A NEW SELF: MHC-CLASS-I-INDEPENDENT NATURAL-KILLER-CELL SELF-TOLERANCE

Vinay Kumar and Megan E. McNerney

Abstract | A fundamental tenet of the immune system is the requirement for lymphocytes to respond to transformed or infected cells while remaining tolerant of normal cells. Natural killer (NK) cells discriminate between self and non-self by monitoring the expression of MHC class I molecules. According to the 'missing-self' hypothesis, cells that express self-MHC class I molecules are protected from NK cells, but those that lack this self-marker are eliminated by NK cells. Recent work has revealed that there is another system of NK-cell inhibition, which is independent of MHC class I molecules. Newly discovered NK-cell inhibitory receptors that have non-MHC-molecule ligands broaden the definition of self as seen by NK cells.

CENTRAL TOLERANCE
Self-tolerance that is created at the level of the central lymphoid organs. Developing T cells, in the thymus, and developing B cells, in the bone marrow, that strongly recognize self-antigen must undergo further rearrangement of antigen-receptor genes to become self-tolerant, or they face deletion. NK cells, which differentiate in the bone marrow, are thought to upregulate the expression of inhibitory receptors until they are self-tolerant and are allowed to migrate to the periphery.

Department of Pathology, Committee on Immunology, University of Chicago, 5841 South Maryland Avenue, S-315 MC3083, Chicago, Illinois 60637, USA.
Correspondence to V.K.
e-mail: vkumar@bsd.uchicago.edu
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Natural killer (NK) cells have several important roles in innate immune responses. Their best-defined effector functions are their ability to kill haematopoietic tumour cells and virally infected cells and to produce pro-inflammatory cytokines, such as interferon-γ (IFN-γ) and tumour-necrosis factor, that activate other immune cells. As cells of the innate immune system, NK cells have an intrinsic and rapid ability to respond to damaged cells. Unlike the adaptive immune cells B cells and T cells, NK cells do not need to be primed, and the molecular mechanisms for this heightened state of alert are now being elucidated[1,2].

The function of NK cells in the elimination of transformed, infected and otherwise stressed cells is an important component of the early immune response. This ability to combat foreign invaders occurs together with tolerance to normal cells (that is, tolerance to self), a property of NK cells that is essential to prevent autoimmunity or destruction of bystander cells. The mechanisms of self-tolerance of NK cells are clearly effective, because there is no known autoimmune state that results from NK-cell dysfunction. Both B and T cells undergo rigorous scrutiny during development, which prevents the maturation of most autoreactive lymphocytes. Any leakage in these CENTRAL-TOLERANCE mechanisms is addressed by PERIPHERAL-TOLERANCE mechanisms. These peripheral mechanisms include monitoring of the way in which antigen is recognized: for example, autoreactive T cells can be tolerized by regulatory T cells or by tolerogenic dendritic cells expressing low levels of co-stimulatory ligands[3]. NK cells are also educated during development to create a population of mature NK cells that are self-tolerant but sensitive to foreign threats[4]. In the periphery, NK cells maintain non-responsiveness to self by being equipped with a large number of inhibitory receptors that recognize normal (self) cells.

Until recently, it was thought that NK-cell inhibitory receptors mainly recognize MHC class I molecules. However, there have now been several reports of NK-cell inhibitory receptors that recognize ligands that are not related to MHC class I molecules (TABLE 1). These ligands are broadly expressed by normal cells, indicating that they have a role in self-tolerance. As we discuss, these findings indicate a novel system that might explain how NK-cell self-tolerance is maintained in MHC-class-I-deficient individuals. Furthermore, novel inhibitory-receptor–ligand pairs provide new targets for the therapeutic manipulation of NK-cell responses. For example, interrupting self-tolerance

PERIPHERAL TOLERANCE
Self-tolerance that is mediated in the peripheral tissues. These mechanisms control potentially self-reactive lymphocytes that have escaped central-tolerance mechanisms.

IMMUNORECEPTOR TYROSINE-BASED INHIBITORY MOTIFS (ITIMs). ITIMs have the amino-acid sequence Ile/Val-X-Tyr-X-X-Leu/Val, where X denotes any amino acid. They recruit inhibitory phosphatases after phosphorylation of their tyrosine residue.

could improve NK-cell activity to haematopoietic malignancies. This overview discusses newly characterized receptor–ligand pairs, focusing on NK-cell inhibitory receptors that recognize widely distributed non-MHC-molecule ligands.

The 'missing-self' hypothesis and MHC receptors

Early studies indicated that NK-cell responses were induced by the absence of expression of MHC class I molecules at the surface of target cells, as described by the missing-self hypothesis[5] (FIG. 1). This hypothesis states that cells that express host MHC class I alleles are protected from NK cells; however, if a target cell fails to express MHC class I molecules, then NK cells kill this cell. The missing-self hypothesis is supported by findings that tumour cells or virally infected cells that have decreased expression of MHC class I molecules become susceptible to NK-cell killing[5,6]. A corollary of this idea is that healthy allogeneic cells are targets for NK cells because they also lack self-MHC alleles[7]. Because MHC class I molecules are expressed by almost all nucleated cells, they are an ideal universal marker of self. In some situations, activation signals can overcome MHC-dependent inhibition[8]. This occurs when cell-surface expression of activating ligands, such as ligands for NKG2D (NK group 2, member D), is induced on target cells by stress, infection or transformation (reviewed in REF. 8).

To monitor MHC class I molecules, NK cells express numerous receptors that recognize polymorphic epitopes of MHC molecules. The inhibitory receptors block NK-cell activation by recruiting protein tyrosine phosphatases that bind IMMUNORECEPTOR TYROSINE-BASED INHIBITORY MOTIFS (ITIMs) in the cytoplasmic domain of the receptors[8]. The human killer-cell immunoglobulin-like receptor (KIR) family and the murine Ly49 C-TYPE-LECTIN-like receptor family evolved to carry out similar functions[8]. The CD94–NKG2A heterodimer of lectin-like molecules is another inhibitory receptor, and it recognizes human HLA-E and mouse Qa1^b, which present MHC class I LEADER PEPTIDES[9–11]. Human NK cells also express the inhibitory receptor

Table 1 | **NK-cell inhibitory receptors that have non-MHC-molecule ligands**

Receptor	Alternative names	Species	Receptor expression	Host ligand	Alternative names	Ligand expression	Refs
2B4	CD244	Mouse and human	NK cells, memory CD8$^+$ T cells and other leukocytes	CD48	–	Nucleated haematopoietic cells and human endothelial cells	28–32, 35–39, 45
CEACAM1	CD66a or BGP	Mouse and human	Ubiquitous	CEACAM1 CEACAM5	CD66a CD66e or CEA	Ubiquitous Some types of epithelial cell	19, 59–61, 63–65
gp49B1	–	Mouse	Activated NK cells, activated T cells, mast cells and macrophages	$\alpha_V\beta_3$-Integrin	Vitronectin receptor or CD51–CD61	Low levels by most cells and high levels by certain cell types	86–90, 92
IRp60	–	Human	NK cells, some subsets of T cells, monocytes and granulocytes	Unknown, probably not an MHC molecule	–	–	133,134
KLRG1	MAFA	Mouse and human	Some subsets of NK cells, activated T cells, mast cells and basophils	Unknown, probably not an MHC molecule	–	–	93–95
LAIR1	–	Mouse and human	NK cells and most leukocytes	Unknown, probably not an MHC molecule	–	–	96
NKR-P1B	CD161b, KLRB1B or Ly55b	Mouse	NK cells	CLR-B	OCIL	Nucleated haematopoietic cells and some types of non-haematopoietic cell	49–52, 55,56
NKR-P1D	CD161d, KLRB1D or Ly55d	Mouse	Most NK cells	CLR-B	OCIL	Nucleated haematopoietic cells and some types of non-haematopoietic cell	49–52, 55,56
SIGLEC7	AIRM1 or p75	Human	NK cells, monocytes and some subsets of CD8$^+$ T cells	Sialic acid	–	Ubiquitous	67, 69–73
SIGLEC9	–	Human	50% of NK cells	Sialic acid	–	Ubiquitous	78
SIGLEC-E	–	Mouse	50% of NK cells	Sialic acid	–	Ubiquitous	79

