IMMUNE FUNCTIONS ENCODED BY THE NATURAL KILLER GENE COMPLEX

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There has been marked progress in our understanding of the role of natural killer (NK) cells in immune responses, mainly due to the identification of NK-cell receptors and their ligands. The genes encoding many NK-cell receptors are located in the NK-gene complex (NKC). Here, we review the properties of NKC-encoded receptors, and provide a genomic and conceptual framework for an insight into NK-cell function and biology.

NATURAL KILLER CELLS (NK cells). Lymphocytes that do not express the T-cell receptor or B-cell receptor and mediate natural killing against prototypical NK-cell-sensitive targets — K562 (human) and YAC-1 (mouse). In humans, NK cells are typically $CD56^+CD3^-$, and they are $NK1.1^+CD3^-$ in the C57BL/6 mouse strain and generally $DX5^+CD3^-$ in other mouse strains. Recent studies indicate that NKG2D might be a useful marker for all $CD3^-$ NK cells in various mouse strains.

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NATURAL KILLER CELLS (NK cells) were initially described on the basis of their 'natural' capacity to kill certain tumour cells *in vitro*, a process known as 'natural killing'[1]. This function does not require host sensitization to the tumour cell and its discovery led to studies of NK-cell activities in tumour surveillance. However, NK cells are also crucial for innate host defence against pathogens, in particular against viral infections. Importantly, NK cells use the same mechanisms to resist tumours and infections.

Many membrane proteins, such as CD2, 2B4, CD11a–CD18 and CD69, can induce or modulate NK-cell cytotoxicity, and cytokines can enhance NK-cell killing. Orchestrated interactions between these molecules contribute to the natural killing of target cells (BOX 1). Despite these complexities, it is useful to categorize NK-cell receptors into two broad categories of inhibitory and activating receptors (FIG. 1).

The susceptibility of tumour targets to natural killing is inversely related to target-cell expression of MHC class I molecules, as determined by crucial studies carried out by Kärre and colleagues[2]. This observation formed the basis for the 'missing-self' hypothesis, which proposes that NK cells survey tissues for expression of MHC class I molecules. In the absence of otherwise ubiquitously expressed MHC class I molecules ('missing self'), NK cells are released from the negative influence of MHC class I molecules and kill the target. 'Missing self' is now explained by the expression of NK-cell inhibitory receptors specific for MHC class I molecules. These receptors include the human killer-cell immunoglobulin-like receptors (KIRs), the rodent Ly49 receptors and the human and rodent CD94–NKG2 molecules[3–7].

In general, the effect of the MHC class-I-specific inhibitory receptors dominates over NK-cell activating receptors. Inhibitory receptors mediate their effect through an IMMUNORECEPTOR TYROSINE-BASED INHIBITORY MOTIF (ITIM) in their cytoplasmic domains[3]. After ligand binding, the ITIM becomes tyrosine phosphorylated by a SRC-family tyrosine kinase, which then recruits and activates SH2-domain-containing protein tyrosine phosphatase 1 (SHP1) and potentially other phosphatases, such as SHP2. Recent studies also implicate a role for SH2-domain-containing inositol polyphosphate 5′ phosphatase (SHIP1), which can also bind to ITIMs[8]. Although the specific targets of the activated phosphatases are as yet unclear, they presumably inhibit NK-cell functions by interrupting the early phosphorylation pathways that are responsible for NK-cell activation — that is, the inhibitory receptors block stimulation by NK-cell activating receptors.

Many NK-cell activating receptors have extracellular domains that are similar to those of the inhibitory receptors but lack the intracellular consensus ITIM[9,10]. Instead, they generally have charged transmembrane residues that facilitate association with signalling chains that are often required for optimal surface expression. On NK cells, these signalling chains include IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIF (ITAM)-containing molecules, such as DNAX-activating protein

Box 1 | **Studies of inhibitory and activating receptors**

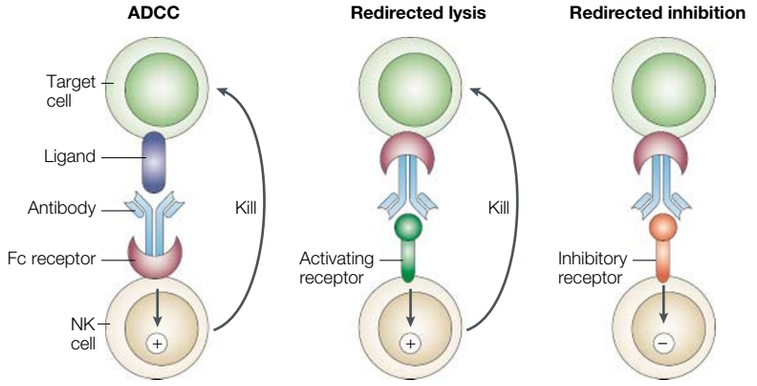
Inhibitory receptors contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic domain, which recruit intracellular phosphatases, such as SH2-domain-containing protein tyrosine phosphatase 1 (SHP1) and also SHP2 and SH2-domain-containing inositol polyphosphate 5′ phosphatase (SHIP). Their inhibitory-receptor function can be demonstrated by several approaches: target cells that express their ligand (either constitutively or by transfection) are killed less well by natural killer (NK) cells that express the specific receptor, whereas target cells remain susceptible to lysis by NK cells that do not display the receptor. Inhibited NK cells might be capable of killing when soluble monoclonal antibodies specific for either the receptor or the ligand are added to the killing assay. If a monoclonal antibody specific for the ligand is added, $F(ab')_2$ fragments should be used to avoid inadvertent stimulation of the NK cell through engagement of the Fc receptor on the NK cell by antibodies coating the target cell. This phenomenon is known as antibody-dependent cellular cytotoxicity (ADCC).

Some non-susceptible targets that express Fc receptors might be killed by 'redirected lysis' or 'reverse ADCC' with use of a monoclonal antibody specific for an activating receptor. In this case, the Fc receptor on the target cell is required presumably for bridging and crosslinking effects. Redirected lysis does not occur with $F(ab')_2$ fragments. By contrast, some susceptible Fc-receptor-expressing targets might be prevented from killing by a monoclonal antibody specific for an inhibitory receptor, a phenomenon known as 'redirected inhibition'.

Alternatively, a monoclonal antibody specific for an activating receptor could be immobilized on plastic and trigger NK-cell release of cytokines or granule components. Of course, if the ligand is known, it can be transfected into otherwise non-susceptible targets and trigger cytolysis. The monoclonal antibody specific for an activating receptor might block this effect if the target does not express an Fc receptor.

Crosslinking of co-stimulatory receptors, such as NKG2D, alone might not trigger the NK cell, but these receptors might markedly enhance NK-cell activation through primary activating receptors.

Finally, it is important to note that an individual NK cell might simultaneously express many, different activating, inhibitory and co-stimulatory receptors.



of 12 kDa (DAP12), also known as killer activating receptor associated protein (KARAP)[9,11], FcεRIγ[12] and CD3ζ. Receptor crosslinking leads to tyrosine phosphorylation of the ITAM on the signalling chain and downstream events that culminate in cytotoxicity and cytokine production. The details of these NK-cell activating and inhibitory pathways are the topics of active investigation (FIG. 1).

Most activating and inhibitory NK-cell receptors are encoded by genes in two genomic regions, the NK-gene complex (NKC) and the leukocyte-receptor complex (LRC)[13]. Whereas molecules encoded in the LRC belong to the immunoglobulin superfamily, are expressed more broadly on a wide variety of haematopoietic cells and have been the topic of recent reviews[3–5], the NKC-encoded molecules have marked differences from LRC-encoded receptors and form the subject of this review. As will become clear, the NKC encodes many molecules with related structures and functions that are important for NK-cell activities.

IMMUNORECEPTOR TYROSINE-BASED INHIBITORY MOTIF (ITIM). This motif is found in the cytoplasmic domains of the inhibitory receptors. After ligand binding, the ITIM (Val/Ile-Xaa-Tyr-Xaa-Xaa-Leu/Val) becomes tyrosine phosphorylated, which recruits and activates phosphatases.

General features of NKC-encoded molecules

The cell-surface molecules NK-cell receptor protein 1 (Nkrp1) and Ly49 were among the first surface molecules to be recognized as being constitutively and selectively expressed by rodent NK cells, functionally active in NK-cell-mediated lysis[14,15] and encoded by genes located on distal mouse chromosome 6 (REFS 16,17). Both molecules have a type II transmembrane protein orientation, share sequence homology with C-type lectins and are expressed as disulphide-linked dimers[18–20]. Subsequent studies resulted in the identification of related human molecules, such as the CD94–NKG2 heterodimers[21] that are encoded in a locus on the SYNTENIC human chromosome 12p13 (REF. 22). It is now recognized that most of these genes are conserved across species, with ORTHOLOGOUS GENES having been identified in mouse, rat and human genomes (FIG. 2). These genetic regions are known as the NKC[6,17]. Whereas the NKC encodes a few molecules that do not seem to be important to NK cells (BOX 2), most molecules encoded by the NKC are receptors that are highly relevant to NK-cell function and have features that were first recognized for Nkrp1 and Ly49 molecules (FIG. 2).

Clusters of genes for lectin-like receptors

The lectin-like molecules can be classified into families, each containing molecules that are highly related, typically with >80% amino-acid identity. So far, gene maps indicate that each family of molecules is encoded by a physical cluster of related genes, separate and distinct from other clusters, with the exception of the intertwined Nkrp1 and Clr (C-lectin related) families. Between families, the molecules have related general structural features (BOX 3) but are only distantly related in sequence (25% amino-acid identity). This distant relatedness is similar to their supergene-family homology with true C-type lectins, such as mannose-binding protein (MBP).

The genomic structures of individual NKC-encoded genes are highly related[23], following a pattern observed for other type II transmembrane-oriented C-type lectin-like receptors (FIG. 3). Frequent transcript splice variants have been observed for some genes[24]. Although their physiological functions have not been determined, alternative spliced forms could be a mechanism for generating molecular diversity from a limited number of germline genes.

NKC-encoded receptors and their ligands

The NKC-encoded receptors expressed by NK cells that have been studied so far have either inhibitory or activating function. (With genome sequencing, database annotation has resulted in assignment of new designations, but most studies continue to use previously adopted names preferentially. For reference, see TABLE 1).

