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Natural Killer Cells: From Bone Marrow to Cytotoxic Cells

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Abstract

Natural killer (NK) cells are lymphoid cells, having central effector functions in immune system without previous sensitization. These cells are related to innate lymphoid cells which are characterized by lymphoid cell morphology with missing antigen-specific receptors. NK cells are defined by lack of expression of CD3 and expression of CD56 (CD3-CD56+). Based on the expression of CD56, human NK cells are classified into two main populations: CD56bright and CD56dim NK cells. Functionally, CD56bright subset is characterized by higher capacity to produce cytokines following activation but has lower cytotoxicity. On the contrary, CD56dim NK cells are more cytotoxic and express higher levels of maturation markers. NK cells have effective cytotoxic activity against virus-infected cells and tumors, and their effector functions are regulated by multiple activating and inhibitory receptors. The activated NK cells are able to kill their target cells in apoptosis process by different mechanisms, including cytolytic granule-dependent exocytosis pathway, death receptor pathway, antibody-dependent cell-mediated cytotoxicity, and the release of cytokines. This review will focus on NK cell differentiation, NK cell receptors as well as their ligands and their role in immune responses. In addition, we will summarize the pathways involved in NK cell effector functions.

Keywords: Natural Killer Cells; NK Cytotoxicity; Inhibitory Receptors; Activating Receptors

Abbreviations: HSCs: Hematopoietic Stem Cells; MPPs: Multipotent Progenitors; CMPs: Common Myeloid Progenitors; CLPs: Common Lymphoid Progenitors; CILCPs: Common Innate Lymphoid Cells Precursors; MAPK: Mitogen Activated Protein Kinase; DD: Death Domain; FADD: Fas-Associated Death Domain; TRADD: TNFR-Associated Death Domain; DISC: Death Inducing Signaling Complex.

Introduction

NK cells are bone marrow-derived lymphocytes arising from the lymphoid lineage which were identified in 1975 as effector lymphocytes due to their ability to lyse virus-infected cells and tumor cells [1,2]. They develop during fetal life as well as after birth from CD34+ hematopoietic stem cells which differentiate into common lymphoid progenitors that can give rise to NK cell progenitor (Figure 1). It has been commonly reported that the bone marrow is considered to be the main site of NK cell development similar to B cells. However, some studies have notified that NK cell development and maturation can take place in other sites such as the thymus [3] and lymph nodes [4]. Despite their lymphoid origin, NK cells are crucial components of the innate immune defense due to the direct kill of their target cells in the absence of specific immunization and lack of antigen-specific cell surface receptors [5,6]. Now, NK cells have been reclassified as a subset of cytotoxic innate lymphoid cells [6,7]. However, they differ from other innate immune cells that they do not mediate phagocytosis and lack bactericidal enzymatic systems. Generally, the effector functions of NK cells are regulated by a balance between activating and inhibitory signals provided by a wide group of activating and inhibitory receptors. When NK cell activating receptors interact with their specific ligands expressed

on the target cells, NK cells become cytotoxic cells and are able to kill their target cells by different mechanisms. In this review, we provide an overview of the characteristics of NK cells, their phenotype, differentiation and maturation, receptors and functions.

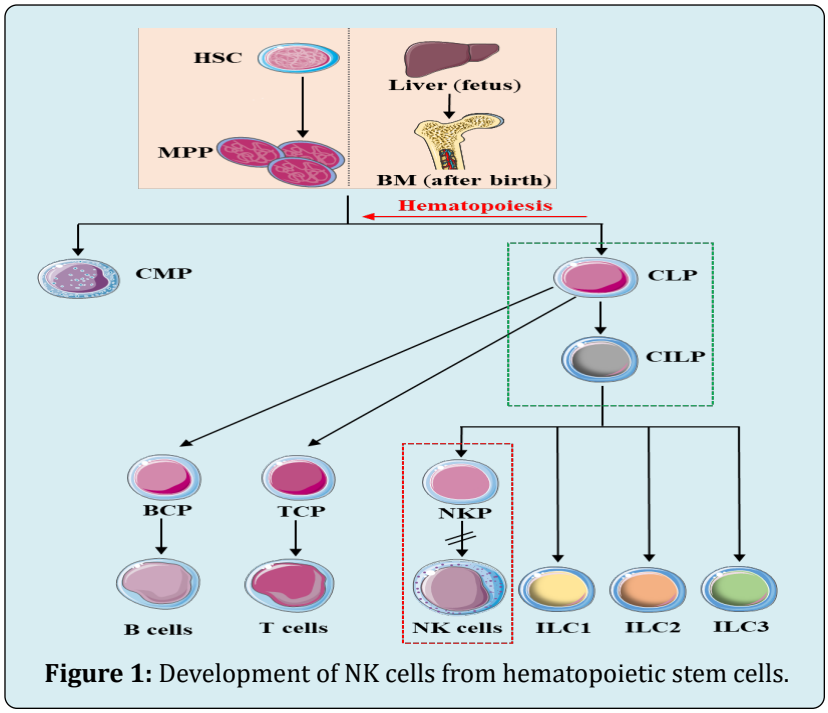

Figure 1: Development of NK cells from hematopoietic stem cells.

Hematopoietic stem cells (HSCs) divide into multipotent progenitors (MPPs) which lead to common myeloid progenitors (CMPs; the precursors of all myeloid cells) and common lymphoid progenitors (CLPs; the precursors of all lymphoid cells). Then CLPs give rise to the precursors of T cells (TCP), B cells (BCP) and common innate lymphoid cells precursors (CILCPs). The CILCPs lead to different groups of innate lymphoid cells including NK cells through their specific precursors. HSC: hematopoietic stem cell; MPPs: multipotential progenitors; BM: bone marrows; CMP: common myeloid progenitor; CLP: common lymphoid progenitor; CILP: common innate lymphoid progenitor; NKP: NK cell progenitor; TCP: T cell progenitor; BCP: B cell progenitor; ILC: innate lymphoid cells.

