Activating and inhibitory receptors and their role in Natural Killer cell function

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Last decade has seen a significant advancement in our understanding of the Natural Killer (NK) cell biology and function. Several receptors present on NK cells have been identified, which are involved in either their activation or inhibition. Similarly, a large number of interacting ligands have been identified on the target cells that upon interaction transmit either activating or inhibitory signals. This review is oriented towards understanding the role of these receptors on the NK cells, which are considered as the first line of defense.

Keywords: NK cells, receptors, ligands, function, inhibition, activation, cytotoxicity

Successful host defense relies on the concerted action of innate and adaptive immune response. Natural killer cells (NK) are an integral component of the innate immunity both in the production of cytokines (IFN-γ, TNF-α) to stimulate other cells of the immune system and in direct destruction of the infected or transformed cells. “Natural killer cells” named first by Herberman et al1, are bone marrow-derived large granular lymphocytes that share a common progenitor with T-cells. They represent a lymphoid population that, in contrast to T or B-lymphocytes, does not express clonally distributed receptors for antigens. Unlike T or B cells, NK cells develop normally in scid mice2 or in mice with disrupted RAG-1 or RAG-2 genes3, indicating that gene rearrangement is not required for their differentiation or function and are thus considered as a key performer of innate immune system4.

NK cell recognition of pathogen-infected cells (viral or bacterial infection) and tumour is based on the expression of multiple cell surface receptors that bind to either MHC-I (major histocompatibility complex) for inhibitory and or non-MHC ligands to transduce activating signals. Thus, NK cells provide a unique defense mechanism to eliminate cells with abnormal MHC class I expression (“Missing self” hypothesis of tumour immunosurveillance)5,6. The mechanisms that regulate NK cell function against tumour and infection have exceeded beyond our expectations in terms of their sophistication and complexity and are based on the regulation of activation/inhibition signals for NK cells.

Two-Receptor Model

NK cells express a number of cell surface receptors that fall into two major categories, namely activation and inhibitory receptors. Activation receptors have a short cytoplasmic tail and a positively charged (basic) amino acid in transmembrane region. It is through this basic amino acid region that the receptor associates with signal transducing molecule such as DAP127, which also have a negatively charged (acidic) amino acid in their transmembrane domain. These signal

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Abbreviations used: NK, natural killer cells; MHC, major histocompatibility complex; ITAM, immunoreceptor tyrosine based activation motif; ITIM, immunoreceptor tyrosine based inhibitory motif; Ig, immunoglobulin; ADCC, antibody dependent cellular cytotoxicity; TCR, T cell antigen receptor; NCRs; natural cytotoxicity receptors, SHP-1, Src homology-1 (SH1) containing phosphatase; CTLD, c-type lectin-like domain, NKR-P1, natural killer cell surface protein 1; KIRs, killer cell immunoglobulin-like receptors; CXCP motif, (cysteine-X-cysteine-proline motif); YXXM motif, (tyrosine-X-X-methionine); where X denotes any amino acid; MICA, [major histocompatibility complex (MHC) class I chain] related gene A; and MICB, [major histocompatibility complex (MHC) class I chain] related chain B; DAP, death associated protein; CTLs, cytotoxic T lymphocytes; HLA, human leukocyte antigen; MCMV, murine cytomegalovirus; XLP, X-linked lymphoproliferative disease; EBV, Epstein barr virus; RAG, recombination activating gene; Rae-1, retenoid acid early transcript 1; ULBPS, UL16 binding proteins; H-60, histocompatibility antigen; m157, a viral gene product; ILT, immunoglobulin-like transcripts; LIR, leukocyte Ig-like receptors; MIR, monocyte/macrophage Ig-like receptors; Mac-1, complement receptor type 3; ICAM, intercellular adhesion molecule; LFA, leukocyte function associated antigen; CD, cluster designation
transducing molecules have a series of amino acids in their cytoplasmic domain known as “ITAM” (immunoreceptor tyrosine based activation motif). Another adapter protein DAP10 acts like co-receptor possessing YXXM motif in its cytoplasmic domain instead of ITAM can co-stimulate NK cells. On engagement of activation receptors with ligand, a signal is sent to the cell that activates a series of kinases (enzymes that add charged groups), eventually leading to activation of the cell’s cytotoxic effector function and IFN-γ production. In contrast to activating receptors, inhibitory receptors have a long cytoplasmic tail that contains an “ITIM” (immunoreceptor tyrosine based inhibitory motif). Upon engagement of ligand, a tyrosine residue in the ITIM is phosphorylated by a kinase, and the phosphotyrosine in turn attracts a phosphatase known as SHP-1, which removes, charged phosphate group. The function of SHP-1 is to antagonize the kinase of activation signal. Because of the requirement for ITAM- associated kinases to phosphorylate the ITIM, and the presence of SHP-1 near the same kinases for their dephosphorylation, the inhibitory signal can only be delivered when the activating and inhibitory receptors are colligated. This is shown schematically in Fig. 1. Thus, when both activating and inhibitory receptors are cross-linked by their ligands on the surface of a potential target cell, the inhibitory signal delivered to the NK cell overrides the activating signal, and the target cell is spared. If only the activating receptor interacts or if more activating receptors than inhibitory receptors are engaged, then the target is killed. Structural and functional characterization of these receptors is currently the most demanding aspect of NK cell biology. This review focuses briefly on the activation and inhibitory receptors on NK cells and their ligands.

