

NATURAL KILLER CELLS: NOW AND FUTURE

ABSTRACT

As the name suggests, natural killer (NK) cells are important cells of the immune system “naturally” primed to “kill” malignant or transformed cells and various infectious pathogens. Like other cells of the immune system, these cells play a major role in immune-regulation; however, their uniqueness lies in blurring the gap between innate and adaptive immune response. Natural killer T cells (NKT), also known as innate lymphocytes are recently discovered cells known for their action against lipid antigens. Properties of NK and NK-T cells have been harnessed in recent years for therapeutic and prophylactic medicine in the form of anti-tumour drugs, immunomodulators and vaccines against HIV, Pox virus, Influenza and many more. Knowledge of the mechanism of action and properties of these cells, brighten the possibility for cure of many diseases, the remedy of which still remain unrevealed!

Keywords: NK cells, NK-T cells, Immune-regulation

INTRODUCTION

Natural killer (NK) cells are large granular lymphocytes derived from the bone marrow and are important component of innate immunity (1-3). They constitute 5-20% of circulating lymphocytes (1,2,4) and are naturally primed to kill altered target cells rapidly until the adaptive immune response comes into action (2,3). They are neither T nor B lymphocyte, therefore lack T- cell receptor (TCR) and the B cell surface immunoglobulin (2). Flowcytometrically, human NK cells are defined by the phenotype CD3⁻ and CD56⁺; additionally they are CD19 and CD14 negative and are commonly divided into two major subpopulations, CD56^{dim} CD16⁺ and CD56^{bright} CD16⁻ (4,5). Their only specific marker is NKp46 (4).

The CD56^{dim} CD16⁺ subset comprises 90% of all peripheral blood NK cells and mediates an early response via direct cellular cytotoxicity. The CD56^{bright} CD16⁻ subset mediates a late but sustained effector function by release of IFN γ , but is poorly cytotoxic (4,5). Natural killer cells were first recognized and named for their ability to kill malignant or transformed cells and are now also known to play an important role in the control of intracellular pathogens including viruses, bacteria, and protozoa (6) along with immunoregulation (7-9).

These cells were first identified in 1971 under Scanning electron microscope by Dr. Thornthwaite in Florida state university while performing the “Plaque cytochrome assay” and were termed “Complement independent plaque forming cells (CIPFC)”. Later, the term Natural Killer cell was coined by Kiessling and Wigzel in 1975 (10).

NK Cell Receptors

NK cells express a diverse array of germ-line encoded inhibitory and activating receptors for Major histocompatibility class I (MHC-1) and Class I-like molecules, classical co-stimulatory ligands, and cytokines (11,12), which allow the NK cells to respond to the antigen without prior sensitization with it, unlike T cells (7). There are few studies which reveal the presence of these receptors on other population of cells like T cells, NK like cells and tumor transformed T cells (7). A small number of pathogen derived ligands can bind to NK cell receptors and directly induce their activation (11).

The common NK cell receptors are Killer Immunoglobulin like receptors (KIRs), Killer lectin like receptors (KLRs) and Natural cytotoxic receptor (NCR)(1-3). KIRs are subset of memory T cells, encoded in leucocyte receptor complex on Chromosome 19q and recognize self MHC-I molecule (HLA-A, -B, -C). KLRs are encoded in NK receptor complex on chromosome 12 and their target ligand

is non classical MHC molecule HLA-E in humans (13).

NK cell response is determined by the balance of all signals transduced by inhibitory and activating receptors (1,14). Inhibitory NK receptors prevent killing by NK cells. They contain immunoreceptor tyrosine-based inhibition motifs (ITIM). The tyrosine component of ITIM gets phosphorylated when it comes in association with its ligand leading to activation of phosphatases which in turn inhibit signalling by other receptors; preventing NK cells from killing host cell (1,3). Few examples are KIR-2DL, KIR-3DL in humans, Ly49A (mice), CD94/NKG2A (man and mice). Inhibitory KIR and Ly49 molecules bind MHC-I with higher affinity as opposed to their activating counterpart (14).

Activating NK receptors are responsible for killing of the target cell. Once the ligand comes in contact with the ITAM (immunoreceptor tyrosine-based activation) bearing DAP12 adapter protein, the tyrosine component gets phosphorylated which turns on the intracellular signalling pathways to activate the NK cells and release of cytotoxic granules to kill the target. The phosphorylated ITAMs bind and activate intracellular tyrosine kinases such as Syk or ZAP-70, leading to further signaling events in the cell (1,7,15). NCR like Nkp46, Nkp44, and Nkp30 are the major activating receptor of human NK cell (14). CD94/NKG2C is the activating counter part of CD94/NKG2A (14). Other activating receptors are NKG2D, KIR-2DS, KIR-3DS, Ly49D, Ly49H (mice)

NK cell regulation

NK cell regulation is important to prevent killing of the self peptide. Also, both the direct and indirect pathways of NK cell activation are likely to contribute to their control of infections (11).

