

## The regulation of NK cell function and development

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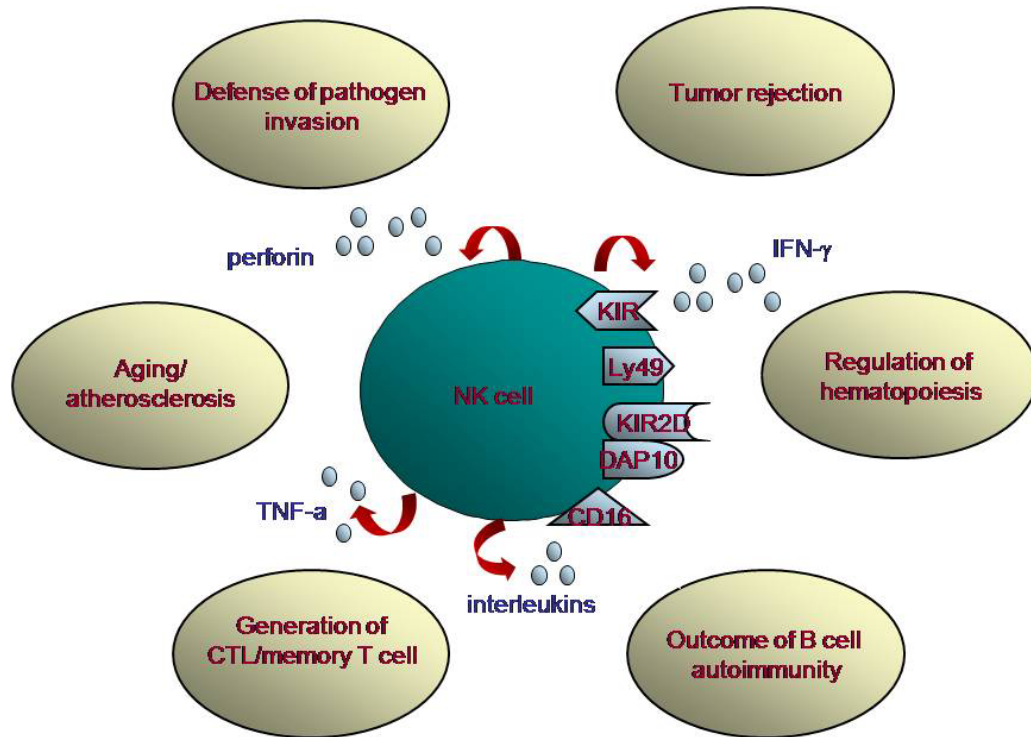
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## 1. ABSTRACT

NK cells are the lymphocytes that are differentiated from hematopoietic stem cells in bone marrow (BM) through sequential acquisition of functional receptors. They are one of the critical components of innate immune system. The process of NK differentiation involves a variety of factors such as cytokines, membrane factors, and transcription factors in addition to BM microenvironment. NK cells express their own activating and inhibitory receptors. The cytolytic functions of NK cells against target cells are determined by the balance between these activating and inhibitory receptors. NK cells discriminate self from non-self by MHC class I-binding inhibitory receptor. Once NK cell inhibitory receptors bound to their MHC class I-ligand, the target cells are protected from NK cell-mediated cytotoxicity. The cytolytic effector functions as well as the characteristic surface phenotypes of NK cells are acquired during the differentiation process. NK cells are emerging to apply as therapeutic agents against a variety of cancers by manipulating differentiation processes and intrinsic activities of the NK cell.

## 2. INTRODUCTION

Natural killer (NK) cells are large granular lymphocyte that are developed from hematopoietic stem cell (HSC) in the bone marrow (BM) (1). NK cells directly destroy pathogen-infected or transformed cells, thus mediating critical functions in innate immunity. They recognize the target cells that are lack of major histocompatibility complex (MHC) class I molecule, and selectively kill the target cells(2-4). This specific elimination is achieved by the common inhibitory CD94-NKG2A complexes and Ly49 family or killer cell immunoglobulin-like receptor (KIR), which are the species-specific inhibitory receptors in mice and humans, respectively. In addition, NK cells express an activating receptor FcR molecule that binds the Fc portion of antibodies, leading to the lysis of the target cells through antibody-dependent cellular cytotoxicity (ADCC). When stimulated, NK cells release perforin and the granzymes. Perforin forms pores in the membrane of the target cells upon release in close proximity to a cell, and the granzymes enter the target cells through the pores, inducing the apoptosis of the cells. In addition, several TNF family



**Figure 1.** Functions of NK cells. NK cells are involved in innate immunity by performing defense against pathogen invasion and tumor rejection. They release several proteins such as perforin, granzymes, and several cytokines, that induce the apoptosis of the target cells. These cytokines are also involved in regulation of other cellular phenomena such as hemtopoiesis and aging. In addition, they stimulate the generation or activities of other lymphocytes such as T or B cells, thus indirectly regulating adaptive immunity.

