[Frontiers in Bioscience 13, 6432-6442, May 1, 2008]

# The regulation of NK cell function and development

**Jin Woong Chung[1], Suk Ran Yoon[1], Inpyo Choi[1]**

[1]*Stem cell Research Center, Korea Research Institute of Bioscience and Biotechnology, Yusong, Taejon 305-333, Republic of Korea*

## TABLE OF CONTENTS

*1. Abstract*
*2. Introduction*
*3. NK development*
*3.1 Developmental stages in NK differentiation*
*3.2 Transcription factors regulating NK differentiation*
*4. NK functions*
*4.1 NK receptor repertoire*
*4.2 Clinical applications of NK cells*
*4.2.1. Active immunotherapy*
*4.2.2. Adoptive immunotherapy*
*4.2.3. Allogenic stem-cell transplantation*
*4.2.4. Donor NK cell infusion*
*4.2.5. Other clinical strategies*
*5. Perspectives*
*6. Acknowledgement*
*7. References*

## 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 theses 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

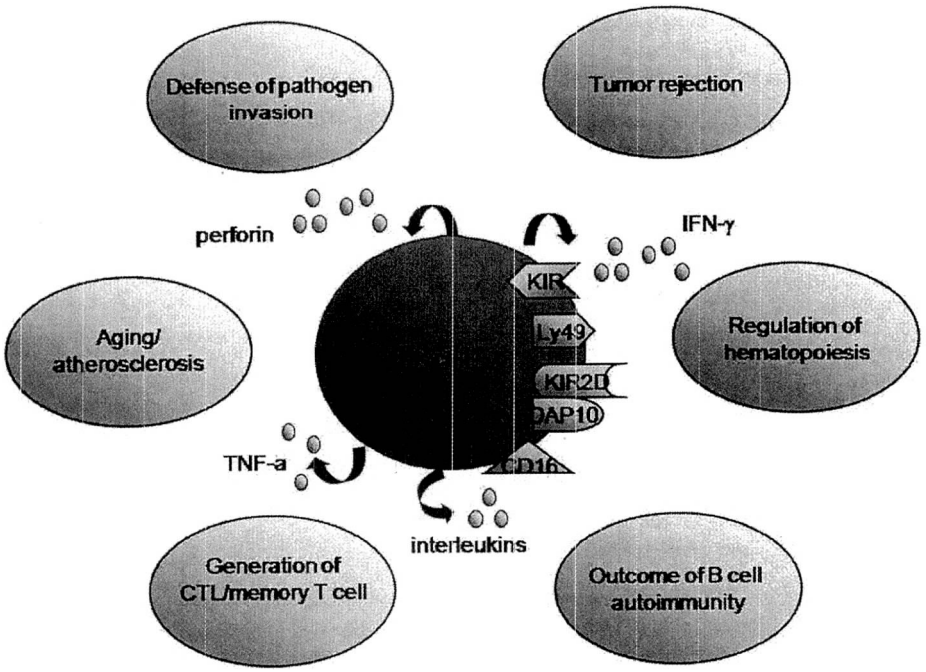


**Figure 1.** Functions of NK cells. NK cells are involved in innate immunity by performing defense against pathogen invasion and tumor rejection. They release several proteins such as perforin, granzymes, and several cytokines, that induce the apoptosis of the target cells. These cytokines are also 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-γ (IFN-γ), 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 β subunit of the IL-2/IL-15 receptor ($lin^{-}CD122^{+}$) in mice, and $CD34^{bright}CD122^{+}CD56^{-}$ 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 CD11β 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).