Klas Karre's Direct Missing self hypothesis

NK cells recognise and eliminate cells that fail to express MHC-I. MHC molecules are recognised by inhibitory receptors present on NK cells which block the ability of NK cells to attack target cells (16). The greater the number of MHC class I molecules on a cell surface, the better protected the cell is against attack by NK cells (3). In the absence of inhibitory signals, signals from activating receptors lead to induction of NK cell cytotoxic function and cytokine release (11). (Figure 1a & 1b)

Killing of target cell through the direct pathway occurs through granule exocytosis leading to release of cytotoxic granule contents namely, perforin, granzymes, granulysin into the intercellular space between NK cell and target cell causing apoptosis or by engagement of death receptors (e.g. Fas/CD95) on target cells by their cognate ligands (e.g. FasL) on NK cells, resulting in classical caspase-dependent apoptosis (17). Following this hypothesis, NKG2D (activating receptor) interacts with MHC-Class-I like ligands induced by cellular distress, infection and malignant transformation. Ly49H receptor in mice and Nkp46 in humans recognise molecules encoded by viruses and displayed on infected cell surface and lyse them. Nkp46 plays a key role in the recognition and clearance of various viral and bacterial pathogens including CMV and Streptococcus pneumonia (7).

Fc γ receptor IIIa (FCYRIIIa) or CD16 are type III receptors for IgG highly expressed on CD56 dim CD16+ NK cell subset and are responsible for antibody dependant cell mediated cytotoxicity (ADCC) (18,19). High affinity antibodies of the subclasses IgG1 and IgG3 bind to FcYRIIIa and induce a potent activating signal which overcomes inhibitory signals and results in both cytotoxicity and a cytokine response, killing the antibody bound antigen. (4,18)

Indirect pathway

NK cell activation results from contact-dependent and soluble signals derived from accessory cells and T cells, transmitted to activating receptors on NK cells. Myeloid accessory cells recognize pathogens via Pathogen recognition receptors (PRR), secrete cytokines, and up regulate co-stimulatory molecules. Accessory cells also present antigen to CD4+T cells and provide T cell co-stimulation; activated T cells secrete IL-2 which synergizes with accessory cell signals to potentiate the activation of NK cells (11). The killing activity is increased 20-100-fold when NK cells are exposed to interferon- α (IFN- α) and IFN- β , or to interleukin-12, which is one of the cytokines produced early in many infections by dendritic cells and macrophages. IL-12, acting in synergy with the cytokine IL-18 produced by activated macrophages, stimulate NK cells to secrete large amounts of IFN γ , and this is crucial in controlling some infections before the IFN γ produced by activated CTL of the adaptive immunity becomes available (1). (Figure 2)

NK cell regulation of T cell differentiation

Infection of a dendritic cell or macrophage by intracellular bacteria or viruses can stimulate the cell to produce IL-12 which triggers NK cells to produce IFN γ which in turn stimulates CD4+ T-cells to differentiate into T-helper-1 (TH1) effector cells and exhibit their cytolytic activity. Similar mechanism is also seen in cases of malignancy (8). Alternatively, infection by certain worms can result in secretion by NK cells of IL-4, which triggers CD4+ T-cell to differentiate to TH2 cells (8). (Figure 3)

Immune regulation of NK cells can be well understood by their role in allergy and inflammation. (20). A robust NK cell response to respiratory viral infection with high levels of IFN γ production and cytolytic activity leads to viral clearance and Th1 environment in the lung and normal tolerance to environmental antigens. An impaired response to viral infection with low IFN γ and cytolytic activity and higher levels of Th2-type cytokines released from NK cells leads to increased inflammation and viral shedding, the recruitment of immature DCs, allergic sensitization with subsequent inflammation (21).