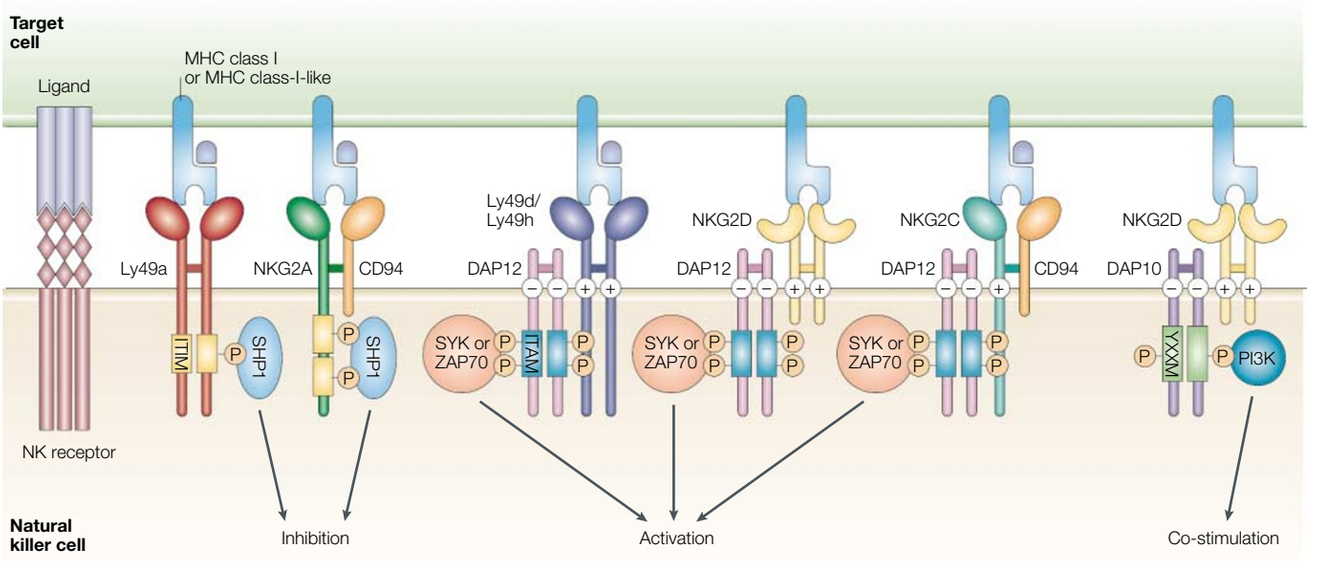


Figure 1 | **NK cells express three types of receptors: activating, inhibitory and co-stimulatory receptors.** After interaction with their target-cell ligands, the inhibitory receptors become tyrosine phosphorylated on their immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and associate with intracellular phosphatases, such as SH2-domain-containing protein tyrosine phosphatase 1 (SHP1), resulting in an inhibitory signal. The activating receptors are associated through positively-charged residues in their transmembrane domains to adaptor molecules, such as DNAX-activating protein of 12 kDa (DAP12). After ligand binding, immunoreceptor tyrosine-based activation motifs (ITAMs) present on the adaptor chain are tyrosine phosphorylated, resulting in cytotoxicity and cytokine production. Interestingly, natural-killer group 2D (NKG2D) molecules can associate with the DAP12 or DAP10 adaptor molecules. After ligand binding, NKG2D associated with a DAP10 molecule seems to deliver a co-stimulatory signal rather than an activating signal. The ligand-receptor interactions are schematically shown and do not reflect interactions as determined by crystallographic studies. NK, natural killer; PI3K, phosphatidylinositol 3-kinase; ZAP70, ζ-chain-associated protein 70 kDa.

However, most NKC-encoded molecules are orphan receptors — their physiological ligands or functions remain undefined or have not been directly determined. Nevertheless, as most belong to families of molecules, one or more of which have been better characterized, the function of the orphan receptors can be predicted.

Ly49 family. The best characterized mouse NKC family is the Ly49 family, of which at least 16 full-length genes and pseudogenes (*Ly49a* to *Ly49q*) have been identified by genomic sequencing in the C57BL/6 strain[23]. Mapping of genomic clones indicates that, except for *Ly49b* (the most distantly related member), their genes are located in a 620-kb cluster telomeric to *Cd69* (REFS 23,25). Marked allelic polymorphism has been noted by genomic analysis of the 129/svJ strain[26].

The prototypic Ly49 receptor, Ly49a, was the first MHC class-I-specific NK-cell inhibitory receptor to be defined at the molecular level[15]. When Ly49a specifically recognizes its ligands, H–2D^d, H–2D^k or H–2D^p, on targets, it delivers signals that prevent killing of the target. Although Ly49a does not have specificity for bound peptides, peptides must be bound in the MHC class I groove for recognition to occur[27]. Despite lectin homology, Ly49a recognizes a carbohydrate-independent epitope on its MHC class I ligand[28]. Similar to Ly49a, Ly49c and Ly49g2 contain cytoplasmic ITIMs and function as MHC class-I-specific inhibitory receptors, although Ly49g2 might have peptide selectivity[29–33]. Ly49e is notable for expression during fetal NK-cell development when other Ly49 molecules are not expressed[34]. Although the ligands are not yet defined for the other Ly49-family members, many have ITIMs and are therefore assumed to function as inhibitory receptors.

By contrast, the Ly49d and Ly49h receptors function as activating receptors[35,36]. Ly49d and Ly49h lack cytoplasmic ITIMs and contain charged transmembrane residues for association with the DAP12 signalling chain[10]. Both Ly49d and Ly49h seem to recognize either mouse or xenogeneic MHC class I molecules, or MHC class-I-like ligands, and they activate NK-cell functions[37–39].

In other species, the Ly49 family is only partially conserved. Many rat Ly49 inhibitory and activating receptors that are specific for MHC class I molecules have been characterized[40,41]. However, the Ly49 'family' exists only as a single gene in humans, known as *LY49L*, which is translated but encodes an apparently non-functional molecule that results from a point mutation[42].

Nkrp1 family. Initially identified as activating receptors on rat NK cells[14,20], the Nkrp1 family includes NK1.1 (Nkrp1c), which is the best known serological marker of NK cells from the C57BL/6 mouse strain[43,44]. Its genes are clustered in a locus close to *Cd69* (REF. 25). Four *Nkrp1* transcripts (*Nkrp1a*, *c*, *d* and *f*) have been identified in NK cells from the C57BL/6 mouse strain[45]. Consistent with the absence of an ITIM and presence of a charged transmembrane residue, mouse Nkrp1c behaves as an activating receptor, by associating with

IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIF (ITAM). B-, T- and natural killer-cell receptors are non-covalently associated with transmembrane proteins that contain one or more ITAMs. The motif (Asp/Glu-Xaa-Xaa-Tyr-Xaa-Xaa-Leu/Ile-Xaa$_{(6–8)}$-Tyr-Xaa-Xaa-Leu/Ile) is tyrosine phosphorylated after engagement of the ligand-binding subunits, which triggers a cascade of intracellular events that result in cell activation.

SYNTENIC REGION
A genomic DNA fragment in another species that contains orthologous genes.

ORTHOLOGOUS GENES
Genes present in a different species that are derived from a common ancestral gene.

NATURAL KILLER T CELLS (NKT cells). A subpopulation of T cells that expresses both NK- and T-cell markers. In the C57BL/6 mouse strain, NKT cells express the NK1.1 (Nkrp1c) molecule and the T-cell receptor (TCR). Some NKT cells recognize CD1d-associated lipid antigens and express a restricted repertoire of TCRs. After TCR stimulation of naive mice, NKT cells rapidly produce interleukin-4 and interferon-γ.

FcεRIγ[12,46]. Nkrp1a and Nkrp1f have related sequences and are also expected to be activating receptors. More closely related to Nkrp1d, Nkrp1b has a cytoplasmic ITIM and is an inhibitory receptor on NK cells from SJL/J and SWR mouse strains[47,48]. This allelic polymorphism is also reflected in serological heterogeneity that is reminiscent of the Ly49 alleles.

Interestingly, only one form of NKRP1 (NKRP1A) is encoded in the human NKC[49] and shares 45% amino-acid identity with mouse Nkrp1. The NKRP1A protein is expressed by only a subset of mature human NK and T cells[49] whereas mouse Nkrp1c is expressed by all NK cells and a specialized subset of T cells, known as NATURAL KILLER T CELLS (NKT cells)[50]. The ligands for NKRP1 receptors have not been identified yet.

NKG2 family and CD94. Similar to the Ly49 and Nkrp1 families, the NKG2 family contains members with activating and inhibitory functions. However, NKG2 molecules, first identified in humans[21], must dimerize with the invariant CD94 molecule for expression on the cell surface and signalling functions[51]. CD94 has a short cytoplasmic domain with no apparent functional motifs. The inhibitory molecule NKG2A has two ITIMs in its intracellular tail and can be alternatively spliced to generate NKG2B[52]. When NKG2C, E or H — NKG2E and NKG2H being generated by alternative splicing[53] — is disulphide-linked to CD94, the heterodimer can function as an activating receptor by associating with DAP12 through its charged transmembrane residue. NKG2F, present in the human NKC, encodes a putative molecule with unusual features: a charged transmembrane residue, a cytoplasmic ITIM-like sequence and no C-type lectin-like domain[54].

The human NKG2A–CD94 and NKG2C–CD94 heterodimers recognize the non-classical MHC class I molecule HLA-E[55]. Mouse Nkg2a–Cd94, Nkg2c–Cd94 and Nkg2e–Cd94 heterodimers recognize the HLA-E-related molecule Qa-1 (REF. 56). The structure of both HLA-E and Qa-1 is similar to that of classical MHC class I molecules, but they mainly display peptides that are derived from the signal peptides of classical MHC class I molecules. Therefore, the interactions of NKG2–CD94 heterodimers with HLA-E or Qa-1 molecules allow NK cells to monitor indirectly the expression of classical MHC class I molecules, as well as the expression of HLA-E or Qa-1 itself, which requires an intact MHC class I assembly pathway.

So far, it seems that the effect of the Nkg2a–Cd94 inhibitory receptor dominates over the Nkg2c–Cd94 activating receptor when an NK cell that expresses both receptors engages Qa-1-expressing targets[57]. This might be explained by the finding that the inhibitory receptor NKG2A–CD94 binds its ligand with higher affinity than the activating receptor NKG2C–CD94 (REF. 58), although other explanations are possible.

NKG2D. NKG2D was first cloned from human NK cells as a complementary DNA that was related to NKG2A and NKG2C[21]. However, NKG2D is distinct from other NKG2 molecules for several reasons. There is only limited sequence homology between NKG2D and other NKG2 molecules (28% amino-acid identity for the lectin-like domain), whereas the other NKG2 molecules are closely related to each other (70% identity). NKG2D is expressed as a disulphide-linked homodimer by NKT cells, γδ T cells, $CD8^+$ T cells and macrophages[59–61].

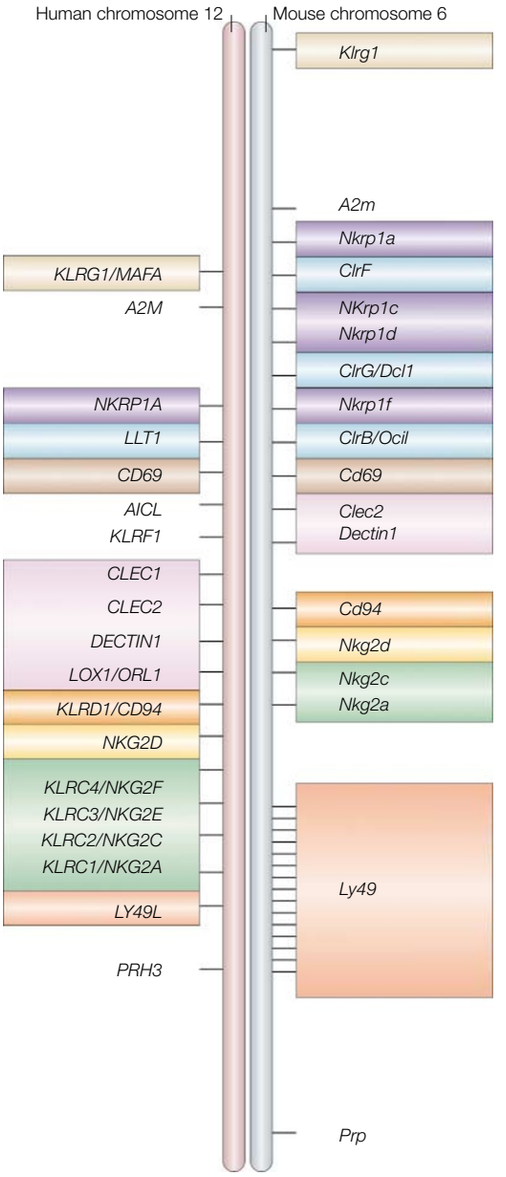


Figure 2 | **Lectin-like molecules encoded in the natural killer gene complex.** The genes for lectin-like receptors are present on human chromosome 12p13.1 and mouse distal chromosome 6. Coloured boxes indicate families of genes that are present in both species. This figure is not drawn to scale. AICL, activation-induced C-type lectin; A2M, α-2 macroglobulin; CLEC, C-type lectin-like receptor; Clr, C-lectin related; DECTIN, dendritic cell-associated C-type lectin; KLR, killer-cell lectin-like receptor; LLT, lectin-like transcript; LOX, oxidized low-density lipoprotein; MAFA, mast cell function-associated receptor; NKG2, natural killer group 2; NKRP, natural killer cell receptor protein; Ocil, osteoclast inhibitory lectin; PRH, proline-rich HaeIII; Prp, proline-rich protein.