NK Cell Phenotype and Subsets

In humans, NK cells are considered as the third largest population of lymphocytes following T and B cells comprising approximately 10 – 15% of all peripheral blood lymphocytes [8,9]. Phenotypically, they are defined as CD3-CD56+ lymphocytes upon their expression of CD56 (Neural Cell Adhesion Molecule, 140 kDa isoform) and lack of expression of the T cell marker CD3 [8]. Moreover, NK cells can be subdivided into various subsets with diverse functions based on the surface expression of CD56, CD16, inhibitory receptors and/or activating receptors. The main two populations of human NK cells are CD56bright CD16– and CD56dim CD16+ [8].

CD56bright CD16– NK cells are immature, cytokine-producing NK cells (5 – 10% of NK cells), being dominant in the lymph nodes and function as immunoregulatory cells by secreting cytokines such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interlukin -10 (IL-10), IL-13 and granulocyte–macrophage colony-stimulating factor (GM-CSF). CD56bright CD16– cells highly express NKG2A inhibitory receptor and have low to absent expression of killer cell immunoglobulin-like receptors (KIRs) [8].

On the other hand, around 90 - 95% of peripheral blood NK cells are CD56dim CD16+ cells which are cytotoxic cells with weak proliferation activity and low levels of cytokines production at resting state [8,10,11]. However, CD56dim NK cells largely secrete proinflammatory cytokines and chemokines more than CD56bright cells after target cell stimulation.

NK Cell Differentiation and Maturation

The differentiation pathway of human NK cell is complex, characterized by losing the expression of specific surface antigens as CD34 and increasing the expression of other surface antigens such as CD56, NKG2A, CD16, KIRs

and CD57 resulting in a heterogeneous CD3−CD56+ NK cell population. Further, a variety of activating and inhibitory receptors are acquired or lost during NK cell maturation. There are many factors regulating NK cell differentiation, including transcription factors and cytokines [12]. Several studies have also shown the importance of microRNAs in the differentiation and maturation of NK cells [13].

Transcription Factors

Transcription factors have a prominent effect on immune cell development and differentiation by regulating gene transcription of specific molecules controlling these processes. There are numerous transcription factors involved in regulation of NK cell differentiation and maturation. For instance, an inhibitor of DNA binding protein (Id2) contains a helix-loop-helix domain which prevents the binding of transcription factors (E2A, E12 and E47) with DNA. Boos, et al. [14] demonstrated that Id2 has a role in NK cell differentiation at later stage because they found that Id2 deficient mice have low number of mature NK cells in the periphery while having normal number of immature NK cells and NK cell progenitors in the bone marrow [14].

Another example is E4-binding protein 4 (E4BP4, also called NFIL3) transcription factor that has a potential regulatory role in the immune system [15]. E4BP4-deficient mice showed a sharp reduction in both immature NK and mature NK numbers, suggesting that E4BP4 is required for the progression from NK cell precursors to immature NK cells and then from immature NK cells to mature NK cells [16,17]. Recently, Male, et al. [18] showed that E4BP4 is necessary for NK cell progenitor development from common lymphoid progenitors and for NK cell development at early stage by controlling the expression of Eomes and Id2 [18].

In addition, T-box transcription factors family including Eomes and T-bet (also known as Tbx21) are defined as basic drivers of immune cell development and cytolytic function. In humans, Knox et al. (2014) have found that Eomes was expressed in both CD56bright and CD56dim NK cells, but it was significantly higher in CD56bright suggesting that Eomes is likely essential for CD56bright maturation [19]. With respect to T-bet, it regulates the expression of sphingosine-1 phosphate receptor 5 (S1P5) which plays an important role in NK cell recirculation. T-bet−/ − mice exhibited a reduced number of NK cells in the spleen, liver and peripheral blood, and an increased number in lymph nodes and bone marrow [20,21]. T-bet−/ − NK cells also showed an impaired cytotoxicity and IFN-γ production [20,21].

Cytokines

Cytokines play an important regulatory role in several processes in the immune system, including development, proliferation, homeostasis, and activation status. In the context of NK cells, IL-2, IL-15 and IL-21 are the major cytokines that regulate NK cell development, differentiation, survival, and function. Their receptors are signaling through three main transduction pathways: the Janus tyrosine kinases (JAK)-STAT pathway, the phosphatidylinositol-3-kinase pathway, and the mitogen activated protein kinase (MAPK) pathway (Figure 2) [22,23]. IL-18 can control NK cell proliferation and cytokine production by binding to IL-18 receptor which activates MAPK and nuclear factor-kappa B (NF-kB) pathways [24]. Table 1 summarizes the key interleukins regulating NK cell differentiation and other biological functions.

