NK Cells Mini Review

Mini Review

Immunology

Introduction

While studying cell-mediated cytotoxicity against tumor cells, Kiessling et al. (1975) discovered a subset of lymphocytes which conveyed a spontaneous selective cytotoxic activity without the need for prior sensitization. This effect was termed “natural cytotoxicity” and the cells thought to be mediating this effect were named Natural Killer (NK) cells. Human and mouse NK cells constitute approximately 15% of all circulating lymphocytes and around 2.5% of splenic leukocytes in mice. Although relatively small in number, NK cells are generally considered to be key components of early innate immune defence. This is achieved by two distinct mechanisms: contact dependent cytotoxicity and cytokine production. Despite the association with innate immunity NK cells exhibit processes such as priming, education and memory, all of which were thought to be characteristic of adaptive immunity.

NK cell development

NK cell development occurs primarily in the bone marrow microenvironment. Hematopoietic stem cells develop into multipotent progenitor (MPP) cells with either a lymphoid or a myeloid bias. A common lymphoid progenitor emerges from these cells, which develops firstly into a precursor NK cell, then to an immature NK cell and finally to a mature NK cell. Precursor NK cells are unable to differentiate into T, B, myeloid or erythroid cells, but are stimulated to form mature NK cells (Rosmaraki et al. 2001). However early stage CD4- CD8- thymocytes can differentiate into NK cells (reviewed in Di Santo 2006).

Human NK cell development

In humans, there are several stages of NK development in the bone marrow, which are based on the expression levels of CD34, CD117, CD56 and CD94. These phenotypes are restricted to the bone marrow and are not found in lymph nodes, spleen or peripheral blood. NK maturation begins with the expression of CD56 followed by simultaneous expression of CD94/NKG2A. Final NK maturation in the bone marrow correlates with a reduction in CD94/NKG2A expression and a $CD56^{low}$ phenotype (stage 5b). It has been proposed that stage 2 cells, which are precursor NK cells, can traffic to lymph nodes, spleen and peripheral blood where they can undergo further but restricted maturation. Further details of marker expression during the different stages of development can be seen in table 1. NK cell developmental stages in human bone marrow.

Also crucial to the development of NK cells from common precursors is the expression of interleukin 2/15 receptor beta (CD122). Precursor NK cells are characterized in humans as $CD34^{+}$ $CD122^{+}$ $CD56^{-}$ that respond to IL-15 and participate in the initiation of the final maturation process. NK cell precursor interaction with stromal cells is also needed for normal NK development (Wu et al. 2001). In addition to requiring stromal cell contact certain cytokines (IL-7, IL-15 (Cooper et al. 2002; Ranson et al. 2003)) and factors (c-kit ligand (CD117), flt-3 ligand (FL)) are also required. It has also been shown that the Tyro3 family of receptors (Tyro3, Mer, Axl) on NK cells and their ligands (Gas6, protein S) on stromal cells are critical for expression of NK cell receptors and functional differentiation *in vitro* and *in vivo* (Caraux et al. 2006).

BIO-RAD

Table 1. NK cell development stages in human bone marrow

	NK development stages in human bone marrow						
Markers	1	2	3a	3b	4	5a	5b
CD133	+/-	+	-	-	-	-	-
CD34	+	+	-	-	-	-	-
CD33	-	+/-	+/-	-	-	-	-
CD117	-	+	+	+	+	-	-
CD244 (2B4)	-	+/-	+/-	+	+	+	+
CD56	-	-	-	+	+	+	+
CD94	-	-	-	-	+	+	-
NKG2A	-	-	-	-	+	+/-	+/-
CD56: bright or dim				$CD56^{dim}$	$CD56^{bright}$	$CD56^{bright<dim}$	$CD56^{bright<<dim}$

Table from Eissens, et al. (2012)

Mouse NK cell development:

NK cell developmental in bone marrow starts with the $CD122^{+}$ precursor NK cell and transitions through to sequential acquisition of the NK cell receptors NKG2, Ly49, and CD117 (c-kit). This leads to a more functionally mature and proliferative NK cell population that is characterized by an up regulation of CD49b and CD11b, expression of CD27 and down regulation of CD51 (Kim et al. 2002). These mature NK cells are also capable of cytotoxicity and inducible cytokine synthesis. Further details of marker expression during the different stages of development can be seen in table 2. NK cell developmental stages in mouse bone marrow.