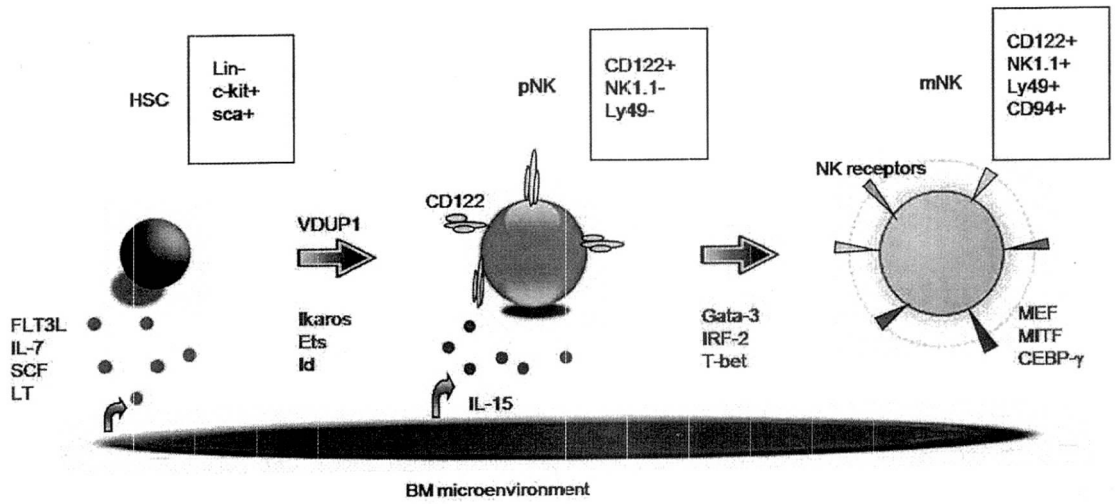


**Figure 2.** Critical factors and developmental pathway of NK cells in Mice. Several factors from microenvironment such as Flt3-ligand, IL-7, SCF and LT induce differentiation of HSC into pNK in BM. These factors generally induce the expression of IL-15 receptor, CD122. In this stage, cytosolic factor 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 LTβreceptor (LTβR) 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 LTβR 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-LTβR 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 LTβR, 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$\alpha^{-/-}$ 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$\alpha^{-/-}$ 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).

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

| Type | Receptors | Ligand(s) | Function | Species |
|---|---|---|---|---|
| C-type lectin receptor | CD94/NKG2C | HLA-E/$Q^a$-$1^b$ | Activation | H, M |
| | CD94/NKG2A | HLA-E/$Q^a$-$1^b$ | Inhibition | H, M |
| | NKG2D | MICA/B,ULBPs | Activation | H, M |
| | NKG2E | HLA-E/$Q^a$-$1^b$ | Activation 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 | | | Activation | |
| | KIR2DS1, KIR2DS2 | HLA-C | Activation Activation | H |
| | KIR2DL4 | HLA-C | Inhibition | 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 | |
| | 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-γ 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 FCγ and/or CD3ζ 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-$Q^a$-$1^b$ (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 allogenic 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 cytolysis (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-Guerin (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 GVDH 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

