Inhibitory receptor mediated regulation of NK cells

Elisenda Alari-Pahissa, Camille Grandclément, Beena Jeevan-Raj

and Werner Held*

Ludwig Center for Cancer Research, Department of Oncology, University of Lausanne, Epalinges, Switzerland

*Correspondence: Werner Held, Ludwig Center for Cancer Research, Department of Oncology, University of Lausanne, Ch. des Boveresses 155, 1066 Epalinges, Switzerland; e-mail: Werner.Held@unil.ch
**ABSTRACT**

Natural Killer (NK) cells are capable of directly recognizing pathogens, pathogen-infected cells as well as transformed cells. NK cells recognize target cells using approximately one hundred germ-line encoded receptors, which display activating or inhibitory function. NK cell activation usually requires the engagement of more than one receptor and these may contribute distinct signaling inputs that are required for the firm adhesion of NK cells to target cells, polarization and the release of cytotoxic granules as well as the production of cytokines. Here we will discuss receptor-mediated mechanisms that counteract NK cell activation. We will first summarize the distinct intracellular inhibitory signaling pathways and how they can dominantly interfere with NK cell activation signaling events. In addition we will discuss mechanisms by which inhibitory receptors modulate cellular activation at the level of receptor-ligand interactions. Receptor-mediated inhibition of NK cell function serves three main purposes: Ensuring tolerance of NK cells to normal cells, enabling NK cell responses to aberrant host cells that have lost inhibitory ligand and finally allowing the recognition of certain pathogens that do not express inhibitory ligands.
Keywords:
Inhibitory receptor, ITIM, SHP-1, Vav1, ITSM, SHIP-1, competitive ligand binding, self tolerance, missing-self recognition

Abbreviations:
ITAM: Immunoreceptor Tyrosine-based Activating Motif; ITIM: Immunoreceptor Tyrosine-based Inhibition Motif; ITSM: Immunoreceptor Tyrosine-based Switch Motif; KIR: Killer Immunoglobulin-like Receptor; LFA-1: Leucocyte Function-associated Antigen-1; NK: Natural Killer; MHC: Major Histocompatibility Complex; SHIP: SH2 domain containing inositol phosphatase 1; SHP-1: SH2 domain containing protein phosphatase 1; SLAM: Signaling Lymphocytic Activation Molecule; SAP: SLAM-associated protein; TAM receptors: TYRO3, AXL and MER receptors.
INTRODUCTION

NK cells are a lymphocyte subpopulation capable of directly recognizing pathogens as well as pathogen-infected cells. In addition, NK cells have long been known to recognize transformed cells and they are increasingly recognized for their immune regulatory roles. NK cells can recognize their targets independent of somatically rearranged antigen-receptors and use around one hundred germ-line encoded receptors, which display activating or inhibitory function (for review see 5,6). While activating receptors are usually expressed on all NK cells, inhibitory receptors often show restricted expression patterns, which creates significant NK cell diversity. Further, NK cells acquire an effector cell program already in their tissue of origin. Bone marrow NK cells constitutively express high levels of Perforin, Granzyme B and IFNγ mRNAs that are, however, not translated into protein. The trigger for the acquisition of this effector program is not known, but IL-17 signaling may play a role.

Subsequent to these developmentally programmed events, NK cells undergo significant phenotypic and functional changes based on environmental cues. Signaling by activation receptors becomes more efficient when NK cells recognize inherited Major Histocompatibility Complex (MHC) class I molecules (licensing) and they undergo further maturation depending on the presence of other cell types. The effector capacity of NK cells is critically impacted by “priming” i.e. exposure to inflammation-induced cytokines, such as IL-15 in the case of viral or IL-12 during bacterial infection. Priming leads to the translation of preformed Perforin and Granzyme B mRNA, enhances IFNγ production, can induce the expression of additional NK cell receptors (NKp44) and renders signaling by activation receptors more effective. The firm adhesion to prospective target cells, the exocytosis of lytic
granules and the production and release of cytokines are then tightly controlled by signals received via inhibitory and activating NK cell receptors. The generally accepted view holds that activating and inhibition signals are integrated and that an excess of activating signals results in an effector response, which includes the release of lytic granules and inflammatory cytokines. A final adaptive process based on the stimulation of NK cells (e.g. by viral ligand such as MCMV m157) is the acquisition of memory-like features, which include increased longevity and improved effector capacities of NK cells.15

1. NK cell activation receptors and signaling

In order to discuss the importance and the mode of action of inhibitory receptors, we will first briefly summarize the various signaling pathways implicated in NK cell activation.