Table 2. NK cell development stages in mouse bone marrow

	NK development stages in mouse bone marrow				
Markers	I	II	III	IV	V
CD122 (IL-2Rbeta)	+	+	+	+	+
CD161 (NK1.1)	-	+	+	+	+
CD94/NKG2, NKG2D	-	+	+	+	+
LY49	-	-	+	+	+
CD117 (c-kit)		-	+	+	+
CD51 (alphav)		Hi	Hi	Lo	-
CD49b (DX5, alpha2)		Lo	Lo	Hi	Hi
CD11b (Mac-1)		Lo	Lo	Lo	Hi
CD43		Lo	Lo	Lo	Hi
IFN-gamma/Cytotoxicity				Lo	Hi
CD27					+

Table adapted from Yokoyama et al. (2004)

NK cell classification

NK cells do not express an antigen-specific receptor (B cell receptor or T cell receptor/CD3 complex) and are traditionally part of the innate immune system. They mediate their effect in an antigen-independent manner that, in general, does not give rise to immunological memory or long-term protective immunity. However, although derived from a separate lineage, NK cells express some common markers with similar functions and morphology to T cells.

Human NK cell classification

Mature human circulating NK cells were initially divided into two subsets according to their relative expression of either high or low levels of CD56. More recently it has been established that NK cell classification can be further refined based on their expression of CD62L, CD57 or CD94/NKG2A. An increase in cytotoxicity has been observed with decreased CD94/NKGA2, CD56 and CD62L expression and increased CD57 expression (Nagler et al. 1989 & Ellis et al. 1989). In addition, interferon (IFN) gamma release is primarily the result of activating receptor stimulation rather than a response to cytokines. Human NK $CD56^{low}$ (around 90%) and $CD56^{high}$ (around 10%) subsets have different homing properties (Cooper et al. 2001), with $CD56^{low}$ predominating in peripheral blood (PB) and $CD56^{high}$ predominating in secondary lymphoid tissues. $CD56^{low}$ NK cell subsets express higher levels of CD16 (FcgammaRIII) and Ig-like receptors. In contrast, the $CD56^{high}$ subset expresses very little or no CD16 ($CD56^{high}$ $CD16^{low}$) and produces type I pro-inflammatory cytokines IFN gamma and tumor necrosis factor (TNF) alpha (Robertson et al. 1990) in response to monokines (IL-12 and IL-15) produced by activated macrophages (Cooper et al. 2001; Fauriat et al. 2010).

Mouse NK cell classification

In mice the expression levels of CD27 ($CD27^+$ and $CD27^-$) define the two major subsets of NK cells (Hayakawa and Smyth, 2006). The $CD11b^{high}CD27^{high}$ NK subset exhibits higher levels of both cytokine production and cytotoxicity. Mouse NK cells are also typically $NK1.1^+$ (Lanier et al. 1986) $FcgammaRIII^+$ (CD16), $CD122^+$ (activation receptor required for NK cells IL-15 responsiveness), and $CD3^-$. They also express low levels of markers found on dendritic cells (CD11c), B cells (CD45R) and T cells (CD2). An NK phenotype of $CD3^-CD49b^+CD122^+$ will identify NK cells in $NK1.1^+$ and $NK1.1^-$ mice. NKp46 (also called NCR1 or CD335) has been put forward as the most specific marker for NK cells in mammalian cells (Westgaard et al. 2004; Walzer et al. 2007). These subsets differ in their expression of activation, inhibitory and chemokine receptors, with the $CD11b^{high}CD27^{high}$ NK subset exhibits higher levels of both cytokine production and cytotoxicity (Hayakawa and Smyth 2006).

NK cell receptors

NK cell receptors function as a detection system, the engagement of which determines the cellular response. There are two functionally distinct types of NK cell receptor whose balance of positive and negative signals control NK cell activity; NK cell inhibitory and NK cell activating receptors. In addition to the competing inhibitory/activating receptors NK cells also possess adhesion receptors which help to localize the cells to sites of injury.

NK cell inhibitory receptors

NK cell inhibitory receptors maintain an inactive state within NK cells through the recognition of constitutively expressed “self molecules” on potential target cells. There are three major types of inhibitory receptors: killer immunoglobulin receptors (KIRs), CD94/NKG2A, Ly49 and Siglecs. Most NK cell inhibitory receptors have immunoreceptor tyrosine-based inhibition motifs (ITIMs) located within their cytoplasmic tails. Table 3. lists NK cell inhibitory markers for mouse and human along with associated ligands.