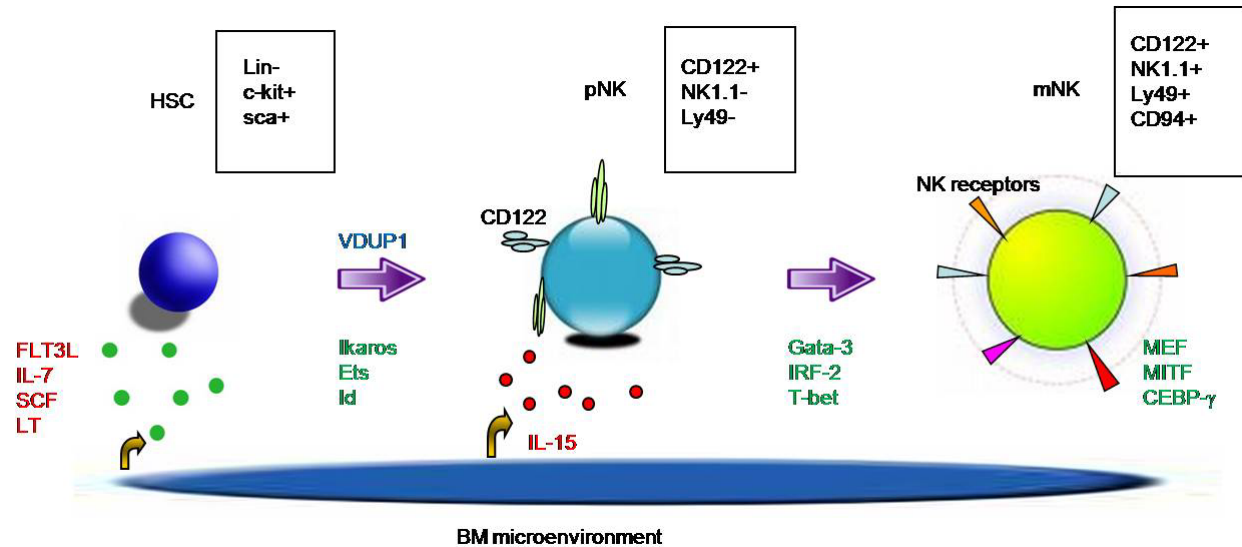
ligands and a variety of cytokines including interferon- $\gamma$  (IFN- $\gamma$ ), IL-1 beta, IL-2, IL-3, IL-4, IL-5, and IL-6(5) also induce the apoptosis of the target cells(6). These proteins from NK cells affect other lymphocytes such as T or B cells, thus indirectly regulating adaptive immunity as well as innate immunity by performing defense of pathogen invasion or tumor rejection (Figure 1). Several clinical consequences such as cancer or infection can be modulated by modifying immune cell activity. In fact, NK cells in the cancer patients generally exhibit abnormal development and functionality. Although NK cells play crucial roles in innate immunity, the differentiation mechanisms of NK cells are relatively not-well understood compared to those of B or T cells. Here, we review the critical factors that regulate NK development and activity. These factors may be the good targets that can be modulated to generate better-behaving NK cells for clinical applications. The clinical application of NK cell to patients suffering from malignant diseases such as leukemia and solid tumors is an approach of current interest in the field of immunotherapy.

## 3. NK DEVELOPMENT

### 3.1. Developmental stages in NK differentiation

Although NK precursors (pNKs) are also found in the thymus, spleen, and lymph node, NK cells primarily develop from HSC in BM through sequential acquisition of specific receptors including NK1.1, DX5, and Ly49 in

mice, and CD161, CD56, CD16, and KIRs in humans(7, 8). Thus, determination of NK differentiation status depends on the surface markers of NK cells. However, the detailed processes of NK maturation from HSC have not been well established. In general, NK differentiation from HSC occurs in two-stages, defined by their surface markers. The first stage is primarily dependent on stromal cell growth factors such as stem cell factor (SCF), FMS-like tyrosine kinase ligand (Flt3L), and IL-7, that induce differentiation of HSC into NK precursor (pNK). pNKs are characterized by the phenotype of lineage-negative and the presence of CD122, a common  $\beta$  subunit of the IL-2/IL-15 receptor ( $\text{lin}^-\text{CD122}^+$ ) in mice, and  $\text{CD34}^{\text{bright}}\text{CD122}^+\text{CD56}^-$  in humans. For the second stage, IL-15 delivers critical signals for maturation of NK cells through the receptor CD122. Since then, they express NK1.1, DX5, and other functional receptors including CD94-NKG2 and Ly49(9) in mice. Fully matured NK cells are characterized by high expression of CD11 $\beta$  and CD43. Also for human NK cells, pNK acquire maturation signal from IL-15 via CD122, and express CD56, CD16, and KIRs to become mature NK cells. Although regulatory factors in NK differentiation are not clearly understood, it is well-known that cell-cell interaction is a critical factor that renders pNK sensitive to the microenvironmental factors. The BM microenvironment is a major source of cytokines that initiates and facilitates NK differentiation. Within BM, stromal cells assist full maturation of NK cells(10, 11).