AIRM1, adhesion inhibitory receptor molecule 1; BGP, biliary glycoprotein; CEA, carcinoembryonic antigen; CEACAM, CEA-related cell-adhesion molecule; CLR-B, C-type-lectin-related B; gp49B1, glycoprotein 49 B1; IRp60, inhibitory receptor protein 60; KLR, killer-cell lectin-like receptor; LAIR1, leukocyte-associated immunoglobulin-like receptor 1; MAFA, mast-cell-function-associated antigen; NK, natural killer; NKR-P1, NK-cell receptor protein 1; OCIL, osteoclast inhibitory lectin; SIGLEC, sialic-acid-binding immunoglobulin-like lectin.

C-TYPE LECTIN
Lectins are carbohydrate-binding molecules, and C-type lectins were named for their ability to bind calcium. C-type-lectin-like molecules, such as many of the natural-killer-cell receptors, are disulphide-linked homodimers that have sequence homology to C-type lectins; however, they do not bind calcium, and they often recognize proteins instead of carbohydrates.

LEADER PEPTIDES
Hydrophobic amino-acid sequences that signal for proteins to translocate to the endoplasmic reticulum. The leader peptide is cleaved before a protein is transported from the cell.

TRANSPORTER ASSOCIATED WITH ANTIGEN PROCESSING (TAP). TAP1 and TAP2 form a heterodimer in the membrane of the endoplasmic reticulum. The TAP1–TAP2 complex transports peptides from the cytoplasm to the endoplasmic reticulum, where peptides can be loaded onto MHC class I molecules. Without these peptides, MHC class I molecules are unstable and are much less likely to transit to the cell surface or to remain there.

β_2-MICROGLOBULIN (β_2m). A single immunoglobulin-like domain that non-covalently associates with the main polypeptide chain of MHC class I molecules. In the absence of β_2m, MHC class I molecules are unstable and are therefore found at very low levels at the cell surface.

leukocyte immunoglobulin-like receptor 1 (LIR1), which engages almost all MHC class I molecules[12]. All of these receptors are expressed by subsets of NK cells, but it is thought that, to remain tolerant to self, each NK cell must express at least one inhibitory receptor that recognizes a self-MHC class I allele[4].

Evidence for MHC-independent inhibition

The missing-self hypothesis elegantly explains much of the biology of NK cells, and it made important predictions, such as the existence of MHC-binding receptors at the surface of NK cells. But there are some aspects of NK-cell biology that cannot be adequately explained solely on the basis of this premise (BOX 1). Most astonishing is the finding that humans and mice with abnormally low levels of MHC class I molecules, owing to a mutation in the TRANSPORTER ASSOCIATED WITH ANTIGEN PROCESSING 2 (TAP2) protein, develop normal numbers of NK cells. These NK cells can kill tumour cells, although suboptimally, and remarkably, they are tolerant of autologous MHC class I^{low} cells[13–16]. These findings were initially thought to be explained by the low levels of residual MHC class I molecules expressed by the hosts, thereby allowing tolerance to be maintained. To address this, investigators analysed mice that were deficient in both TAP1 and β_2-MICROGLOBULIN (β_2m), which express undetectable amounts of MHC class I molecules at the cell surface. NK cells from these mice were found to function similarly to those from mice that are deficient in either TAP or β_2m, indicating that there are non-MHC-dependent mechanisms of NK-cell self-tolerance[17,18].

To account for these observations, two new explanations for NK-cell self-tolerance have been proposed: first, NK cells from MHC-class-I-deficient hosts have a lower activation potential, owing to decreased activating-receptor expression and/or function; or second, NK cells are kept self-tolerant by interactions between non-MHC-dependent receptor–ligand pairs. Considering the first possibility, there is both evidence that supports it and evidence that repudiates it[19–21]. In some TAP2-deficient patients, the expression of activating receptors is lower than in healthy control individuals, and of those NK-cell clones that express activating receptors, the receptors function normally[19]. In other patients and in β_2m-deficient mice, activating receptors are expressed at normal levels, and these receptors are also functional[20,21]. So, it is not clear to what extent activating-receptor modulation accounts for NK-cell self-tolerance in MHC-class-I-deficient hosts. As will become apparent here, there is an increasing body of evidence that supports the second possibility, that non-MHC-mediated inhibition regulates NK cells.

Another independent piece of evidence that supports the idea of non-MHC-dependent self-tolerance is the finding that not all NK cells express an inhibitory receptor for a self-MHC class I allele[22]. In the C57BL/6 strain of inbred mouse, which is of the haplotype H–2^b, the known H–2^b-binding NK-cell inhibitory receptors are Ly49C, Ly49I and CD94–NKG2A[9,23,24]. So, it would be expected that every NK cell in a C57BL/6 mouse would express at least one of these receptors, yet 10% of NK cells in these mice do not express any of these receptors[22]. It has not been ruled out that other H–2^b-binding inhibitory receptors exist, but an

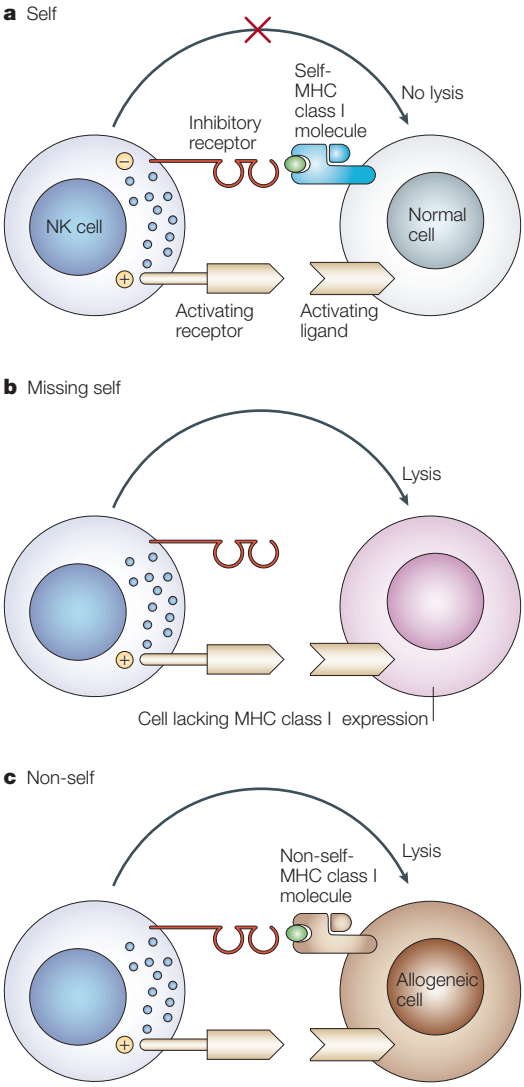

Figure 1 | **NK-cell self-tolerance and the 'missing-self' hypothesis. a** | On interacting with a normal, autologous target cell, a natural killer (NK) cell might receive activating signals; however, because the target cell expresses the appropriate self-MHC class I alleles, the NK cell does not lyse the target cell. This is a consequence of inhibitory signals from the ligated MHC-binding receptor at the surface of the NK cell. **b** | If the target cell loses expression of MHC class I molecules, owing to viral infection or transformation, then the MHC-binding inhibitory receptor at the surface of the NK cell is not engaged. In this way, the NK cell does not receive inhibitory signals and therefore lyses the target cell. In this case, the target cell is perceived by the NK cell to be missing self. **c** | In the setting of an allogeneic transplant, host NK cells interact with donor target cells that express foreign MHC class I alleles. In most cases, the foreign, non-self-MHC class I molecules do not engage all of the inhibitory receptors at the surface of a host NK cell. This leads to lysis of the allogeneic cells by host NK cells.