**Activation Receptors involved in Cytotoxicity**

**CD16 (FcγRIII)**

The most extensively studied membrane receptor on majority of NK cells is the transmembrane ~70 kDa glycoprotein of the immunoglobulin (Ig) superfamily, which possesses low affinity for IgG, CD16 (FcγRIII). CD16 is non-covalently associated with the γ subunit of the high affinity IgE receptor (FcεRI-γ) in mouse NK cells and with FcεRI-γ or the ζ subunit of T cell antigen receptor (TCR) complex in human NK cells. Upon CD16 mediated activation, NK cells secrete cytokines, mediate ADCC (antibody dependent cellular cytotoxicity) and induce apoptosis as a consequence of Fas ligand (CD95L) interaction. While CD16 is responsible and necessary for ADCC, this receptor has not been implicated in other modes of NK cell-mediated cytotoxicity.

**CD2 (LFA-2, E-Rosette Receptor)**

CD2 is a ~50 kDa membrane glycoprotein of Ig superfamily expressed on NK and T cells. The biochemical events accompanying CD2 activation are similar to those initiated by CD16 ligation. In particular, CD2 stimulation of NK cells results in the activation of src-family kinases and the generation of inositol-triphosphates (IP3). The predominant ligand of human CD2 is a membrane glycoprotein, CD58 (LFA-3, lymphocyte function associated antigen-3). However, expression of CD58 alone is not sufficient to confer cells sensitive to NK mediated lysis suggesting that CD2 may serve as co-stimulatory receptor that augments, but does not initiate the primary activation of NK cells.

**CD28**

The CD28 receptor/B7 ligand system is the dominant co-stimulatory pathway affecting T cell immune responses. Although CD28 is expressed on human fetal NK cells, it is lost upon maturation and is absent on peripheral blood NK cells in adults. YT
(immature NK) cell line\textsuperscript{17}, which expresses CD28, killed target cells expressing either CD80 or CD86. Splenic NK cells express CD28, and ligation of CD28 with B7 ligand augments NK cell proliferation, and tumour cell lysis\textsuperscript{18}.