**Figure 2.** Critical factors and developmental pathway of NK cells in Mice. Several factors from microenvironment such as Flt3-ligand, IL-7, SCF and LT induce differentiation of HSC into pNK in BM. These factors generally induce the expression of IL-15 receptor, CD122. In this stage, cytosolic factor VDUP1 and transcription factors including Ikaros, Ets, and Id families are involved. Via CD122, IL-15 regulates NK commitment, and the expression of functional receptors in NK cells via regulation of TFs such as of Gata-3, IRF-2 and T-bet. Once committed to NK cells, TFs including MEF, MITF and CEBP-g regulate the activity of NK cells via induction of their surface expression of various receptors. Surface markers for each stage of NK development are labeled in boxes. Soluble, cytosolic, and transcription factors are written in red, blue, and green, respectively.

Thus, the BM stroma itself is one of the critical factors for differentiation of NK cell. However, several early-acting cytokines such as SCF, Flt3L, and IL-7 can replace the stromal cell requirements *in vitro* (9, 12) (Figure 2). These cytokines induce the expression of CD122 through their receptors, thus leading to the higher responsiveness to IL-15. Recently, Vitamin D3 upregulated protein 1 (VDUP-1), a stress-response gene, was reported to regulate NK differentiation by control of CD122 expression and IL-15 responsiveness(13). During NK maturation, VDUP1 seems to regulate CD122 expression in a transcription level. In addition, VDUP1 may be involved in the redox regulation during NK cell development. In fact, levels of intracellular ROS were reduced in VDUP1-deficient mice(13). NK cells are susceptible to ROS during development, because its presence produces mature NK cells that are defective in cytolytic activity (14). It has been suggested that dysregulation of intracellular ROS might affect NK differentiation by interfering with IL-2/CD122 expression. Interactions between lymphotoxin(LT) $\alpha_1\beta_1$  from HSC and LT $\beta$ receptor (LT $\beta$ R) in stromal cells are also known to participate in the generation of pNK(15, 16). In addition, interaction between LT $\alpha_1\beta_2$  on lymphoid progenitor cells and LT $\beta$ R in stromal cells has been known as a critical factor in the later stage of NK maturation(17), although it has been revealed that LT-LT $\beta$ R interaction is not essential for the formation of Ly49 and CD94/NKG2 repertoire (18). pNKs express LT $\alpha_1\beta_2$ , and activate stromal cells via LT $\beta$ R, which in turn induce IL-15R expression on pNKs. Presentation of IL-15 is sufficient to drive subsequent maturation processes such as the expression of

NK1.1(19). For example, culture of LT $\alpha$ <sup>-/-</sup> BM cells with exogenous IL-15 without stromal cells generates comparable numbers of NK cells as cultured WT BM cells *in vitro*, indicating that IL-15 itself can overcome the defect in NK differentiation of LT $\alpha$ <sup>-/-</sup>. IL-15 has been known to be indispensable for NK development(20, 21), generation of pNK from HSC is an IL-15-independent mechanism(22). After commitment to the NK lineage by signals from stromal factors, pNKs begin serial acquisition of the phenotypic and functional characteristics of mature NK cells by IL-15 stimulation, as determined by their specific surface receptors. Interestingly, although IL-2 can also augment the acquisition of CD19 and KIRs on pNKs or immature NK cells in humans(23), IL-2 seems to be dispensable for the development of NK cells in mice as IL-2 deficient mice exhibit normal development of NK cells(24).

### 3.2. Transcription factors regulating NK differentiation

In addition to the cytokines described above, several transcription factors are involved in NK differentiation, and each transcription factor (TF) regulates the developmental processes in a stage-specific manner (Figure 2). Ets family members such as PU.1 and Ets-1, and Ikaros family such as Ikaros, Helios, and Aiolos(25, 26) belong to the early-acting TF in the NK differentiation that are involved in the lineage-commitment or maintenance of pNKs by regulation of key cytokine receptors. For example, PU.1 directly induces the expression of the receptor for IL-7(27). Since IL-7 is a cytokine that induces the generation of pNK, PU.1-deficient mice show defects in NK cell development(28).