Box 1 | Evidence for non-MHC-binding inhibitory receptors

Before non-MHC-binding natural killer (NK)-cell inhibitory receptors were identified and characterized, there were paradoxes in NK-cell biology that could not be explained by regulation of self-tolerance through MHC class I molecules alone. These anomalies pointed to the existence of non-MHC-binding inhibitory receptors. The newly identified inhibitory receptors have widely expressed non-MHC-molecule ligands, and their identification might allow us to answer some of the following outstanding questions.

- **Humans and mice that are deficient in transporter associated with antigen processing (TAP) have NK cells that are not autoreactive. So, how are NK cells kept self-tolerant in the absence of MHC class I molecules?**
- **In mice, some NK cells do not express any of the known self-MHC-binding inhibitory receptors. How are these NK cells kept self-tolerant?**
- **During development, there might be a window in which NK cells have effector functions (because at this stage they form lytic granules and express interferon-γ), but these cells do not yet express Ly49-family members and killer-cell immunoglobulin-like receptors (KIRs). What prevents these cells from being autoreactive to other cells in the bone marrow?**

alternative explanation for how these NK cells remain self-tolerant is that they express non-MHC-binding inhibitory receptors.

An additional observation that points to the existence of non-MHC-binding inhibitory receptors is that, during NK-cell development, some effector functions are acquired before the appearance of MHC-binding receptors[1,25–27]. This implies that there are unregulated and armed NK cells in the bone marrow, which could potentially be autoaggressive. Again, this paradox could be resolved if immature NK cells express an inhibitory receptor that recognizes a non-MHC-molecule self-ligand, and there is evidence for this[28,29].

Non-MHC-binding NK-cell inhibitory receptors

Inhibition of NK cells by 2B4. Of the non-MHC-binding receptors, 2B4 (also known as CD244) is one of the best characterized (FIG. 2). 2B4 is expressed by all human and mouse NK cells, as well as by memory αβ T cells, γδ T cells, monocytes, granulocytes and mast cells[30–32]. It is a member of the SIGNALLING LYMPHOCYTIC ACTIVATION MOLECULE (SLAM; also known as CD150) subfamily of the CD2 family of immunoglobulin receptors. Members of the SLAM subfamily have two or more cytoplasmic IMMUNORECEPTOR TYROSINE-BASED SWITCH MOTIFS (ITSMs). Signalling through molecules that contain ITSMs involves many of the same SRC HOMOLOGY 2 (SH2)-DOMAIN-containing molecules as signalling through molecules that contain ITIMs or IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIFS (ITAMs), including the protein tyrosine phosphatases SHP1 (SH2-domain-containing protein tyrosine phosphatase 1) and SHP2 and kinases such as LCK and FYN[33,34] (FIG. 3). ITSMs are implicated in the pathogenesis of X-LINKED LYMPHOPROLIFERATIVE SYNDROME (XLP), which is caused by a mutation in the ITSM-binding protein SLAM-associated protein (SAP)[34]. SAP binds ITSMs in human 2B4 and mediates activating signals by recruiting FYN; in the absence of functional SAP, the activating signals are abrogated, and human 2B4 either fails to signal or recruits phosphatases that lead to inhibitory outcomes[33].

The ligand for 2B4 is CD48, a GLYCOSYLPHOSPHATIDYLINOSITOL (GPI)-LINKED member of the CD2 family[35]. CD48 is expressed by all nucleated haematopoietic cells and by human endothelial cells; this broad expression provides numerous opportunities for interaction between 2B4-expressing immune cells and CD48-expressing immune cells[30]. In fact, many of these interactions are beginning to be characterized in normal and diseased states. In mice, 2B4 has been considered to be an activating receptor, but recent evidence indicates that it can also inhibit NK cells[29,36–39]. The initial characterization in mice used 2B4-specific antibodies[37]; when added to cytotoxicity assays, these antibodies increase target-cell lysis, indicating that 2B4 is an activating receptor[37]. More recent studies indicate that such 'activation' is a consequence of interrupting negative signals from CD48 expressed by target cells[29,38,39]. Consistent with this, antibody-mediated enhancement of lysis is seen only when CD48-expressing target cells are used[29,37]. The most unambiguous data regarding the function of 2B4 are provided by recent studies of 2B4-deficient mice[29,38,39]. NK cells from 2B4-deficient mice show higher levels of killing of $CD48^+$ tumour cells than wild-type NK cells[29,38,39]. Moreover, *in vivo*, 2B4-deficient mice eliminate $CD48^+$ tumour cells more efficiently than wild-type mice[29,39]. Together, these studies indicate that mouse 2B4 mainly functions as an inhibitory receptor. In addition to inhibiting cytotoxicity, 2B4 also inhibits NK-cell production of IFN-γ when it is engaged by targets that express CD48 (REF. 29). Furthermore, unlike the human form of 2B4, inhibition by mouse 2B4 occurs regardless of the presence or absence of SAP[29,38]. Despite the dominant role of 2B4 being inhibition of NK-cell function, there are specific situations in which 2B4 can activate mouse NK cells. These include the crosslinking of 2B4 at the surface of NK cells, using plate-bound antibodies[37], homotypic 2B4–CD48 interactions among NK cells and among $CD8^+$ T cells, and 2B4–CD48 interactions among NK cells and $CD8^+$ T cells[40–42].

In contrast to mouse 2B4, human 2B4 seems to be mainly an activating receptor[36]. This conclusion is based on studies in which 2B4 was crosslinked by antibodies, as well as studies that involved $CD48^+$ and $CD48^-$ target cells[36,43,44]. In general, CD48-expressing target cells (unlike those in mice) are more susceptible to NK-cell killing than cells that do not express CD48 (REFS 43,44). 2B4 can also be inhibitory in humans but only in the absence of functional SAP, as occurs in patients with XLP and during the development of human NK cells[28,45].

In mice, the inhibitory effect of 2B4 extends beyond tumour-cell targets. 2B4-deficient mouse NK cells show increased killing of $CD48^+$ allogeneic and syngeneic splenocytes, indicating that, even in the presence of the powerful inhibitory effect of self-MHC class I molecules, 2B4 has an important role in the inhibition of NK cells[29]. These data show that the mouse 2B4–CD48 pair provides a system for NK-cell self-tolerance that is independent of MHC class I molecules. So, could these findings resolve the paradoxes

SIGNALLING LYMPHOCYTIC ACTIVATION MOLECULE (SLAM). A receptor that is expressed by several types of immune cell. Receptors in the SLAM subfamily of CD2 proteins, which includes 2B4, have similar sequences, have immunoreceptor tyrosine-based switch motifs (ITSMs) and bind SLAM-associated protein (SAP).

IMMUNORECEPTOR TYROSINE-BASED SWITCH MOTIFS (ITSMs). ITSMs have the amino-acid sequence Thr-X-Tyr-X-X-Val/Ile, where X denotes any amino acid. They recruit many of the same signalling molecules as immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and immunoreceptor tyrosine-based activation motifs (ITAMs), but they also recruit SAP (signalling lymphocytic activation molecule (SLAM)-associated protein).