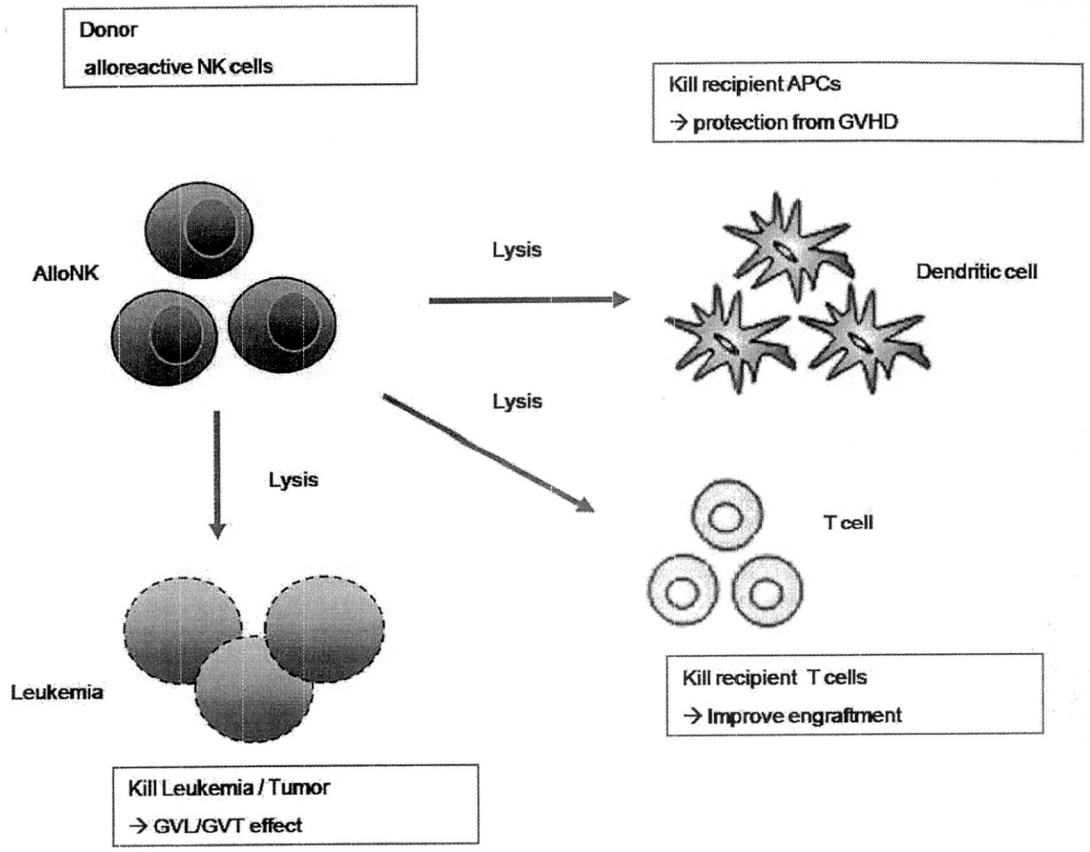


**Figure 3.** Natural killer cells in allogenic stem cell transplantation. Allogenic NK cells from donor can directly kill recipient T cells as well as inhibit T cell mediated GVHD through killing of recipient antigen presenting cells such as dendritic cells that may initiate GVHD. Furthermore, NK cells 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

Jin Woong Chung and Suk Ran Yoon contributed equally to this work. This work was partially

supported by a grant SC13040 of 21C Frontier Stem Cell Research Project, GRL project from the Ministry of Science and Technology, and Catholic Institute of Cell therapy Basic Science Programs Foundation, Republic of Korea.