NKT cells

In the mid to late 1980s and early 1990s, an unusual population of T-cell was identified (8). They share phenotypic and functional characteristics of both T cells and NK cells and express a TCR- $\alpha\beta$ along with NK markers, NK1.1 or CD161 (3,9,22). However, not all NKT cells express CD161 and few conventional T cells may also express CD161 (8,22). NKT cells make up 0.01-2% of human peripheral blood mononuclear cells and link innate and adaptive immune response. (23)

Unlike most conventional T cells, NKT cells do not recognize peptide antigens bound to MHC class I or MHC class II molecules. Instead, these cells are known to respond to lipid antigens, which they recognize with their TCRs, as ligands bound to non classical MHC class Ib molecule, CD1d. (8,9, 20).

TYPES OF NKT cells

Type I/ invariant (iNKT)/ classical NKT/ V α 14iNKT (mouse), V α 24iNKT (human)

These are the most widely studied cells in recent times also known as the innate lymphocytes (8) expressing an invariant TCR α chain (V α 14J α 18 in mice, V α 24J α 18 in human) which pairs with limited number of TCR β chain (V β 2, 7 and 8.2 in mice and V β 11 in human) (9). Virtually, all the iNKT cells recognize a specific type of ceramide glycolipid (α -galactosylceramide) when bound to CD1d and get activated (8,23).

KRN7000 is currently the only form of α -galactosylceramide (α -GalCer) that has been tested in human subjects, and is also the best studied activator of iNKT cells in mouse models of disease (8).

As seen with NK cells, iNKT cells also have a role to play in immune regulation. Interaction between iNK TCR and CD1d on DCs, triggers IL-4 and IFN- γ production by iNKT cells and up-regulates iNKT expression of CD40 ligand (CD40L). IFN- γ and CD40L induce DCs to produce IL-12. IL-12 further activates iNKT cells, inducing a second wave of IFN- γ production by iNKT cells.(8,20,23). The activation of iNKT cells by α -GalCer may also have an impact on other leukocytes, such as the activation of bystander CD8+ T cells or the recruitment of DCs and neutrophils (8).

Similar to the action of NK cells, specific activation of iNKT cells induce apoptosis of CD1d-expressing target cells. However, the physiologic role of iNKT cells is more consistent with activation of other cytolytic cells, such as NK cells, rather than with direct killing (8). (Figure 4)

Type II/ non-classical

Like iNKT these cells are CD1d restricted but unlike them, they express a diverse TCR repertoire. These cells are less well defined compared to iNKT(8). Few studies suggest a counter-regulatory action of Type I and TypeII NKT, where iNKT cells enhance anti tumor activity and Type II cells suppress the proliferation of iNKT cells (9,24).

NKT like cells

Cd1D independent T cells expressing CD161/CD3/ CD 56. Most of these are MHC I/II restricted $\alpha\beta$ or MHC unrestricted $\gamma\delta$ cells.(8,23,24)

Uterine NK cells (uNK) and pregnancy

uNK cells are derived from a subset of peripheral NK cell (pNK) (13,25) which under hormonal influence of pregnancy get recruited to the uterus. They express CD 56 and other activating and inhibiting receptors but lack CD 16. In addition, in contrast to the majority of peripheral NK cells, uNK cells express CD69, an early activation marker. uNK cells are believed to play an important role in the development of placental vasculature, promoting placental and trophoblast growth and as immunomodulators at the maternal-fetal interface (13,26). Dysregulation of uNK and pNK cells during pregnancy are known to cause recurrent pregnancy loss (13,25).

Memory NK cells- blurring the line between innate and adaptive response

More mature a NK cell, the more virus-specific its surface molecules, suggesting they are able to recall prior viral infections. Secondary NK cell responses are virus-specific, and are independent of T and B lymphocytes, but are restricted to a specific population of hepatic NK cells. Upon reinfection these “primed” or “memory” NK cells proliferate, degranulate, and secrete IFN- γ to a much greater extent than “naïve” NK cells and confer protective immunity. (11)

Functions of NK cells

It is a well known fact that NK and NKT cells play a protective role against malignancy, viral infections like influenza, pox, herpes, dengue west Nile fever , hepatitis B infection (12,23,27); bacterial infections like Mycobacterium tuberculosis and Mycobacterium bovis,

Listeria monocytogenes, *Staphylococcus aureus*, *Lactobacillus johnsonii*, *Pseudomonas aeruginosa* and *Nocardia* species (11); parasitic infections, autoimmune disorders (20) and graft versus host diseases (28), by induction of cytotoxic activity. (8,11)

iNKT cells have anti-HIV activity demonstrated by its depletion during the infection and variable recovery following highly active anti-retroviral therapy (HAART)(23).