SRC-HOMOLOGY-2 DOMAIN (SH2 domain). A domain that is found in signalling molecules. It binds phosphorylated tyrosine residues and thereby mediates protein–protein interactions.

IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIFS (ITAMs). ITAMs have the amino-acid sequence Asp/Glu-X-X-Tyr-X-X-Leu/Ile-X_{6-8}-Tyr-X-X-Leu/Ile, where X denotes any amino acid. They recruit activating signalling molecules after tyrosine phosphorylation.

X-LINKED LYMPHOPROLIFERATIVE SYNDROME (XLP). Patients with XLP have complicated immune dysfunctions, often triggered by infection with Epstein–Barr virus. Many patients develop fatal B-cell lymphoproliferation. The gene that encodes SAP (signalling lymphocytic activation molecule (SLAM)-associated protein) has been found to be mutated in these patients.

(BOX 1) of MHC-class-I-dependent inhibition? For the question of how NK-cell self-tolerance is maintained during development, when MHC-binding receptors are absent, the answer is yes; human and mouse 2B4 function to protect autologous cells from autoaggressive, developing NK cells[28,29]. Concerning the other mysteries of MHC molecules and NK-cell self-tolerance, emerging data indicate that 2B4–CD48 interactions maintain self-tolerance in the absence of regulation by MHC class I molecules. NK cells from β_2m-deficient mice are prevented from killing syngeneic cells by CD48 (V.K. and M.E.M., unpublished observations). Furthermore, mature NK cells that lack self-MHC-binding inhibitory receptors are kept self-tolerant by 2B4 (V.K. and M.E.M., unpublished observations). These findings strongly support the idea that, both in the presence and in the absence of MHC-class-I-mediated regulation, 2B4–CD48 interactions restrict NK-cell autoreactivity.

Why are there differences in the function of 2B4 expressed by mouse and human NK cells? One explanation is that the studies of human and mouse NK cells were carried out differently. The studies of mouse cells examined 2B4-deficient NK cells *in vivo*, whereas the studies of human cells used antibody crosslinking of 2B4 *in vitro*. A second potential explanation is that human NK cells have evolved a different requirement for SAP, which provides an activating signal in human NK cells.

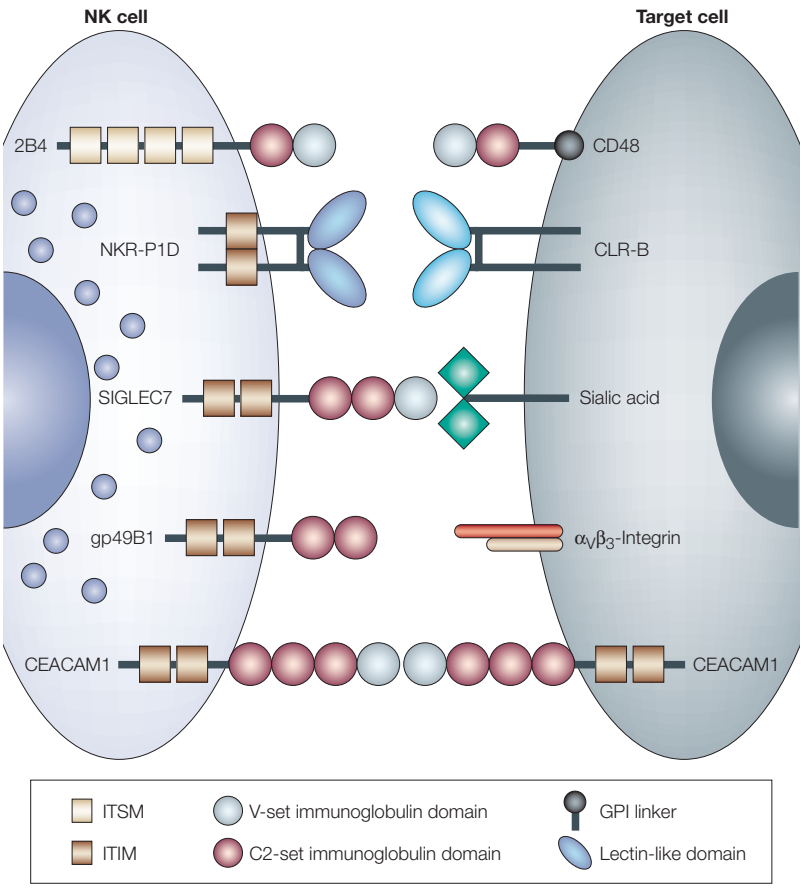

Figure 2 | **NK-cell inhibitory receptors that have non-MHC-molecule ligands.** As well as MHC class I molecules, there are several other types of molecule that inhibit natural killer (NK) cells. As depicted, these ligands are structurally diverse, and they are widely expressed by both haematopoietic and non-haematopoietic cells. Unlike killer-cell immunoglobulin-like receptors (KIRs) and Ly49-family members, which are expressed by subpopulations of NK cells, the non-MHC-binding inhibitory receptors are expressed by most (and in some cases, all) NK cells. The human form of 2B4 is shown; in mice, 2B4 also has a short isoform with two immunoreceptor tyrosine-based switch motifs (ITSMs). Both forms interact with CD48. Both NK-cell receptor protein 1 (NKR-P1)-family members and their ligands C-type-lectin-related (CLR)-family members form homodimers and have lectin-like extracellular domains. Sialic-acid-binding immunoglobulin-like lectin 7 (SIGLEC7) binds sialic-acid linkages that are present on the exposed portion of its ligand sialic acid. Glycoprotein 49B1 (gp49B1) is found in mice and is structurally similar to KIRs. It interacts with $\alpha_V\beta_3$-integrins. Carcinoembryonic-antigen-related cell-adhesion molecule 1 (CEACAM1) has many isoforms, which are generated by alternative splicing. These have varying numbers of immunoglobulin domains, and immunoreceptor tyrosine-based inhibitory motifs (ITIMs) can be either present or absent from their cytoplasmic domain. The most common form expressed by leukocytes is shown. GPI, glycosylphosphatidylinositol.

Inhibitory receptors of the NK-cell-receptor protein 1 family. In mice, the NK-cell-receptor protein 1 (NKR-P1; also known as killer-cell lectin-like receptor B1, KLRB1) family of receptors has five members: NKR-P1A, NKR-P1B, NKR-P1C, NKR-P1D and NKR-P1F. Similar to the Ly49 family, NKR-P1-family members are homodimeric C-type-lectin-like molecules. NKR-P1C was the first to be identified and is also known as NK1.1, which is a commonly used marker for identifying the NK cells of C57BL/6 mice[46,47]. NKR-P1C has a charged transmembrane residue that associates with the γ-chain of the high-affinity receptor for IgE (FcεRI), and ligation of NKR-P1C activates mouse NK cells[48]. NKR-P1A and NKR-P1F have similar sequences and are thereby thought to be activating receptors. By contrast, NKR-P1B and NKR-P1D contain ITIMs, and ligation of these receptors inhibits NK cells in functional assays[49–52]. In humans, only one NKR-P1 molecule, NKR-P1A, has been identified, and its function is unclear, because it lacks an ITIM or a charged transmembrane residue[53].