## 7. REFERENCES

1. Freud, A. G. & M. A. Caligiuri: Human natural killer cell development. Immunol Rev, 214, 56-72 (2006)
2. Petersson, M. G., A. Gronberg, R. Kiessling & M. T. Ferm: Engagement of MHC class I proteins on natural killer cells inhibits their killing capacity. Scand J Immunol, 42, 34-8 (1995)
3. Ljunggren, H. G. & K. Karre: Host resistance directed selectively against H-2-deficient lymphoma variants. Analysis of the mechanism. J Exp Med, 162, 1745-59 (1985)
4. Ljunggren, H. G., C. Ohlen, P. Hoglund, T. Yamasaki, G. Klein & K. Karre: Afferent and efferent cellular interactions in natural resistance directed against MHC class I deficient tumor grafts. J Immunol, 140, 671-8 (1988)
5. Saito, S., K. Nishikawa, T. Morii, M. Enomoto, N. Narita, K. Motoyoshi & M. Ichijo: Cytokine production by CD16-CD56bright natural killer cells in the human early pregnancy decidua. Int Immunol, 5, 559-63 (1993)
6. Maki, G., H. G. Klingemann, J. A. Martinson & Y. K. Tam: Factors regulating the cytotoxic activity of the human natural killer cell line, NK-92. J Hematother Stem Cell Res, 10, 369-83 (2001)
7. Rosmaraki, E. E., I. Douagi, C. Roth, F. Colucci, A. Cumano & J. P. Di Santo: Identification of committed NK cell progenitors in adult murine bone marrow. Eur J Immunol, 31, 1900-9 (2001)
8. Ikawa, T., S. Fujimoto, H. Kawamoto, Y. Katsura & Y. Yokota: Commitment to natural killer cells requires the helix-loop-helix inhibitor Id2. Proc Natl Acad Sci U S A, 98, 5164-9 (2001)
9. Williams, N. S., J. Klem, I. J. Puzanov, P. V. Sivakumar, M. Bennett & V. Kumar: Differentiation of NK1.1+, Ly49+ NK cells from flt3+ multipotent marrow progenitor cells. J Immunol, 163, 2648-56 (1999)
10. van den Brink, M. R., S. S. Boggs, R. B. Herberman & J. C. Hiserodt: The generation of natural killer (NK) cells from NK precursor cells in rat long-term bone marrow cultures. J Exp Med, 172, 303-13 (1990)
11. Ogasawara, K., S. Hida, N. Azimi, Y. Tagaya, T. Sato, T. Yokochi-Fukuda, T. A. Waldmann, T. Taniguchi & S. Taki: Requirement for IRF-1 in the microenvironment supporting development of natural killer cells. Nature, 391, 700-3 (1998)
12. Mrozek, E., P. Anderson & M. A. Caligiuri: Role of interleukin-15 in the development of human CD56+ natural killer cells from CD34+ hematopoietic progenitor cells. Blood, 87, 2632-40 (1996)
13. Lee, K. N., H. S. Kang, J. H. Jeon, E. M. Kim, S. R. Yoon, H. Song, C. Y. Lyu, Z. H. Piao, S. U. Kim, Y. H. Han, S. S. Song, Y. H. Lee, K. S. Song, Y. M. Kim, D. Y. Yu & I. Choi: VDUP1 is required for the development of natural killer cells. Immunity, 22, 195-208 (2005)
14. Nakamura, K. & K. Matsunaga: Susceptibility of natural killer (NK) cells to reactive oxygen species (ROS) and their restoration by the mimics of superoxide dismutase (SOD). Cancer Biother Radiopharm, 13, 275-90 (1998)
