cells have more potent anti-infection roles. Studies have shown that mice lacking IFN-γ receptors are more susceptible to infection by Cytomegalovirus, *Listeria monocytogens* and protozoa like *Toxoplasma gondii* [67].

Wang Y et al., observed that IL-28B when overexpressed by the hydrodynamic gene delivery, facilitates the proliferation of NK-cells and aid in protection against influenza virus [68]. Gandini M et al., studied the protective role of NK-cells against dengue infection. The authors observed up-regulation of CD107a and TLR3 (markers of NK-cells activation) in patients with dengue compared to healthy volunteer and concluded that activation of NK-cells by dengue virus limits the viral spread, inflammatory response, and severity of the disease [69].

Pathogens can directly activate NK-cells by binding with specific receptors on the surface of the cells. Activated NK-cells promote the killing of such pathogens by inactivation of proteins involved in defence against cellular oxidative stress and inhibition of various metabolic pathways of pathogens [70]. According to Walch M et al, the load of *Listeria monocytogenes* is significantly reduced in granulysin transgenic mice compared to wild type [71]. Using the murine model, Clark SE et al., investigated the effect of NK-cell on host susceptibility to *Listeria monocytogenes* infections and concluded that activated NK-cells exert pro-bacterial effects which were not dependent on TFN-α production. The authors further added that during *Listeria monocytogenes* infection, NK-cell switch from IFN-γ to IL-10 secretion that diminish resistance to infection [72].

Djaoud Z et al., studied the effect of NK-cells in 24 patients with EBV infection type 1. They found that 13 patients developed potent NK-cell and γdT cell responses while 11 showed mild γdT and strong NK-cell responses. This indicated the efficacy of NK-cell on eradicating EBV infection type 1 [73].

As per Vitenshtein A et al., removal of *Cryptococcus neoformans* requires activation of NKp30 receptors of NK-cells. The authors also stated that decreased efficacy of NK-cells against fungal infection in HIV patients is associated with NKp30 and administration of IL-12 can restore the efficacy. Other receptors of NK-cells such as NKp46 in human and NCR1 in mouse eliminate the fungal infections by recognition of virulence proteins such as Epa1 or Epa6 [74].

Venkatasubramanian S et al., studied the mouse model and cells of patients both infected with *Mycobacterium tuberculosis* and reported the presence of memory like cells whose expansion was IL-21 dependent. According to the authors, during BCG vaccination IFN-γ producing memory-like NK-cells (CD3-NKp46+CD27+KLRG1+) develop, expand and provide immunity against the bacteria [75].

Schuch A et al., also reported the presence of Fragment Crystalisable-Epsilon-Receptor-1-Gamma (FCERIC) memory-like NK-cells in chronic hepatitis B patients co-infected with cytomegalovirus [76]. However, the role of NK-cell in hepatitis B viral infection is controversial. Some authors suggested the limited contribution of NK-cells on the initial clearance of hepatitis B viral infection [77]. Rather, CD8 T cells were shown the controller of viral clearance. In contrast, some experimental studies established the requirement of NK-cell to eradicate the viral infection [78]. A study involving two blood donor, in whom the presence of HBsAg was accidentally identified, reported that NK-cells are capable of mounting an early and effective immune response against HB virus [79]. Similarly, in another two independent experimental studies, it was shown that the level of IFN-γ and cytolytic ability increases in the HBV infection thereby suggesting the participation of NK-cells in the initial stage of HB viral infection. However, most of the studies are conducted in experimental animals, the results of these studies may not fully be correlated with that of human and since limited studies are available in human beings, further studies are still required to demonstrate the exact role played by NK-cells against HBV infections in Human beings [80,81].

When an infection develops in the body, NK-cells may be activated directly or indirectly. NK-cells can directly target the infected cells in response to the down-regulation of MHC-I molecules and up-regulation of stress-induced ligands on the surface of target cells. Indirectly NK-cells may be activated by cytokines and co-stimulatory signals aggregated from macrophages, monocytes and dendritic cells which are activated in response to infections [82].