NKG2D does not have known cytoplasmic motifs and was first shown to associate preferentially with a signalling chain known as DAP10, which is encoded by a gene located 130 bp away from the *DAP12* gene[62]. DAP10 does not have any ITAMs; instead, it contains a site for the recruitment of phosphatidylinositol 3-kinase[62] (similar to CD28), and functional studies indicate that NKG2D might behave as a co-stimulatory molecule on T cells[63].

It was less clear whether the function of NKG2D on NK cells was primary activation or co-stimulation[64,65]. However, recent studies have shown that NKG2D isoforms can be expressed by NK cells in the absence of DAP10 and can associate with DAP12. These isoforms give rise to the unusual circumstance whereby a receptor with the same extracellular domain (and presumably ligand specificity) has different functional outcomes (primary activation versus co-stimulation)[66,67].

In humans, the NKG2D ligands are MHC class-I-chain-related protein A (MICA), MICB and the UL16-binding protein (ULBP) family[59,68]. The *MICA* and *MICB* genes are linked to the HLA region and have limited expression by epithelial and vascular endothelial cells, whereas the ULBP molecules seem to be constitutively expressed by a broader array of tissues. Although the reciprocal relationships for these ligands in mice are not yet clearly defined, mouse Nkg2d interacts with the minor histocompatibility molecule H-60 and members of the Rae1 (retinoic acid early transcript 1) family[60,61]. In addition, another ligand for mouse Nkg2d, known as mouse ULBP-like transcript 1 (Mult1), has been recently identified and, similar to ULBP molecules, its transcripts are constitutively expressed by many tissues[69].

Despite marked sequence diversity, the NKG2D ligands have several common features. The NKG2D ligands have sequences and structures related to MHC class I molecules, although they do not associate with β_2-microglobulin (β_2m) or bind peptides[70,71]. Moreover, the expression of many NKG2D ligands seems to be inducible. Expression of human MICA and MICB by epithelial tissues is markedly enhanced in inflammatory bowel disease[72]. In mice, *Rae1* transcripts are induced by retinoic-acid treatment of embryonal-carcinoma cells and by phorbol-ester stimulation and transformation of skin cells[73]. So, NKG2D might facilitate the triggering of immune cells in the context of inflammatory reactions, although much work on this subject is still required, as the nature of the stimuli and the role of the constitutively expressed ligands, human ULBP and mouse Mult1, remain to be determined.

KLRF1. Killer cell lectin-like receptor subfamily F1 (KLRF1), also known as NKp80, is another human NKC-encoded, lectin-like molecule. Expression of KLRF1 seems to be restricted to mature leukocytes, NK cells and NKT cells[74,75]. Crosslinking of KLRF1 stimulates both resting and activated NK cells[75]. Unlike other NKC-encoded molecules, it contains cytoplasmic tyrosine residues that are not typical of an ITAM or ITIM, and has no charged transmembrane residues. The ligands for NKp80 have not been described.

KLRG1. Mast cell function-associated antigen (MAFA) or KLRG1 was originally identified as an inhibitory C-type lectin-like molecule expressed on the surface of the rat mucosal mast-cell line RBL-2H3 (REF. 76). Human and mouse orthologues of KLRG1 have a broader expression pattern, including NK- and T-cell subsets, but not mast cells[77–79]. The mouse protein contains a conserved cytoplasmic sequence that might function as an ITIM[76,79]. Interestingly, expression of Klrg1 by NK cells is downregulated in MHC class I-deficient mice, in contrast to the Ly49 inhibitory receptors which are upregulated[79]. However, no binding between classical MHC class I molecules and KLRG1 has been demonstrated[79]. Whereas expression of KLRG1 correlates with the ability of NK cells to produce interferon-γ (IFN-γ) in the course of infection[80], the physiological role of KLRG1 and its ligand *in vivo* remains to be discovered. Interestingly, the location of the mouse *Klrg1* gene is relatively distant from the NKC, compared with human *KLRG1*, which is consistent with gene duplication and chromosomal inversion events.

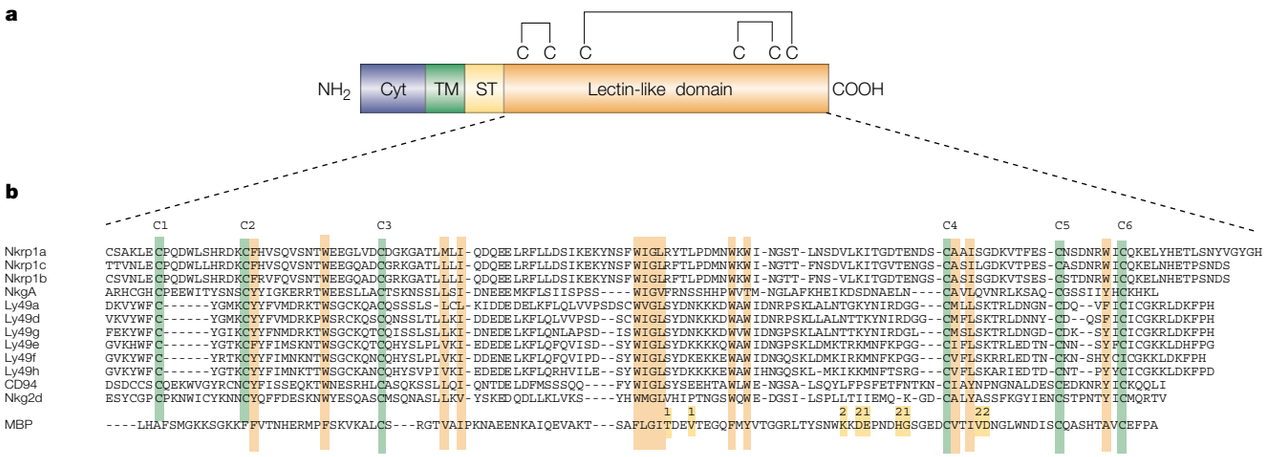
CD69 and other NKC molecules. Other molecules encoded by the NKC have been identified, including human lectin-like transcript 1 (LLT1)[81], human activation-induced C-type lectin (AICL)[82] and mouse C-lectin-related protein (Clr)[83]. These molecules form another family of molecules, related to CD69, which are among the earliest cell-surface molecules to be induced during lymphocyte activation[84,85]. CD69 is generally absent on resting lymphocytes (including mouse NK cells[46]), but activation leads to the rapid induction of CD69 expression, and crosslinking of CD69 with monoclonal antibodies in the presence of phorbol ester results in marked lymphocyte activation. Similarly, expression of LLT1 and AICL can be upregulated by stimulation of peripheral-blood lymphocytes[86]. The inducible and more widespread expression of these CD69-related molecules contrasts with other NKC-encoded molecules. The physiological functions of CD69 and related molecules remain elusive.

Box 2 | **Non-NK-cell receptors in the natural killer gene complex**

The natural killer gene complex (NKC) encodes molecules with atypical expression patterns compared with other molecules expressed by natural killer (NK) cells. The human *LOX1* or oxidized low-density lipoprotein lectin-like receptor 1 (*ORL1*) gene is located in the middle of the NKC, ~200 kb telomeric of *CD69* (REF. 137). In contrast to the other genes in the NKC, *LOX1* is expressed by vascular-rich tissues and some non-vascular cells[138]. Whereas early studies of LOX1 indicated that its main function was binding of oxidized low-density lipoproteins (LDL), recent studies indicate that LOX1 is a scavenger receptor involved in dendritic-cell (DC) uptake of heat-shock protein-associated antigens for cross-presentation[139]. Approximately 30 kb telomeric to *LOX1*, a cluster of related genes (*DECTIN1, CLEC1* and *CLEC2*) has been identified[140]. This family has distinct, but partially overlapping, expression by endothelial and haematopoietic subpopulations, including DCs. DECTIN1 binds β-glucan and is the main receptor involved in activation of macrophages by zymosan particles[141]. β-glucan binding is cation independent, which is consistent with the absence of consensus residues for Ca^{2+} binding in DECTIN1 and which indicates a different mode for carbohydrate binding of NKC-related receptors.

Box 3 | **Structure of the lectin-like molecules encoded in the natural killer gene complex**

All lectin-like molecules described so far are disulphide-linked dimers with a type II orientation and sequence homology to C-type lectins. Where crystallographic data are available, these receptors also have structures related to those of the C-type lectins. Six invariant cysteine residues are observed in the extracellular domain of all the lectin-like natural killer (NK)-cell receptors (see figure part a). Pairs of these residues are believed to form intra-chain disulphide bonds. A stalk (ST) region of variable length is present between the transmembrane domain (TM) and the ligand-binding domain. The ST region might provide a flexible connection to the lectin-like domain. The amino-acid alignment of the lectin-like domains in NK-cell receptors with the lectin-domain prototype, rat mannose-binding protein (Mbp), shows partial conservation of the residues that are characteristic for a lectin carbohydrate-recognition domain (CRD) (part b). Most obvious is the conservation of two pairs of cysteine residues that form two disulphide bonds (C3–C6) and (C4–C5) (green). Many of the remaining residues of the CRD prototype are aromatic, aliphatic or hydrophobic (orange). However, the residues forming the Ca^{2+}-bindings sites of Mbp (1) and (2) (yellow) are completely absent in the NK-cell receptors, indicating that the lectin-like molecules encoded in the NKC are not able to bind sugar residues in a manner analogous to the C-type lectins.



Recent progress has been reported on other NKC molecules that are expressed by accessory cells (BOX 2). These studies indicate that the NKC receptors might control other aspects of innate immune functions.

Structural analysis of NKC-encoded molecules

The NKC-encoded receptors, their ligands and their interactions have been analysed at the biophysical and structural levels[87]. Binding affinities of NKG2A–CD94 with HLA-E, and Ly49a with H–2D^d are comparable to those of other immune molecules that are involved in cell–cell interactions (K_D ~2 mM to 56 mM)[58,88]. By contrast, NKG2D has a higher affinity for some ligands, ranging from 5 nM to 1 mM (TABLE 2).

The crystal structures of several NKC-encoded receptors showed similarities to the classical C-type lectin fold. However, amino acids that correspond to the Ca^{2+}-binding residues of MBP are not conserved, which renders the potential Ca^{2+}-binding loop in the NKC-encoded receptors non-functional[70,71,89–92]. This feature indicates that these molecules are not authentic Ca^{2+}-dependent lectins.