15. Iizuka, K., D. D. Chaplin, Y. Wang, Q. Wu, L. E. Pegg, W. M. Yokoyama & Y. X. Fu: Requirement for membrane lymphotoxin in natural killer cell development. Proc Natl Acad Sci U S A, 96, 6336-40 (1999)
16. Lian, R. H., R. K. Chin, H. E. Nemeth, S. L. Libby, Y. X. Fu & V. Kumar: A role for lymphotoxin in the acquisition of Ly49 receptors during NK cell development. Eur J Immunol, 34, 2699-707 (2004)
17. Kather, A., S. Chantakru, H. He, K. Minhas, R. Foster, U. R. Markert, K. Pfeffer & B. A. Croy: Neither lymphotoxin alpha nor lymphotoxin beta receptor expression is required for biogenesis of lymphoid aggregates or differentiation of natural killer cells in the pregnant mouse uterus. Immunology, 108, 338-45 (2003)
18. Stevenaert, F., K. Van Beneden, V. De Colvenaer, A. S. Franki, V. Debacker, T. Boterberg, D. Deforce, K. Pfeffer, J. Plum, D. Elewaut & G. Leclercq: Ly49 and CD94/NKG2 receptor acquisition by NK cells does not require lymphotoxin-beta receptor expression. Blood, 106, 956-62 (2005)
19. Wu, Q., Y. Sun, J. Wang, X. Lin, Y. Wang, L. E. Pegg, A. Futterer, K. Pfeffer & Y. X. Fu: Signal via lymphotoxin-beta R on bone marrow stromal cells is required for an early checkpoint of NK cell development. J Immunol, 166, 1684-9 (2001)
20. Carson, W. E., J. G. Giri, M. J. Lindemann, M. L. Linett, M. Ahdieh, R. Paxton, D. Anderson, J. Eisenmann, K. Grabstein & M. A. Caligiuri: Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. J Exp Med, 180, 1395-403 (1994)
21. Budagian, V., E. Bulanova, R. Paus & S. Bulfone-Paus: IL-15/IL-15 receptor biology: a guided tour through an expanding universe. Cytokine Growth Factor Rev, 17, 259-80 (2006)
22. Vosshenrich, C. A., T. Ranson, S. I. Samson, E. Corcuff, F. Colucci, E. E. Rosmaraki & J. P. Di Santo: Roles for common cytokine receptor gamma-chain-dependent cytokines in the generation, differentiation, and maturation of NK cell precursors and peripheral NK cells *in vivo*. J Immunol, 174, 1213-21 (2005)
23. Ferlazzo, G., D. Thomas, S. L. Lin, K. Goodman, B. Morandi, W. A. Muller, A. Moretta & C. Munz: The abundant NK cells in human secondary lymphoid tissues require activation to express killer cell Ig-like receptors and become cytolytic. J Immunol, 172, 1455-62 (2004)
24. Klebb, G., I. B. Autenrieth, H. Haber, E. Gillert, B. Sadlack, K. A. Smith & I. Horak: Interleukin-2 is indispensable for development of immunological self-tolerance. Clin Immunol Immunopathol, 81, 282-6 (1996)
25. Warren, L. A. & E. V. Rothenberg: Regulatory coding of lymphoid lineage choice by hematopoietic transcription factors. Curr Opin Immunol, 15, 166-75 (2003)
26. Singh, H., K. L. Medina & J. M. Pongubala: Contingent gene regulatory networks and B cell fate specification. Proc Natl Acad Sci U S A, 102, 4949-53 (2005)
27. DeKoter, R. P., H. J. Lee & H. Singh: PU.1 regulates expression of the interleukin-7 receptor in lymphoid progenitors. Immunity, 16, 297-309 (2002)