2. Autoimmune diseases

In various autoimmune diseases, the occurrences of NK-cell have been reported in the target organ where they provide first line defence. In the case of autoimmune reaction caused by infections, NK-cells are noted to suppress autoimmunity and facilitate elimination of microorganisms as well as prevention of tissue damage by autoimmune reactions [83]. The involvement of NK-cells in auto-immunisation is depicted to be associated with different Killer-cell Immunoglobulin Like Receptor (KIR) haplotypes. KIR2DS1 gene was frequently observed in the case of systemic lupus erythematosus while it was not common in atopic dermatitis [84]. Similarly, KIR3DS1 was more frequently found in multiple sclerosis [85].

Studies have also shown that in several autoimmune diseases, there is not only decreased count of NK-cells but also reduction on their cytotoxic functions. As for example, multiple sclerosis of animals also called autoimmune encephalitis showed depletion in NK-cell count with increased mortality risk [86]. However, the cytotoxic roles of NK-cells also have been indicated to augment autoimmune diseases. One of the common examples is type 1 diabetes mellitus (T1DM). NK-cells are not observed in pancreatic islets in healthy individuals while they are present in case of patients with T1DM [87]. According to the preclinical data, NK-cells have important roles in the development of T1DM [88]. Moreover, some studies explained impaired or decreased functions of NK-cells in patients with T1DM. As per Gur C et al, degranulation of NK-cells cause binding of NK-cell receptors with ligands on β-cells of the pancreas and destroy them [88]. They also found the involvement of NKp46 in the development of T1DM. Similarly, in another study, it was shown that there is a low expression of NKp30 and Nkp46 as the duration of disease increases [89]. The NKG2D expression also decreases but it is not associated with disease duration [90]. Lorini R et al., showed a decreased cytolytic activity of NK-cell in chronic diabetes [91].

In autoimmune diseases like rheumatoid arthritis, NK-cells are shown to have disease promoting roles [92]. However, in the case of systemic lupus erythematosus, there is a decrease in NK-cell count and cytolytic functions, in addition, to decrease in $CD4^{+}Cd25^{+}$treg cells [93]. Therefore the abnormality in NK-cell functions plays a significant role in various autoimmune disorders.

As per Fogel LA et al., NK-cells rather than being the consequences of the autoimmune process, facilitate its initiation [94]. However, as per Folci M, NK-cell protects against autoimmune disease by inhibiting dendritic cells, down-regulating antigen presentation and T-cell proliferation. NK-cells can also lyse activated macrophages and reduce unwanted phagocytic activity [95]. Luo Q et al., concluded that TIGIT (T-cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibitory domains) acts as a potent down-regulator of NK-cells in the cases of SLE and hence TIGIT signalling pathway blockade via anti-TIGIT monoclonal antibody could be used as a novel therapeutic approach for treating SLE [96].

3. Tumours

Immunosurveillance is the primary function of NK-cells. They not only recognise tumour cells but also control the growth and metastasis. However, the cytotoxic action of NK-cells is greatly influenced by the tumour microenvironment, cytokine profile, and genetics of cancer patients. This indicates that NK-cell phenotypes vary according to the type and location of tumours.

In some cancers, pNK-cells are shown to exhibit different phenotypes comparable to those NK-cells present at the tumour site. In the case of metastatic malignant melanoma, it was observed that pNK-cells showed decreased activity with reduced IFN-α production and increase in CD16. The molecular mechanism is rather unclear, but

it has been hypothesised that increased serum concentration of tumour receptors may block the receptors on the surface of pNK-cells leading to their decreased activity. It is though undoubtful that NK-cells possess efficient tumour fighting potential, further research works are needed to fully understand NK-cell anti-tumour potential in different cancers [97].

The tumour microenvironment is mostly populated by $CD56^{bright}CD16^{dim}$ NK-cells [98]. In the presence of suitable stimuli, NK-cells produce a large amount of TNF-α and IFN-γ. Production of IFN-γ is further stimulated by STAT 4 (signal transducer activator of transcription factor 4) under the influence of IL-12 [99]. IL-18 enhances IFN-γ production via IL-1Rrp (interleukin 1 receptor-related protein) [100]. IFN, TNF and other cytokines from NK-cells are important in cancerous cell elimination.

NK-cells exhibit various features that make them an ideal option for immunotherapy.

- Recognition and lysis of target cell by NK-cell do not require prior immunisation.
- NK-cells are easily isolated and expanded ex vivo.
- They have a shorter life span.
- Lyse tumours cell without affecting healthy tissue.
- Reduces the risk of cytokine storm form T cells as in case of other immunotherapies [101].