The mechanism of activation of NK cells by fungi has still not been clear; however there are few studies which suggest role of NK cells against fungal infections. NK cells are the main source of IFN- γ in the early phase of *Aspergillus fumigatus* infection and are required to induce killing by macrophages. NK cells are thought to mediate perforin- dependent killing of *Cryptococcus neoformans*.

Patients with progressive malignant disease and autoimmune diseases have a marked deficiency in NK cytolytic activity, which is not observed in patients with stable non-progressive disease (5,8). Studies in mice have shown that activation of these cells prevent disease progression.

In a study conducted by Jeanning et al, immune dysfunction in type 2 Diabetes mellitus was attributed to down regulation of NKp46 and NKG2D positive NK cells predisposing the individuals to various infections and malignancies (29).

Future prospects

Personalised medicine

Study of the proteins normally found in a healthy NK cell and its deviation in case of several diseases/malignancies is a major step forward in patient analysis. In the future, intervention using targeted drug therapy whenever a patient's NK cells deviate from the standard protein may become a first step towards personalized medicine (30).

Therapeutics:

1. Human iNKT cells that are activated by α -GalCer or α - GalCer-pulsed dendritic cells, mediate cytotoxic anti-tumor activity by production of IFN γ , against a number of cell lines derived from hematologic malignancies of myeloid, B and T cell lineages, melanoma, glioma, lung, breast, colorectal, liver, kidney, prostate, and head and neck cancers that express CD1d. IFN γ produced also causes inhibition of tumor angiogenesis (8,9,20).
2. Modified forms of α GalCer with altered lipid tails are Th2 biased compounds which reduce IFN- γ production and induces strong IL-4 production and have shown to prevent diabetes better than the KRN7000 form of α -GalCer which elicits a more mixed cytokine response of both IL-4 and IFN- γ (8, 20).
3. Majority of the immunomodulators like Thalidomide, linalidomide, promalidomide, imatinib, sorafenib , azacytidine etc. function by influencing the NK cells (31).
4. Therapeutic antibodies: Tumor-targeted monoclonal antibodies that initiate NK cell ADCC have been used clinically (28). Antibodies targeting CD20, Her2/neu, epidermal growth factor receptor (EGFR), and disialoganglioside (GD2) are examples of clinically successful antibodies whose mechanisms include NK cell mediated ADCC. (4,19). ADCC-mediating therapeutic antibodies currently FDA approved for cancer therapy are Rituximab for non hodgkins lymphoma (NHL), Ofatumumab and Alemtuzumab for CLL, Transtuzumab in breast cancer, Cetuximab for colorectal carcinoma.
5. Trials: Therapeutic trials for autoimmune disorders aim at either expanding the NK cell population or usage of monoclonal antibodies for ADCC by NK cells. Few examples of such ongoing trials for Multiple sclerosis are with Daclizumab, Alemtuzumab, Rituzimab (5).

Vaccine

1. NK cells respond much more quickly than CD8+ T cells after re-exposure to the vaccine antigen and represent more than 70% of IFN- γ -secreting and cytotoxic cells during the first 24h after re-exposure to Rabies virus. Therefore, antigen-specific IL-2 secretion from T cells may recruit NK cells as effectors of adaptive immunity and, thus, NK cell responses can be potentiated by vaccination (6).
2. α GalCer has been shown to be an effective mucosal adjuvant to activate iNKT cells; for inducing antigen-specific immune responses following administration of HIV peptides(9), influenza virus antigen (23) and for inducing protective immunity against sexually transmitted HSV-2 infection in mice (23).
3. Poxvirus-based vaccine vectors or immune-therapies, through their oncolytic property have shown to prevent post surgical metastasis by eliciting NK activity (12).
4. NK cells force immune escape in HIV infection through both direct Killer-immunoglobulin-like receptor mediated killing as well as through facilitating ADCC. There is an opportunity to harness these immune responses in the design of more effective HIV vaccines (18,32,33).

CONCLUSION

NK and NK T cells have become one of the most studied cells in recent times. From being regarded as “Null Cells” a few decades ago to being regarded as major cells responsible for immune response, numerous studies are still being done to discover various mechanisms of actions and their importance not only in the immune response but also in therapeutic medicine. Therefore, knowledge of these cells and their function becomes one of the important aspects in human immunology.