28. Colucci, F., S. I. Samson, R. P. DeKoter, O. Lantz, H. Singh & J. P. Di Santo: Differential requirement for the transcription factor PU.1 in the generation of natural killer cells versus B and T cells. Blood, 97, 2625-32 (2001)
29. Boggs, S. S., M. Trevisan, K. Patrene & K. Geogopoulos: Lack of natural killer cell precursors in fetal liver of Ikaros knockout mutant mice. Nat Immun, 16, 137-45 (1998)
30. Kee, B. L. & C. Murre: Induction of early B cell factor (EBF) and multiple B lineage genes by the basic helix-loop-helix transcription factor E12. J Exp Med, 188, 699-713 (1998)
31. Zhuang, Y., P. Soriano & H. Weintraub: The helix-loop-helix gene E2A is required for B cell formation. Cell, 79, 875-84 (1994)
32. Spits, H., F. Couwenberg, A. Q. Bakker, K. Weijer & C. H. Uittenbogaart: Id2 and Id3 inhibit development of CD34 (+) stem cells into predendritic cell (pre-DC)2 but not into pre-DC1. Evidence for a lymphoid origin of pre-DC2. J Exp Med, 192, 1775-84 (2000)
33. Samson, S. I., O. Richard, M. Tavian, T. Ranson, C. A. Vosshenrich, F. Colucci, J. Buer, F. Grosveld, I. Godin & J. P. Di Santo: GATA-3 promotes maturation, IFN-gamma production, and liver-specific homing of NK cells. Immunity, 19, 701-11 (2003)
34. Lohoff, M., G. S. Duncan, D. Ferrick, H. W. Mittrucker, S. Bischof, S. Prechtl, M. Rollinghoff, E. Schmitt, A. Pahl & T. W. Mak: Deficiency in the transcription factor interferon regulatory factor (IRF)-2 leads to severely compromised development of natural killer and T helper type 1 cells. J Exp Med, 192, 325-36 (2000)
35. Lian, R. H. & V. Kumar: Murine natural killer cell progenitors and their requirements for development. Semin Immunol, 14, 453-60 (2002)
36. Townsend, M. J., A. S. Weinmann, J. L. Matsuda, R. Salomon, P. J. Farnham, C. A. Biron, L. Gapin & L. H. Glimcher: T-bet regulates the terminal maturation and homeostasis of NK and Valpha14i NKT cells. Immunity, 20, 477-94 (2004)
37. Taki, S., S. Nakajima, E. Ichikawa, T. Saito & S. Hida: IFN regulatory factor-2 deficiency revealed a novel checkpoint critical for the generation of peripheral NK cells. J Immunol, 174, 6005-12 (2005)
38. Colonna, M.: Immunoglobulin superfamily inhibitory receptors: from natural killer cells to antigen-presenting cells. Res Immunol, 148, 169-71 (1997)
39. Vance, R. E., J. R. Kraft, J. D. Altman, P. E. Jensen & D. H. Raulet: Mouse CD94/NKG2A is a natural killer cell receptor for the nonclassical major histocompatibility complex (MHC) class I molecule Qa-1 (b). J Exp Med, 188, 1841-8 (1998)
40. Lanier, L. L., B. C. Corliss, J. Wu, C. Leong & J. H. Phillips: Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. Nature, 391, 703-7 (1998)
41. Wagtmann, N., S. Rajagopalan, C. C. Winter, M. Peruzzi & E. O. Long: Killer cell inhibitory receptors specific for HLA-C and HLA-B identified by direct binding and by functional transfer. Immunity, 3, 801-9 (1995)
42. Moretta, A., C. Bottino, S. Sivori, E. Marcenaro, R. Castriconi, M. Della Chiesa, S. Carlomagno, R. Augugliaro, M. Nanni, M. Vitale & R. Millo: Natural killer lymphocytes: "null cells" no more. Ital J Anat Embryol, 106, 335-42 (2001)
43. Farag, S. S., T. A. Fehniger, L. Ruggeri, A. Velardi & M. A. Caligiuri: Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. Blood, 100, 1935-47 (2002)
44. Steinle, A., P. Li, D. L. Morris, V. Groh, L. L. Lanier, R. K. Strong & T. Spies: Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. Immunogenetics, 53, 279-87 (2001)
45. Cosman, D., J. Mullberg, C. L. Sutherland, W. Chin, R. Armitage, W. Fanslow, M. Kubin & N. J. Chalupny: ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. Immunity, 14, 123-33 (2001)
46. Ljunggren, H. G. & K. Karre: In search of the 'missing self': MHC molecules and NK cell recognition. Immunol Today, 11, 237-44 (1990)
47. Valiante, N. M., M. Uhrberg, H. G. Shilling, K. Lienert-Weidenbach, K. L. Arnett, A. D'Andrea, J. H. Phillips, L. L. Lanier & P. Parham: Functionally and structurally distinct NK cell receptor repertoires in the peripheral blood of two human donors. Immunity, 7, 739-51 (1997)
48. Draghi, M., N. Yawata, M. Gleimer, M. Yawata, N. M. Valiante & P. Parham: Single-cell analysis of the human NK cell response to missing self and its inhibition by HLA class I. Blood, 105, 2028-35 (2005)
49. Fernandez, N. C., E. Treiner, R. E. Vance, A. M. Jamieson, S. Lemieux & D. H. Raulet: A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. Blood, 105, 4416-23 (2005)
50. Kim, S., J. Poursine-Laurent, S. M. Truscott, L. Lybarger, Y. J. Song, L. Yang, A. R. French, J. B. Sunwoo, S. Lemieux, T. H. Hansen & W. M. Yokoyama: Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature, 436, 709-13 (2005)
51. Vitale, M., J. Zimmer, R. Castriconi, D. Hanau, L. Donato, C. Bottino, L. Moretta, H. de la Salle & A. Moretta: Analysis of natural killer cells in TAP2-deficient patients: expression of functional triggering receptors and evidence for the existence of inhibitory receptor (s) that prevent lysis of normal autologous cells. Blood, 99, 1723-9 (2002)
52. Yokoyama, W. M. & S. Kim: Licensing of natural killer cells by self-major histocompatibility complex class I. Immunol Rev, 214, 143-54 (2006)
53. Uhrberg, M., P. Parham & P. Wernet: Definition of gene content for nine common group B haplotypes of the Caucasoid population: KIR haplotypes contain between seven and eleven KIR genes. Immunogenetics, 54, 221-9 (2002)
54. Makrigiannis, A. P., A. T. Pau, P. L. Schwartzberg, D. W. McVicar, T. W. Beck & S. K. Anderson: A BAC contig map of the Ly49 gene cluster in 129 mice reveals extensive differences in gene content relative to C57BL/6 mice. Genomics, 79, 437-44 (2002)
55. Williams, N. S., T. A. Moore, J. D. Schatzle, I. J. Puzanov, P. V. Sivakumar, A. Zlotnik, M. Bennett & V. Kumar: Generation of lytic natural killer 1.1+, Ly-49- cells