Receptors associated with membrane bound signaling adaptors

Similar to antigen receptors, multiple activation receptors expressed by NK cells depend on non-covalently associated, membrane anchored adaptors for signal transduction. Some of these adaptors (DAP12, FcRγ CD3ζ) contain one or three ITAMs (Immunoreceptor Tyrosine-based Activating Motif). NK cell receptors associated with DAP12 include human NKp44 and mouse NKG2D. Those associated with FcRγ include mouse CD16 and NK1.1, while human CD16 or NKp30 are associated with CD3ζ FcRγ. Receptor engagement activates Src family tyrosine kinases (including Lck and Fyn), which phosphorylate ITAMs in NK cells and allow the recruitment of the non-receptor tyrosine kinases Syk and Zap70. Subsequently, both trans membrane (Linker of activated T cells (LAT) and
Non-T cell activation linker (NTAL)) and cytosolic adaptors (SLP-76) form platforms for the activation of downstream molecules (such as phospholipase C (PLC)-γ1 and -γ2, Vav2, Vav3 and Ras-family members) and signaling pathways (such as the mitogen-activated protein kinases (MAPK), c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and p38) that trigger NK cell mediated cytotoxicity and cytokine production.6

In addition to ITAM bearing adaptors, NK cells also express DAP10, a homodimeric membrane-anchored signaling adaptor with an YxxM motif (in single-letter amino acid code, with x indicating non-conserved positions). DAP10 is associated with NKG2D or murine Ly49D and Ly49H. Similar to Ly49D and H, a short NKG2D isoform, which is only present in activated mouse NK cells, can pair with either DAP10 or DAP12. The DAP-10 YxxM motif is also present in receptors that co-stimulate T cells, such CD28 or ICOS. Phosphorylated DAP10 recruits the p85 subunit of phosphoinositide 3-kinase (PI3K) and the small adaptor Grb2 in association with Vav1.16

**NK cell activation independent of membrane bound signaling adaptors**

NK cells express additional activating cell surface receptors that function independent of an association with membrane-anchored adaptors. SLAM (Signaling Lymphocytic Activation Molecule) family receptors activate NK cells using semi-conserved S/T/I/VYxxV/I motifs (termed) present in the cytoplasmic portion of the receptor. The best understood receptor is 2B4 (CD244) but the function of other SLAM family receptors seems to be regulated in similar ways.17 Upon ligand binding and phosphorylation, the ITSM associates with small cytoplasmic adaptors such as SAP (SLAM-associated protein (SAP, also termed SH2D1A), EAT-2 and ERT. SAP
mediates activating function by coupling SLAM family receptors to the Src tyrosine kinase Fyn, which links SAP to the exchange factor Vav1. SAP-Fyn contributes to the formation of conjugates between NK cells and target cells.\textsuperscript{18} EAT-2 does not promote conjugate formation but accelerates polarization and exocytosis of cytotoxic granules,\textsuperscript{19} providing an explanation why both adaptors are needed for normal NK cell effector function. However, as detailed below, in certain cases SLAMs can act as inhibitory receptors. Additional receptors, including CD44, CD137, the TNF receptor family member CD27, CD160 and DNAM-1 contribute to NK cell activation signaling. However, the signaling events induced by these receptors are not well defined.