Recently, ligands for NKR-P1 molecules have been identified: NKR-P1B and NKR-P1D recognize C-type-lectin-related B (CLR-B; also known as OCIL), and NKR-P1F binds CLR-G (also known as OCILrP2)[51,52]. The CLR family has three members: CLR-B, CLR-F and CLR-G. The genes encoding these are located, together with the genes encoding NKR-P1-family members, on human chromosome 12 and mouse chromosome 6, in the NK complex of lectin-like molecules[54]. Similar to 2B4 and CD48, the NKR-P1 family and the CLR family are tightly genetically linked, indicating that co-inheritance of the receptor and self-ligand might be biologically important[30,54].

Similar to MHC class I molecules, CLR-B protein is broadly expressed, by all nucleated haematopoietic cells and by some non-haematopoietic cells[52]. This finding is consistent with the observation that *Clr-b* mRNA is widely expressed[55,56]. NKR-P1D protein is expressed by most NK cells, and it inhibits NK-cell lysis of CLR-B-expressing target cells[51]. Interaction between NKR-P1D and CLR-B also inhibits the lytic activity of NK cells against syngeneic MHC class I^+ and MHC class I^{low} tumour cells[51], showing that, similar

GLYCOSYLPHOSPHATIDYL-INOSITOL LINKED (GPI linked). A lipid modification of a protein that anchors the protein to the plasma membrane.

to the 2B4–CD48 system, NKR-P1D–CLR-B-mediated inhibition occurs in parallel with MHC-class-I-mediated inhibition. Intriguingly, NK cells from MHC class I^{low}, β_2m-deficient mice are inhibited by NKR-P1D, indicating that NKR-P1D can regulate NK cells in the absence of normal levels of MHC class I molecules. Despite this *in vitro* data, the *in vivo* significance of NKR-P1B- and NKR-P1D-mediated inhibition has yet to be determined.

So far, a human homologue of these inhibitory NKR-P1 molecules has not been identified. A human CLR-like molecule, lectin-like transcript 1 (LLT1), is known and is closely related to mouse CLR-G[57]. LLT1 protein is broadly expressed by peripheral-blood cells, including NK cells[58]. Surprisingly, ligation of LLT1 at the surface of NK cells activates IFN-γ production, indicating that CLR and CLR-like molecules might signal bidirectionally, functioning both as ligands and as signalling molecules themselves[58]. Identifying the cognate interacting proteins for LLT1 and other orphan lectin-like receptors encoded in the human NK complex[54] will shed light on human NK-cell regulation by the NKR-P1 family of receptors.

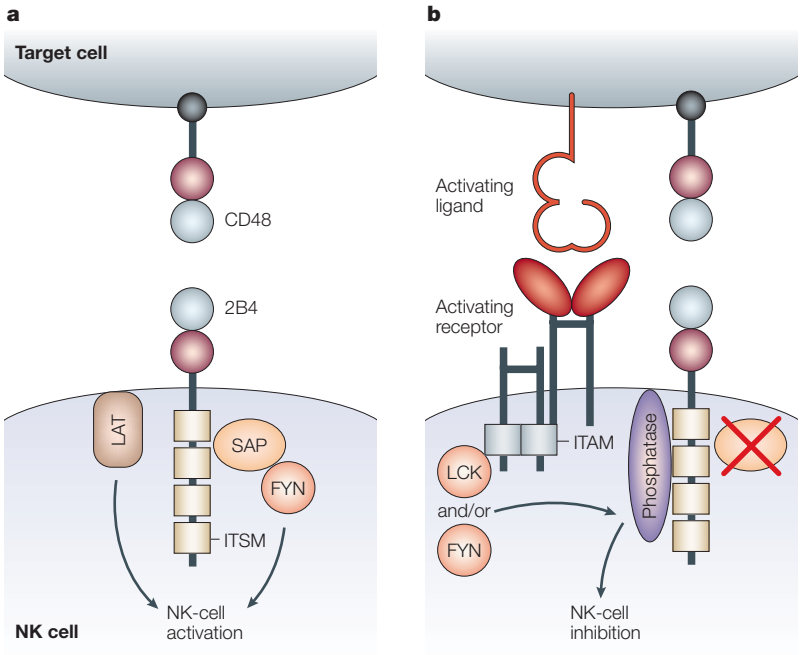

Figure 3 | **Proximal signalling pathways of 2B4: activating and inhibitory.** Immunoreceptor tyrosine-based switch motifs (ITSMs) are more versatile than traditional immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and immunoreceptor tyrosine-based activation motifs (ITAMs), because ITSMs recruit kinases in some situations and phosphatases in others. **a** | Ligation of 2B4 leads to activation of mature human natural killer (NK) cells. When 2B4 is engaged by CD48, ITSMs in the cytoplasmic tail of 2B4 bind the SRC homology 2 (SH2) domain of SAP (signalling lymphocytic activation molecule (SLAM)-associated protein). SAP then recruits the kinase FYN, through the SH3 domain of FYN. Linker for activation of T cells (LAT) molecules present in lipid-raft domains also associate with 2B4, and in conjunction with FYN, this interaction leads to the activation of NK cells. **b** | In mouse NK cells, as well as in NK cells from patients with X-linked lymphoproliferative syndrome (XLP) and in immature human NK cells, 2B4 transmits inhibitory signals. In humans, this inhibition occurs in the absence of functional SAP, as is the case for patients with XLP and for immature NK cells. When 2B4 and an activating receptor are co-engaged by a potential target cell, the ITSMs of 2B4 are phosphorylated by the SRC-family kinases LCK and/or FYN. This leads to the recruitment of phosphatases, such as SHP1 (SH2-domain-containing protein tyrosine phosphatase 1) and SHP2, which in turn inhibit activation by dephosphorylating molecules downstream of the activating receptor. In mice, 2B4 transmits inhibitory signals regardless of the presence or absence of SAP. ITAM, immunoreceptor tyrosine-based activation motif.

Carcinoembryonic-antigen-related cell-adhesion molecule 1 mediates immune self-tolerance. The carcinoembryonic antigen (CEA) family is large and multifunctional[59]. Its founding member, CEA-related cell-adhesion molecule 5 (CEACAM5; also known as CEA), is most renowned for its use as a marker of colon cancer[59]. CEAs are members of the immunoglobulin superfamily and can be divided into two subgroups: the CEACAM subgroup and the pregnancy-specific glycoprotein subgroup. The CEACAM subgroup has seven members. CEACAM1 (also known as CD66a or BGP) is the only CEACAM that is expressed by NK cells[60]. The ligand for CEACAM1 is CEACAM1 itself, binding in a homophilic interaction[61]. CEACAM1 also takes part in heterophilic interactions with the GPI-linked molecule CEACAM5 (REF. 61). CEACAM1 has several isoforms, which are generated by alternative splicing; these include two cytoplasmic tail derivatives: a long form that contains two ITIMs, and a short form that lacks ITIMs. Humans and mice preferentially express the ITIM-containing form[62]. In addition to NK cells, CEACAM1 is expressed by granulocytes, dendritic cells, lymphocytes, endothelial cells and epithelial cells[59,63].

Recent investigations have established the importance of CEACAM1-mediated negative regulation of NK cells[19,64,65]. One study examined the role of CEACAM1 in the prevention of NK-cell autoreactivity in TAP2-deficient patients[19]. Remarkably, whereas 12% of NK-cell clones from healthy donors express CEACAM1, 80% of NK-cell clones from TAP2-deficient patients express CEACAM1; the TAP2-deficient NK cells also express CEACAM1 at higher levels than normal NK cells. So, CEACAM1 expression seems to be upregulated to compensate for abnormal MHC-class-I-mediated NK-cell inhibition. Importantly, blocking the engagement of CEACAM1 at the surface of TAP2-deficient NK cells allows the killing of autologous cells (which express CEACAM1), showing that, in the absence of self-MHC class I molecules, CEACAM1 can prevent NK-cell autoaggression[19].