KIRs

Most KIRs are inhibitory, in that their recognition of the major histocompatibility complex (MHC) suppresses the cytotoxic activity of their NK cell. KIRs (15 genes) are encoded in the leukocyte receptor complex (LRC) on human chromosome 19q13.4 where other Ig-like receptors are also encoded. Their nomenclature is based on whether the receptor has two or three Ig-like external domains (KIR2D or KIR3D) with short (S; without ITIM) or long (L; with one or two ITIM sequences) cytoplasmic domains (Colonna M et al. 1995). The S forms are activating receptors associated with DAP12 (immunoreceptor tyrosine based activation motif, ITAM, positive adapter molecule), whereas L forms are inhibitory receptors that contain ITIMs. Different KIRs have different specificity for HLAs. KIR2DL1 (CD158a) and KIR2DL2 (CD158b) are both specific for HLA-C; whereas, KIR3DL1 (originally called NKB1) and KIR3DL2 (previously named P140) are specific for HLA-Bw4 and HLA-A, respectively.

CD94/NKG2A

CD94/NKG2A is a family of C-type lectin receptors that are expressed predominantly on the surface of NK cells and a subset of $CD8^+$ T-lymphocyte. The CD94/NKG2 family includes seven members: NKG2A, B, C, D, E, F and H. Genes encoding these receptors are clustered in the natural killer complex (NKC) on human chromosome 12 and mouse chromosome 6 together with Clr (C-lectin related) genes. CD94/NKG2A is capable of being either inhibitory or activating depending on the members of the complex.

NKG2 receptors are transmembrane type II and specifically dimerize with the CD94 molecule to form heterodimers. CD94 contains a short cytoplasmic domain and it is responsible for signal transduction. Receptors of the CD94/NKG2 family bind non classical MHC class I glycoproteins (HLA-E in human and Qa-1 molecules in the mouse).

Ly49, Siglecs and other NK cell receptors

The Ly49 is an NK cell receptor more prominent in mice than in humans. The Ly49 family of genes is encoded in the NKC on mouse chromosome 6. The Ly49a receptor was originally identified on a mouse T cell tumor cell (Nagasawa et al. 1987). Ly49b recognizes MHC class I molecules H-2Dd, H-2Dk and H-2Dp and Ly49c binds to H-2Kb.

Non classical inhibitory receptors, include the LILR family of genes (also called LIR, ILT and CD85) and the CD33-related sialic acid binding Ig-like lectins (CD33rSiglecs); in particular human CD33rSiglec-7 (p75, adhesion inhibitory receptor 1 or AIRM1) (Falco et al. 1999; Nicoll et al. 1999). Only one of the LILR genes, LILB1 (ILT2/LIR1), encodes an inhibitory receptor on NK cells. LILB1 expression is variable on peripheral NK cells, ranging from negligible to about 75% (Yokoyama et al. 2003; Yokoyama 1995; Long 1999; Parham 2005; Cosman et al. 1997).

These receptors (regardless of MHC restriction) have inhibitory motifs (ITIMs) in their cytoplasmic domains which blunt activation signals. CD33-related Siglecs are largely inhibitory and widely expressed on human and mouse NK cells, dendritic cells, neutrophils, monocytes, eosinophils, basophils and B cells (Crocker and Varki 2001). There are ten human CD33-related Siglecs: Siglec-3 (CD33), Siglec-5 (CD170), Siglec-6 (CD327), Siglec-7 (CD328), Siglec-8, Siglec-9 (CD329), Siglec-10, Siglec-11, Siglec-14 and Siglec-16. In contrast, mice have five CD33-related Siglecs: Siglec-3 (CD33), Siglec-E, Siglec-F, Siglec-G and Siglec-H (Crocker et al. 2007; Cao et al. 2010).

Table 3. NK cell inhibitory markers

Inhibitory Receptor	Ligand	Species
CD85 (ILT-2)	HLA-A, -B, -G	H
CD94/NKG2A	HLA-E (H), Qa-1b (M)	H, M
CD161	Clr-b (NKR-P1D)	M
CD244 (2B4)	CD48	H, M
KIR2DL, KIR3DL	HLA class I	H
Ly49 A-C, E-G, I-O	H-2 class I	M
KLRG1	cadherins *	H, M
TGF-betaR	TGF-beta family	H, M

H = Human, M = Mouse

Table adapted from Di Santo, 2006; *Li et al. 2009

NK cell activating receptors

NK cells also express a variety of activating receptors which can be grouped into several categories. The main activating receptor groups on NK cells include CD16, NKR-P1 (NK1.1, CD161), NKG2D (KLRK1, CD314), NCR (NKp30, NKp44, NKp46, NKp80); and activating isoforms of human KIRs. These molecules function as activating receptors because they lack ITIMs and instead have ITAM positive adaptor molecules (DAP12). Table 4. lists NK cell activating markers for mouse and human along with associated ligands.