**Leucocyte Function-associated Antigen-1 (LFA-1)**

The $\beta_2$ integrin LFA-1 (CD11a/CD18) plays a key role for NK cell function by mediating adhesion to target cells. To do so, LFA-1 must first undergo a conformational change to achieve a high-affinity binding state. In NK cells, LFA-1 activation occurs by inside out signals from co-activating receptors such as NKG2D, DNAM-1 or 2B4,\textsuperscript{20} which then allows firm adhesion. Furthermore, in NK cells LFA-1 has the ability to signal on its own and to function as a co-activating receptor. The engagement of LFA-1 by its ligand ICAM-1 on target cells is sufficient to polarize (but not release) lytic granules in human NK cells.\textsuperscript{21} Ligation of LFA-1 leads to phosphorylation and activation of Vav1 and the kinase Pyk2, which together with the Wiskott-Aldrich syndrome protein (WASp) regulate actin polymerization.\textsuperscript{22} While binding of ICAM-1-coated beads to mouse NK cells is also sufficient to induce reorganization of the actin cytoskeleton, lytic granules do not polarize.\textsuperscript{23} Thus
increased adhesive function of LFA-1 is an additional feature common to productive NK cell activation.

**Synergy and integration of NK cell activation signaling**

NK cell receptors have been classified into activation and co-activation receptors depending on whether an individual receptor is sufficient to trigger an effector response or whether receptor co-engagement is required. Based on stimulations of resting human NK cells using natural ligands, only CD16 qualified as an activation receptor. For all other receptors, pairwise engagement was needed to induce target cell lysis.\(^\text{24}\) A common consequence of the engagement of activating NK cell receptors is the phosphorylation of Vav family members. ITAM containing adaptors activate Vav2 and Vav3, while the YxxM containing adaptor DAP10 \(^\text{25}\) and switch motifs activate Vav1.\(^\text{18}\) Activation of Vav-1 is negatively regulated by the E3 ubiquitin ligase c-Cbl and engagement of a single coactivation receptor is not sufficient to the inhibitory effect.\(^\text{26}\) The basis for synergy is that co-engagement of receptors (e.g. human 2B4 and NKG2D) phosphorylates two distinct tyrosine residues (Y113 and Y128) in the adaptor protein SLP76. Indeed, receptor combinations that do not synergize phosphorylate only one of the two tyrosines.\(^\text{27}\) Through the phosphorylation of two tyrosines, SLP76 integrates signals from distinct co-activation receptors and this is essential for Vav1 activation.\(^\text{26}\)

Although a corresponding analysis of murine NK cells is currently lacking, based on antibody-mediated stimulation, several receptors, including NK1.1, NKG2D, NKp46, Ly49H and CD16 can be considered activating in NK cells from naive mice.\(^\text{10}\) Species-specific differences in adaptor usage and/or signal transduction may also contribute to this difference. Irrespective of this, the need for receptor co-engagement
to activate NK cells is reduced when NK cells have been primed in vivo or exposed to cytokines in vitro.\textsuperscript{14}

2. Receptor mediated inhibition of NK cell function

Inhibitory receptors specific for MHC class I molecules

The existence of inhibitory receptors was predicted based on negative effects of MHC class I molecules expressed by target cells on NK cell effector responses. Inhibitory receptors specific for classical MHC class I molecules expressed by human NK cells belong to the Killer Immunoglobulin-like Receptor (KIR) family. In addition, human NK cells express Leukocyte Immunoglobulin-like receptor B2 (LILRB1 also termed LIR-1), a pan MHC class I receptor and CD94/NKG2A, a receptor for non-classical HLA-E. Mouse NK cells also express CD94/NKG2A, which is specific for Qa-1b (the orthologue of human HLA-E), as well as Ly49 family receptors, which recognize classical and certain non-classical MHC class I molecules, such as H2-M3.\textsuperscript{28} In addition, NK cells express a significant number of additional inhibitory receptors, which recognize non-MHC class I ligands such as Sialic acid side chains (SIGLEC7 and 9 in humans and SIGLEC-E in mice), CD155 (TIGIT), Cadherins (KLRG1) and Ceacam1 (homophilic). In general, these receptors prevent NK cell mediated damage of “normal-self” tissues and allow responses to aberrant cells that lack relevant markers of self.