NK-cells mediate tumour invasion by 3 mechanisms:

- The release of cytolytic granules (perforin and granzymes), and stimulating cell lysis. A recent clinical trial conducted in perforin-deficient mice, demonstrated reduced tumour cell lysis suggesting the indispensable role of perforins in NK-cell-mediated cytotoxicity. Several studies also have emphasised that perforins can be helpful in tracking the risk of cancer relapse. The roles of granzymes are yet to be understood [102].
- TNF ligand-mediated apoptosis of target cells. These ligands bind with Fas receptors expressed on target cells and induce apoptosis [103].
- Activation of IFN-α, that stimulate NK-cell to secrete cytokines with anti-tumour activity (IL-10, IL-13, TNF-α) [103].

NK-cell functions are exploited via several clinical therapies in order to effectively treat cancer patients. Adaptive NK-cell therapy is most commonly used. It may be autologous or allogeneic NK-cell therapy. The NK-cells for these therapies are obtained from a suitable donor or the peripheral blood of patients. They may also be obtained from embryonic stem cells, bone marrow or umbilical cord blood [104].

In the case of autologous NK-cell therapy, the NK-cells isolated from the patients are first activated and expanded in the presence of IL-2. Incorporation of IL-12, IL-15 and IL-18 in the medium, further result in the generation of NK-cells with a memory and more effective functions. These activated and expanded NK-cell are then transferred back to the patients.

In the case of allogeneic NK-cell therapy, NK-cells are obtained from a suitable donor (Haploidentical or HLA matched donor). These cells are activated and expanded by the mechanism similar to that of autologous transfer but in this case, T cells are removed.

Both autologous and allogeneic NK-cells can be engineered with Chimeric Antigen Receptors (CARs) that facilitate specific binding of NK-cell with overexpressed antigens on tumour cells. When CARs bind with the tumour antigen, a potent signal is generated that enhances rapid activation of NK-cell resulting in the elimination of tumour cells. Several studies reported successful treatment of tumours using CAR-engineered NK-cells [105]. Transduction of NK-cells with CARs may overcome the resistance posed by many B-cell dependent acute or chronic leukaemia to killing by NK-cell alone [106].

NK-92 cell lines have been transduced with CARs specific for disialoganglioside (GD2, in case of neuroblastoma) CD19 and CD20 (in case of B-cell malignancies) [107].

Intracranial transfer of NK92-EGFR-CAR cells represented a promising therapeutic modality in glioblastoma [81]. Similarly, administration of Cs1 (surface protein over-induced in multiple myeloma cells) specific CAR NK-cell could be a novel strategy in case of multiple myeloma [108].

Ilander M et al., assessed the relation of T and NK-cells with successful Tyrosine Kinase Inhibitor (TKI) cessation among the patients with Chronic Myeloid Leukaemia (CML). They found that NK-cells were associated with relapse-free survival since the patients with a higher percentage of NK-cells showed the better possibility to remain in remission while such association was not observed in case of T and B-cells [109].

Liu E et al., in their study isolated NK-cells from cord blood and using retroviral vector transduced them with CAR-CD19 gene, IL-15 gene and iC9 gene (inducible caspase-9- based suicide gene). These genetically engineered cord blood-NK-cells when transferred in the murine model exhibited efficient cytolytic functions in primary leukemic cells in-vitro and prolonged the survival [110].

4. Allergic Reactions

NK-cells stimulate T cell to mediate inflammatory and allergic responses associated with respiratory tract [111]. In both normal and pathological cases, NK-cells can interact with other cells of the immune system. NK-cells in conjugation with macrophages and dendritic cells lead to the generation of cytokines or effector molecules that mediate immunity and severity of allergic diseases [112]. The exact roles of NK-cell in allergies are poorly understood [113]. However, a reduced count of $CD56^{+}CD16^{+}$ NK-cells is observed in poly-allergic patients [114]. In most of the cases of allergic dermatitis, increased levels of $CD3^{-}CD16^{-}CD56^{+}$ NK-cells are observed [115].

NK-cells are also involved in the control of allergic airway disease. During allergic lung inflammation, depletion of NK-cells delays the clearance of eosinophils and CD4 T-lymphocytes from the respiratory tract [116].