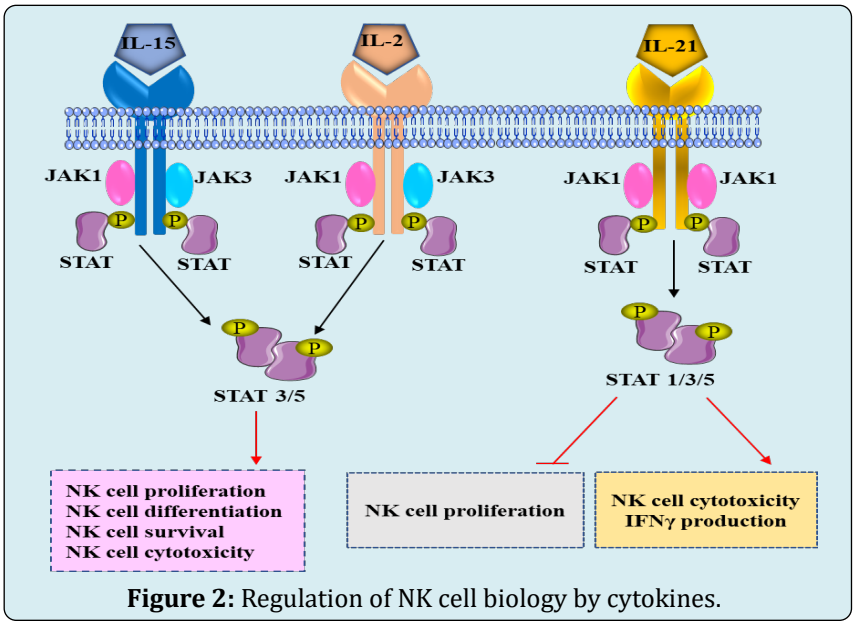

Figure 2: Regulation of NK cell biology by cytokines.

Different cytokines can control NK cells through their receptors. When the cytokines bind with their specific receptors, they can activate specific signaling pathways regulating NK cell biology. IL: interleukin; JAK: Janus kinase; STAT: signal transducer and activator of transcription; IFNγ: interferon-γ.

Cytokine	Source	Signaling	Function
IL-2	T cells	JAK1/3	Cytokine production, proliferation, survival, enhanced cytotoxicity
		STAT3/5	
		MAPK	
IL-7	Stromal cells	JAK1/3	Development of thymic NK cells, proliferation
		STAT5	
IL-12	DCs	JAK2	Differentiation, proliferation, cytokine production, enhanced cytotoxicity
	Macrophages	STAT3/4/5	
IL-15	DCs	JAK1/3	Cytokine production, proliferation, survival, maturation, enhanced cytotoxicity
	Macrophages	STAT3/5	
	Stromal cells	MAPK	
IL-18	DCs	MyD88	
	Macrophages	MAPK	Cytokine production, proliferation
		NF-kB	
IL-21	T cells	JAK1/3	Differentiation, enhanced cytotoxicity, cytokine production, limits proliferation
		STAT1/3/5	

Table 1: Major interleukins regulating NK cell differentiation and biological functions.
IL: Interlukin; JAK: Janus tyrosine kinase; STAT: Signal transducer and activator of transcription; DCs: dendritic cells; MAPK: Mitogen activated protein kinase

MicroRNAs

MicroRNAs are a large family of short non-coding (22 nucleotides) RNA molecules that regulate gene expression post-transcriptionally by targeting the 3' untranslated regions of mRNAs, resulting in translational inhibition and/or mRNA degradation. There are several microRNAs affecting the development and maturation of NK cells. For example, a consistent increase in the expression levels of microR-181a/b is associated within different stages of NK cell development, suggesting that microR-181 has an impact on human NK cells development from CD34+ hematopoietic progenitors [25]. MicroR-181 establishes its effect on NK cells development through downregulation of nemo-like kinase (a protein kinase that negatively regulates Notch-dependent transcriptional activation pathway). Another example is microRs-15/16 which are highly expressed in NK cells and are involved in regulation of NK cell development by controlling the level of Myb (also known as c-Myb) [26]. Myb is a transcriptional activator factor related to myeloblastosis family that is highly expressed in immature NK cells (CD56bright NK cells), however, its expression is decreased during NK cell maturation (CD56dim NK cells). It has been found that immature NK cells are accumulated, while mature NK cells are reduced in the absence of microR-15/16 [26]. Further, it was noted by Mundy-Bosse, et al. [27] that immature NK cells were extremely reduced in acute myeloid leukemia patients due to a disruption in NK cell differentiation [27]. They also found low levels of T-bet and Eomes transcription factors as a result of elevation level of microR-29b in NK cells of leukemia patients (microR-29b is a regulator of T-bet and Eomes). As a consequence of microR-29b deletion in NK cells, the immature NK subset is returned to appear again [27].

NK Cell Receptors

During NK cell maturation, they acquire a series of cell surface molecules that regulate NK cell effector functions. These molecules can be classified into different groups: inhibitory receptors, activating receptors, adhesion molecules, cytokine and chemotactic receptors.

Inhibitory Receptors

NK cell functions are negatively controlled by many inhibitory receptors where CD94/NKG2A and inhibitory KIRs are the major inhibitory receptors [28,29]. Inhibitory

receptors transmit their inhibitory signals through intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs), located in the cytoplasmic tail of these receptors. Following binding the ligand with its receptor, the tyrosine residues in ITIMs are phosphorylated, and thus recruit and activate Src homology 2 domains of the intracytoplasmic protein tyrosine phosphatases SHP-1 and SHP-2. Then, these phosphatases inhibit the activation of NK cells by dephosphorylation of specific intracellular signaling molecules at multiple stages of signaling cascade [30,31].

CD94/NKG2A

NKG2A is a transmembrane protein structurally characterized by C-type lectin extracellular domains, covalently coupled to CD94 subunit [32,33]. CD94/NKG2A receptor is found as a heterodimer that recognizes non-classical human leukocyte antigen (HLA) class I molecule, HLA-E [34]. It is naturally expressed on about half of all NK cells, and its expression is not stable and can be affected by cytokines in the surrounding environment. CD94 has a short cytoplasmic domain lacking signaling function, whereas NKG2A contains ITIM domain in its intracellular structure, which upon tyrosine phosphorylation can recruit SHP-1 or SHP-2 providing inhibitory signal transduction [32,33].