from multipotential murine bone marrow progenitors in a stroma-free culture: definition of cytokine requirements and developmental intermediates. J Exp Med, 186, 1609-14 (1997)
56. Williams, N. S., A. Kubota, M. Bennett, V. Kumar & F. Takei: Clonal analysis of NK cell development from bone marrow progenitors *in vitro*: orderly acquisition of receptor gene expression. Eur J Immunol, 30, 2074-82 (2000)
57. Roth, C., J. R. Carlyle, H. Takizawa & D. H. Raulet: Clonal acquisition of inhibitory Ly49 receptors on developing NK cells is successively restricted and regulated by stromal class I MHC. Immunity, 13, 143-53 (2000)
58. Held, W., J. D. Coudert & J. Zimmer: The NK cell receptor repertoire: formation, adaptation and exploitation. Curr Opin Immunol, 15, 233-7 (2003)
59. Raulet, D. H., R. E. Vance & C. W. McMahon: Regulation of the natural killer cell receptor repertoire. Annu Rev Immunol, 19, 291-330 (2001)
60. Freud, A. G. & M. A. Caligiur: Human natural killer cell development. Immunological Reviews, 214, 56-72 (2006)
61. Yokoyama, W. M.: Natural killer cell receptors. Curr Opin Immunol, 7, 110-20 (1995)
62. Ryan, J. C. & W. E. Seaman: Divergent functions of lectin-like receptors on NK cells. Immunol Rev, 155, 79-89 (1997)
63. Raulet, D. H., W. Held, I. Correa, J. R. Dorfman, M. F. Wu & L. Corral: Specificity, tolerance and developmental regulation of natural killer cells defined by expression of class I-specific Ly49 receptors. Immunol Rev, 155, 41-52 (1997)
64. Dorfman, J. R. & D. H. Raulet: Acquisition of Ly49 receptor expression by developing natural killer cells. J Exp Med, 187, 609-18 (1998)
65. Moretta, L. & A. Moretta: Killer immunoglobulin-like receptors. Curr Opin Immunol, 16, 626-33 (2004)
66. Lanier, L. L.: Face off--the interplay between activating and inhibitory immune receptors. Curr Opin Immunol, 13, 326-31 (2001)
67. Uhrberg, M., N. M. Valiante, B. P. Shum, H. G. Shilling, K. Lienert-Weidenbach, B. Corliss, D. Tyan, L. L. Lanier & P. Parham: Human diversity in killer cell inhibitory receptor genes. Immunity, 7, 753-63 (1997)
68. Trowsdale, J., R. Barten, A. Haude, C. A. Stewart, S. Beck & M. J. Wilson: The genomic context of natural killer receptor extended gene families. Immunol Rev, 181, 20-38 (2001)
69. Ljunggren, H. G. & K. J. Malmberg: Prospects for the use of NK cells in immunotherapy of human cancer. Nat Rev Immunol, 7, 329-39 (2007)
70. Smyth, M. J., E. Cretney, M. H. Kershaw & Y. Hayakawa: Cytokines in cancer immunity and immunotherapy. Immunol Rev, 202, 275-93 (2004)
71. Becknell, B. & M. A. Caligiuri: Interleukin-2, interleukin-15, and their roles in human natural killer cells. Adv Immunol, 86, 209-39 (2005)
72. Farag, S. S. & M. A. Caligiuri: Cytokine modulation of the innate immune system in the treatment of leukemia and lymphoma. Adv Pharmacol, 51, 295-318 (2004)
73. Rosenberg, S. A.: Interleukin-2 and the development of immunotherapy for the treatment of patients with cancer. Cancer J Sci Am, 6 Suppl 1, S2-7 (2000)
74. Weiss, J. M., J. J. Subleski, J. M. Wigginton & R. H. Wiltrout: Immunotherapy of cancer by IL-12-based cytokine combinations. Expert Opin Biol Ther, 7, 1705-21 (2007)
75. Hayashi, T., T. Hideshima, M. Akiyama, K. Podar, H. Yasui, N. Raje, S. Kumar, D. Chauhan, S. P. Treon, P. Richardson & K. C. Anderson: Molecular mechanisms whereby immunomodulatory drugs activate natural killer cells: clinical application. Br J Haematol, 128, 192-203 (2005)
76. Brandau, S., J. Riemensberger, M. Jacobsen, D. Kemp, W. Zhao, X. Zhao, D. Jocham, T. L. Ratliff & A. Bohle: NK cells are essential for effective BCG immunotherapy. Int J Cancer, 92, 697-702 (2001)
77. Link, B. K., Z. K. Ballas, D. Weisdorf, J. E. Wooldridge, A. D. Bossler, M. Shannon, W. L. Rasmussen, A. M. Krieg & G. J. Weiner: Oligodeoxynucleotide CpG 7909 delivered as intravenous infusion demonstrates immunologic modulation in patients with previously treated non-Hodgkin lymphoma. J Immunother (1997), 29, 558-68 (2006)
78. Rosenberg, S. A., M. T. Lotze, L. M. Muul, S. Leitman, A. E. Chang, S. E. Ettinghausen, Y. L. Matory, J. M. Skibber, E. Shiloni, J. T. Vetto & *et al.*: Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med, 313, 1485-92 (1985)
79. Rosenberg, S.: Lymphokine-activated killer cells: a new approach to immunotherapy of cancer. J Natl Cancer Inst, 75, 595-603 (1985)
80. Rosenberg, S. A., M. T. Lotze, L. M. Muul, A. E. Chang, F. P. Avis, S. Leitman, W. M. Linehan, C. N. Robertson, R. E. Lee, J. T. Rubin & *et al.*: A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med, 316, 889-97 (1987)
81. Miller, J. S., Y. Soignier, A. Panoskaltsis-Mortari, S. A. McNearney, G. H. Yun, S. K. Fautsch, D. McKenna, C. Le, T. E. Defor, L. J. Burns, P. J. Orchard, B. R. Blazar, J. E. Wagner, A. Slungaard, D. J. Weisdorf, I. J. Okazaki & P. B. McGlave: Successful adoptive transfer and *in vivo* expansion of human haploidentical NK cells in patients with cancer. Blood, 105, 3051-7 (2005)
82. Tam, Y. K., J. A. Martinson, K. Doligosa & H. G. Klingemann: *Ex vivo* expansion of the highly cytotoxic human natural killer-92 cell-line under current good manufacturing practice conditions for clinical adoptive cellular immunotherapy. Cytotherapy, 5, 259-72 (2003)
83. Klingemann, H. G.: Natural killer cell-based immunotherapeutic strategies. Cytotherapy, 7, 16-22 (2005)
84. Shlomchik, W. D., M. S. Couzens, C. B. Tang, J. McNiff, M. E. Robert, J. Liu, M. J. Shlomchik & S. G. Emerson: Prevention of graft versus host disease by inactivation of host antigen-presenting cells. Science, 285, 412-5 (1999)
85. Ruggeri, L., M. Capanni, E. Urbani, K. Perruccio, W. D. Shlomchik, A. Tosti, S. Posati, D. Rogaia, F. Frassoni,