Notably, even in the presence of self-MHC class I molecules, CEACAM1 prevents NK-cell autoreactivity[19]. Specifically, CEACAM1 inhibits TAP2-deficient NK cells from killing $TAP2^+$ cells. Even NK-cell clones from healthy donors killed autologous cells when CEACAM1 was blocked. Both $TAP2^+$ and $TAP2^-$ NK cells showed maximal killing of MHC class I^+ target cells only when MHC class I molecules and CEACAM1 were concomitantly disrupted[19]. Therefore, CEACAM1 and MHC class I molecules at the surface of target cells provide layers of inhibition that are not redundant. Inexplicably, $CEACAM1^-$ NK-cell clones from TAP2-deficient patients did not kill autologous cells; these NK cells

V-SET IMMUNOGLOBULIN DOMAIN
An immunoglobulin domain is a characteristic protein fold that is present in all members of the immunoglobulin superfamily. On the basis of size and sequence, V-set immunoglobulin domains are similar to the variable regions of antibody molecules.

C2-SET IMMUNOGLOBULIN DOMAINS
C2-set immunoglobulin domains are similar to the constant regions of antibody molecules, as defined on the basis of size and sequence.

might be regulated by decreased activating-receptor expression or by expression of another receptor that recognizes self[19].

Soluble CEACAM1 is found in normal serum and is present at increased levels in various pathological states[66]. In comparison with healthy control individuals, TAP2-deficient patients have much less serum CEACAM1 (REF. 65). Soluble CEACAM1 blocks signalling through cell-surface CEACAM1, as shown by the increase in NK-cell activity *in vitro* caused by the addition of CEACAM1–immunoglobulin fusion proteins or serum from healthy control individuals. So, although the biological function of soluble CEACAM1 remains unknown, a decreased level of soluble CEACAM1 might ensure that the inhibitory effect of CEACAM1 in the absence of MHC class I molecules is maximized[65].

Role of sialic-acid-binding immunoglobulin-like lectins and sugar residues in self-recognition. Sialic-acid-binding immunoglobulin-like lectins (SIGLECs) have a V-SET IMMUNOGLOBULIN DOMAIN, which binds sialic acid, and varying numbers of C2-SET IMMUNOGLOBULIN DOMAINS[67]. Humans have 11 SIGLECs, and human NK cells express SIGLEC7 (also known as p75 or AIRM1) and SIGLEC9 (REF. 67). Mice have eight SIGLECs, and mouse NK cells express SIGLEC-E[68]. SIGLEC9 and SIGLEC-E each have an ITSM (similar to 2B4) and an ITIM, whereas SIGLEC7 has two ITIMs[67]. Sialic acids are a large, diverse family of nine-carbon α-keto-acid monosaccacharides[68]. They are ubiquitously expressed and are often bound to the exposed portions of other carbohydrates that are components of cell-surface lipids and proteins, through α2,3- and α2,6-sugar linkages, or bound to each other through α2,8-sugar linkages[68].

SIGLEC7 was originally identified as an inhibitory receptor expressed by all NK cells and monocytes and some $CD8^+$ T cells[69,70]. The cytoplasmic region of SIGLEC7 binds SHP1, and SIGLEC7 inhibits NK-cell killing when its extracellular domain is crosslinked by antibody[69]. One ligand that has been identified for SIGLEC7 is the ganglioside GD3, a glycosphingolipid that contains an α2,8-linked disialic acid[71,72], and NK cells do not lyse GD3-synthase-transfected P815 tumour cells, as a result of inhibition by SIGLEC7 (REF. 73). Interestingly, GD3 is expressed by central-nervous-system cells, by melanoma cells and by some T cells[74,75]. Of the other SIGLECs expressed by NK cells, SIGLEC9 inhibits both T-cell lines and basophil cell lines[76,77], and it is expressed by 50% of NK cells[78]. SIGLEC-E, which is expressed by mouse NK cells, is similar to SIGLEC7 and SIGLEC9; 50% of mouse NK cells express SIGLEC-E[79], and it is also inhibitory[80]. There is little known about the function of these receptors; however, as discussed later, SIGLECs might have a role in discrimination between self and non-self in pathogenic states.

Other non-MHC-binding NK-cell inhibitory receptors. Five signal-regulatory proteins (SIRPs) have been identified in humans, and one, SIRP-α[81], has been identified in mice. SIRPs are immunoglobulin-superfamily members; they are polymorphic and are also generated by variations in splicing. SIRPs are thought to be the primordial adaptive immune receptor, because they have joined V–J immunoglobulin domains[81]. SIRP-α and SIRP-β2 recognize the ubiquitously expressed molecule CD47 (also known as integrin-associated protein, IAP)[81–83]. SIRP-α inhibits phagocytes from eliminating normal, $CD47^+$ self-cells; CD47-deficient haematopoietic cells are promptly cleared by phagocytes when transplanted into normal, syngeneic hosts[84,85]. This innate system of self-recognition resembles the regulation of NK cells by MHC class I molecules, so it is not surprising that NK cells express SIRP-β2 (REFS 82,83). SIRP-β2 is expressed by all activated NK cells and by most T cells. What is surprising is that SIRP-β2-specific antibody does not influence NK-cell lysis[82], but this is probably a consequence of the lack of ITIMs or charged transmembrane residues in the cytoplasmic region of the SIRP-β2 protein. The presence of SIRP-β2 at the surface

Table 2 | **Numerous pathogens encode potential ligands for non-MHC-binding NK-cell inhibitory receptors**

Pathogen	Microbial product or effect	NK-cell receptor	Outcome	References
Epstein–Barr virus	Increased host expression of CD48	2B4	Possibly activation of human NK cells	97
Neisseria spp., *Salmonella typhimurium*, *Haemophilus influenzae*, *Moraxella catarrhalis* and mouse hepatitis virus	Bind CEACAM1 for entry to host cells	CEACAM1	Inhibition of T cells; unknown effect on NK cells	113–117
Hepatitis C virus	Envelope protein E2	CD81	Inhibition of NK cells	108,109
Fowlpox virus, cowpox virus, vaccinia virus, myxoma virus, African swine fever virus and rat cytomegalovirus	C-type lectin homologue	Possibly NKR-P1-family member	Unknown	100–105
Poxviruses: variola virus, vaccinia virus and myxoma virus	CD47 homologue	Possibly SIRP-β2	Unknown	103,106,107
Neisseria meningitidis, *Haemophilus influenzae*, *Escherichia coli*, *Trypanosoma cruzi* and others	Sialic acid	Possibly SIGLEC7 and/or SIGLEC9	Unknown	68

CEACAM1, carcinoembryonic-antigen-related cell-adhesion molecule 1; NK, natural killer; NKR-P1, NK-cell receptor protein 1; SIGLEC, sialic-acid-binding immunoglobulin-like lectin; SIRP-β2, signal-regulatory protein-β2.

of T cells promotes their adhesion to CD47$^+$ cells, and it is possible that SIRP-β2 expressed by NK cells might function in a similar manner[82].

Glycoprotein 49 B1 (gp49B1) is an inhibitory receptor in mice, and it has two C2-set immunoglobulin domains and two ITIMs[86]. No human homologue has been identified as yet, but it is structurally similar to the killer-cell immunoglobulin-like receptors (KIRs)[87]. gp49B1 is expressed by most activated NK cells, as well as by mast cells, macrophages and activated T cells[86–88]. gp49B1 binds $\alpha_V\beta_3$-integrin (a heterodimer of CD51 and CD61), which is expressed at low levels in most tissues and at high levels by osteoclasts, macrophages, some types of granulocyte, platelets, endothelial cells, endometrial tissue, inflammatory sites and invasive tumour cells[89,90]. The NK cells of gp49B1-deficient mice are mostly functional[91], but after *in vivo* infection with virus, gp49B1-deficient NK cells produce more IFN-γ *ex vivo*[92]. In light of the identification of the ligand for gp49B1, it will be important to readdress the role of gp49B1 in NK-cell killing of $\alpha_V\beta_3$-integrin$^+$ target cells.