Inhibitory KIRs

KIRs (CD158) are transmembrane glycoproteins, encoded on chromosome 19q13.4, and belong to immunoglobulin (Ig) superfamily. KIRs family are classified into 14 groups (2DL1 to 2DL5, 3DL1 to 3DL3, 2DS1 to 2DS5, and 3DS1) according to the number of extracellular Ig-like domains, and cytoplasmic tail length (Table 2) [35]. They are monomeric receptors, structurally characterized by either 2 extracellular Ig-like domains (designated D1 and D2 in KIR2D) or 3 extracellular Ig-like domains (designated D0, D1 and D2 in KIR3D). However, these receptors have differences in their cytoplasmic domains and are subdivided into KIRs with long (L) cytoplasmic tails (KIR2DL and KIR3DL) and others with short (S) cytoplasmic tails (KIR2DS and KIR3DS). Based on these variances, KIRs are classified to two functionally distinct groups: inhibitory KIRs as KIR2DL and KIR3DL where the long tails generate an inhibitory signal, and activating KIRs with short tails as KIR2DS and KIR3DS [36].

KIR		Ligand
Inhibitory KIRs	**2DL1**	HLA-C group 2
	2DL2/3	HLA-C group 1, B46, B73 and some HLA-C group 2
	3DL1	HLA-Bw4
	3DL2	HLA-A3, A11
	3DL4	HLA-G
	3DL5	Not known
	3DL3	Not known
Activating KIRs	**2DS1**	HLA-C group 2
	2DS2	Not known
	2DS3	Not known
	2DS4	HLA-A11 and subsets of HLA-C
	2DS5	Not known
	3DS1	Not known

Table 2: Activating and inhibitory KIRs and their ligands.

Inhibitory KIRs signal through their long tails and ITIMs in the cytoplasmic domain. Following engagement with their ligands, the tyrosine residues of ITIMs are phosphorylated and then recruit protein tyrosine phosphatases as SHP-1, which are critical for mediating inhibitory function. The known ligands for inhibitory KIRs are HLA-class I (HLA-A, B and C) molecules. In contrast to other inhibitory KIRs, engagement of KIR2DL4 by its ligand results in increasing IFN-γ production without activation of cytotoxicity [37].

Activating Receptors

NK cells express a wide group of activating receptors, which upon ligation transmit activating intracellular signals to initiate the effector functions of NK cells. The major activating receptors are NKG2D (Natural-Killer Group 2, member D), natural cytotoxicity receptors (NCRs), DNAX accessory molecule-1 (DNAM-1), CD16 (Fcγ RIII) and activating KIRs [28]. In addition, several co-receptors act in

coordination with the activating receptors to activate NK cells such as NKp80, CD2, CD160, and 2B4 [28]. Commonly, NK activating receptors signal through two prominent signaling pathways: immunoreceptor tyrosine-based activating motifs (ITAMs) pathway, and DNAX-activation protein-10 (DAP-10) pathway.

NKG2D

NKG2D is one of the most important activating receptors expressed by NK cells. It is a C-type lectin surface receptor which was identified in 1991 [38]. NKG2D binds to induced-self ligands overexpressed on the abnormal cells, including MHC class I-chain-related proteins A and B (MICA and MICB), and UL16-binding proteins (ULBPs) [39,40]. There is no ITAM signaling motif in the intracellular domain of NKG2D, therefore, it signals through its association with adaptor protein called DAP10. When the receptor is engaged, this initiates downstream signaling pathways that promote NK cell degranulation and production of cytokines and chemokines such as IFN-γ, TNF-α, GM-CSF, CCL4 and CCL1 [41].

Natural Cytotoxicity Receptors (NCRs)

Natural cytotoxicity receptors (NCRs) are exclusively expressed by NK cells and mainly involved in the mechanisms by which NK cells kill their targets. These receptors, are non-MHC class-I activating receptors and belong to Ig superfamily. In humans, three NCRs (NKp46, NKp44, and NKp30) have been recognized. NKp46 (NCR1 or CD335) and NKp30 (NCR3 or CD337) are constitutively expressed by all peripheral blood NK cells [42,43], while NKp44 (NCR2 or CD336) is expressed on NK cells mainly after IL-2 stimulation [44]. NCRs include an extracellular ligand-binding domain and a transmembrane domain, while they lack functional cytoplasmic tails. So, each receptor is coupled with single dimeric ITAM-containing adaptor proteins (CD3ζ, FcRIγ and DAP12) to generate their activating signals. Among NCRs, NKp30 and NKp46 associates with FcRγ and/or CD3ζ, whereas DAP12 is connected with NKp44 [45,46]. Engagement of NCRs by their ligands results in phosphorylation of tyrosine residue in ITAMs of the adaptor molecules by Src family kinases. The phosphorylated ITAMs recruit and activate tyrosine kinases as zeta chain-associated protein kinase 70 (ZAP70). Consequently, these kinases phosphorylate other signaling molecules which lead to increasing cytokines secretion such as IFN-γ and TNF-α and activation of cellular cytotoxicity [47,48].