**NKR-P1 (CD161)**

NKR-P1, first identified in the rat is expressed by almost all NK cells as a disulfide-linked homodimer. The polypeptide is a type II integral membrane protein with a deduced external carboxy-terminal domain homologous to Ca\textsuperscript{2+}-dependent (C-type) lectin superfamily\textsuperscript{19}. The rat NKR-P1 cDNA was cloned by using a monoclonal antibody (mAb) selected for its ability to activate rat NK cells\textsuperscript{20}. Three highly related genes NKR-P1A, B and C have been identified in mice and rats. These genes display allelic polymorphism and the C57BL/6 and BALB/c allelic forms differ by 1-10\%\textsuperscript{21}. The prototype mouse NK cell antigen, NK1.1 molecule, long considered to be most specific serological determinant on murine NK cell, is encoded by \textit{Mus NKR-P1C} in C57 BL/6 mice\textsuperscript{22}. A single human NKR-P1 homolog has been found as disulfide-linked homodimer of C-type lectin superfamily on the subsets of NK and T-cells.

The NKR-P1 genes are present on rat chromosome 4, mouse chromosome 6, and human chromosome 12p12-p13 in a region designated as the “NK gene complex”. The mouse NKR-P1 genes are clustered and genetically linked to the Ly-49 gene family in the NK gene complex\textsuperscript{23}. The NKR-P1 receptor is more promiscuous in its carbohydrate specificity than other C-type lectins and there are several known NKR-P1 genes each with potential allelic forms. These observations may explain the confusing array of effects that different carbohydrates have on natural killing\textsuperscript{24}. Interestingly, expression of NKR-P1 in the rat has also been detected on activated peripheral blood granulocytes, activated monocytes and splenic dendritic cells which killed NK-sensitive YAC-1 cells \textit{in vitro} mediated by Ca\textsuperscript{2+}-dependent pathways\textsuperscript{25}.

NKR-P1 proteins in all rodents express the CXCP motif, which is also found in the cytoplasmic domains of CD4 and CD8 that interacts with phosphorylated p56 lck; however, human NKR-P1 lacks this motif. Another remarkable feature is the presence of ITIM in the cytoplasmic domain of rat and mouse NKR-P1B, but not in other NKR-P1 isoforms. Because of ITIM motif some isoforms have been found to be inhibitory rather than having activation effector function\textsuperscript{26}. NKR-P1 crosslinking and inhibition studies suggest a role for this receptor in target cell recognition and in cytotoxicity. However, NKR-P1 has not been found essential for NK cell development and function\textsuperscript{27}.

**CD94/NKG2**

CD94/NKG2 receptors are expressed on human NK cells and on subset of T-cells. NKG2 are members of type II, C-type lectin superfamily, which heterodimerize with CD94, except NKG2D, which is an activation receptor. NKG2A and CD94 form a heterodimer\textsuperscript{28} (70 kDa) composed of \textasciitilde30 kDa CD94 glycoprotein disulfide bonded to a 43 kDa NKG2A glycoprotein; it can recognize the MHC non-classical class I HLA-E ligand coexpressed with peptides derived from the leader sequences of certain HLA-A, B, C and non-classical HLA-G chains\textsuperscript{29}. Upon ligand engagement, inhibition signal is sent to NK cell through ITIM motif on cytoplasmic tail of NKG2A. Whereas CD94:NKG2C heterodimer, upon interaction with activation molecule can activate NK cells\textsuperscript{30}. Even after transfection of NKG2A/B with strong promoters, the proteins are unable to be expressed on the cell membrane unless they are disulfide bonded to CD94 glycoprotein. Thus, CD94 might assist in the transport of NKG2 receptors to the cell surface\textsuperscript{31}. Human NKG2A possess two ITIMs that have been shown to be capable of interacting with SHP-1 and SHP-2; like KIR (Ig superfamily), ITIMs are required for mediating the maximal inhibitory signal, but opposite to KIR, the membrane distal ITIM is of primary importance rather than the membrane-proximal ITIM, for inhibitory function\textsuperscript{32}.