Structural analysis of NKC receptor–ligand complexes indicates several modes for ligand binding. The Ly49a homodimer binds H–2D^d through two distinct sites. The first site (site 1) corresponds to one side of the MHC class I peptide-binding groove but not the peptide itself. The second site (site 2) is a cavity beneath the peptide-binding platform of H–2D^d, which consists of the α_2- and α_3-domains of the MHC heavy chain and β_2m, and partially overlaps the CD8-binding site. Mutational studies indicated that residues at site 2 but not site 1 are important for Ly49a recognition, as shown by monitoring binding to soluble Ly49a and inhibition of cytotoxicity by NK cells that express Ly49a[93–95]. The involvement of β_2m in site 2 might explain the specificity of Ly49a for mouse compared with human β_2m[94,96]. Therefore, site 2 is the functional binding site of Ly49a to its MHC class I ligand, which is distinct from the binding site of the T-cell receptor (TCR) to an H–2D^d-restricted complex, thereby being more analogous to the binding of CD8 to MHC class I molecules. Despite some differences, especially in terms of dimerization, structural and mutational studies of other Ly49-family members and their interactions with MHC class I ligands generally provide comparable results[94,97,98].

By contrast, crystallographic studies show that the NKG2D homodimer straddles both the α_1 and α_2 domains of MICA in a manner analogous to the interaction between a peptide-bound MHC class I molecule and its specific TCR[70]. Analyses of NKG2D–ULBP3 and Nkg2d–Rae1β complexes indicate a similar mode of interaction[71,92]. So, NKG2D uses a single binding site to recognize its sequence-diverse ligands, which shows the conformational plasticity of its ligand-binding site as highlighted by workers in the field.

So, the crystal structures of NKC-encoded molecules illustrate that these structurally related molecules recognize MHC class-I-related ligands at different sites. These findings might explain the inter-relationships between activating and inhibitory receptors that have the same ligands. Although this topic requires further investigation, future studies might show that an inhibitory receptor

and an activating receptor might bind the same ligand at different sites. Alternatively, the different outcomes could result from differences in affinities or association–dissociation constants of the same MHC class I (or MHC class-I-related) ligand for inhibitory and activating receptors.

Physiological function of activating receptors

The inhibitory NKC-encoded receptors seem to explain the 'missing-self' hypothesis, but the physiological roles of most putative activating receptors remain unclear, as they were often first identified as a result of antibody-crosslinking or reverse-genetics studies. Nevertheless, progress has been made towards clarifying the physiological roles of several activating receptors.

NKG2D: tumour and virus immunity. Some ligands for NKG2D are not well expressed by normal tissues, yet they are constitutively expressed by many *in vitro*-adapted tumour-cell lines and carcinogen-induced tumours, indicating that expression of these ligands is induced after tumorigenesis[73]. However, expression of these ligands enhances susceptibility to NKG2D-dependent NK-cell cytotoxicity[60,61] and also primes tumour-specific cytotoxic T-cell responses[99]. The induced expression of NKG2D ligands on tumours seems to be counterproductive from the tumour viewpoint[100,101]. Recent studies indicate that soluble MIC can be found in patients with MIC-expressing tumours and that the soluble MIC can downregulate expression of NKG2D and potentially block ligand recognition[100] (FIG. 4). Soluble MIC seems to be derived from metalloproteinase cleavage of membrane MIC[102]. So, soluble MIC molecules are an example of a new tumour-evasion strategy that might be applicable to other NK-cell activating or co-stimulatory receptors.

Interestingly, expression of NKG2D ligands is also induced after virus infection, allowing co-stimulation of virus-specific cytotoxic T lymphocytes (CTLs) and efficient killing of infected cells, even when the virus has markedly downregulated expression of MHC class I molecules and, therefore, antigen presentation[63,103]. Conversely, human cytomegalovirus (HCMV) contains an open reading frame (ORF), known as UL16, that binds the ULBP family of NKG2D ligands and blocks their interaction, and that of MIC, with NKG2D[68]. Furthermore, mouse cytomegalovirus (MCMV) expresses a molecule, known as gp40 or m152, that downregulates expression of both MHC class I molecules and ligands for Nkg2d[104]. These data indicate that NKG2D is important in anti-viral defence, as well as in tumour immunity.

Ly49h: resistance to MCMV. Studies also implicate other NKC loci in innate immune control of virus infections. Scalzo *et al.*[105] found that the NKC-linked autosomal dominant genetic locus known as *Cmv1* is responsible for the genetic resistance of certain mouse strains to MCMV infections. Recently, *Cmv1* was shown to encode the NK-cell activating receptor Ly49h in the C57BL/6 mouse strain[106–108]. MCMV-infected mice genetically deficient in Ly49h expression or treated with a monoclonal antibody specific for Ly49h have uncontrolled virus replication and markedly increased mortality[106]. An otherwise resistant mouse, which expresses Ly49h, can develop susceptibility to MCMV when the ITAMs of DAP12 are mutated[109]. These data support the hypothesis that the activating-receptor function of Ly49h is required for NK-cell mediated resistance to MCMV.

Recently, the ligand for Ly49h was identified as an MCMV-encoded molecule known as m157, which is expressed with early-phase kinetics in infected cells[110,111]. m157 specifically triggers freshly isolated Ly49h-expressing NK cells to produce IFN-γ within a few hours *in vitro*[111], even though the first few days of the *in vivo* response are dominated by a nonspecific phase of NK-cell activation, presumably resulting from systemically produced cytokines[112]. Thereafter, there is specific proliferation of Ly49h-expressing NK cells, indicating a 'clonal expansion' of NK cells *in vivo*.

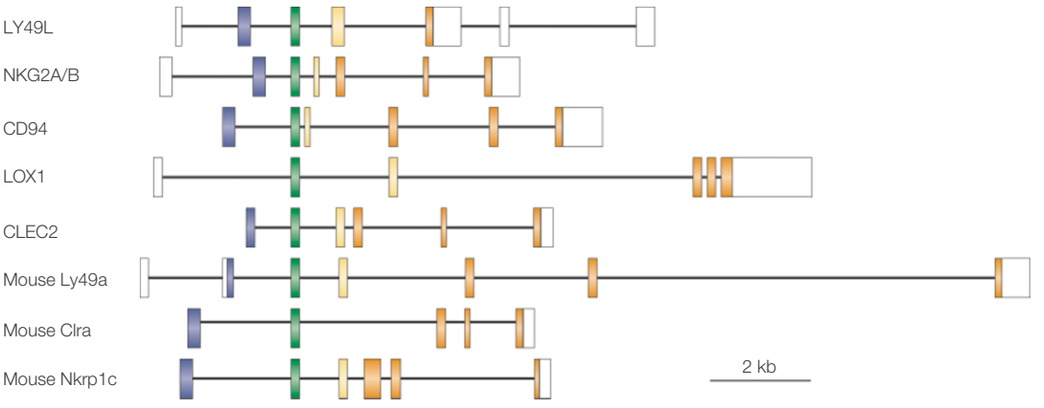


Figure 3 | **Genomic structure of lectin-like receptors encoded in the natural killer gene complex.** All genes are divided into exons, which are depicted in boxes. The cytoplasmic region is depicted in blue, the transmembrane domain in green, the stalk region in yellow and the three exons encoding the lectin-like domain in orange. Exon and intron sizes are drawn to approximate scale. Untranslated sequences are represented by white boxes. CLEC, C-type lectin-like receptor; Clr, C-lectin related; LOX, oxidized low-density lipoprotein; NKG2, natural killer group 2; Nkrp, natural killer cell receptor protein.

Interestingly, m157, similar to other ligands for NKC-encoded activating receptors[113], also binds to an NKC-encoded inhibitory receptor in the 129 mouse strain[110], indicating that it might have evolved to block NK-cell responses against infected cells. In addition, m157 is predicted to have folds that resemble those of MHC class-I-like molecules. Moreover, MCMV has at least 11 other ORFs with predicted MHC-like folds[111], even though only one (m144) was known from sequence alignment and seems to be a ligand for an as yet unidentified inhibitory receptor[114]. At least one of these molecules (m152) inhibits the expression of host MHC class I molecules[115], indicating that the putative MHC-like molecules in the MCMV genome might also have other

Table 1 | **Functions and ligands of NKC receptors**

Receptor	Gene	Species*	Function	Ligands‡	References
KLRG1§ (MAFA-L)	*Klrg1/KLRG1*	M/H	Not known	Not known	76–78
NKRP1A (KLRB1§)	*NKRP1A*	H	Activation	Not known	49
Nkrp1a	*Nkrp1a*	M	Activation	Not known	45
Nkrp1b	*Nkrp1b*	M (SJL, SWR)	Inhibition	Not known	48, 142
Nkrp1c (NK1.1)	*Nkrp1c*	M	Activation	Not known	44
Nkrp1d	*Nkrp1d*	M	Inhibition	Not known	45
Nkrp1f	*Nkrp1f*	M	Activation	Not known	45
Clrb, Clrf, Clrg	*Clrb*, *Clrf*, *Clrg*	M	Not known	Not known	83
LLT1	*LLT1*	H	Not known	Not known	81
AICL	*AICL*	H	Not known	Not known	82
KLRF1	*KLRF1*	H	Activation	Not known	74
CLEC1	*CLEC1*	H	Not known	Not known	143
CLEC2	*Clec2/CLEC2*	M/H	Not known	Not known	143
DECTIN1	*Dectin1/ DECTIN1*	M/H	Not known	β-glucan	141
LOX1 (ORL1)	*LOX1*	M/H	Not known	LDL, HSPAP	138,139
NKG2D (KLRK1§)	*Nkg2d/NKG2D*	M/H	Activation, co-stimulation	Rae1/60, Mult1/MIC, ULBP	59–61,68,69
CD94‖–NKG2A (KLRC1§)	*CD94–Nkg2a/NKG2A*	M/H	Inhibition	Qa-1/HLA-E	55,56
CD94‖–NKG2C (KLRC2§)	*CD94–Nkg2c/NKG2C*	M/H	Activation	Qa-1/HLA-E	55,56
CD94‖–NKG2E (KLRC3§)	*CD94–Nkg2e/NKG2E*	M/H	Activation	Qa-1/HLA-E	55,56
CD94‖ –NKG2F (KLRC4§)	*CD94–NKG2F*	H	Not known	Not known	54
Ly49a	*Ly49a*	M	Inhibition	H–2D^d, H–2D^k, H–2D^p	15
Ly49c	*Ly49c*	M	Inhibition	H–2K^b, H–2D^b, H–2K^d, H–2D^d, H–2D^k	29,32
Ly49d	*Ly49d*	M	Activation	H–2D^d, Hm1-C4	37,39,144
Ly49e	*Ly49e*	M	Inhibition	Not known	34
Ly49g (LGL1)	*Ly49g*	M	Inhibition	H–2D^d	30–32
Ly49h	*Ly49h*	M	Activation	m157	110,111

*H, human; M, mouse. Rat and tunicate receptors are mentioned in the text. ‡Note that most receptors are orphan receptors and many Ly49 receptors are not listed owing to space constraints. For mouse receptors, ligand specificity is reported for the C57BL/6 allele unless otherwise noted. Ligand specificity has been assessed by several different approaches, including killing of ligand-transfected target cells, soluble ligand (tetramer) binding, reporter-cell assays and biophysical and structural interactions of soluble (often refolded) molecules. For many receptor–ligand pairs, data are available from only one or a few approaches and conflicting data have been reported. This list is meant to be illustrative and to provide some guidance and comparisons between receptors, rather than being exhaustive or resolving any conflicting findings. §The KLR gene nomenclature is derived from the HUGO (Human Gene organization) Gene Nomenclature Committee. ‖The CD94 molecule is also known as KLRD1. AICL, activation-induced C-type lectin; CLEC, C-type lectin-like receptor; Clr, C-lectin related; DECTIN, dendritic-cell-associated C-type lectin; HSPAP, heat-shock protein-associated proteins; KLR, killer-cell lectin-like receptor; LDL, low-density lipoprotein; LLT, lectin-like transcript; LOX, oxidized low-density lipoprotein; MAFA, mast cell function-associated antigen; MIC, MHC class-I-chain-related protein; Mult1, mouse ULBP-like transcript 1; NKC, natural killer gene complex; NKG2, natural-killer group 2; NKRP, NK-cell receptor protein; Rae1, retinoic acid early transcript 1; ULBP, UL16-binding protein.