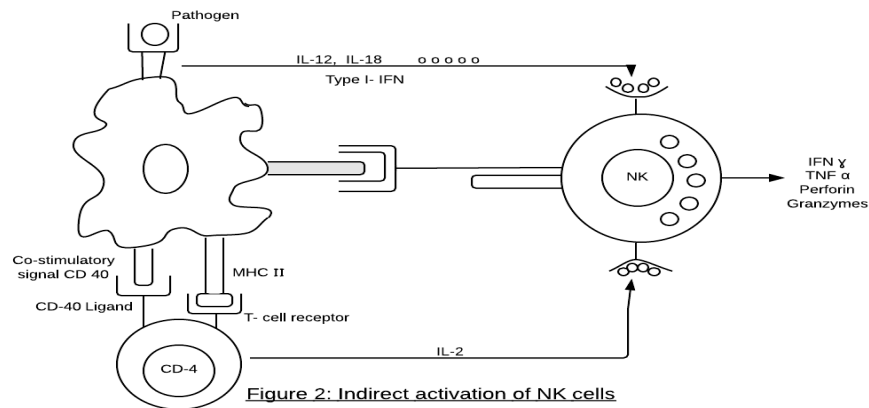
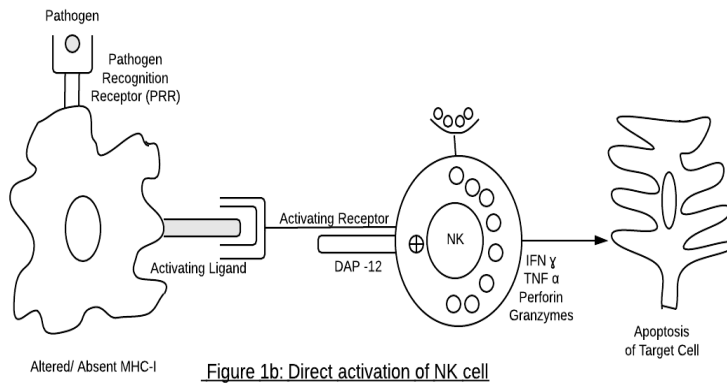
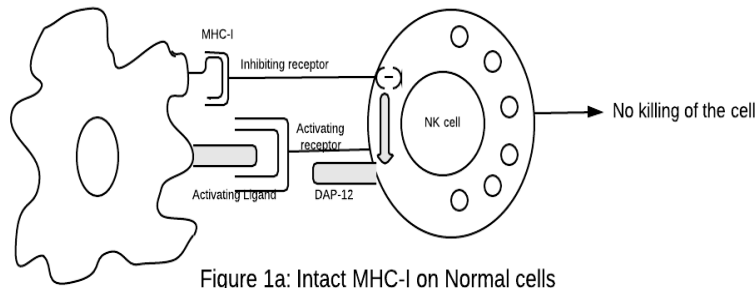
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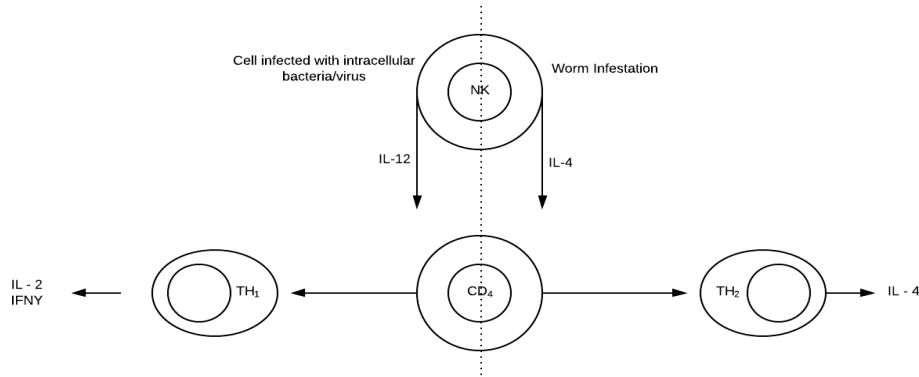
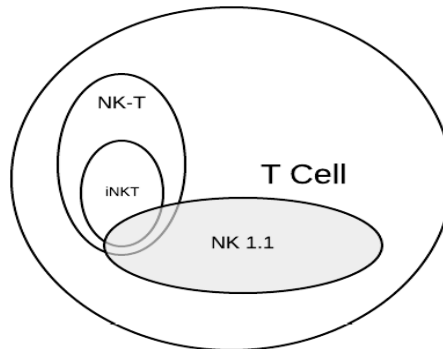


Figure: 3 NK cell regulation of T- cell Differentiation

Figure 4
NK-T cells: subset of T cell;
iNKT cells : subset of CD1d
restricted NKT cells(<1% of
circulating T cells); NK 1.1/
CD 161 cells: may comprise
25 % of circulating T cells.



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