F. Aversa, M. F. Martelli & A. Velardi: Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science, 295, 2097-100 (2002)
86. Ruggeri, L., A. Mancusi, K. Perruccio, E. Burchielli, M. F. Martelli & A. Velardi: Natural killer cell alloreactivity for leukemia therapy. J Immunother (1997), 28, 175-82 (2005)
87. Miller, J. S., S. Cooley, P. Parham, S. S. Farag, M. R. Verneris, K. L. McQueen, L. A. Guethlein, E. A. Trachtenberg, M. Haagenson, M. M. Horowitz, J. P. Klein & D. J. Weisdorf: Missing KIR-ligands is associated with less relapse and increased graft versus host disease (GVHD) following unrelated donor allogenic HCT. Blood (2007)
88. Giebel, S., F. Locatelli, T. Lamparelli, A. Velardi, S. Davies, G. Frumento, R. Maccario, F. Bonetti, J. Wojnar, M. Martinetti, F. Frassoni, G. Giorgiani, A. Bacigalupo & J. Holowiecki: Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. Blood, 102, 814-9 (2003)
89. Ruggeri, L., A. Mancusi, M. Capanni, E. Urbani, A. Carotti, T. Aloisi, M. Stern, D. Pende, K. Perruccio, E. Burchielli, F. Topini, E. Bianchi, F. Aversa, M. F. Martelli & A. Velardi: Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. Blood (2007)
90. Ruggeri, L., A. Mancusi, E. Burchielli, F. Aversa, M. F. Martelli & A. Velardi: Natural killer cell alloreactivity and haplo-identical hematopoietic transplantation. Cytotherapy, 8, 554-8 (2006)
91. Soiffer, R. J., E. P. Alyea, E. Hochberg, C. Wu, C. Canning, B. Parikh, D. Zahrieh, I. Webb, J. Antin & J. Ritz: Randomized trial of CD8+ T-cell depletion in the prevention of graft-versus-host disease associated with donor lymphocyte infusion. Biol Blood Marrow Transplant, 8, 625-32 (2002)
92. Passweg, J. R., A. Tichelli, S. Meyer-Monard, D. Heim, M. Stern, T. Kuhne, G. Favre & A. Gratwohl: Purified donor NK-lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation. Leukemia, 18, 1835-8 (2004)
93. Sentman, C. L., M. A. Barber, A. Barber & T. Zhang: NK cell receptors as tools in cancer immunotherapy. Adv Cancer Res, 95, 249-92 (2006)
94. Long, E. O., D. F. Barber, D. N. Burshtyn, M. Faure, M. Peterson, S. Rajagopalan, V. Renard, M. Sandusky, C. C. Stebbins, N. Wagtmann & C. Watzl: Inhibition of natural killer cell activation signals by killer cell immunoglobulin-like receptors (CD158). Immunol Rev, 181, 223-33 (2001)
95. Sutherland, C. L., B. Rabinovich, N. J. Chalupny, P. Brawand, R. Miller & D. Cosman: ULBPs, human ligands of the NKG2D receptor, stimulate tumor immunity with enhancement by IL-15. Blood, 108, 1313-9 (2006)
96. Skov, S., K. Rieneck, L. F. Bovin, K. Skak, S. Tomra, B. K. Michelsen & N. Odum: Histone deacetylase inhibitors: a new class of immunosuppressors targeting a novel signal pathway essential for CD154 expression. Blood, 101, 1430-8 (2003)
97. Olszewski, A. J. & M. L. Grossbard: Empowering targeted therapy: lessons from rituximab. Sci STKE, 2004, pe30 (2004)
98. Adams, G. P. & L. M. Weiner: Monoclonal antibody therapy of cancer. Nat Biotechnol, 23, 1147-57 (2005)
99. Marcus, R. & A. Hagenbeek: The therapeutic use of rituximab in non-Hodgkin's lymphoma. Eur J Haematol Suppl5-14 (2007)
100. Hartmann, F., C. Renner, W. Jung, L. da Costa, S. Tembrink, G. Held, A. Sek, J. Konig, S. Bauer, M. Kloft & M. Pfreundschuh: Anti-CD16/CD30 bispecific antibody treatment for Hodgkin's disease: role of infusion schedule and costimulation with cytokines. Clin Cancer Res, 7, 1873-81 (2001)
101. Suck, G.: Novel approaches using natural killer cells in cancer therapy. Semin Cancer Biol, 16, 412-8 (2006)
102. Krause, S. W., R. Gastpar, R. Andreesen, C. Gross, H. Ullrich, G. Thonigs, K. Pfister & G. Multhoff: Treatment of colon and lung cancer patients with *ex vivo* heat shock protein 70-peptide-activated, autologous natural killer cells: a clinical phase I trial. Clin Cancer Res, 10, 3699-707 (2004)

**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

**Key Words:** Hematopoietic stem cells, Natural killer development, NK cell activity, NK cell therapy , Review

**Send correspondence to:** Inpyo Choi, Stem cell Research Center, Korea Research Institute of Bioscience and Biotechnology, Yusong, Taejon 305-333, Republic of Korea, Tel: 82-42-860-4223, Fax: 82-42-860-4593, E-mail: ipchoi@kribb.re.kr

http://www.bioscience.org/current/vol13.htm