## NK cell functions and development

**Table 1.** NK receptors and their ligands

Type	Receptors	Ligand(s)	Function	Species
C-type lectin receptor	CD94/NKG2C	HLA-E/Q <sup>a</sup> -1 <sup>b</sup>	Activation	H, M
	CD94/NKG2A	HLA-E/Q <sup>a</sup> -1 <sup>b</sup>	Inhibition	H, M
	NKG2D	MICA/B, ULBPs	Activation	H, M
	NKG2E	HLA-E/Q <sup>a</sup> -1 <sup>b</sup>	Activation	H, M
	Ly49C	H-2D/H-2K	Activation	M
	Ly49D	H-2D	Activation	M
	Ly49H	MCMC m157	Inhibition	M
	Ly49A	H-2D	Inhibition	M
	Ly49E	Unknown	Inhibition	M
	Ly49G	H-2D		M
Killer Ig receptors	KIR2DS1, KIR2DS2	HLA-C	Activation	H
	KIR2DL4	HLA-C	Activation	H
	KIR2DL1	HLA-G	Inhibition	H
	KIR3DL1	HLA-Cw4	Inhibition	H
	KIR3DL2	HLA-Bw4		H
		HLA-A3 and A11	Activation	H
Natural cytotoxicity receptor	NKp30		Activation	H
	NKp44	Unknown	Activation	H
	NKp46	Unknown		H
		Unknown		H, M

Abbreviations: MIC, MHC class I chain related antigens; ULBPs, UL16 binding protein; KIR, killer immunoglobulin like receptor; HLA, human leukocyte antigen; H, human; M; Mouse.

pNKs without Ikaros are also shown to fail to express CD122, making themselves not being able to respond to IL-15, thus having defect in subsequent NK maturation(29). In addition to these TF families, the inhibitors of DNA binding (Id proteins) and the basic HLH E-box TFs, including E2A, E2-2, and HEB also appear to regulate cell fate decisions in the early stage of NK development. The E-proteins are highly expressed in the BM, spleen, and thymus, playing a crucial role in lineage commitment(30, 31). Id proteins regulate the activities of these E-proteins through sequestration by binding to them(32). Thus, interaction between E-proteins and Id proteins is a critical event in the development of lymphocytes including NK cells.

The second group of TFs that regulates the final maturation of NK cells includes Gata-3, IRF-2, and T-bet. The absence of these TFs have shown the incomplete development of functional NK cells or immature phenotypes of NK cells(33-36). These TFs mainly regulate the expression of functional receptors in NK cells. Thus, NK cells still can be generated in the absence of these TFs, but having immature phenotypes. Interestingly, these TFs are known to affect not only the development but also the peripheral homeostasis or homing of NK cells. For example, T-bet-deficient mice display defects in the number of peripheral NK cells. T-bet overexpression restores normal peripheral NK numbers due to recovery of NK cell homeostasis within the periphery (33-36). In addition, IRF-2-deficient mice exhibit differential NK cell deficiency in the periphery and BM, due to premature death in the periphery in the absence of IRF-2(37). The absence of Gata-3 leads to the defect of homing of NK cells specifically to the liver, in addition to maturation and IFN- $\gamma$  production in NK cells(33). Thus, Gata-3 seems to be an essential factor not only in NK maturation but also in specific homing of NK cell to the liver, and specify distinct effector phenotypes in this lineage. NK cells from mice deficient with these TFs exhibit the similar phenotypes, thus it is likely that all these TFs may affect the

development of NK cells with a sequential cascade in a hierarchy despite that each TF seems to specifically regulate unique characteristics of NK cells(36, 37). The third group of TFs includes MEF, MITF, and C/EBP, which regulate the functional capacity of mature NK cells. Lack of these TFs does not directly affect the normal development of NK cells. Rather, the absence of the TFs gives rise to reduced cytolytic capacity and cytokine production of NK cells. Although the developmental pathways of NK cells are relatively unclear compared to those of other lymphocytes such as T or B cells, it is likely that NK cells acquire a characteristic surface phenotype as well as cytolytic effector functions during this differentiation process. The process of NK cell differentiation is a coordinated mechanism that involves many cytokines, surface receptors, and transcription factors. By the cooperation of these factors a variety of NK cells repertoires are generated, that express distinct activating and inhibitory receptors.