HAPLOTYPE
A chromosomal segment from similar or related ancestors that contains identical alleles in a region of genetically linked loci.

functions, such as blocking host MHC class-1-mediated presentation or binding other NK-cell receptors.

Although human NK cells do not express Ly49 molecules, it is possible that a similar virus-recognition strategy exploits molecules encoded in the LRC. Whereas DAP12-deficient humans do not have increased susceptibility to virus infections[116], HCMV has at least two ORFs that encode ligands for both structural types of inhibitory receptor, indicating that it has evolved mechanisms to specifically evade NK-cell activating receptors[117]. Moreover, other documented virus-evasion strategies seem to be directed against NK cells, indicating that human NK cells express virus-recognition receptors. So, analogous to the relationship between inhibitory rodent Ly49 and human KIRs, it is probable that human NK cells use virus-control mechanisms that are conceptually similar to mouse Ly49h, even though the specific receptor and signalling-chain counterparts might be divergent.

Role of NKC-encoded molecules on T cells. Although the NKC was originally defined because of the selective expression of NKC-encoded molecules by conventional NK cells, a subpopulation of T cells constitutively expresses these molecules[50]. Indeed, the commonly used NKT designation for these cells is derived from their expression of NK1.1, which is otherwise a marker of mouse $CD3^-$ NK cells. NKT cells have not been reported to express the Ly49 activating receptors but do express inhibitory Ly49 molecules.

Other T cells might also express NKC-encoded molecules under certain conditions. Qa-1 tetramers bind to antigen-specific T cells after they respond to polyomavirus infection, owing to the expression of CD94–Nkg2a heterodimers[57]. T-cell antigen-specific function is inhibited by CD94–NKG2 during virus clearance and oncogenesis. Although this functional inhibition might not be evident in responses to every virus, marked T-cell expression of CD94–NKG2 can be found in other infections[118,119]. Emerging data also indicate that primed T cells might express other NKC molecules, including the Ly49 family and KLRG1, that might modulate the function of antigen-specific T cells[32,80,120,121], a possibility that requires further evaluation. Similarly, NKG2D molecules are also expressed by T cells, where they associate with DAP10. NKG2D can provide co-stimulatory function under conditions when peptide–MHC class I stimulation might be downregulated[59]. (T cells do not express DAP12 so NKG2D provides co-stimulatory function only on T cells[66,67]). NKG2D might also function in situations where CD28 co-stimulation is not available[63]. So, there are emerging data indicating that NKC molecules might modulate the function of antigen-specific T cells during the immune response.

Table 2 | **Summary of equilibrium dissociation constants**

Receptor	Ligand	K_D	References
Mouse Nkg2d	H60	18–26 nM	145,146
Mouse Nkg2d	Rae1α	690 nM	145
Mouse Nkg2d	Rae1β	345 nM	145
Mouse Nkg2d	Rae1γ	486–586 nM	145
Mouse Nkg2d	Rae1δ	726 nM	145,146
Mouse Nkg2d	Rae1ε (B6)	28 nM	146
NKG2D	MICA	300 nM	70
Ly49a	$H–2D^d$	6–26 μM	88
CD94–NKG2A	HLA-E	2.1–56.6 μM	58
CD94–NKG2C	HLA-E	11.9–107 μM	56
KIR	MHC class I	10 μM	147
LIR	MHC class I	15–100 μM	148

KIR, killer-cell immunoglobulin-like receptor; LIR, leukocyte immunoglobulin-like receptor; MICA, MHC class-I-chain related protein A; NKG2, natural-killer group 2; Rae, retinoic acid early transcript.

Evolution of NK receptors

Allelic polymorphism of the NKC. Phenotypic variations have been genetically mapped to the NKC. These variants include resistance to MCMV (*Cmv1*, see above) that might be related to other NKC-associated loci involved in resistance to viral infections. *Rmp1* is associated with resistance to mousepox virus infection and *Rhs1* with resistance to herpes simplex virus infection[122,123]. These phenotypes might result from alleles for other NKC-encoded receptors, as present data indicate that they segregate from *Cmv1* (*Ly49h*). At least two other phenotypically defined loci have been associated with the NKC: *Chok* (*Ly49d*), which controls target killing by mouse NK cells[124]; and *Nka*, which controls rat NK-cell lysis of allogeneic lymphocytes[125]. So, the NKC has marked allelic polymorphism that is manifested by marked differences in NK-cell recognition and function and could result from the presence or absence of a relevant gene (*Ly49h*), point mutations that alter receptor specificity or expression, small deletions or insertions, or even 'chimeric' genes[113].

Allelic polymorphism among NKC loci has been demonstrated at the DNA level by several approaches. A comprehensive list of NKC alleles[126,127] indicates the presence of mouse NKC HAPLOTYPES that are conserved among pedigree-related inbred strains. More detailed analysis of the genomic organization of the Ly49 cluster in two different strains of mice (C57BL/6 and 129/svJ)[26] show a plasticity in gene content between haplotypes, similar to that described for the KIR gene family[128,129]. These loci are composed of common genes interspersed by variable regions where some genes might be absent. Better knowledge of allelic polymorphism of the NKC and related haplotypes might provide insight into inherent differences in NK-cell functions between individuals.

Co-evolution of the MHC and NKC. Whereas human NK cells express certain NKC molecules that are functional orthologues, such as CD94–NKG2, to mouse NKC receptors, efforts to identify human *Ly49* genes have detected only a single *Ly49*-related gene, *LY49L*, that does not seem to encode a functional protein[42]. Instead, functionally analogous receptors to rodent Ly49 are encoded by the KIR genes located in the LRC on human chromosome 19q13.4 (REF. 13). These receptors are highly polymorphic immunoglobulin-like molecules that recognize HLA class I determinants[3]. Despite their structural divergence from the rodent lectin-like

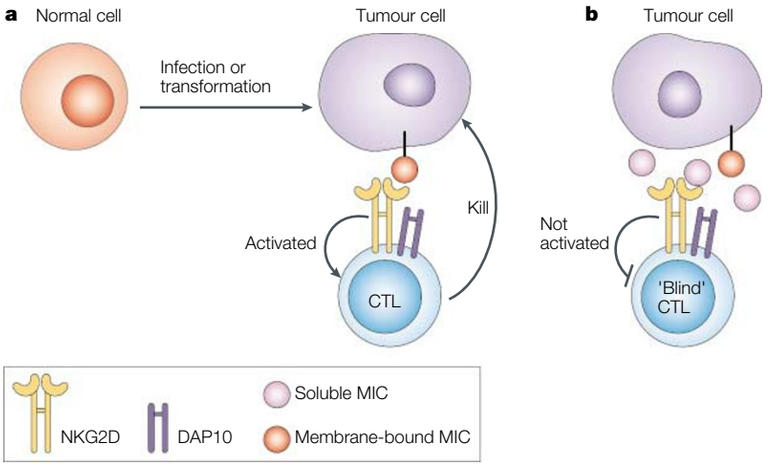


Figure 4 | **A new tumour-evasion strategy. a** | Cells that are stressed, as a result of virus infection or transformation, often express natural-killer group 2D (NKG2D) ligands, such as MHC class-I-chain-related protein (MIC). Such cells should be susceptible to killing by cytotoxic T lymphocytes (CTLs), because of the co-stimulatory signal given to the CTL through the NKG2D–DAP10 (DNAX-activating protein of 10 kDa) complex. **b** | A recent study[101] has shown that a soluble form of MIC is detectable in the blood of patients with MIC-expressing tumours. Soluble MIC blocks the interaction of NKG2D with membrane-bound MIC, so the CTLs never receive the co-stimulatory signal and, therefore, cannot detect the presence of the tumour cells. Adapted, with permission from Nature © (2002) Macmillan Magazines Ltd., from REF. 101.

receptors, the inhibitory KIR molecules mediate MHC class I inhibition by the same mechanism, through cytoplasmic ITIMs that recruit and activate SHP1. There are also molecules lacking ITIMs that have charged transmembrane residues that facilitate association with ITAM-containing signalling chains, such as DAP12. These molecules are known as killer-activating receptors (KARs)[130]. Furthermore, genes in both the KIR and lectin-like NK-cell receptor superfamilies have been duplicated many times, with marked individual variability and allelic polymorphism[129].

In parallel with the expansion of the human *KIR* genes, but not the human *Ly49* cluster, and the expansion of the rodent *Ly49* genes, there are relatively few examples of NK-cell receptors that are encoded by the mouse LRC on chromosome 7. Furthermore, the finding of lectin-like molecules expressed by monocytes and dendritic cells is reminiscent of LRC molecules[128], in which the *KIR* gene family of NK-cell receptors is flanked by the immunoglobulin-like transcript (ILT) genes, which are more broadly expressed by other leukocytes[5]. So, the conceptual frameworks derived from analysis of either receptor system have been useful in understanding the properties of the corresponding receptors.

Interestingly, another common property of the NKC and LRC is their independent segregation from the MHC. The genes encoding NK-cell receptors with specificity for MHC class I molecules would have to generate sufficient diversity of receptors to interact with their ligands, which are among the most polymorphic of molecules that are encoded in the mammalian genome. Alteration in MHC class I molecules would impose pressure on the evolution of NK-cell receptors for changes that would maintain interactions with their MHC class I ligands. New receptors that are better suited to recognize these modified ligands also might evolve and they might interact at a different site. Interestingly, therefore, the NKC and LRC receptors do not bind their MHC ligands in the same manner[87]. Independent segregation also indicates that a selection process would be required to provide NK cells with the appropriate repertoire of receptors that recognize self-MHC[131], which is a topic of current investigations.

Given the extreme polymorphic nature of MHC molecules, it is not surprising that analysis of the *Ly49* cluster has shown parallel allelic polymorphism for individual molecules. This allelic polymorphism is also complicated by serological heterogeneity. For example, some monoclonal antibodies specific for a given Ly49 or Nkrp1 molecule in one inbred strain might react with another family member in a different inbred strain[47,48,113]. Furthermore, the functions of these reactive molecules could be totally opposite in the different mouse strains and the ligand specificity might be altered[132]. By contrast, other NKC-encoded receptors, such as NKG2D, show very little allelic polymorphism[65], perhaps related to the polymorphism that is displayed by its ligands or its plasticity in ligand binding.

Despite the marked polymorphism of the NKC and LRC receptors and the MHC, the repertoire of expressed NK-cell receptors is limited and markedly less than that generated by the TCR rearrangement machinery. However, it should be noted that Ly49 receptors are quite promiscuous in MHC class I recognition and the ligands for a given Ly49 receptor cannot be categorized in any other way, such as on the basis of MHC haplotypes or antibody reactivity. Similar promiscuous specificity has been noted for the KIRs. This broader, yet specific, recognition might help to explain other NK-cell specificities, such as in bone-marrow transplantation[133].