A standard model of receptor mediated inhibition

Inhibitory receptors block NK cell function by interfering with activation signals. They are thought to act locally and block only signals from activation receptors with which they are co-aggregated. An illustration of this notion is that the proper
integration of activating and inhibitory receptor signals depends on the receptor-ligand complexes to have similar dimensions. Indeed, activation and inhibitory ligand pairs co-localize when their respective sizes match, whereas they are segregated when their sizes differ.\textsuperscript{29,30} In addition, despite the diversity of early activating signaling pathways and events, inhibitory receptors interfere globally with NK cell activation.

Typical receptors with inhibitory function are characterized by the presence of conserved I/VxYxxL/V motifs (Immunoreceptor Tyrosine-based Inhibition Motif (ITIM)) in the cytoplasmic portion of the receptor. MHC class I receptor complexes contain two ITIMs, either in a tandem arrangement in monomeric KIRs or a single ITIM per subunit in homodimeric Ly49 receptors. Receptor engagement leads to ITIM phosphorylation by Src family kinases, including Lyn or Lck.\textsuperscript{31} Phosphorylated ITIMs recruit and activate the tandem SH2 protein phosphatases SHP-1 and SHP-2, whereby SHP-1 plays the essential role for the inhibitory function of MHC class I receptors.\textsuperscript{32} A key substrate of SHP-1 downstream of inhibitory KIR is Vav1\textsuperscript{33} (Fig. 1A). As discussed above Vav1 is phosphorylated by NK cell activation and Vav1 dephosphorylation prevents the formation of downstream c-Cbl, Crk, p130CAS, C3G complexes,\textsuperscript{34} which have essential roles in inducing cell adhesion and cytolysis. If Vav2 and Vav3 proteins were also ITIM-SHP-1 targets, this would explain how inhibitory receptors could interfere globally with NK cell activation pathways. However this remains to be shown.

Available evidence suggests that inhibitory signals block NK cell activation upstream of LFA-1 function and upstream of actin-dependent signals required for efficient receptor clustering and signaling. As mentioned above, signals delivered by co-activation receptors are needed to improve LFA-1-dependent adhesion of resting NK cells to target cells.\textsuperscript{18} Such inside-out signals are blocked when the inhibitory CD94-
NKG2A receptor is co-engaged.\(^{35}\) In addition, imaging analyses show that the engagement of inhibitory MHC class I receptors leads to a rapid formation of microclusters, which prevents microclustering of activating receptors.\(^{36}\) Moreover, inhibitory signaling efficiently prevents the polarization of lytic granules.\(^{37}\) Thus, rather than allowing activation signals to be fully established before acting on them, inhibitory receptors prevent activation signals from being propagated.

**An alternative mechanism of ITIM-dependent inhibition**

The analysis of biochemical events following target cell encounter by NK cells unexpectedly revealed that inhibitory receptor engagement not only prevents but also triggers unique signaling events, which are not detected when only activation signals are transduced. KIR or CD94/NKG2A engagement activated the tyrosine kinase c-Abl, which lead to the phosphorylation of Crk.\(^{34}\) Further experiments showed that phosphorylated Crk is present at inhibitory, but not activating synapses, and that Crk phosphorylation is needed for NK cell inhibition (Fig. 1B). This is explained by a role for unphosphorylated Crk in the clustering and the signaling by activating NK cell receptors such as CD16.\(^{38}\) Even more surprisingly, counter to an assumed need for co-aggregation with activation receptors, engagement of CD94-NKG2A by HLA-E alone was sufficient to induce Crk phosphorylation,\(^{38}\) suggesting that at least CD94/NKG2A receptor complexes can exert autonomous signaling function.