## 4. NK FUNCTIONS

### 4.1. NK cell receptor repertoire

NK cells express their own repertoire of cell surface receptors, and these receptors interact with MHC class I or class I-like molecules on target cells. The end result of such interactions is either activating or inhibiting the NK cell. Unlike B cells and T cells, NK cells do not have the capacity to rearrange genes encoding antigen recognition receptors.

NK cell receptors are divided into two structural types: killer cell immunoglobulin-like receptors (KIRs in human) and C-lectin-like receptors (Ly49 in mouse, CD94/NKG2 receptors in both species) (38, 39) (Table 1). Each receptor family contains members that either activate or inhibit NK cell activity. Upon binding of an MHC determinant, inhibitory receptors act through immunoreceptor tyrosine-based inhibition motifs (ITIMs), whereas activating receptors have immunoreceptor

tyrosine-based activation motifs (ITAMs) that contains the adaptor molecule DAP-12 in place of the ITIMs (40).

Both the activating and inhibitory receptors bind to MHC class I molecules or MHC class I-related molecules (non-classical MHC class I) on target cells, and the balance between these two opposing signals regulates the NK cell effector function (41). NK cell activating receptors include natural cytotoxicity receptors (NCRs) and NKG2D, and signaling through these receptors results in NK cytotoxicity and/or cytokine production. NCR and NKG2D mediate cytotoxicity against MHC class I-deficient or negative targets. Some NCRs, including NKp30, NKp44, and NKp46 are involved in NK cell-mediated lysis of tumor cells, mediated through their association with DAP12- or ITAM-containing FC $\gamma$  and/or CD3 $\zeta$  adaptor molecules (42, 43). The NKG2D homodimer also triggers activating signals, along with DAP10 or DAP12 transmembrane signaling adaptor molecules. Targets of NKG2D are generally MHC-class I polypeptide related sequence A/B (MICA and MICB) and UL16-binding proteins (ULBPs) (44, 45). In contrast, inhibitory receptors prevent NK cell activity directed against cells expressing self-MHC class I molecules, preventing auto-aggression of NK cells. When target cells fail to present self-MHC class I molecules, NK cells no longer receive inhibitory signal via these molecules, and they kill the target cells. This process is referred to as *missing-self recognition* (46). Therefore, early exposure to host MHC class I molecules is what mediates development of NK tolerance. It has been shown that every NK cell expresses at least one self MHC-specific inhibitory receptor at both the clonal and polyclonal levels (47, 48). One recent study in mice found that some NK cells do not express self-MHC class I-specific inhibitory receptors, but these cells are not fully functional (49, 50). Likewise, NK cell function is also reduced in humans with a genetic deficiency in transporter-associated antigen processing (TAP), which renders them unable to present self-MHC class I molecules (51). The current model holds that NK cells lacking expression of a self-MHC class I-specific inhibitory receptor are not fully functional and hypo-responsive due to a lack of “licensing” by the appropriate MHC class I ligands. “Licensing” refers to a hypothesis that NK cells need to recognize MHC class I molecules during their development in order to acquire full effector function (50, 52).

The NK cell receptor repertoire refers to the group of receptors used by an individual or a mouse strain, as well as the combination of NK receptors expressed by individual NK cells. The number of *KIR* and *Ly49* genes varies between different individuals and mouse strains (53, 54), and *in vitro* differentiation experiments indicate that NK cells acquire Ly receptors and KIRs in a pre-determined order during development (55-59). Additionally, individual NK cells also express different combinations of KIR (or Ly49) and CD94/NKG2 receptors. Such combinatorial expression of NK receptors may involve the expression of CD94:NKG2A followed by KIRs late in NK cell development (60). The *CD94:NKG2DA* genes are relatively conserved and are constant in the self-tolerance mechanism, whereas the *KIRs* provide some level of variety in the receptor repertoire.

Certain KIRs and Ly49 receptors are specific for allelic determinants on classical MHC-class I molecules, while CD94-NKG2 receptors are specific for non-classical MHC molecules. In mouse, Ly49 recognizes the MHC class Ia molecules H-2D and/or H-2K (61, 62). Specifically, Ly49A, Ly 49G, and Ly49D recognize H-2D; Ly49C/I has a strong affinity for H-2Kb, but it can also bind H-2Kd to a lesser extent (63, 64). In contrast, mouse CD94-NKG2 receptors recognize non-classical MHC-Q<sup>a</sup>-1<sup>b</sup> (59). The human KIRs display a greater degree of complexity than mice Ly49 receptors. KIRs recognize varying groups of HLA-A, HLA-B, and HLA-C alleles, and CD94-NKG2A recognizes HLA-E (58, 65). KIR2DL2, KIR2DL3, KIR2DS2, and KIR2DS3 recognize HLA-C alleles, whereas KIR3DL1 recognizes HLA-Bw4 and KIR3DL2 recognizes HLA-A alleles. It therefore appears that HLA-C is the predominant class I isotype involved in inhibitory and activating regulation. KIRs are encoded by a family of genes on chromosome 19 that exhibits extensive haplotypic variation and allelic polymorphism (66, 67). Individuals differ in the number and type of inherited *KIR* genes and the *KIR* gene products that are clonally distributed in the NK cell receptor repertoire (68). Because of this individual specificity and distribution of KIRs, KIR-ligand mismatched NK cells from a donor have alloreactivity to recipient cells. Researchers are now making use of this property and applying NK cells to allogeneic stem cell transplantation.