In contrast to rodents and humans, the avian MHC contains genes for lectin-like receptors that resemble most closely the rodent NKC molecules[83,134]. The C-type lectin-like molecule B-NK1 is expressed by chicken NK-cell lines, carries an ITIM and is the first non-mammalian NK-cell receptor to be characterized[134]. The physical linkage of putative NK-cell receptors and avian MHC genes supports a linked co-evolutionary relationship. It will be of interest to determine whether the specificity of the avian lectin-like receptors for MHC ligands is related to their genetic linkage with the MHC.

Recent studies also indicate that the NKC might be found as far back as tunicates (invertebrate chordates) that display allorecognition phenomena that influence colony formation[135]. The cDNA for a lectin-like molecule was isolated from *Botryllus schlosseri* and shown to be most related to NKC-encoded molecules, particularly CD94 and NKRP1. This molecule is expressed by a subpopulation of granulated blood cells. Using Southern-blot analysis, its gene shows allelic polymorphism and might belong to a family of related genes. So, the NKC seems to antedate the evolution of adaptive immunity.

Finally, nearly all orthopoxvirus genomes sequenced so far contain ORFs for lectin-like receptors that most closely resemble the NKC-encoded receptors. Fowlpox

virus, for example, contains eight such ORFs[136]. These large DNA viruses might have captured related host genes to evade the host immune system.

Concluding remarks

This overview of the NKC highlights important concepts about NK-cell receptors. Many genes encoding NK-cell receptors are found in clusters that are located in the NKC. Inhibitory, activating and co-stimulatory receptors are encoded by families of highly related genes; most of these receptors are still orphan receptors because little is known about their physiological ligands and functions. Present knowledge indicates that NKC-encoded receptors bind MHC class I or MHC class-I-like ligands and account for the genetic basis of phenotypes involving NK cells. Furthermore, these studies provide a conceptual framework for NK-cell activities that include 'missing-self', tumour specificity and anti-pathogen effects, which indicates that further dissection of the NKC and its gene products will continue to provide important insights into NK-cell biology.

1. Yokoyama, W. M. in *Fundamental Immunology* (ed. Paul, W. E.) 575–603 (Lippincott–Raven, New York, 1999).
2. Kärre, K., Ljunggren, H. G., Piontek, G. & Kiessling, R. Selective rejection of H–2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* **319**, 675–678 (1986).
This paper describes studies that led to the 'missing-self' hypothesis.
3. Long, E. O. Regulation of immune responses through inhibitory receptors. *Annu. Rev. Immunol.* **17**, 875–904 (1999).
4. Moretta, L., Biassoni, R., Bottino, C., Mingari, M. C. & Moretta, A. Human NK-cell receptors. *Immunol. Today* **21**, 420–422 (2000).
5. Colonna, M., Nakajima, H. & Cella, M. A family of inhibitory and activating Ig-like receptors that modulate function of lymphoid and myeloid cells. *Semin. Immunol.* **12**, 121–127 (2000).
6. Yokoyama, W. M. & Seaman, W. E. The Ly49 and NKRP1 gene families encoding lectin-like receptors on natural killer cells: the NK gene complex. *Annu. Rev. Immunol.* **11**, 613–635 (1993).
7. Lopez-Botet, M., Llano, M., Navarro, F. & Bellon, T. NK cell recognition of non-classical HLA class I molecules. *Semin. Immunol.* **12**, 109–119 (2000).
8. Wang, J. W. *et al.* Influence of SHIP on the NK repertoire and allogeneic bone marrow transplantation. *Science* **295**, 2094–2097 (2002).
9. Tomasello, E. *et al.* Gene structure, expression pattern, and biological activity of mouse killer cell activating receptor-associated protein (KARAP)/DAP-12. *J. Biol. Chem.* **273**, 34115–34119 (1998).
10. Smith, K. M., Wu, J., Bakker, A. B., Phillips, J. H. & Lanier, L. L. Cutting edge: Ly49D and Ly49H associate with mouse DAP12 and form activating receptors. *J. Immunol.* **161**, 7–10 (1998).
11. Lanier, L. L., Cortiss, B. C., Wu, J., Leong, C. & Phillips, J. H. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. *Nature* **391**, 703–707 (1998).
12. Arase, N. *et al.* Association with FcR-γ is essential for activation signal through NKRP1 (CD161) in natural killer (NK) cells and NK1.1$^+$ T cells. *J. Exp. Med*. **186**, 1957–1963 (1997).
13. Wende, H., Volz, A. & Ziegler, A. Extensive gene duplications and a large inversion characterize the human leukocyte receptor cluster. *Immunogenetics* **51**, 703–713 (2000).
14. Chambers, W. H. *et al.* Monoclonal antibody to a triggering structure expressed on rat natural killer cells and adherent lymphokine-activated killer cells. *J. Exp. Med.* **169**, 1373–1389 (1989).
15. Karlhofer, F. M., Ribaudo, R. K. & Yokoyama, W. M. MHC class I alloantigen specificity of Ly49$^+$ IL-2-activated natural killer cells. *Nature* **358**, 66–70 (1992).
This was the first description of an MHC class-I-specific inhibitory receptor (Ly49a) on natural killer (NK) cells.
16. Yokoyama, W. M., Kehn, P. J., Cohen, D. I. & Shevach, E. M. Chromosomal location of the Ly49 (A1, YE1/48) multigene family. Genetic association with the NK1.1 antigen. *J. Immunol*. **145**, 2353–2358 (1990).
17. Yokoyama, W. M. *et al.* cDNA cloning of mouse NKRP1 and genetic linkage with Ly49. Identification of a natural killer cell gene complex on mouse chromosome 6. *J. Immunol.* **147**, 3229–3236 (1991).
The initial description of the NK gene complex (NKC).
18. Chan, P. Y. & Takei, F. Molecular cloning and characterization of a novel murine T cell surface antigen, YE1/48. *J. Immunol.* **142**, 1727–1736 (1989).
19. Yokoyama, W. M., Jacobs, L. B., Kanagawa, O., Shevach, E. M. & Cohen, D. I. A murine T lymphocyte antigen belongs to a supergene family of type II integral membrane proteins. *J. Immunol.* **143**, 1379–1386 (1989).
20. Giorda, R. *et al.* NKRP1, a signal transduction molecule on natural killer cells. *Science* **249**, 1298–1300 (1990).
21. Houchins, J. P., Yabe, T., McSherry, C. & Bach, F. H. DNA sequence analysis of NKG2, a family of related cDNA clones encoding type II integral membrane proteins on human natural killer cells. *J. Exp. Med.* **173**, 1017–1020 (1991).
22. Renedo, M. *et al.* The human natural killer gene complex is located on chromosome 12p12–p13. *Immunogenetics* **46**, 307–311 (1997).
23. Wilhelm, B. T., Gagnier, L. & Mager, D. L. Sequence analysis of the Ly49 cluster in C57BL/6 mice: a rapidly evolving multigene family in the immune system. *Genomics* **80**, 646–661 (2002).
24. Silver, E. T., Elliott, J. F. & Kane, K. P. Alternatively spliced LY49D and H transcripts are found in IL-2-activated NK cells. *Immunol. Today* **44**, 14–17 (1996).
25. Brown, M. G. *et al.* A 2-Mb YAC contig and physical map of the natural killer gene complex on mouse chromosome 6. *Genomics* **42**, 16–25 (1997).
26. Makrigiannis, A. P. *et al.* 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**, 437–444 (2002).
27. Correa, I. & Raulet, D. H. Binding of diverse peptides to MHC class I molecules inhibits target cell lysis by activated natural killer cells. *Immunity* **2**, 61–71 (1995).
28. Matsumoto, N., Ribaudo, R. K., Abastado, J.-P., Margulies, D. H. & Yokoyama, W. M. The lectin-like NK cell receptor Ly49A recognizes a carbohydrate-independent epitope on its MHC class I ligand. *Immunity* **8**, 245–254 (1998).
29. Stoneman, E. R. *et al.* Cloning and characterization of 5E6(Ly49C), a receptor molecule expressed on a subset of murine natural killer cells. *J. Exp. Med.* **182**, 305–313 (1995).
30. Mason, L. H. *et al.* Cloning and functional characteristics of murine large granular lymphocyte-1: a member of the Ly49 gene family (Ly49G2). *J. Exp. Med.* **182**, 293–303 (1995).
31. Brennan, J., Mahon, G., Mager, D. L., Jefferies, W. A. & Takei, F. Recognition of class I major histocompatibility complex molecules by Ly49: specificities and domain interactions. *J. Exp. Med.* **183**, 1553–1559 (1996).
32. Hanke, T. *et al.* Direct assessment of MHC class I binding by seven Ly49 inhibitory NK cell receptors. *Immunity* **11**, 67–77 (1999).
33. Franksson, L. *et al.* Peptide dependency and selectivity of the NK cell inhibitory receptor Ly49C. *Eur. J. Immunol.* **29**, 2748–2758 (1999).
34. Van Beneden, K. *et al.* Expression of Ly49E and CD94/NKG2 on fetal and adult NK cells. *J. Immunol.* **166**, 4302–4311 (2001).
35. Mason, L. H. *et al.* The Ly49D receptor activates murine natural killer cells. *J. Exp. Med.* **184**, 2119–2128 (1996).
36. Smith, H. R. *et al.* Nonstochastic coexpression of activation receptors on murine natural killer cells. *J. Exp. Med.* **191**, 1341–1354 (2000).
37. Nakamura, M. C. *et al.* Mouse Ly49D recognizes H-2D^d and activates natural killer cell cytotoxicity. *J. Exp. Med.* **189**, 493–500 (1999).
38. George, T. C., Ortaldo, J. R., Lemieux, S., Kumar, V. & Bennett, M. Tolerance and alloreactivity of the Ly49D subset of murine NK cells. *J. Immunol.* **163**, 1859–1867 (1999).
39. Furukawa, H., Iizuka, K., Poursine-Laurent, J., Shastri, N. & Yokoyama, W. M. A ligand for the murine NK activation receptor Ly49D: activation of tolerized NK cells from β_2-microglobulin-deficient mice. *J. Immunol.* **169**, 126–136 (2002).
40. Naper, C. *et al.* Ly49i2 is an inhibitory rat natural killer cell receptor for an MHC class Ia molecule (RT1-A1c). *Eur. J. Immunol.* **32**, 2031–2036 (2002).
41. Naper, C. *et al.* Ly49s3 is a promiscuous activating rat NK cell receptor for nonclassical MHC class I-encoded target ligands. *J. Immunol.* **169**, 22–30 (2002).
References 40 and 41 are recent studies on rat NKC receptors.
42. Westgaard, I. H., Berg, S. F., Orstavik, S., Fossum, S. & Dissen, E. Identification of a human member of the Ly49 multigene family. *Eur. J. Immunol.* **28**, 1839–1846 (1998).
43. Hackett, J. Jr *et al.* Origin and differentiation of natural killer cells. II. Functional and morphologic studies of purified NK1.1$^+$ cells. *J. Immunol.* **136**, 3124–3131 (1986).
44. Ryan, J. C., Turck, J., Niemi, E. C., Yokoyama, W. M. & Seaman, W. E. Molecular cloning of the NK1.1 antigen, a member of the NKRP1 family of natural killer cell activation molecules. *J. Immunol.* **149**, 1631–1635 (1992).
45. Plougastel, B., Matsumoto, K., Dubbelde, C. & Yokoyama, W. M. Analysis of a 1-Mb BAC contig overlapping the mouse Nkrp1 cluster of genes: cloning of three new Nkrp1 members, Nkrp1d, Nkrp1e, and Nkrp1f. *Immunogenetics* **53**, 592–598 (2001).
46. Karlhofer, F. M. & Yokoyama, W. M. Stimulation of murine natural killer (NK) cells by a monoclonal antibody specific for the NK1.1 antigen. IL-2-activated NK cells possess additional specific stimulation pathways. *J. Immunol.* **146**, 3662–3673 (1991).
47. Kung, S. K., Su, R. C., Shannon, J. & Miller, R. G. The NKRP1B gene product is an inhibitory receptor on SJL/J NK cells. *J. Immunol.* **162**, 5876–5887 (1999).
48. Carlyle, J. R. *et al.* Mouse NKRP1B, a novel NK1.1 antigen with inhibitory function. *J. Immunol.* **162**, 5917–5923 (1999).
49. Lanier, L. L., Chang, C. & Phillips, J. H. Human NKR-P1A. A disulfide-linked homodimer of the C-type lectin superfamily expressed by a subset of NK and T lymphocytes. *J. Immunol.* **153**, 2417–2428 (1994).
50. Bendelac, A., Rivera, M. N., Park, S. H. & Roark, J. H. Mouse CD1-specific NK1 T cells: development, specificity, and function. *Annu. Rev. Immunol.* **15**, 535–562 (1997).
51. Lazetic, S., Chang, C., Houchins, J. P., Lanier, L. L. & Phillips, J. H. Human natural killer cell receptors involved in MHC class I recognition are disulfide-linked heterodimers of CD94 and NKG2 subunits. *J. Immunol.* **157**, 4741–4745 (1996).
This paper describes that CD94 forms heterodimers with NKG2.
52. Plougastel, B., Jones, T. & Trowsdale, J. Genomic structure, chromosome location, and alternative splicing of the human *NKG2A* gene. *Immunogenetics* **44**, 286–291 (1996).
53. Bellon, T. *et al.* Triggering of effector functions on a CD8$^+$ T cell clone upon the aggregation of an activatory CD94/kp39 heterodimer. *J. Immunol.* **162**, 3996–4002 (1999).
54. Plougastel, B. & Trowsdale, J. Cloning of NKG2F, a new member of the NKG2 family of human natural killer cell receptor genes. *Eur. J. Immunol.* **27**, 2835–2839 (1997).
55. Braud, V. M. *et al.* HLA-E binds to natural-killer-cell receptors CD94/NKG2A, B and C. *Nature* **391**, 795–799 (1998).
56. Vance, R. E., Kraft, J. R., Altman, J. D., Jensen, P. E. & Raulet, D. H. 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**, 1841–1848 (1998).
57. Moser, J. M., Gibbs, J., Jensen, P. E. & Lukacher, A. E. CD94–NKG2A receptors regulate antiviral CD8$^+$ T cell responses. *Nature Immunol.* **3**, 189–195 (2002).
This paper shows the effect of NKC-encoded receptors on T-cell immunity to an oncogenic virus.