**Inhibition via immune receptor tyrosine based switch motifs (ITSM)**

Inhibitory receptor signaling can also occur in the absence of a consensus ITIM. Selected examples of such receptors and their mode of action are discussed below. Even though SLAM family receptors, including 2B4, have been introduced as
activating receptors, under certain circumstances these receptors can exert inhibitory roles. For example, in NK cells from patients with X-linked lympho proliferative (XLP) disease, 2B4 functions as an inhibitory receptor. XLP patients have inactivating mutations in the SAP-encoding SH2D1A gene. In the absence of SAP, SLAM receptors interact with SHP-1, SHP-2, and SHIP-1 (SH2 domain containing inositol phosphatase 1). Functional experiments using a SAP-deficient B cell line, showed that 2B4-dependent inhibition of BCR signaling was mediated by SHIP-1 (Fig. 1C). While 2B4 engagement induced SHIP-1 phosphorylation, it is unclear how 2B4 recruits SHIP-1. Once activated by phosphorylation and recruited to the plasma membrane, SHIP-1 hydrolyzes the 5' phosphate of phosphatidylinositol (3,4,5)-triphosphate (PIP₃), and thus prevents the activation of AKT and PLCγ. While it is clear that SLAMs have inhibitory function when SH2D1A is mutated, an intriguing unresolved question is how SLAMs receptors can act as inhibitory receptors when NK cells express SAP. According to one model, the inhibitory versus activating function of 2B4 in NK cells is regulated by the expression levels of 2B4, the availability of intracellular SAP and the density of CD48 ligand.

Another prominent example of a receptor using an ITSM for cellular inhibition is PD1. This receptor is known for its role in limiting T cell mediated damage in situations of chronic stimulation and its presence is a hallmark feature of exhausted T cells. PD-1 is not expressed on normal NK cells but is induced by chronic stimulation such as the presence of multiple myeloma or Hepatitis C Virus infection. The cytoplasmic tail of PD-1 contains both an ITIM and an ITSM, whereby the latter is required for PD-1-mediated inhibition. PD-1 engagement does not recruit SAP but significantly enhances the recruitment of SHP-2. Further, proximity of PD-1 to the T cell receptor is essential for the inhibitory function, which chiefly impacts PI3K/Akt
activity.\textsuperscript{45} Thus, ITSM-mediated inhibition is mediated via more than one pathway. It remains to be seen whether distinct types of ITSM-mediated inhibition are operative in NK cells.

**ITIM and switch motif independent NK cell inhibition**

There is also emerging evidence for inhibitory effects that operate independent of ITIM and ITSM motifs. A recent study reported a novel Cbl-b/TAM (TYRO3, AXL and MER) receptor-dependent inhibitory pathway for NK cell activation.\textsuperscript{46} Engagement of TAM receptor tyrosine kinases with their endogenous ligand Gas6 suppressed proliferation and IFN-\(\gamma\) production by NK cells induced by the NKG2D receptor. Gas6 induced the recruitment of the ubiquitin ligase Cbl-b to the cytoplasmic domain of TAM receptors, which lead TAM ubiquitination and suppression of NK cell activation by NKG2D and other receptors (Fig. 1D). Indeed, \(Cbl-b^{-/-}\) NK cells were resistant to negative regulation of NK cells by Gas6, demonstrating that Cbl-b acts downstream of TAM receptors. A possible developmental basis for these defects could be excluded, since a small-molecule TAM kinase inhibitor readily abolished the inhibitory effect of Gas6, and enhanced NK cell cytotoxicity towards cancer cells.\textsuperscript{46} How the inhibitory TAM/Cbl-b pathway counteracts the function of NK cell activation receptors remains to be determined.

**3. Modification of NK cell activation at the level of receptor-ligand interactions**

**Inhibitory ligands expressed in \textit{trans} and \textit{cis}**

Inhibitory receptors strongly counteract effector responses when interacting with ligands present on opposing cell membranes (\textit{trans} interaction). However, some inhibitory receptors can also interact with ligand present on the NK cell’s own cell
membrane (cis interaction). This was first described for MHC class I-specific Ly49 receptors in the mouse. A considerable fraction of inhibitory Ly49 receptors is constitutively associated with MHC class I in cis. These cis complexes appear relatively stable and are not thought to contribute to inhibitory signaling. Thus, cis binding effectively reduces the availability of inhibitory receptors to bind MHC class I on target cells. Consequently, the function of inhibitory receptors is reduced, which lowers the threshold for NK cell activation (Fig. 2A). Cis/trans binding modes have been reported for additional inhibitory NK cell receptors including human LILRB1 and Siglecs.