CD94 (Kp43) essentially lacks a cytoplasmic domain, thus deficient in intrinsic signal transduction capacity. CD94 is a single gene with limited or no allelic polymorphism, The NKG2 family comprises four genes, designated as NKG2, -A, -C, -E, and F. CD94 and the four NKG2 genes are closely linked on human chromosome 12\textsuperscript{25}-p13 in the ‘Human NK-gene complex’. NKG2D/F is an unusual gene, which shows homology with the other NKG2 genes only in the 5′ region\textsuperscript{33,34}. NKG2-D/F is a recently characterized potent activation molecule conserved among human, mouse and rat (NKR-P2) NK and CTLs, with a C-terminal lectin-like domain (CTLD) and is the most characterized receptor till date on NK cells. Unlike other activating NK receptors, NKG-2D does not appear to signal through ITAM containing signaling polypeptide. Instead, it selectively pairs with KAP/DAP10 (killer cell activating–receptor associated polypeptide/DNAX activating protein,
transmembrane adapters), which can recruit the p85 subunit of PI3-kinase through YXXM motif present in its cytoplasmic domain. This signaling motif is related to a similar functional motif contained in the cytoplasmic domain of CD28 (co-stimulatory receptor), thereby suggesting that NKG2D may co-stimulate rather than directly activate effector function.  

Recently, two splice variants of NKG-2D have been identified: a long form (NKG-2DL) that has a 13-amino acid extension at the amino terminus, and a short form (NKG-2DS). Resting NK cells express mRNA encoding NKG2D-L, and after activation, mRNA encoding NKG2D-S is upregulated. Immunoprecipitation studies showed that NKG2D-L associates only with DAPI0, whereas NKG2D-S can associate with both DAPI0 and DAPI2. Differential signaling ability of NKG2D depends both on the cell type and on the activation state, which is determined by the differential expression of DAPI0 versus DAPI1, on the two different NKG-2D isoforms. Thus, NKG-2D has been found to deliver a direct stimulatory signal to NK cells, but only a co-stimulatory signal to T cells, because the specificity to T cell signaling would be compromised by direct stimulation of T cells by NKG2D. NKG2D is unique in the sense that it is relatively non-polymorphic. It forms a homodimer, whereas the other NKG2 molecules (NKG2, -A, -C, E) heterodimerize with CD94. NKG-2D shows minimal amino acid sequence differences between four mouse strains. These molecules most likely represent alleles with the same function because of conservation of transmembrane and cytoplasmic domains. Arginine at residue 69 is conserved in all four-mouse strains, which is responsible for NKG-2D interaction with DAPI0.  

The limited polymorphism of NKG-2D is somewhat surprising because the ligands for NKG-2D are numerous and heterogenous and display significant allelic polymorphism. A number of NKG-2D target ligands have been identified. The most interesting of these are a pair of closely related hNKG2D ligands called MICA and MICB [major histocompatibility complex (MHC) class I chain-related]38. MICA/B are structurally similar to MHC-class I and possess α1, α2 and α3 domains, but they share only 27% amino acid identity with human MHC class I. The transcriptional control regions of MICA and MICB genes contain sequences that are highly homologous to heat shock elements of hsp70 genes. Expression of MIC proteins is upregulated under conditions of “stress” such as heat shock, viral infection and allograft rejection. ULBPs (UL16 binding proteins) are another group of hNKG2D ligands, distantly related to MHC class I in their α1 and α2 domains, and are anchored to the membrane via a GPI (glycophosphatidyl inositol)-linkage, whereas UL16 bears no sequence or structural similarity to NKG-2D.  

Natural Cytotoxicity Receptors  
A true NK receptor involved in natural cytotoxicity should mediate NK cell triggering independent of the engagement of other surface molecules. In addition, its surface expression may be expected to be mostly restricted to NK cells. The triggering surface molecules with both of these properties have been identified in humans, based upon the screening of monoclonal antibodies capable of inducing NK-mediated killing of Fc receptor positive tumour target cells (in a redirected killing assay). This approach led to the identification of several molecules, which mediate NK cell activation and are referred to as natural cytotoxicity receptors (NCRs).  