KLRG1 (also known as MAFA) is expressed by NK cells and other leukocytes[93]. It is thought to be inhibitory, as it contains an ITIM and as crosslinking with specific antibody decreases cytokine production and lytic activity by an NK-cell line[94]. Its ligand is unknown, but interestingly, in contrast to Ly49 molecules, KLRG1 expression is decreased in MHC-class-I-deficient mice[95].

Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1) is also expressed by most human leukocytes, including NK cells[96]. LAIR1 contains two ITIMs, binds SHP1 and SHP2, and inhibits NK cells when crosslinked with specific antibody[96]. Its ligand is unknown, but it is thought to have a non-MHC molecule as a ligand[96].

Pathogens and non-MHC inhibitory ligands

It is clear that pathogens exploit MHC class I molecules to their advantage[6]. Some microorganisms have evolved mechanisms to inhibit the presentation of peptides by MHC class I molecules so that they remain concealed from the adaptive immune system. Furthermore, some pathogens express MHC-class-I-like molecules to ensure their protection from host NK-cell responses. Given the influence of non-MHC-binding inhibitory receptors, non-MHC-molecule markers of self are apt to be exploited by pathogens for immune protection (TABLE 2).

For example, the ligand for 2B4, CD48, was originally identified as a protein that is induced by infection of B cells with Epstein–Barr virus (EBV)[97]. Perhaps, increased expression of CD48 originally inhibited NK-cell antiviral activity. EBV infections occur worldwide, and according to the Centers for Disease Control and Prevention (United States), EBV infects at least 95% of people in the United States. It is conceivable that, under such pressure from EBV, SAP or SAP-like molecules evolved to convert 2B4 from an inhibitory receptor that prevents responses to self in mice to an activating receptor that recognizes pathogens in humans. Supporting this hypothesis, patients with abnormal signalling through 2B4 or with insufficient NK-cell activity are susceptible to fulminant infections with EBV and other herpesviruses[34,98]. Moreover, this occurrence might have a precedent in the evolution of mouse resistance to mouse cytomegalovirus (MCMV)[99]. Ly49I is an inhibitory receptor for MHC class I molecules, and the MCMV-encoded protein m157 engages Ly49I, thereby inhibiting NK-cell antiviral function. Some strains of mice resist infection with MCMV by expressing Ly49H, which transmits activating signals when bound to m157 (REF. 99). Many NK-cell receptors have similar activating and inhibitory counterparts, which might have evolved from a pathogen–host 'arms race'.

C-type-lectin-like molecules are encoded by several viruses, and it would be interesting to determine whether these engage inhibitory NKR-P1-family members[100–105]. In addition, CD47 homologues are expressed by the poxviruses variola virus, vaccinia virus and myxoma virus[103,106,107]. Could these virally encoded proteins mimic ligands for SIRPs, the inhibitory receptors that are expressed by NK cells, T cells and other leukocytes? The role of these proteins in the pathogenesis of viral diseases is unknown, but the range of microorganisms that have independently acquired genes encoding C-type-lectin-like molecules or CD47-like proteins indicates that this area is worth pursuing. One example of this possibility has been documented: the hepatitis C virus envelope protein E2 binds CD81 expressed by NK cells and, in this way, inhibits NK-cell antiviral activity[108,109].

Numerous microorganisms produce or acquire sialic acid, including *Neisseria meningitidis*, *Haemophilus influenzae*, *Escherichia coli* and *Trypanosoma cruzi*[68]. It will be important to determine whether sialic acid also protects bacteria by inhibiting SIGLEC-expressing NK cells. SIGLECs can also be masked by the binding of sialic acid in *cis*[73], and they can be unmasked by cellular activation[110,111]. Numerous pathogens express sialidases[68], which could unmask SIGLECs at a local area of infection, thereby providing another method of increasing inhibitory signalling.

In one example, a pathogen receptor for host-cell entry also contributes to immunosuppression[112]. CEACAM1 is used as a receptor for host-cell entry by *Neisseria* spp., *Salmonella typhimurium*, *H. influenzae*, *Moraxella catarrhalis* and mouse hepatitis virus[113–117]. The binding of *Neisseria gonorrhoeae* to CEACAM1 at the surface of CD4$^+$ T cells also downregulates T-cell activation. Whether *Neisseria* spp. or other CEACAM1-binding microorganisms similarly inhibit NK cells remains to be investigated[112].

Non-MHC molecules and tumour immunity

Tumour cells often have decreased expression of MHC class I molecules[118], but it has been found that MHC allotypes that inhibit NK cells are preferentially maintained by transformed cells[119]. One might predict that, in the same way, tumour variants that induce or upregulate expression of non-MHC-molecule

inhibitory ligands would be selected for. This is true for SIGLEC7 ligands, which are highly expressed by renal-cell carcinomas[71] and melanomas[74], and in the case of the former, higher expression of SIGLEC7 ligands correlates with increased metastatic potential[120]. Increased metastasis could be a consequence of a non-immune mechanism, such as increased cell adhesion to a SIGLEC-expressing metastatic site, and/or a consequence of immunosuppression of NK cells, although the latter has not been tested.

CEACAM1, which can interact with CEACAM1 at the surface of NK cells, is expressed at higher levels in several tumour types than in their normal tissue counterparts, including in cancer of the breast, prostate, colon and endometrium[121]. Melanoma cells also express CEACAM1, and increased CEACAM1 expression by melanoma and NK cells correlates with poor prognosis[64] and increased metastasis[122]. CEACAM1 expression is also a prognostic factor in adenocarcinoma of the lung[123]. It is expressed at only low levels by renal-cell carcinomas, but it is upregulated by these cells after interaction with lymphocytes or exposure to IFN-γ[124]. Although CEACAM1 has well-characterized roles in carcinogenesis *in vivo*, including tumour-suppressor activity and angiogenesis- and metastasis-promoting activity[121], CEACAM1 does block NK-cell killing of tumour cells *in vitro*[19,64]. Its dampening effect on the immune system *in vivo* deserves further attention.

In humans, leukaemic cells have decreased expression of CD48 compared with non-transformed lymphocytes[125]. This might reflect immune selection as a result of the activating nature of 2B4 expressed by human NK cells. Moreover, patients with XLP are prone to lymphomas; this could stem, in part, from dysregulated 2B4 signalling and NK-cell tumour surveillance.

CLR-B expression by tumour cells inhibits NK-cell cytotoxicity[51,52], yet counter-intuitively, it is downregulated by mouse tumour cell lines[52]. The researchers who made this finding ventured that downregulation might stem from tumour overexpression of casein kinase 2 (CK2), which promotes both growth of cells and internalization of receptors that have sites for phosphorylation by CK2 (as does CLR-B). They also proposed an alternative explanation, for which there was some evidence: that CLR-B is pro-apoptotic and thereby unfavourable to tumour cells. In both cases, downregulation of CLR-B expression might be a novel signal of cell stress that is perceived by NK cells as missing self[52].