58. Vales-Gomez, M., Reyburn, H. T., Erskine, R. A., Lopez-Botet, M. & Strominger, J. L. Kinetics and peptide dependency of the binding of the inhibitory NK receptor CD94/NKG2A and the activating receptor CD94/NKG2C to HLA-E. *EMBO J.* **18**, 4250–4260 (1999).
59. Bauer, S. *et al.* Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* **285**, 727–729 (1999).
The authors describe how NKG2D and its ligands affect NK- and T-cell functions.
60. Cerwenka, A. *et al.* Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity* **12**, 721–727 (2000).
61. Diefenbach, A., Jamieson, A. M., Liu, S. D., Shastri, N. & Raulet, D. H. Ligands for the murine NKG2D receptor: expression by tumour cells and activation of NK cells and macrophages. *Nature Immunol.* **1**, 119–126 (2000).
62. Wu, J. *et al.* An activating immunoreceptor complex formed by NKG2D and DAP10. *Science* **285**, 730–732 (1999).
63. Groh, V. *et al.* Costimulation of CD8αβ T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nature Immunol.* **2**, 255–260 (2001).
64. Pende, D. *et al.* Role of NKG2D in tumour cell lysis mediated by human NK cells: cooperation with natural cytotoxicity receptors and capability of recognizing tumours of nonepithelial origin. *Eur. J. Immunol.* **31**, 1076–1086 (2001).
65. Ho, E. L. *et al.* Co-stimulation of multiple NK cell activation receptors by NKG2D. *J. Immunol.* **169**, 3667–3675 (2002).
66. Gilfillan, S., Ho, E. L., Cella, M., Yokoyama, W. M. & Colonna, M. NKG2D recruits two distinct adapters to trigger natural killer cell activation and costimulation. *Nature Immunol.* **3**, 1150–1155 (2002).
67. Diefenbach, A. *et al.* Selective associations with signaling molecules determine stimulatory versus costimulatory activity of NKG2D. *Nature Immunol.* **3**, 1142–1149 (2002).
References 66 and 67 are recent studies showing that NKG2D isoforms can associate with different signalling chains.
68. Cosman, D. *et al.* ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* **14**, 123–133 (2001).
69. Carayannopoulos, L., Naidenko, O., Fremont, D. & Yokoyama, W. M. Cutting edge. Murine UL16-binding protein-like transcript 1: a newly described transcript encoding a high-affinity ligand for murine NKG2D. *J. Immunol.* **169**, 4079–4083 (2002).
70. Li, P. *et al.* Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. *Nature Immunol.* **2**, 443–451 (2001).
71. Radaev, S., Rostro, B., Brooks, A. G., Colonna, M. & Sun, P. D. Conformational plasticity revealed by the cocrystal structure of NKG2D and its class I MHC-like ligand ULBP3. *Immunity* **15**, 1039–1049 (2001).
72. Groh, V. *et al.* Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. *Proc. Natl Acad. Sci. USA* **93**, 12445–12450 (1996).
73. Girardi, M. *et al.* Regulation of cutaneous malignancy by γδ T cells. *Science* **294**, 605–609 (2001).
74. Roda-Navarro, P. *et al.* Human KLRF1, a novel member of the killer cell lectin-like receptor gene family: molecular characterization, genomic structure, physical mapping to the NK gene complex and expression analysis. *Eur. J. Immunol.* **30**, 568–576 (2000).
75. Vitale, M. *et al.* Physical and functional independency of p70 and p58 natural killer (NK) cell receptors for HLA class I: their role in the definition of different groups of alloreactive NK cell clones. *Proc. Natl Acad. Sci. USA* **93**, 1453–1457 (1996).
76. Guthmann, M. D., Tal, M. & Pecht, I. A secretion inhibitory signal transduction molecule on mast cells is another C-type lectin. *Proc. Natl Acad. Sci. USA* **92**, 9397–9401 (1995).
77. Butcher, S., Arney, K. L. & Cook, G. P. MAFA-L, an ITIM-containing receptor encoded by the human NK cell gene complex and expressed by basophils and NK cells. *Eur. J. Immunol.* **28**, 3755–3762 (1998).
78. Blaser, C., Kaufmann, M. & Pircher, H. Cutting edge. Virus-activated CD8 T cells and lymphokine-activated NK cells express the mast cell function-associated antigen, an inhibitory C-type lectin. *J. Immunol.* **161**, 6451–6454 (1998).
79. Corral, L., Hanke, T., Vance, R. E., Cado, D. & Raulet, D. H. NK cell expression of the killer cell lectin-like receptor G1 (KLRG1), the mouse homolog of MAFA, is modulated by MHC class I molecules. *Eur. J. Immunol.* **30**, 920–930 (2000).
80. Robbins, S. H. *et al.* Cutting edge: inhibitory functions of the killer cell lectin-like receptor G1 molecule during the activation of mouse NK cells. *J. Immunol.* **168**, 2585–2589 (2002).
81. Boles, K. S., Barten, R., Kumaresan, P. R., Trowsdale, J. & Mathew, P. A. Cloning of a new lectin-like receptor expressed on human NK cells. *Immunogenetics* **50**, 1–7 (1999).
82. Hamann, J., Montgomery, K. T., Lau, S., Kucherlapati, R. & van Lier, R. A. W. AICL: a new activation-induced antigen encoded by the human NK gene complex. *Immunogenetics* **45**, 295–300 (1997).
83. Plougastel, B., Dubbelde, C. & Yokoyama, W. M. Cloning of Clr, a new family of lectin-like genes localized between mouse *Nkrp1a* and *Cd69* genes. *Immunogenetics* **53**, 209–214 (2001).
84. Cebrian, M. *et al.* Triggering of T cell proliferation through AIM, an activation inducer molecule expressed on activated human lymphocytes. *J. Exp. Med.* **168**, 1621–1637 (1988).
85. Yokoyama, W. M. *et al.* Characterization of a cell surface-expressed disulfide-linked dimer involved in murine T cell activation. *J. Immunol.* **141**, 369–376 (1988).
86. Eichler, W., Ruschpler, P., Wobus, M. & Drossler, K. Differentially induced expression of C-type lectins in activated lymphocytes. *J. Cell. Biochem.* **81**, 201–208 (2001).
87. Natarajan, K., Dimasi, N., Wang, J., Mariuzza, R. A. & Margulies, D. H. Structure and function of natural killer cell receptors: multiple molecular solutions to self, nonself discrimination. *Annu. Rev. Immunol.* **20**, 853–885 (2002).
88. Natarajan, K. *et al.* Interaction of the NK cell inhibitory receptor Ly49A with H–2D^{d}: identification of a site distinct from the TCR site. *Immunity* **11**, 591–601 (1999).
89. Boyington, J. C. *et al.* Structure of CD94 reveals a novel C-type lectin fold: implications for the NK cell-associated CD94/NKG2 receptors. *Immunity* **10**, 75–82 (1999).
90. Tormo, J., Natarajan, K., Margulies, D. H. & Mariuzza, R. A. Crystal structure of a lectin-like natural killer cell receptor bound to its MHC class I ligand. *Nature* **402**, 623–631 (1999).
This study describes a structural analysis of the complex formed between Ly49a and its MHC class I ligand.
91. Wolan, D. W. *et al.* Crystal structure of the murine NK cell-activating receptor NKG2D at 1.95 Å. *Nature Immunol.* **2**, 248–254 (2001).
92. Li, P., McDermott, G. & Strong, R. K. Crystal structures of RAE-1β and its complex with the activating immunoreceptor NKG2D. *Immunity* **16**, 77–86 (2002).
93. Matsumoto, N., Yokoyama, W. M., Kojima, S. & Yamamoto, K. The NK cell MHC class I receptor Ly49A detects mutations on H–2D^{d} inside and outside of the peptide binding groove. *J. Immunol.* **166**, 4422–4428 (2001).
94. Michaelsson, J., Achour, A., Rolle, A. & Karre, K. MHC class I recognition by NK receptors in the Ly49 family is strongly influenced by the β_2-microglobulin subunit. *J. Immunol.* **166**, 7327–7334 (2001).
95. Wang, J. *et al.* Binding of the natural killer cell inhibitory receptor Ly49A to its major histocompatibility complex class I ligand. Crucial contacts include both H–2D^{d} and β_2-microglobulin. *J. Biol. Chem.* **277**, 1433–1442 (2002).
96. Matsumoto, N., Mitsuki, M., Tajima, K., Yokoyama, W. M. & Yamamoto, K. The functional binding site for the C-type lectin-like natural killer cell receptor Ly49A spans three domains of its major histocompatibility complex class I ligand. *J. Exp. Med.* **193**, 147–158 (2001).
97. Dimasi, N. *et al.* Crystal structure of the Ly49I natural killer cell receptor reveals variability in dimerization mode within the Ly49 family. *J. Mol. Biol.* **320**, 573–585 (2002).
98. Sundback, J., Achour, A., Michaelsson, J., Lindstrom, H. & Karre, K. NK cell inhibitory receptor Ly49C residues involved in MHC class I binding. *J. Immunol.* **168**, 793–800 (2002).
99. Diefenbach, A., Jensen, E. R., Jamieson, A. M. & Raulet, D. H. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature* **413**, 165–171 (2001).
100. Groh, V., Wu, J., Yee, C. & Spies, T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature* **419**, 734–738 (2002).
This paper identifies soluble MIC as a new strategy for tumour evasion.
101. Yokoyama, W. M. Catch us if you can. *Nature* **419**, 679–680 (2002).
102. Salih, H. R., Rammensee, H. G. & Steinle, A. Cutting edge: down-regulation of MICA on human tumours by proteolytic shedding. *J. Immunol.* **169**, 4098–4102 (2002).
103. Tortorella, D., Gewurz, B. E., Furman, M. H., Schust, D. J. & Ploegh, H. L. Viral subversion of the immune system. *Annu. Rev. Immunol.* **18**, 861–926 (2000).
104. Krmpotic, A. *et al.* MCMV glycoprotein gp40 confers virus resistance to CD8^{+} T cells and NK cells *in vivo*. *Nature Immunol.* **3**, 529–535 (2002).
105. Scalzo, A. A., Fitzgerald, N. A., Simmons, A., La Vista, A. B. & Shellam, G. R. *Cmv1*, a genetic locus that controls murine cytomegalovirus replication in the spleen. *J. Exp. Med.* **171**, 1469–1483 (1990).
106. Brown, M. G. *et al.* Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science* **292**, 934–937 (2001).
107. Daniels, K. A. *et al.* Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49h. *J. Exp. Med.* **194**, 29–44 (2001).
108. Lee, S. H. *et al.* Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C-type lectin superfamily. *Nature Genet.* **28**, 42–45 (2001).
109. Sjolin, H. *et al.* Pivotal role of KARAP/DAP12 adaptor molecule in the natural killer cell-mediated resistance to murine cytomegalovirus infection. *J. Exp. Med.* **195**, 825–834 (2002).
110. Arase, H., Mocarski, E. S., Campbell, A. E., Hill, A. B. & Lanier, L. L. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* **296**, 1323–1326 (2002).
111. Smith, H. R. *et al.* Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc. Natl Acad. Sci. USA* **99**, 8826–8831 (2002).
References 106–108, 110 and 111 are studies that identify Ly49h as being the factor involved in genetic resistance to MCMV and its ligand.
112. Dokun, A. O. *et al.* Specific and nonspecific NK cell activation during virus infection. *Nature Immunol.* **2**, 951–956 (2001).
113. Mehta, I. K., Smith, H. R. C., Wang, J., Margulies, D. H. & Yokoyama, W. M. A 'chimeric' C57L-derived Ly49 inhibitory receptor resembling the Ly49D activation receptor. *Cell. Immunol.* **209**, 29–41 (2000).
114. Farrell, H. E. *et al.* Inhibition of natural killer cells by a cytomegalovirus MHC class I homologue *in vivo*. *Nature* **386**, 510–514 (1997).
115. Kavanagh, D. G., Gold, M. C., Wagner, M., Koszinowski, U. H. & Hill, A. B. The multiple immune-evasion genes of murine cytomegalovirus are not redundant: m4 and m152 inhibit antigen presentation in a complementary and cooperative fashion. *J. Exp. Med.* **194**, 967–978 (2001).
116. Paloneva, J. *et al.* Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. *Nature Genet.* **25**, 357–361 (2000).
117. Orange, J. S., Fassett, M. S., Koopman, L. A., Boyson, J. E. & Strominger, J. L. Viral evasion of natural killer cells. *Nature Immunol.* **3**, 1006–1012 (2002).
118. McMahon, C. W. *et al.* Viral and bacterial infections induce expression of multiple NK cell receptors in responding CD8^{+} T cells. *J. Immunol.* **169**, 1444–1452 (2002).
119. Miller, J. D. *et al.* CD94/NKG2 expression does not inhibit cytotoxic function of lymphocytic choriomeningitis virus-specific CD8^{+} T cells. *J. Immunol.* **169**, 693–701 (2002).
120. Peacock, C. D., Lin, M. Y., Ortaldo, J. R. & Welsh, R. M. The virus-specific and allospecific cytotoxic T-lymphocyte response to lymphocytic choriomeningitis virus is modified in a subpopulation of CD8^{+} T cells coexpressing the inhibitory major histocompatibility complex class I receptor Ly49G2. *J. Virol.* **74**, 7032–7038 (2000).
121. Assarsson, E. *et al.* CD8^{+} T cells rapidly acquire NK1.1 and NK cell-associated molecules upon stimulation *in vitro* and *in vivo*. *J. Immunol.* **165**, 3673–3679 (2000).
122. Brownstein, D. G. & Gras, L. Differential pathogenesis of lethal mousepox in congenic DBA/2 mice implicates natural killer cell receptor NKRP1 in necrotizing hepatitis and the fifth component of complement in recruitment of circulating leukocytes to spleen. *Am. J. Pathol.* **150**, 1407–1420 (1997).
123. Pereira, R. A., Scalzo, A. & Simmons, A. Cutting edge: a NK complex-linked locus governs acute versus latent herpes simplex virus infection of neurons. *J. Immunol.* **166**, 5869–5873 (2001).
124. Idris, A. H. *et al.* The natural killer cell complex genetic locus, *Chok*, encodes Ly49D, a target recognition receptor that activates natural killing. *Proc. Natl Acad. Sci. USA* **96**, 6330–6335 (1999).
125. Dissen, E., Ryan, J. C., Seaman, W. E. & Fossum, S. An autosomal dominant locus, Nka, mapping to the Ly49 region of a rat natural killer (NK) gene complex, controls NK cell lysis of allogeneic lymphocytes. *J. Exp. Med.* **183**, 2197–2207 (1996).
126. Brown, M. G. *et al.* Natural killer gene complex (NKC) allelic variability in inbred mice: evidence for NKC haplotypes. *Immunogenetics* **53**, 584–591 (2001).
127. Lee, S. H. *et al.* Haplotype mapping indicates two independent origins for the Cmv1s susceptibility allele to cytomegalovirus infection and refines its localization within the Ly49 cluster. *Immunogenetics* **53**, 501–505 (2001).