NKp46 is a 46 kDa type I transmembrane glycoprotein belonging to the Ig superfamily and has been found in immature NK cells undergoing in vitro maturation starting from CD34+ cells. Cross-linking of NKp46 resulted in increased cytotoxicity, Ca2+ mobilization, and cytokine production. Analysis of cell distribution indicates that NKp46 is expressed by all NK cells including the CD56-bright CD16− subset. Its cytoplasmic modules are coupled to intracytoplasmic transduction machinery by their association with CD3ζ and FcεRIγ adapter proteins that contain ITAM. Recent work by Tomasello et al. indicated that in double knock-out mice lacking CD3ζ and FcεRIγ chains the cytolytic activity against most target cells (including YAC, BW15.02, RMA, RMA-S and C4.4.25) is profoundly reduced. These findings indicate that in mice NKp46 (or other, still undefined, murine receptors associated with ζ/γ polypeptide) may also play a prominent role in natural cytotoxicity mediated by NK cells.  

NKp30, another triggering receptor (Ig superfamily) of 30 kDa, functionally similar to NKp46 and CD16 has been found to be associated with CD3ζ chain that is phosphorylated at tyrosine following treatment of cells with pervanadate and thus takes part in activation. Similar to NKp46 and
NKp30, NKp44 also triggers NK-mediated cytotoxicity upon cross-linking by specific monoclonals. Expression of NKp44 alone in IL-2 activated NK cells, gave it a status to be considered as the first marker for activated human NK cells. NKp44 is a glycoprotein of 29 kDa that associates with the ITAM-bearing KARAP/DAP12 signal transducing molecule. A direct correlation has been found between the surface density of NCRs and the ability of NK cells to kill various tumours. NKG2D (another potent triggering receptor) plays either a complementary or a synergistic role with NCRs.

Several other membrane receptors have been implicated in NK cell activation based on the ability of specific monoclonal antibodies to permit lysis of Fc-receptor bearing target cells (mAb redirected cytotoxicity assay). Structures implicated with this idea include: Ly 6, mouse and human CD69, mouse and human 2B4, human, CS1 (CRACC), DNAM-1, and CD44. The ligands for various activation receptors are listed in Table 1.

**Inhibitory Receptors**

The inhibitory receptors superfamily (IRS) describes an expanding group of receptors that attenuate activation of a number of different cell types in the immune system. By definition, IRS members must act in trans, be able to recruit phosphatase (such as SHP-1) through an ITIM. Inhibitory receptors mediate this function only upon their clustering with an activating counter part on the cell surface.

Generally, the activating and inhibitory receptors recognize different ligands; however, under certain circumstances, cells simultaneously express pairs of activating and inhibitory receptors with closely related extracellular domains binding similar ligands. When both activating and inhibitory receptors are engaged by their respective ligands, the net outcome is determined by the relative strength of these opposing signals. Thus, a central paradigm has emerged in which pairing of activation and inhibition is necessary to modulate immune response.

**Ly-49 Receptors**

The Ly-49 receptors are type II transmembrane, C-type lectin-like molecules that are expressed as homodimers. Mouse NK cells express at least seven inhibitory Ly-49 receptors. These are responsible for the recognition of polymorphic MHC-I molecules on potential target cells and successful recognition of MHC class I by Ly-49 requires α1 and α2 domains of MHC class I as well as the presence of a peptide in the peptide binding groove. Expression of Ly-49 family of receptors is a relatively late event in NK cell development. Seven well described Ly-49 family members containing inhibitory motifs in their cytoplasmic domains (Ly49A, -B, -C, -E, F, G, and -I) are known to be expressed at least at the mRNA level by NK cells from C57BL/6 (B6) mice. Diversity within Ly-49 family is provided by alternate mRNA splicing and allelic polymorphism. Expression of Ly-49 genes is monoallelic and each NK cell can co-express several Ly-49 receptors at one time.