**Competition between activating and inhibitory receptors**

Activation can also be reduced based on a competition between activating and inhibitory receptors for the same ligand expressed on other cells. The most prominent model is the competitive inhibition of CD28 activation by CTLA-4. Here, the co-stimulatory CD28 and the co-inhibitory CTLA-4 expressed by T cells compete for CD80/CD86 on antigen presenting cells (APC). Inhibition is based on the up regulation of CTLA-4 upon (chronic) T cell activation and on the fact that the affinity of CTLA-4 for CD80/CD86 exceeds that of the co-stimulatory CD28. CD28 and CTLA-4 expression is also observed on NK cells, at least in the mouse and following in vitro culture in IL-2. NK cell activation by plate-bound CD80 induced IFNγ production, which was strongly reduced when NK cells lacked CD28. Conversely, IFNγ production was significantly increased when NK cells lacked CTLA-4, indicating that CD28 and CTLA-4 have opposing roles also in NK cells. Thus, competitive effects, similar to the ones operating in T cells, may directly reduce
NK cell activation. However, the precise contribution of competition and signaling to the inhibitory function of CTLA-4 remains to be addressed, not only in NK cells.

Similar to the situation described above, NK cells co-express the co-activation receptor DNAM-1 (CD226) and the ITIM-bearing TACTILE (CD96) that both bind CD155. Since CD96 has a higher affinity for CD155 as compared to the activating DNAM-1,$^{51}$ DNAM-1 function is likely reduced due to competition for CD155 binding $^{52}$ (Fig. 2B). However, since CD96 can deliver inhibitory signals, the relative contribution of competition and signaling remains to be determined. One additional aspect of CD96 function may be worth mentioning. CD96 engagement reduced cytokine production but not killing.$^{51}$ This is similar to the absence of CD45, a membrane-anchored protein tyrosine phosphatase, which blunted cytokine production but had a minor effect on cytolytic activity. Selective effects of inhibitory receptors on a subset of effector functions may be explained by a lower activation threshold for cytotoxicity as compared to IFN$\gamma$ production.$^{53}$

4. Non-conventional roles of inhibitory NK cell receptors

The response of activation receptors to stimulation can vary significantly between individual NK cells from the same donor. NK cells, which can recognize MHC class I, respond efficiently to stimulation, while NK cells, which cannot recognize MHC class I respond poorly.$^{10,54,55}$ The improved responsiveness of NK cell activation receptors, which depends on ITIM-dependent signals from MHC class I receptors, is referred to as “licensing”.$^{10}$ The molecular mechanism for licensing is still debated. One possible mechanism, referred to as “disarming”, is that activation receptors become by default responsive to stimulation at some point during NK cell development. ITIM-dependent inhibitory signals would neutralize activation signaling
and this would keep the activation pathway competent to respond to subsequent stimulation. If NK cells cannot bind MHC class I, persistent stimulation would eventually render activation pathways non-functional.\textsuperscript{56} An alternative “arming” scenario proposes that NK cell activation receptors are by default poorly responsive to stimulation and signals from MHC class I receptors are needed to render activation receptors competent to signal.\textsuperscript{56} Here, inhibitory receptors and consequently ITIM-dependent signals instruct NK cells. The finding that CD94-NKG2A engagement can induce the activity of c-Abl and phosphorylate Crk\textsuperscript{38} is consistent in principle with such a scenario. Irrespective of the mechanism, the improved functionality of activation receptors is essential for efficient NK cell responses to cells that lack MHC class I expression.