References 126 and 127 describe NKC haplotypes in mice and contain a compendia of markers and typing regimens for distinguishing NKC alleles of many NKC genes in many laboratory mouse strains.

128. Wilson, M. J. *et al.* Plasticity in the organization and sequences of human KIR/ILT gene families. *Proc. Natl Acad. Sci. USA* **97**, 4778–4783 (2000).
129. Vilches, C. & Parham, P. KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. *Annu. Rev. Immunol.* **20**, 217–251 (2002).
130. Olcese, L. *et al.* Human killer cell activatory receptors for MHC class I molecules are included in a multimeric complex expressed by natural killer cells. *J. Immunol.* **158**, 5083–5086 (1997).
131. Raulet, D. H., Vance, R. E. & McMahon, C. W. Regulation of the natural killer cell receptor repertoire. *Annu. Rev. Immunol.* **19**, 291–330 (2001).
132. Mehta, I. K., Wang, J., Roland, J., Margulies, D. H. & Yokoyama, W. M. Ly49A allelic variation and MHC class I specificity. *Immunogenetics* **53**, 572–583 (2001).
133. Yokoyama, W. M. Hybrid resistance and the Ly49 family of natural killer cell receptors. *J. Exp. Med.* **182**, 273–277 (1995).
134. Kaufman, J. *et al.* The chicken B locus is a minimal essential major histocompatibility complex. *Nature* **401**, 923–925 (1999).
This study provides evidence for the apparent linkage of the NKC with the MHC in chickens.
135. Khalturin, K., Becker, M., Rinkevich, B. & Bosch, T. C. Urochordates and the origin of natural killer cells: identification of a CD94/NKR-P1-related receptor in blood cells of *Botryllus*. *Proc. Natl Acad. Sci. USA* **100**, 622–627 (2003).
This recent paper identifies the presence of an NKC-related molecule in tunicates.
136. Afonso, C. L. *et al.* The genome of fowlpox virus. *J. Virol.* **74**, 3815–3831 (2000).
This is one of many poxvirus genome papers that highlights the presence of open reading frames for NKC-related molecules.
137. Bull, C. *et al.* The centromeric part of the human NK gene complex: linkage of LOX1 and LY49L with the CD94/NKG2 region. *Genes Immunol.* **1**, 280–287 (2000).
138. Sawamura, T. *et al.* An endothelial receptor for oxidized low-density lipoprotein. *Nature* **386**, 73–77 (1997).
139. Delneste, Y. *et al.* Involvement of LOX1 in dendritic cell-mediated antigen cross-presentation. *Immunity* **17**, 353–362 (2002).
140. Sobanov, Y. *et al.* A novel cluster of lectin-like receptor genes expressed in monocytic, dendritic and endothelial cells maps close to the NK receptor genes in the human NK gene complex. *Eur. J. Immunol.* **31**, 3493–3503 (2001).
141. Brown, G. D. *et al.* Dectin1 is a major β-glucan receptor on macrophages. *J. Exp. Med.* **196**, 407–412 (2002).
References 139 and 141 describe innate non-NK-cell functions of NKC molecules.
142. Kung, S. K., Su, R. C., Shannon, J. & Miller, R. G. The NKRP1B gene product is an inhibitory receptor on SJL/J NK cells. *J. Immunol.* **162**, 5876–5887 (1999).
143. Colonna, M., Samaridis, J. & Angman, L. Molecular characterization of two novel C-type lectin-like receptors, one of which is selectively expressed in human dendritic cells. *Eur. J. Immunol.* **30**, 697–704 (2000).
144. George, T. C., Mason, L. H., Ortaldo, J. R., Kumar, V. & Bennett, M. Positive recognition of MHC class I molecules by the Ly49D receptor of murine NK cells. *J. Immunol.* **162**, 2035–2043 (1999).
145. O'Callaghan, C. A., Cerwenka, A., Willcox, B. E., Lanier, L. L. & Bjorkman, P. J. Molecular competition for NKG2D: H60 and RAE1 compete unequally for NKG2D with dominance of H60. *Immunity* **15**, 201–211 (2001).
146. Carayannopoulos, L. N. *et al.* Ligands for murine NKG2D display heterogeneous binding behaviour. *Eur. J. Immunol.* **32**, 597–605 (2002).
147. Vales-Gomez, M., Reyburn, H. T., Mandelboim, M. & Strominger, J. L. Kinetics of interaction of HLA-C ligands with natural killer cell inhibitory receptors. *Immunity* **9**, 337–344 (1998).
148. Chapman, T. L., Heikeman, A. P. & Bjorkman, P. J. The inhibitory receptor LIR1 uses a common binding interaction to recognize class I MHC molecules and the viral homolog UL18. *Immunity* **11**, 603–613 (1999).

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