Acquisition of Ly-49 receptor is thought to be a stochastic process in which NK cells will continue to express different Ly-49 receptors in a cumulative manner until they express one that recognizes self.

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**Table 1—Activating receptors and their ligands in human and mouse NK cells**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
</tr>
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<tbody>
<tr>
<td><strong>Shared between human and mice</strong></td>
<td></td>
</tr>
<tr>
<td>CD11b (Mac-1, αMβ2, C3R)</td>
<td>CD54 (ICAM-1), CD102 (ICAM-2)</td>
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<tr>
<td>CD16 (FcγIII)</td>
<td>IgG</td>
</tr>
<tr>
<td>CD28</td>
<td>B7-1, B7-2</td>
</tr>
<tr>
<td>CD44</td>
<td>Hyaluronic Acid</td>
</tr>
<tr>
<td>CD69</td>
<td>-</td>
</tr>
<tr>
<td>CD244 (2B4)</td>
<td>CD48</td>
</tr>
<tr>
<td>IFN-αβR</td>
<td>IFN-α, IFN-β</td>
</tr>
<tr>
<td>NKG2C, NKG2E</td>
<td>HLA-E (human), Qa1b (mouse)</td>
</tr>
<tr>
<td>NKG2D</td>
<td>ULBP1, ULBP2, ULBP3 (human)</td>
</tr>
<tr>
<td></td>
<td>MICA, MICB (human)</td>
</tr>
<tr>
<td></td>
<td>Rae-1, H-60 (mouse)</td>
</tr>
<tr>
<td>Nkp46</td>
<td>Tumour Ligands (?), Influenza virus Hemagglutinin (human), ligands on normal cells (?) (human)</td>
</tr>
</tbody>
</table>

| **Human** | |
| CS1(CRACC) | - |
| ILT2(LIR-1) | HLA class I |
| KIR3DS | HLA class I |
| Nkp44 | Tumour ligands (?), Influenza virus Hemagglutinin |
| Nkp30, Nkp80 | Tumour ligands (?), ligands on normal cells (?) |

| **Mouse** | |
| gp49A | - |
| LY49D | H-2 class I |
| LY49H | m157 |
MHC-class I. The resulting diverse array of receptors displayed by each NK cell creates the divergent repertoire that monitors cells from the selective loss of class I molecules. Based on the functional studies, Ly-49A was initially attributed to be specific for D\textsuperscript{d} but not K\textsuperscript{b} or K\textsuperscript{s}. Ly-49A also binds to H-2\textsuperscript{k} cells, but binding of Ly-49A to H-2\textsuperscript{d} was consistently higher than H-2\textsuperscript{k}. Ly-49A transfectants bound significantly to H-2\textsuperscript{s} cells, albeit weakly, and to H-2\textsuperscript{eq,ly}. These results indicate that Ly-49A exhibits a broad and often strong MHC reactivity. Ly-49C recognizes the H-2\textsuperscript{d} molecule. In cell adhesion experiments, Ly-49C has been found to possess affinity with H-2\textsuperscript{b}, H-2\textsuperscript{s}, H-2\textsuperscript{k} and H-2\textsuperscript{r} gene products. Ly-49C exhibits allelic diversity, differing in four amino acids between the B6 and BALB/c strains. However, later activity difference between Ly-49C\textsubscript{BALB} and Ly-49C\textsubscript{B6} could not be detected showing no evidence for difference in the specificity of these allelic Ly-49C receptors. Ly-49C shares sequence homology with Ly-49I and Ly-49F. By the tetramer binding studies, Ly-49C has been found to exhibit little, if any, capacity to discriminate peptides bound to K\textsuperscript{b} or D\textsuperscript{b}.60