CD16

The first and best characterized activating receptor identified on NK cells is CD16, a low affinity Fc receptor for IgG (FcgammaRIII) (Perussia et al. 1983). NK cells can mediate antibody-dependent cellular cytotoxicity through FcgammaRIII, which binds the Fc portion of IgG coating a target (Anegon et al. 1988). Although there are several Fc receptors for IgG, NK cells express only FcgammaRIII. In addition, despite their ability to initiate antibody-dependent cell-mediated cytotoxicity (ADCC), $CD16^{-}CD3^{-}$ human NK cells can still mediate natural killing (Lanier et al. 1988).

NKR-P1 (NK1.1 and CD161)

NKRP1 (Kirb1) belongs to a family of lectin like molecules with type II orientation encoded in mice (NK1.1) (Ryan et al. 1992; Giorda et al. 1991). Its expression is relatively selective for NK cells. NKR-P1A or CD161 is classified as a type II membrane protein because it has an external C terminus. NKR-P1A, the receptor encoded by the KLRB1 gene, recognizes lectin like transcript-1 (LLT1) as a functional ligand. In humans, there is only a single gene (NKRP1A) expressed on a subpopulation of NK cells (Lanier et al. 1994).

NKG2D (KLRK1 and CD314)

The NKG2D receptor binds to ligands structurally homologous to MHC class I (e.g. human ligands MICA, MICB and mouse ligands RAE-1alpha, RAE-1beta (reviewed by Radaev et al. 2003). NKG2D is expressed as a disulfide-linked homodimer on all human and mouse NK cells. It is distinct from other NKG2 molecules in that it shares very little homology (28% instead of 70%) and does not heterodimerize with CD94. In both mice and humans NKG2D expression is not restricted to NK cells. In humans it is also found on gamma delta TCR^{+} T cells and $CD8^{+}$ T cells. In mice it is found on most NKT cells and on activated $CD8^{+}$ T cells (Bauer et al. 1999). NKG2D does not have a cytoplasmic motif and preferentially associates with the signaling chain DAP10 via an YxxM motif for recruitment of PI3K (Wu et al. 1999), suggesting that NKG2D, when associated with DAP10 acts as a co-stimulatory molecule. In mice, there are two isoforms of NKG2D, a long form (NKG2D-L) and a short form (NKG2D-S). Although both forms are present on resting NK cells, the longer form is predominately expressed and preferentially associates with DAP10. Other NK activation receptors of NKG2 are heterodimeric NKG2A-CD94 and NKG2E-CD94 (Lazetic et al. 1996).

NCR (NKp30, NKp44, NKp46)

NCRs are type I TM receptors that, unlike T cell receptors (TCRs) and immunoglobulins, do not undergo recombination in order to become functionally active. NCRs possess ITAMs which activate NK cells while NKp44 also has an ITIM. Originally identified as receptors with the ability to mediate the killing of tumor-transformed cells NCRs have also been implicated in the control and elimination of several pathogens (Magri et al. 2011). NCRs also have a role in immune homeostatis by regulating the expression of several immune cell types. The ligands for these receptors include self derived molecules as well as pathogen components (Hudspeth et al. 2013).

Human KIRs

Human killer cell immunoglobulin-like receptors are a family of transmembrane glycoproteins expressed on NK cells. They have the capability to be either activating or inhibitory. Activating KIRs possess truncated cytoplasmic domains, while inhibitory KIRs have long cytoplasmic domains. KIRs are key regulators of the development, tolerance and activation of NK cells. The major ligands for activatory KIRs are MHC molecules and upon binding signaling is mediated KARAP/DAP12. Evidence suggests that this signaling may contribute to the "education" of NK cells, an observation blurring the lines between adaptive and innate immunity (Ivarsson et al. 2015).

Other activating receptors

Ly49d and Ly49h in mice do not contain cytoplasmic ITIMs. They are instead activation receptors associated with DAP12 (Smith et al. 1998). Ly49d has no known role in viral defence, but controls NK cell specificity for killing of a xenogeneic target, as in Chinese hamster ovary cells, due to recognition of Chinese hamster MHC class I molecules (Furukawa et al. 2002). Ly49h is involved in viral infection and can be selectively activated to produce IFN gamma and chemokines in response to virally infected macrophages (Dorner et al. 2004).