Activating KIRs

In contrast to inhibitory KIRs, KIRs with short cytoplasmic tail are activating KIRs. These receptors are associated with ITAM-bearing DAP12 adaptor protein. When activating KIRs are ligated, src family kinases phosphorylate the ITAM-containing adaptor molecule DAP12. Consequently, DAP12 activates ZAP70 and spleen tyrosine kinase which generate the downstream activation cascade [49]. There are several activating KIRs such as KIR2DS1 which has similar Ig-like domains to inhibitory KIR2DL1 and also binds HLA-C2 [50]. KIR2DS4 is the oldest and most predominant activating KIR receptor, having the ability to bind with C1 and C2 epitopes of HLA-C allotypes and HLA-A11 [51].

CD16A (FcγRIIIA)

In humans, several constant fragment gamma receptors (FcγRs) are found on the surface of NK cells, dendritic cells, neutrophils, monocytes, macrophages, B cells and some CD8+ T cells including activating receptors such as (FcγRIIA, FcγRIIC, FcγRIIIA and FcγRIIIB) and a single inhibitory receptor, FcγRIIB [52]. These receptors bind to Fc portion of IgG to initiate intracellular signaling pathways. CD16 is a low-affinity IgG Fc receptor (FcγRIII) that is expressed on the surface of NK cells, neutrophils, dendritic cells, monocytes and macrophages [52]. It is responsible on a mechanism of immune defense called antibody-dependent cell-mediated cytotoxicity (ADCC). There are two isoforms of CD16, FcγRIIIA (CD16A) which is expressed mainly on majority of CD56dim NK cells, and FcγRIIIB (CD16B) which is found on neutrophils. Once CD16A binds to the Fc region of IgG, NK cells release cytotoxic granules containing perforin and granzymes, causing lysis of target cells as tumors and virus-infected cells.

DNAM-1

DNAM-1 (CD226) is an activating receptor of the immunoglobulin superfamily expressed on NK cells, CD8+ T cells, and other immune cells. DNAM-1 recognizes two ligands related to nectin family: poliovirus receptor (CD155, also known as nectin-like molecule 5) and Nectin-2 (CD112) which are expressed by various healthy tissues as well as tumor cells [53,54]. The interaction between DNAM-1 and its ligands boosts cytokines production and cell-mediated cytotoxicity against dendritic cells, tumor cells and virus infected cells [55-57].

Acquisition of NK Cell Functions

NK cells play a key role in immuno-surveillance and host defense against certain infected or transformed cells mediated by direct cytolysis via perforin/granzyme, death receptors (Fas, TRAIL) and/or ADCC pathways [58]. Alongside, NK cells secrete cytokines and chemokines which

influence the host's immune response by regulating other immune cells [59,60]. NK cells have to be educated and primed to be able to recognize their target cells.

NK Cell Education

NK cells express many inhibitory receptors that recognize diverse self-molecules to prevent self-reactivity against healthy cells, however, self-major histocompatibility complex (MHC) class I molecules recognized by NK inhibitory receptors are essential for NK cell education [61,62]. NK cell education, also known as licensing, is a process developed during NK cell maturation to acquire the effector functions that are adapted to the host in which they develop. It has become clear by several reports that binding of inhibitory receptors on NK cells by self-MHC class I molecules is important to determine whether an NK cell will be functionally capable of mediating missing-self recognition, or it will be hyporesponsive following stimulation. Interestingly, Fauriat, et al. [63] demonstrated that NK cell education can be also mediated by activating KIRs. Their results showed that the expression of KIR2DS1 (an activating KIR) and its ligand (HLA-C2) reduces the responsiveness of NK cells against their targets in both presence and absence of NKG2A [63]. They also found that hyporesponsiveness is limited to target cell recognition because KIR2DS1+ NK cells are stimulated by exogenous cytokines [63].

NK Cell Priming

Resting human NK cells show little cytotoxic functions when incubated in vitro with tumor target cells, suggesting that resting NK cells require additional signals for their complete activation [64]. Resting NK cells should be activated in a process called priming to achieve their effector functions. Several in vitro studies have demonstrated the role of myeloid cells in activation of resting NK cells. In this context, dendritic cells play a critical role in priming resting NK cells through secretion of cytokines as IL-15 [65], IL-18 [66] and IL-27 [67].

Inhibitory/Activating Signals Balance

Under normal circumstances, NK cells can discriminate between normal healthy cells and abnormal cells (infected or transformed) through MHC class I molecules. Healthy cells constitutively express MHC class I molecules which bind to NK cell inhibitory receptor (CD94/NKG2A) to avoid NK cell-mediated lysis (Figure 3A) [68]. On contrary, NK cells are able to recognize and kill their target cells due to the imbalance between inhibitory and activating signals via two models: "missing-self" recognition where the abnormal cells lose the expression of MHC class I ligands to escape the cytotoxic T cells (Figure 3B), and "stress-induced recognition" in which the abnormal cells show an upregulation of damage-associated proteins (Figure 3C).