Two of the Ly-49 receptors Ly-49D and Ly-49H appear to be activating rather than inhibitory receptors. Ly-49D shows activation in response to target cells expressing the D\textsuperscript{b} class I-molecule. Ly-49D and Ly-49H lack ITIM and associated non-covalently with ITAM bearing membrane adaptor protein DAP12 via a charged residue in the transmembrane region. A viral gene product (m157) is expressed on MCMV (murine cytomegalovirus) infected cells can bind to Ly-49I and gives an inhibitory signal in MCMV susceptible strains, whereas m157 has been found to give activation signal in MCMV resistant strain after its binding to Ly-49 H on NK cells.63 Ly-49E is very similar in sequence to other MHC-reactive Ly-49 receptors (Ly-49C, I and F), suggesting a role in MHC recognition. Weak binding of Ly-49E to H-2\textsuperscript{d} has been reported in cell-cell adhesion assays. Ly-49F binds weakly but consistently to MHC molecules of the (H-2\textsuperscript{b}) haplotype. Strong inhibitory action of Ly-49G2 with H-2\textsuperscript{d} has been reported despite weak binding; though comparatively equal extent of inhibitory effect was found to be associated with Ly-49A after strong binding because of avidity differences or strong signaling in the former.54 Ly-49I binds to H-2\textsuperscript{b,d,k,r,s,v} with variable strength and can discriminate peptides presented by K\textsuperscript{b}. In humans, members of Ly-49 multigene family have been identified; however the encoded transcript is probably not functional. The human Ly-49L gene is located approximately 150 kb from NKG-2A gene.64 Interacting ligands (HLA subgroup) for various Ly 49 receptors are shown in Table 2.

**Killer Cell Immunoglobulin (Ig)-like Receptors (CD158)**

Killer cell immunoglobulin-like receptors (KIRs) are type I transmembrane molecules with either two or three Ig domains. In humans, the KIR genes encode glycoprotein of Ig superfamily that bind HLA class-I as ligands and can inhibit or activate NK cells, which is dependent on the presence of ITIM or ITAM motifs in their cytoplasmic domain.65 KIR genes are located on human chromosome 19q 13.4, where about 12 genes are estimated to be located based on the analysis of existing cDNA sequences. Two subfamilies of the KIR are identified, depending on the number of Ig-like domains in the extracellular regions of the molecules that are KIR2D (2 Ig-like domains) and KIR3D (3 Ig-like domains). Within both subfamilies, there is diversity throughout the extracellular, transmembrane and intracellular domains. Like Ly-49, KIRs are encoded by about 12 polymorphic genes, recognize polymorphic epitope on human leukocyte antigen classes HLA-A, HLA-B and HLA-C and are expressed on overlapping subsets of NK cells and memory T cells. Half of the KIR molecules have two ITIMs in their cytoplasmic domain that bind predominantly SHP-1 and inhibit cell mediated cytotoxicity and cytokine secretion.