CD244 is a member of the signaling lymphocyte-activation molecule (SLAM) that binds CD48 on target cells. CD244 is found on human chromosome 1q22 and mouse chromosome 1 and its receptor mediates non-MHC restricted killing (Velikovsky et al. 2007). Its expression is restricted to cells that mediate NK-like killing as it is expressed on all human and mouse NK cells, most gamma delta TCR^+ T cells, and $CD8^+$ T cells (Garni-Wagner et al. 1993).

Table 4. NK cell activating markers

Activation Receptors	Ligand	Species
CD16 (FcgammaRIII)	Immune complexes	H, M
CD25 (IL-2Ralpha)	IL-2	H
CD27	CD70	H, M
CD28	CD80, CD86	H, M
CD69	*	H, M
CD94/NKG2C, E	HLA-E (H), Qa-1b (M)	H, M
CD122 (IL-2Rbeta)	IL-2, IL-15	H, M
CD161	Clr-g (NKR-P1F)	H, M
CD266 (DNAM-1)	CD112 (Nectin-2), CD155	H
CD244 (2B4, SLAMF4)	CD48	H, M
NKG2D	MICA, B, ULBs (H), Raels (H, M), H60 (M)	H, M
KIR2S, KIR3S	HLA class I	H
Ly49D, H, P	H-2 class I, MCMV m157 (Ly49H	M
NCR (NKp30, 44, 46)	Viral hemagglutinins*	H, M (NKp46)
ILT-1 (Ig-like transcript 1)	*	H
IFN-alpha/beta R	Type I interferons	H, M
Gp49A	*	M

H=human; M= mouse. * Ligands on tumor cells which activate NK cells expressing the NCR family of activation molecules which include NKp46, NKp44 and NKp30 are still unknown. Table adapted from Di Santo, 2006

Adhesion receptors

For NK cells to efficiently carry out their effector functions, they must be able to migrate to the site of injury. Adhesion receptors are a key group of molecules that contribute to this function, by increasing their levels of expression. Table 5. below lists the main adhesion molecules and their ligands in human and mouse.

Table 5. NK cell adhesion receptors in human and mouse

Adhesion Receptors	Ligand	Species
CD2 (LFA-2)	CD48, CD58 (LFA-3)	H, M
CD11a (LFA-1)	CD54 (ICAM-1), CD102 (ICAM-2)	H, M
CD11b (Mac-1) H, M	CD54 (ICAM-1)	H, M
CD43 (Leukosialin)	CD54 (ICAM-1)*	H, M
CD44	Hyaluronic acid	H, M
CD49b (DX5)	**collagen, laminin, fibronectin	M
CD56 (N-CAM)	N-CAM, heparin sulphate, FGFR	H
Lag3 (CD223)	HLA class II	H

H=human; M= mouse.

Table adapted from Di Santo, 2006, *Nieto et al. 1999, **Garrod et al. 2007

Mechanisms of action of NK cells

NK cells can lyze virally infected cells and tumor cells without prior sensitization. This lysis or cytolytic function is controlled by inhibitory NK receptors that specifically bind to MHC (HLA) molecules on healthy cells and NK cell activation receptors that detect stressed cells. When MHC class I molecules are down regulated or lost on tumor cells or in viral infections, inhibitory signals from inhibitory receptors are lost resulting in NK cell activation. This is called “missing-self” triggered NK activation. NK cell activation receptors (e.g. NKG2D) can detect self molecules up regulated at higher levels on damaged cells. This is called “stress-induced self recognition.” Cell surface receptors control inhibition and activation; proliferation and effector functions (cytotoxicity and cytokine production) (reviewed by Spits et al. 1998; Colucci et al. 2003). Once activated by NK cell receptors NK cells can use several methods to exert their cytotoxic effects. These include cytolytic granule mediated cell apoptosis and ADCC. When activated by cytokines or interferons NK cells secrete interferon gamma and TNF alpha which promote phagocytosis.

NK cells and adaptive immunity

The ability to generate memory cells following a primary infection and the consequent rapid immune activation and response to succeeding infections by the same antigen is fundamental to the role T and B cells play in the adaptive immune response. For many years, NK cells have been considered to be a part of the innate immune system. However, recently, increasing evidence suggests that NK cells can display several features that are usually attributed to adaptive immune cells (e.g. T cell responses) such as expansion and contraction of subsets, increased longevity and a form of immunological memory, characterized by a more potent response upon secondary challenge with the same antigen. The role of NK cells in both the innate and adaptive immune responses is becoming increasingly important in both basic research and targeted drug development.

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