However, pathogenesis of asthma, allergic lung inflammation and the involvement of NK-cells in these conditions are still a question of debate. As per some studies, NK-cell activity increases in asthma and it contributes to allergic lung inflammation. There is an increased level of $IL\text{-}4^{+}CD56^{+}$ NK-cells in the circulation of asthmatic patients [117]. Likewise, in paediatric patients with acute asthma, the levels of $CD3^{-}CD56^{+}$ NK-cell are significantly increased. Conversely, Tubby C et al., could not find any difference between NK-cells obtained from asthmatic patients and those without asthma, in terms of number, expression of receptors, expression of cytotoxic mediator and functional efficacy [118]. Hence, further studies on the involvement of NK-cells in allergic airway disease are necessary to resolve controversial and inconclusive results.

The Therapeutic Scope of NK-cells

NK-cells as therapeutic approaches are most commonly used in cancer immunotherapy. Initially, researches were mostly focused on autologous NK-cell therapies but due to some drawback and issues related to efficacy, allogenic NK-cell therapies were developed in clinical trials. Adaptive NK-cell therapies could eliminate the problems such as graft rejection, relapse or graft vs host diseases [119]. Also, allogenic NK-cell therapy was found safe and effective against cancers like renal cancer, metastatic melanoma, acute myeloid lymphoblastosis, Hodgkin's disease, breast and ovarian cancer [120].

NK-cells are also useful in refractory cases of Highly Active Antiretroviral Therapy (HAART). Similarly, in autoimmune diseases like T1DM, blockade of activation receptor NKp46 using specific

antibody may prevent activation of NK-cells. Likewise, blockade of NKG2A inhibitory receptors and RANKL (receptor activator of NF κ and β ligand) can be useful in rheumatoid arthritis [121].

Now NK-cells are successfully derived from embryonic stem cells and pluripotent stem cells. Such functional NK-cells on adoptive transfer may be useful in the treatment of a wide range of diseases. However, their efficacy in cancer therapy should experimentally be determined. Additionally, NK-cells are considered more reliable agent for immunotherapies compared to T-lymphocytes. Researches conducted in recent past have shed light on some genetic and pharmacological technique to increase NK-cells activity.

i. Administration of cytokine

IL-2 has the potential to increase NK-cell activity in-vitro. Clinical trials are being conducted on patients with renal carcinoma and metastatic melanoma. Trials conducted on primates also demonstrated increased cytotoxicity in the presence of IL-2 [122].

ii. Use of monoclonal antibody (mAb)

Administration of mAb such as anti-CD20 (rituximAb), CetuximAbTm, anti-Her2 etc., to a tumour cell may stimulate rapid NK-cell degranulation and cell lysis [123, 124].

iii. Blocking inhibitory receptors

Blocking receptors such as Ly 49 and KIR have shown to improve anti-tumour activity both in-vitro and in-vivo [125].

Some recent ongoing clinical trials on NK-cell-mediated immunotherapy include:

- Comparative study of the efficacy of combined therapy in patients with advanced pancreatic cancer. In this study, some patients received both NK-cell immunotherapy and irreversible electroporation while the rest received only NK-cell immune therapy.
- Infusion of NK along with trastuzumab in patients with gastric and breast cancer so as to determine the efficacy of expanded autologous NK-cells infused in patients after the administration of Trastuzumab.
- Combined therapy involving NK-cells and Bortezomib. This ongoing clinical trial is focused on the idea of whether Bortezomib when pre-administered increases sensitivity of NK-cells to TRAIL in-vivo as in in-vitro cases.
- Study of efficacy of activated NK-cells in the treatment of solid tumours among children and adolescents.

CONCLUSION

NK-cells which are derived from bone marrow stem cells are an integral part of an immune system. They play important roles in several diseases including infections, tumours, allergies, and autoimmune disorders, which suggest that NK-cells can be used as an ideal therapeutic approach in such cases. Recent development on the futuristic scope of NK-cell therapy involves clinical trials with the use of allogeneic NK-cells or haploidentical stem cells. Though the success rate is not up to the mark, further understanding of NK-cell biology and pathophysiology in-vivo may lead to the generation of more effective and novel therapeutic modalities against human disorders.

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