It is clear that NK cells participate in the first line of defense against various diseases. As a part of the innate immune response, NK cells are able to kill virus-infected cells or transformed cells without previous priming. Tumor cells often lose or reduce expression of the MHC class I ligand for the NK cell inhibitory receptors, resulting in NK cell-mediated cytotoxicity (46). Researchers are beginning to utilize this function of NK cells as a mechanism for anti-cancer immunotherapy against a broad range of cancers. Several such strategies for NK-based therapies have been proposed and are discussed below (69).

## 4.2. Clinical application of NK cells

### 4.2.1. Active immunotherapy

Cancer patients frequently present with low-functioning NK cells, but exogenous NK activators could be used to enhance their endogenous NK response to tumors. Cytokines including IL-2, IL-12, IL-15, IL-18, IL-21, and the Type I IFNs can activate endogenous NK cells directly or indirectly (70-72). Administration of IL-2 activates and expands the NK cell population in some cancer patients (72, 73), but these trials have generated mixed results; success of this intervention apparently depends of the type of tumor being treated and/or is limited by severe toxic side effects. To address these concerns, combination therapy using IL-2 together with IL-12, IL-21 might be more useful therapeutically (74).

Broad-spectrum immunomodulatory drugs, such as thalidomide and its derivative lenalidomide (75), have also been used for myeloma patients. Thalidomide may increase NK cell activity during myeloma by stimulating T cells to produce IL-2 (75). Similarly, adjuvant intravesical

*Bacillus Calmette-Guérin* (BCG) therapy has been reported as a successful immunotherapy in the treatment of superficial bladder cancer (76). CpG oligodeoxynucleotides, a group of immunostimulatory DNA complexes, also enhanced antitumor activity in non-Hodgkin's lymphoma, partially through the activation of NK cells (77).

### 4.2.2. Adoptive immunotherapy

Adoptive immunotherapy refers to the introduction of *ex vivo* manipulated cells for clinical applications. Adoptive transfer of NK cells to cancer patients dates back to the 1980's, originating from the trials by Rosenberg *et al.* that utilized a combination of *ex vivo*-expanded autologous lymphokine-activated killer (LAK) cells and exogenous IL-2 as an anti-tumor therapy (78, 79). This trial induced only a 20% response, which included partial and complete responses; a similar anti-tumor effect was achieved with a high dose of IL-2 alone (80). In another trial, Miller *et al.* combined haploidentical NK-cell infusion with IL-2 administration to cancer patients in a non-transplantation setting (81). In general, donor NK cell infusions were well tolerated, with no induction of graft-versus-host disease (GVHD). Using this protocol, 5 out of 9 AML patients achieved complete remission.

The NK cell line NK-92 expresses several activating (but no inhibitory) KIRs and shows significant cytotoxicity to several tumor cell lines. Adoptive transfers of this cell line are currently being applied to cancer treatment (82); to date, infusion of NK-92 cells has proved to be safe and has generated antitumor effects in a few cases (83).

### 4.2.3. Allogenic stem-cell transplantation

Allogenic stem cell transplantation (SCT) from unrelated or sibling donors is a current established protocol for several hematological malignancies, such as AML and ALL (84, 85), but like any transplantation procedure, it can present a serious side effect: graft-versus-host disease (GVHD). Since T cell receptors are highly individualized and sensitive to non-self presentation, recipient antigen-presenting cells (APC), such as dendritic cells, may aberrantly activate donor-derived T cells. These activated T cells mediate the severe damage characteristic of GVHD. On the other hand, donor-derived T cells also recognize and kill residual tumor cells in the recipient, which is referred to as the graft-versus-tumor (GVT) or the graft-versus-leukemia (GVL) reaction. Current strategies to improve the outcome of allogenic hematopoietic SCT in cancer therapy involve minimizing harmful GVHD reactions while enhancing beneficial GVT reactions.