<table>
<thead>
<tr>
<th>Receptor isotype</th>
<th>Ligand</th>
<th>Function</th>
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<tbody>
<tr>
<td>Ly-49A</td>
<td>H2d, H2k, H2s</td>
<td>Inhibitory</td>
</tr>
<tr>
<td>Ly-49B</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ly-49C</td>
<td>H2b, H2d, H2k, H2s</td>
<td>Inhibitory</td>
</tr>
<tr>
<td>Ly-49D</td>
<td>H2k, H2d</td>
<td>Activation</td>
</tr>
<tr>
<td>Ly-49E</td>
<td>H2r</td>
<td>Inhibitory</td>
</tr>
<tr>
<td>Ly-49F</td>
<td>H2d</td>
<td>Inhibitory</td>
</tr>
<tr>
<td>Ly-49G2</td>
<td>H2d</td>
<td>Inhibitory</td>
</tr>
<tr>
<td>Ly-49H</td>
<td>m157</td>
<td>Activation</td>
</tr>
<tr>
<td>Ly-49I</td>
<td>H2b,d,r,s,v</td>
<td>Inhibitory</td>
</tr>
</tbody>
</table>
Activating isoforms in KIR, lacking ITIM, associate with the ITAM-bearing DAP-12 adaptor protein. Simultaneous expression of activating and inhibitory KIRs that recognize different HLA-class II ligands would provide signal for elimination of cells that had lost the expression of ligands for the inhibitory receptor, but retained expression of the ligands for the activating receptor. Xenocompatibility (NK cell receptors of one species functionally recognize MHC class I ligands of other) has been detected in chimpanzee and human. The KIR families are divergent, with only three conserved between chimpanzee and humans. The receptors for polymorphic class I being divergent, whereas those for non-polymorphic class I are conserved. Although chimpanzee and human NK cells exhibit identical receptor specificities for MHC-C, they are mediated by non-orthologous KIR. This evolutionary progress demonstrates the rapid evolution of NK cell receptor systems and implies that “catching up” with class I is not the only force driving this evolution.

Mouse NK cell gp49 (type I) is the first example of an Ig-related molecule with the ability to inhibit mouse NK cell activity and is closely related to a newly identified human gene than it is to KIR, indicating that gp49 and KIR are not immediate homologs gp49A and gp49B cloned from murine mast cells. The identification of gp49 as a receptor with inhibition potential in mast cells and NK cells suggests that inhibition may be a more wide spread means of regulation in the lymphoid and myeloid system than was previously recognized.

All NK cells do not express all the inhibitory receptors, but it has been suggested that all NK cells must express at least one of the inhibitory receptors to prevent the killing of autologous cells. This interaction between the MHC and its receptor would bring about the shutdown of the NK cells.

**In vivo function of NK Receptors**

As the NK receptor biology is in progress, its relation to the pathological conditions is proving its significance; one of the severe disorders, X-linked lymphoproliferative disease (XLP) is characterized by abnormal immune responses to EBV (Epstein barr virus). After exposure to EBV, XLP males either succumb to fulminant infectious mononucleosis or develop lymphoma, hypo or dys-gammaglobulinemia, and or histiocytic infiltration leading to marrow aplasia. This disease is associated with the altered function of 2B4 molecule, owing to the lack of SH2D1A/Sap, which is an intracytoplasmic molecule.

In bone marrow transplantation, elimination of parental bone marrow cells by subsets of NK cells due to lack of inhibitory receptor for parental H-2 antigen proves its involvement in hybrid histocompatibility phenomenon. Recent clinical data suggest that mismatch of NK receptors and ligands during allogeneic bone marrow transplantation may be used to prevent leukemia relapse.

In placental decidual tissue, the number of NK cells have been found to be more than T cells, which is the site of exposure to alloantigen. The population of NK cells in the decidual tissue differs from those in circulation with respect to the repertoire of NK cell receptors on these cells. The biological significance of NK cells in placenta is an intriguing issue, which may redefine the maternal-fetal interactions. However, the most important role of NK cells is the host defense in antiviral and cancer immunity.

In conclusion, NK cells have shown great promise for the immunotherapy of cancer for several decades. However, till now only self-effacing clinical success has been achieved by manipulating the NK cell compartment in patients with malignancies. Progress in the field of NK receptors has revolutionized our concept of how NK cells selectively recognize and lyse tumours and virally infected cells while sparing normal cells. Major families of cell surface receptors that inhibit and activate NK cells to lyse target cells have been characterized, including KIR, NCR, and C-type lectins. Further identification of NK receptor ligands and their expression on normal and transformed cells is needed to begin development of rational clinical approaches to manipulate receptor/ligand interaction for clinical benefits. Identification of diverse receptors regulating the NK cell function may help us to understand its physiology, thereby offering novel therapeutic tools.

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