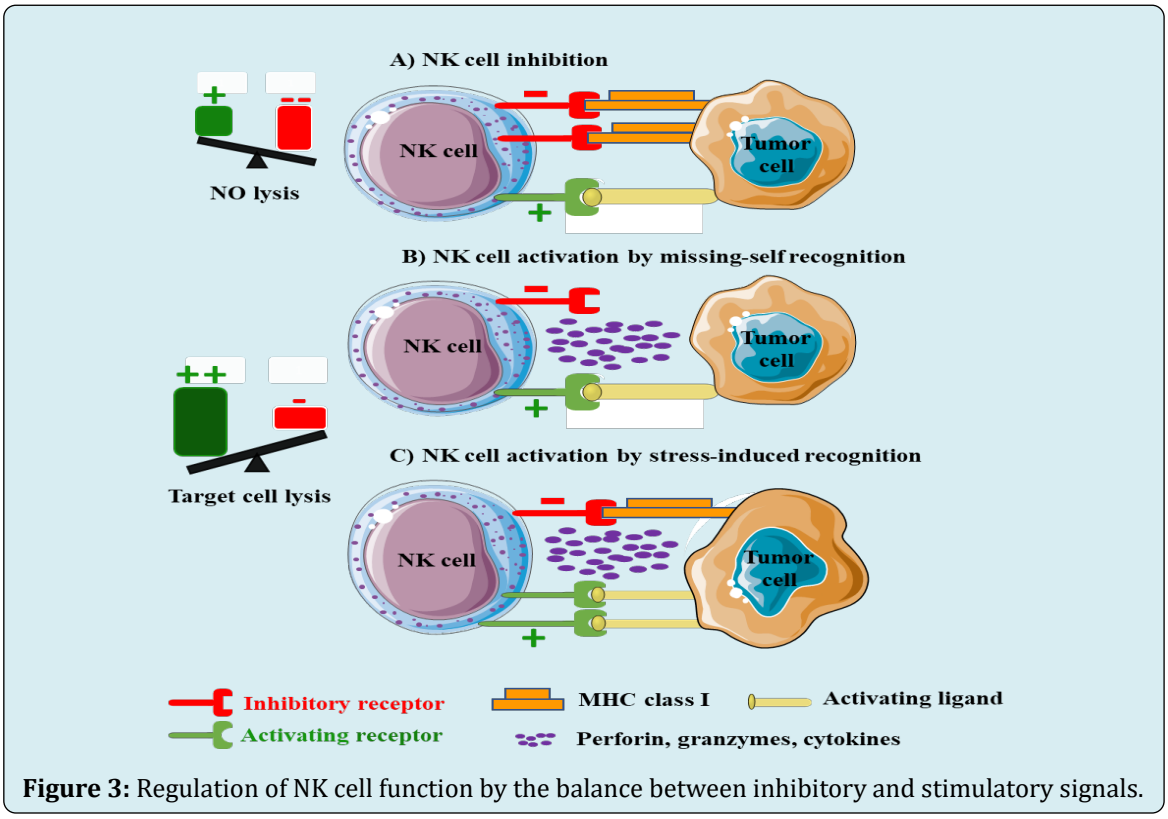

Figure 3: Regulation of NK cell function by the balance between inhibitory and stimulatory signals.

A) NK cells are tolerant to healthy host cells as healthy cells constitutively express MHC class I molecules which bind to NK cell inhibitory receptor to avoid NK cell-mediated lysis. B) Activation of NK cells by missing-self status in which tumor cells lose expression of MHC class I molecules. C) NK cells are activated by stressed cells, upregulating the expression of activating ligands for NK cell activating receptors (stress-induced recognition).

NK Cell Effector Functions

NK cells are key players in the effector arm of the immune system that maintain the homeostasis via recognition and killing abnormal and pathogen-infected cells. Several damage-associated proteins have been found in tumor cells such as MICA, MICB, ULBPs binding with NKG2D, ligands of NKp30 (B7-H6 and HLA- BAT3), mixed-lineage leukemia protein (a ligand of NKp44), and CD155 and CD112 which interact with DNAM-1 [69]. Beside its activation by tumor cells and pathogens, NK cells can directly or indirectly receive signals from other immune cells (Figure 4). For instance, dendritic cells can regulate NK cell proliferation and function by secreting IL-12, type I IFNs, and IL-15 [69]. Monocytes/ macrophages also play a role in NK cell activation and cytotoxicity by secreting cytokines such as IL-2, IL-12, IL-18, IL-1β and IFN-β. CD4+ T cells can secrete IL-2, which is critical for NK cell survival and proliferation [69].