**Perspectives**

The mode of action of inhibitory receptors is still incompletely understood. This applies both to the understanding of intracellular signaling events as well as the relative importance of competitive inhibition of cellular activation. Further, it was thought that inhibitory receptors act globally but recent work has raised the possibility that some inhibitory receptors may act on specific activation pathways and selected effector functions. In addition, there is some evidence that receptors identified based on their inhibitory function can perform additional roles. Examples include NK cell licensing, a possible co-stimulatory role of KIR in CD4 T cells\textsuperscript{57} and evidence that inhibitory receptors improve NK cell survival.\textsuperscript{58,59} The central role of these inhibitory pathways is to ensure self-tolerance i.e. to prevent NK cell responses to cells that normally express specific markers of “self”, such as MHC class I molecules, thereby allowing responses to aberrant self cells that have lost relevant markers of self.
Moreover, recent data raise the possibility that inhibitory receptors play a role for the detection of “non-self” cells. NK cells can kill Cryptococcus and Candida based on NKp30-dependent activation\(^1\). It seems reasonable to assume that NKp30-mediated NK cell activation can proceed since the fungal cells lack MHC class I molecules and likely other inhibitory ligands characteristic of human cells. Since NK cells exert protective roles in other fungal infections, absence of receptor-mediated inhibition may be of more general relevance for the recognition of pathogens by NK cells.

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References


Figure Legends

Fig. 1. Receptor-mediated activating and inhibitory signaling

A  Signaling by the activating NKG2D-DAP-10 receptor complex (via YxxM motifs) leads to the phosphorylation of Vav1, which is essential for firm adhesion to target cells and the execution of an effector response. Co-engagement of inhibitory KIR (Killer Immunoglobulin-like receptors) leads to the phosphorylation of ITIMs (ITIM, Immunoreceptor tyrosine-based inhibition motif), which mediate inhibitory signaling by recruiting the protein tyrosine phosphatase SHP-1. Activated SHP-1 dephosphorylates and thus inactivates Vav1.

B  Signaling by the activating CD16-CD3ζ receptor complex (via ITAMs) depends on the adaptor protein Crk. Inhibitory KIR activates the c-Abl tyrosine kinase, which phosphorylates and consequently inactivates Crk.

C  In the absence of the cytosolic adaptor SAP (SLAM-associated protein), the ITSM (Immunoreceptor tyrosine-based switch motif) present in 2B4 can initiate inhibitory signaling by recruiting (directly or indirectly) SHIP-1, an inositol phosphatase. SHIP hydrolyzes the 5' phosphate from phosphatidylinositol (3,4,5)-triphosphate (PIP3), thus preventing the activation of AKT and PLCγ.

D  The engagement of TAM receptors (TYRO3, AXL and MER) induces the recruitment of Cbl-b and the ubiquitinylation (ubi) of the protein tyrosine kinase (PTK) domain of TAM. This suppresses NK cell activation by NKG2D receptors by unknown mechanisms.
Fig. 2. Modification of NK cell activation at the level of receptor-ligand interactions

A  A significant fraction of inhibitory Ly49 receptors is constitutively associated with MHC class I molecules in the plane of the NK cells’ membrane (in cis). These cis complexes do not contribute to inhibitory signaling. Thus, cis binding reduces the abundance of inhibitory receptors available to bind MHC class I on target cells, thereby lowering the threshold for NK cell activation. The absence of cis binding increases the threshold for NK cell activation.

B  NK cells are activated by the interaction of the DNAM-1 (CD226) receptor with CD155 ligand, which is counteracted by competitive binding of inhibitory TACTILE (CD96) to CD155. The activating receptor is disfavored due to a lower affinity for CD155 (indicated by a faint arrow) as compared to TACTILE (CD96). In addition, signaling likely contributes to the inhibitory effects.
Fig. 1

A  NKG2D  KIR  
   ↓
   DAP-10  
   ↓
   Vav1  
   ↓
   Adhesion Kill

B  CD16  KIR  
   ↓
   SHIP  
   ↓
   SHIP  
   ↓
   Akt

C  2B4  
   ↓
   PIP  
   ↓
   PiP  
   ↓
   NKG2D activation

D  TAM  
   ↓
   Ubi

- ITAM  - YxxM  - ITIM  - ITSM  - PTK

Fig. 2

A  Target cell  
   MHC-I
   ↓
   Ly49
   Act  Inhib

B  CD155  
   CD155  
   DNAM-1 (CD226)
   Act  Inhib