Since NK cells have a less diverse receptor repertoire than T cells, they can kill normal missing-self targets and still respond to target cells expressing foreign MHC class I alleles. For this reason, contrary to traditional transplantation strategies, NK alloreactivity can be beneficially exploited in order to direct donor NK cells to recipient leukemic cells. A prerequisite for NK cell alloreactivity is that the recipient's MHC class I allele must not match the KIR of donor-derived NK cells; i.e., the recipient must lack one or more KIR ligands present in the

donor. Veladi *et al.* have shown that infusion of allogenic KIR-ligand mismatched NK cells improved the survival rate and reduced the relapse rate for haploidentical SCT in AML cases (85, 86). Other studies have confirmed a beneficial effect of a KIR-ligand mismatch on the relapse rate and the survival of the recipient (87, 88).

### 4.2.4. NK-cell based donor lymphocyte infusion

As discussed above, unlike T cells, NK cells mediate GVT effects without implication of GVHD. GVT reactions can be mediated by donor-derived NK cells, as well as NK cells developing from donor hematopoietic stem cells (85, 89, 90). The decrease in GVHD was presumably mediated by donor NK cell alloreactivity against the cells ordinarily responsible for GVHD; namely, recipient APCs such as dendritic cells. Furthermore, allo-NK cells can also kill recipient T cells and improve engraftment (Figure 3).

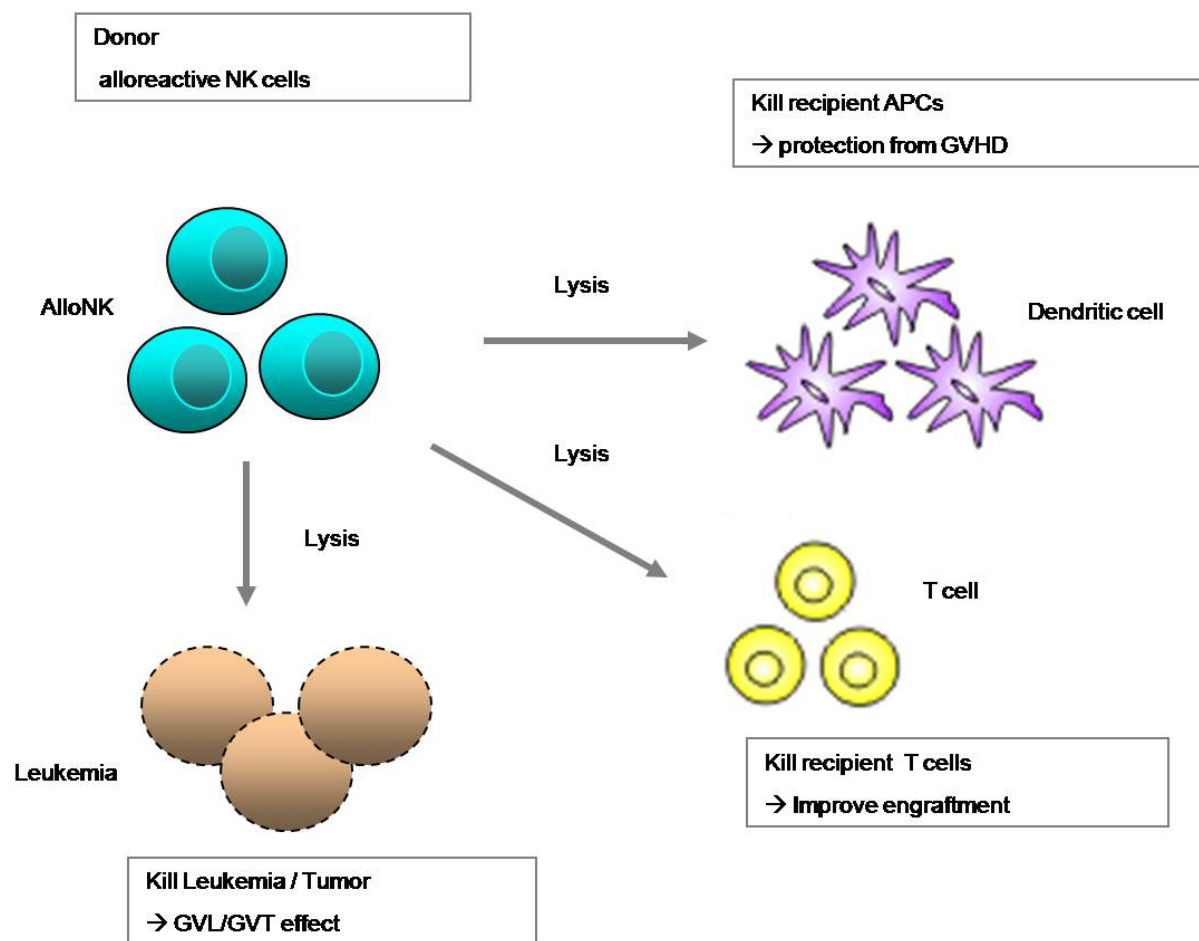
Unfortunately, many patients relapse after hematopoietic SCT. Donor lymphocyte infusion (DLI) can induce potent GVT effect in some patients. DLI, however, may cause GVHD induced by T cells, which can be a severe complication leading to death. To minimize the risk of GVHD, modified strategies have been developed such as T-cell depleted DLI. Introduction of T-cell depleted donor lymphocytes containing alloreactive NK cells has shown to improve the survival of patients by enhancing GVL and reducing GVHD (86, 91). Purified donor NK cells have also been used in DLI to facilitate engraftment and induce GVT effects in haploidentical SCT (92). NK-cell infusions are relatively safe and generate long term remission in some patients with leukemia relapse. NK-cell based DLI could be applied to treat patients with tumor relapse after haploidentical SCT or patients for conditioning regimen prior to SCT.