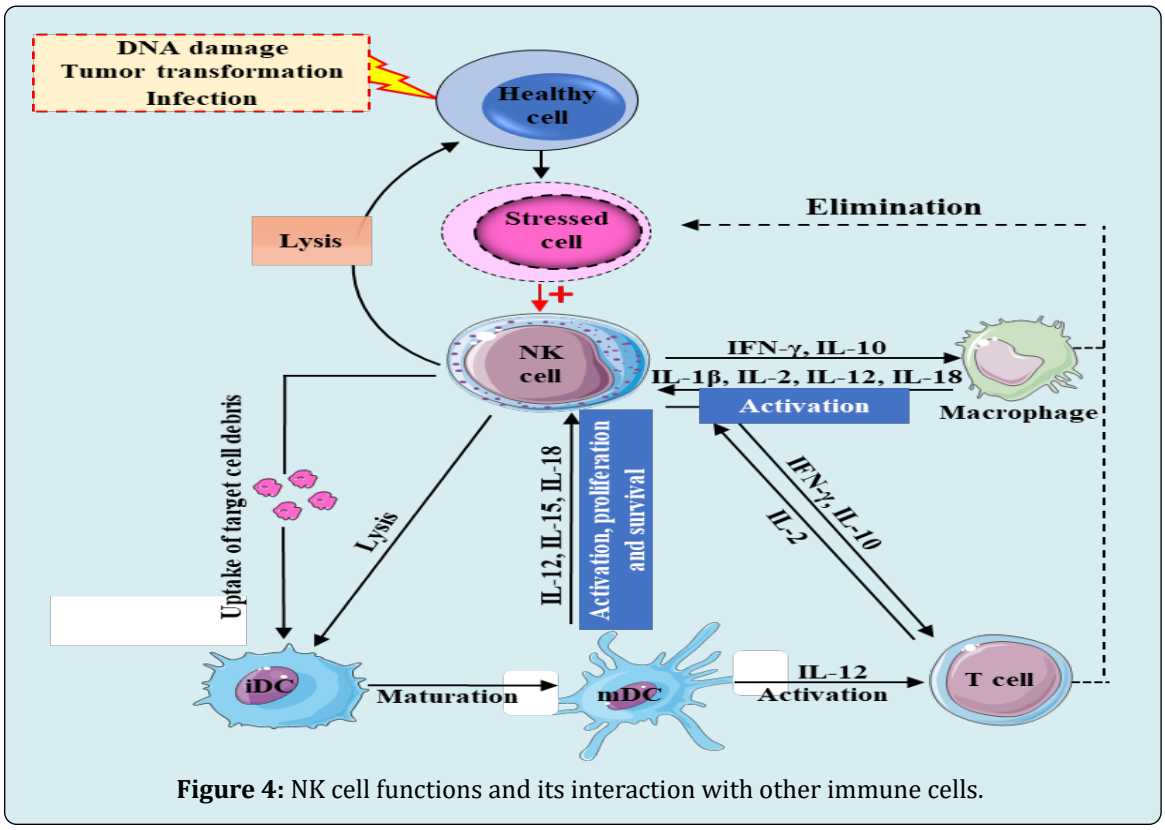

Figure 4: NK cell functions and its interaction with other immune cells.

NK cells can recognize and kill stressed cells either directly after their activation by the stressed cells or indirectly by stimulation of other immune cells through cytokine secretion. In addition, other immune cells are able to regulate and activate NK cells. IL: interleukin; IFNγ: interferon-γ; iDC: immature dendritic cell; mDC: mature dendritic cell.

Otherwise, Treg cells can suppress NK cell proliferation and activity by secreting transforming growth factor- β (TGF-β) and IL-10 [69]. Activated NK cells are able to kill their target cells in a process called apoptosis (a process of programmed cell death) though a variety of mechanisms, including cytolytic granule-dependent exocytosis pathway, signaling through the TNF death receptor family members such as FAS (CD95) and TRAIL (TNF-related apoptosis-inducing ligand), ADCC via CD16, and the release of cytokines IFN-γ and TNF-α [70].

NK Cell Cytotoxicity

Cytolytic Granule-Dependent Exocytosis

The cytotoxic granules of NK cells are specific secretory lysosomes contain a mixture of cytotoxic mediators

(perforin, granzymes, and granulysin) that cause death of the target cells. Moreover, the membrane of these granules comprise highly glycosylated membrane proteins called lysosomal-associated membrane protein-1 (LAMP-1 or CD107a) and LAMP-2 or CD107b [71]. CD107a expression on NK cell surface has been described as a marker of NK cell degranulation and is upregulated on the cell surface following NK cell [72].

Perforin

Perforin is a pore-forming protein (67 kDa), where its gene is constitutively transcribed in NK cells and is regulated by receptor activation signals and cytokines (IL-2 and IL-15). The exact molecular mechanism induced by perforin remains indefinable, but there are two suggested hypotheses describe its function. The first one proposes that perforin makes pores in the membrane of target cell, so this allows granzymes to diffuse into the target cell and also allows an ionic exchange, which causes an osmotic unbalance. The second suggests that perforin binds to the target-cell membrane (through electrostatic or possibly receptor-mediated interactions) and is internalized into its endosomes (containing granzymes) to disrupt them; thereby granzymes are released into the cytosol.

Granzymes

Granzymes are proteases related to serine proteases family. In humans, there are five granzymes (A, B, H, K and M) have been defined where granzymes A and B are the most abundant granzymes [73]. Granzymes are synthesized as pro-enzymes and are activated by cathepsin C and H. Although the most accepted suggestion is that the perforin pores can serve as passive conductors of granzymes through the target cell membrane, Motyka, et al. [74] have shown that in the absence of perforin, granzyme B can form a complex with the mannose-6-phosphate receptor on the surface of target, and then the complex is internalized by endocytosis. Compared with other granzymes, granzyme B has the strongest pro-apoptotic activity. It induces targets death based on caspase-dependent apoptosis rapidly through two pathways. In the first pathway, granzyme B directly activates caspase 3, which enhances DNA destruction [75]. The second is characterized by promotion of permeability of the mitochondrial outer membrane and cleaving of a molecule from the Bcl-2 family called BID (BH3-interacting-domain death agonist). In turn, BID induces cytochrome-C release from mitochondria triggering the activation of caspase 9 which enhances caspase 3 activation [76].

Granulysin

Granulysin is a cationic protein related to the saposin-like protein family which is converted to its active form by proteolytic cleavage from 3 to 5 days after cell activation. It makes pores in the mitochondrial membrane of target cell, disrupting the transmembrane potential ($\Delta\psi$) in mitochondria [77]. Moreover, granulysin induces cell death through activation of caspase-3 [77].

Death Receptor Pathway

Death receptors are cell surface receptors related to TNF super family that transmit apoptotic signals initiated by specific ligands such as Fas ligand (FasL; CD178), TNF-α and TRAIL (CD253). They play an important role in apoptosis via activation of caspase cascade. Death receptors contain an intracellular death domain (DD), which upon ligand binding associates directly with an adaptor protein called Fas-associated death domain (FADD) or indirectly via TNFR-associated death domain (TRADD). FADD also contains death effector domain which interacts with pro-caspase-8 to form a complex at the receptor called the death inducing signaling complex (DISC). The final step in this process is mobilization of caspase 8 to the DISC causing its activation and initiation of apoptosis [78].

Fas (CD95)

Fas and its ligand (FasL) play an important role in killing targets such as virus-infected cells or cancer cells by NK cells. NK cells express FasL on their surface to suppress tumor growth [79]. In some cases, tumor cells do not express Fas, but NK cells have the ability to induce Fas expression on these cells via IFN-γ secretion [80]. Binding of Fas with FasL promotes receptor clustering, DISC formation and activation of caspase 8 by auto-proteolysis process [81]. Accordingly, caspase 8 converts procaspase 3 to its active form (caspase 3) which initiates apoptosis process via DNA cleavage [81]. Further, caspase 8 can also hydrolyze BID, which destroys the mitochondrial membrane and triggers cytochrome c release [81].

Trail

TRAIL, also known as Apo2 ligand, is a member of TNF superfamily which is capable to induce NK cell-mediated apoptosis for many tumors and transformed cells. Five receptors have been recognized for TRAIL in humans where two of them, TRAIL-R1 (death receptor DR4) and TRAIL-R2 (DR5), are able to initiate an apoptotic signal [82]. Ligation of TRAIL to its receptor (DR4 and DR5) results in engagement of FADD proteins in their cytoplasmic domain, and then formation of DISC. As a result, caspase-8 is activated which is able to trigger the apoptosis process as mentioned above in Fas.

Antibody-Dependent Cell-Mediated Cytotoxicity

Antibody-dependent cell-mediated cytotoxicity (ADCC), also called antibody-dependent cellular cytotoxicity, is an immune mechanism whereby the effector cells bearing Fc receptors can recognize and lyse antibody-coated target cells (opsonized cells). Activating low affinity FcγRIIIA receptor (CD16A) is highly expressed on the cytotoxic CD56dim CD16+ NK cell subset, and mediates ADCC by binding to the Fc portion of IgG antibody. After recognition of their targets, CD56dim CD16+ NK cells interact with the Fc region of IgG antibody coating the target cell which is followed by phosphorylation of ITAMs via cellular src kinases to activate the signaling pathways in NK cells. As a result, cytotoxic granules secrete their contents as perforin and granzymes, causing lysis of target cells [83,84].

NK Cell Cytokines Secretion

Beside their cytotoxicity, NK cells also have important regulatory function mediated by cytokines and chemokines secretion following their stimulation [85,86]. Moreover, NK cells participate in a complex interaction network with other lymphocytes, dendritic cells, and macrophages to effectively control immune responses [87]. For instance, NK cells are considered as a major producer of IFN-γ in response to their stimulation, which has immune regulatory activity as well as direct effector activity [88,89]. Activated NK cells also secrete TNF-α which has both direct cytotoxic activity by triggering caspase-8-mediated apoptosis and immune regulatory activity by inducing dendritic cells maturation [90,91]. Additionally, NK cells have the ability to secrete other factors as GM-CSF, immunoregulatory cytokines (IL-5, IL-10, IL-13), and chemokines (MIP-1α, MIP-1β, IL-8, and RANTES) allowing NK cells to attract and co-localize with other immune cells at inflammation sites.

NK Cells and Cancer

NK cells play a major role in the immunosurveillance of malignancy (hematological or solid tumors) by direct killing of malignant cells and/or releasing a number of cytokines that regulate both innate and adaptive immune responses. The cytotoxic activity of NK cell against tumors have been firstly confirmed in animal models, where transplanted hematopoietic tumors or chemically-induced tumors were rejected in various mice models due to NK activity. In contrast, eradicating NK cells in such models often led to an aggressive tumor growth and metastasis [92].

In humans, several studies have shown a relationship between low levels of NK cell activity and the increase in the risk of cancer. For instance, a prospective epidemiological follow-up study has performed in Japan for 11 years and included 3625 individuals showed that a low NK cell cytotoxicity is associated with an increased risk of cancer occurrence [93]. In addition, the infiltration of NK cells into tumors is associated with good prognosis in patients with colorectal carcinoma, gastric carcinoma and lung cancer. Many clinical trials have been used NK cells as adoptive immunotherapy based on the alloreactivity of donor's NK cells to treat the hematological malignancies and solid tumors [94-96]. More recently, a variety of ex vivo expansion and activation methods have been used to increase both number and function of the infused NK cells to improve their antitumor activity [97-99]. However, tumor cells can develop various strategies to escape immunosurveillance of NK cell and other effector cells, which can be explained by general mechanisms such as saturation of the immune system by rapid growth of the tumor as well as alterations of NK cells and other immune cells leading to dysfunction of the immune system [100-107].

Conclusion

NK cells are distinct lymphocytes playing a prominent role in the innate immune responses against different types of cancer and virus-infected cells. Their effector functions are controlled by activating and inhibitory signals of a wide group of inhibitory and activating receptors expressed on their surface. Phenotypically, NK cells are categorized into two subsets: CD56bright and CD56dim NK cells based on the expression of CD56 molecules, where each subset has characteristic maturation profile and functions. In order to be able to recognize their targets, NK cells must be educated by host MHC class I molecules, and primed via specific cytokines. After that, NK cells can be activated by their target cells as well as signals from other immune cells. Consequently, the target cells are destroyed via the activated NK cells in a process called apoptosis occurred though different mechanisms. Based on their ability to sense the altered expression of MHC molecules and stress markers through various receptors, tumor cells become reachable targets for NK cell lysis due to down-regulation of MHC expression as well as enhancement of stress ligands expression. Many attempts and clinical trials have been previously and still performed to utilize NK cells as immunotherapy to treat solid and hematopoietic tumors (https://clinicaltrials.gov).

Declarations of Interest

None

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