### 4.2.5. Other clinical strategies

Several other strategies have been approached to develop NK cell-mediated immunotherapy. Some tactics take advantage of NK receptor biology to selectively block or activate NK receptors to modulate immunity (93). Blocking CD94/NKG2A or inhibitory KIRs might reduce the NK inhibitory signal (94). This strategy is relevant to allogenic NK activity in KIR-HLA mismatched stem cell transplantation (85). Upregulating the expression of activating receptors (e.g., NKG2D) on NK cells, or their ligands (ULBPs and MICA/B) on tumor cells could mediate an increase in the NK activating signal (95). In support of this idea, histone deacetylase inhibitors act as anticancer agents by increasing levels of MICA and MICB, the ligands for NKG2D, rendering tumor cells more susceptible to NK-cell mediated lysis (96).

Since NK cells also mediate ADCC through the CD16 receptor (Fc gamma receptor) on their surface, administration of an antibody against CD20 (rituximab) has been used to target NK cells to B-cell lymphomas (97). CD20 is expressed on mature B cells, but not on immature B cells, and approximately 93% of B cell lymphoma patients express CD20 on their tumor cells (98). The Fc domain of rituximab induces ADCC of B cells through





**Figure 3.** Natural killer cells in allogeneic stem cell transplantation. Allogeneic NK cells from donor can directly kill recipient T cells as well as inhibit T cell mediated GVHD through killing of recipient antigen presenting cells such as dendritic cells that may initiate GVHD. Furthermore, NK cells can provide anti-tumor effects by killing residual cancers in the recipient.

activation of the Fc gamma receptor on the surface of NK cells (99). In a related approach, bispecific antibodies promote NK cell recognition and cytolysis of tumor cells; antibodies specific for CD16 on NK cells and for CD30 on non-Hodgkin's lymphoma mediate such a response (100). Exploitation of heat shock protein 70 (Hsp70) has also been used to direct NK cells against tumors. Hsp70 is highly expressed in various types of tumors, and these Hsp70-expressing tumors can be clinically targeted using autologous NK cells that have been pre-stimulated with Hsp70 peptide (101, 102).

## 5. PERSPECTIVES

Although some aspects of the molecular NK differentiation mechanisms and regulation of NK activity still remain a mystery, interest in unraveling these mechanisms for therapeutic purposes has greatly increased in recent years. The process of NK cell differentiation involves many soluble factors, transcription factors, and elements of the BM microenvironment. These factors cooperate during NK development to generate diversity of

the receptor repertoires. Despite their obvious immunotherapeutic potential, clinical trials in cancer patients treated with activated NK cells have yielded only a limited number of successful reports. In order to develop successful NK cell-based immunotherapy in the future, we must keep several considerations in mind, including necessary criteria for donor selection, conditioning of recipients, and identification of tumor susceptibilities of NK cells. Further elucidation of how these factors affect the success of clinical NK cell therapy should be coupled to studies that examine the modulation of inhibitory and activating receptors on NK cells. As we gain understanding of the NK developmental processes and accumulate knowledge of how these processes regulate expression of cell surface receptors responsible for NK cell activity, NK cells will continue to emerge as effective immunotherapeutic agents for cancer treatment.

## 6. ACKNOWLEDGMENT

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**Abbreviation:** NK: natural killer; BM: bone marrow; pNK: precursor NK; KIR: killer cell immunoglobulin-like receptor; GVT: graft-versus-tumor; GVL: graft-versus-leukemia; GVHD: graft versus host disease

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