Regulation of NK-cell alloreactivity

One hope of NK-cell researchers is that alloreactive NK cells could be used for the treatment of haematopoietic malignancies, although this research area is still developing. Donor-derived alloreactive NK cells can increase graft-versus-leukaemia responses, decrease graft-versus-host disease and decrease host-versus-graft rejection in the setting of an allogeneic bone-marrow transplant for the treatment of leukaemia[126]. These functions arise from mismatches between donor KIRs and host MHC class I molecules[126]. Could blocking non-MHC-binding inhibitory receptors further increase the activity of donor NK cells?

As yet, the function of non-MHC-binding receptors in alloreactivity has not been studied in detail. The maternal–fetal interface is one example of an allograft in which the self-tolerance of NK cells is important, and this tolerance is delicately balanced[127]. Unlike peripheral-blood NK cells, CEACAM1 is found to be expressed by most activated decidual NK cells[128]. Extravillous trophoblasts, which are derived from fetal tissue and are therefore allogeneic, also express CEACAM1, and *in vitro*, CEACAM1 ligation is inhibitory for decidual NK cells and other lymphocytes[128]. In conjunction with the other reports on CEACAM1-mediated regulation of NK cells, this supports the idea that CEACAM1 dampens NK-cell alloreactivity.

2B4-deficient mouse NK cells show increased alloreactivity *in vitro*[29]. *In vivo*, CD48-specific antibody blockade decreases the engraftment of allogeneic bone-marrow transplants, which is consistent with the idea that host NK cells reject transplants in the absence of 2B4 ligation[129]. Indeed, 2B4-deficient mice show greater rejection of allogeneic bone-marrow transplants (V.K. and M.E.M., unpublished observations). This might be relevant in the case of haematopoietic-stem-cell transplantation, in which donor-derived human NK-cell alloreactivity is desirable, but at present, developing donor NK cells are inhibited by 2B4 during the first month after transplantation[130].

Regulation of NK-cell autoreactivity

Several non-MHC-dependent NK-cell receptors have been associated with autoimmunity. Clearly, 2B4 has a role in the self-tolerance of autologous cells *in vitro*: 2B4 present at the surface of mouse cells inhibits the killing of syngeneic cells by NK cells[29], and at the surface of human cells, 2B4 inhibits the killing of syngeneic cells by immature NK cells[28]. *In vivo*, soluble CD48 is detectable at high levels in patients with rheumatoid arthritis[131]. The association between soluble CD48 and human arthritis is worthy of further research, particularly because polymorphisms in 2B4-family members have also been associated with a mouse model of systemic lupus erythematosus[132].

A genetic susceptibility locus for psoriasis has recently been mapped in humans[133]. One gene on this locus encodes inhibitory receptor protein 60 (IRp60), a protein that is expressed by all NK cells and by monocytes, granulocytes and a subset of T cells[133,134]. IRp60 is an inhibitory receptor that is thought to have a non-MHC molecule as a ligand[134]. It will be interesting to determine whether polymorphisms in IRp60 decrease its function and therefore result in decreased inhibition in an autoimmune setting. In a different model, increased inhibitory signalling prevents pathological inflammation: *in vivo* crosslinking of CEACAM1 with specific antibody inhibits the development of T-helper-1-cell-mediated colitis in mice[135].

Finally, cells of the central nervous system express low levels of MHC class I molecules[136] but express GD3 (REF. 137), which inhibits NK cells through binding

SIGLEC7 (REF. 73). We speculate that a ligand that is not an MHC molecule, such as GD3, might contribute to the differential sensitivity of neurons to syngeneic NK cells[138] and might contribute, in part, to the immune privilege that neural tissues are thought to have.

Conclusions

Analysis of MHC-class-I-deficient hosts has yielded valuable information for understanding NK cells; however, this line of study is also fraught with unexplained phenomena and unanswered questions. The accumulated data that are reviewed here strongly indicate that another, previously underappreciated system regulates NK cells. Certainly, further understanding of non-MHC-dependent inhibition of NK cells will yield valuable insights into NK-cell biology and therapeutic opportunities. Unlike the various types of receptor that recognize MHC class I molecules, which are expressed by subsets of NK cells, many of the non-MHC-binding receptors are expressed by most NK cells and might be more conserved between individuals, potentially providing a unique therapeutic opportunity to manipulate an entire NK-cell population. Many of these receptors are also expressed by T cells and myeloid cells, so further knowledge in this field will impact on our understanding of many aspects of the immune system.

1. Stetson, D. B. *et al*. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J. Exp. Med.* **198**, 1069–1076 (2003).
2. Tato, C. M. *et al*. Innate production of IFN-γ by NK cells is independent of epigenetic modification of the IFN-γ promoter. *J. Immunol.* **173**, 1514–1517 (2004).
3. Walker, L. S. & Abbas, A. K. The enemy within: keeping self-reactive T cells at bay in the periphery. *Nature Rev. Immunol.* **2**, 11–19 (2002).
4. Raulet, D. H., Vance, R. E. & McMahon, C. W. Regulation of the natural killer cell receptor repertoire. *Annu. Rev. Immunol.* **19**, 291–330 (2001).
5. Karre, 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).
6. Tay, C. H., Szomolanyi-Tsuda, E. & Welsh, R. M. Control of infections by NK cells. *Curr. Top. Microbiol. Immunol.* **230**, 193–220 (1998).
7. Yu, Y. Y., Kumar, V. & Bennett, M. Murine natural killer cells and marrow graft rejection. *Annu. Rev. Immunol.* **10**, 189–213 (1992).
8. Lanier, L. L. NK cell recognition. *Annu. Rev. Immunol.* **23**, 225–274 (2005).
9. 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).
10. Lee, N. *et al*. HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. *Proc. Natl Acad. Sci. USA* **95**, 5199–5204 (1998).
11. Braud, V. M. *et al*. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* **391**, 795–799 (1998).
12. Chapman, T. L., Heikeman, A. P. & Bjorkman, P. J. The inhibitory receptor LIR-1 uses a common binding interaction to recognize class I MHC molecules and the viral homolog UL18. *Immunity* **11**, 603–613 (1999).
13. Liao, N. S., Bix, M., Zijlstra, M., Jaenisch, R. & Raulet, D. MHC class I deficiency: susceptibility to natural killer (NK) cells and impaired NK activity. *Science* **253**, 199–202 (1991).
14. Hoglund, P. *et al*. Recognition of β_2-microglobulin-negative (β_2m^-) T-cell blasts by natural killer cells from normal but not from β_2m^- mice: nonresponsiveness controlled by β_2m^- bone marrow in chimeric mice. *Proc. Natl Acad. Sci. USA* **88**, 10332–10336 (1991).
15. de la Salle, H. *et al*. Homozygous human TAP peptide transporter mutation in HLA class I deficiency. *Science* **265**, 237–241 (1994).
16. Zimmer, J. *et al*. Activity and phenotype of natural killer cells in peptide transporter (TAP)-deficient patients (type I bare lymphocyte syndrome). *J. Exp. Med.* **187**, 117–122 (1998).
17. Dorfman, J. R., Zerrahn, J., Coles, M. C. & Raulet, D. H. The basis for self-tolerance of natural killer cells in β_2-microglobulin$^-$ and TAP-1$^-$ mice. *J. Immunol.* **159**, 5219–5225 (1997).
18. Salcedo, M. *et al*. Fine tuning of natural killer cell specificity and maintenance of self tolerance in MHC class I-deficient mice. *Eur. J. Immunol.* **28**, 1315–1321 (1998).
19. Markel, G. *et al*. The mechanisms controlling NK cell autoreactivity in TAP2-deficient patients. *Blood* **103**, 1770–1778 (2004).
This study shows that increased CEACAM1 expression prevents NK-cell autoreactivity in TAP2-deficient patients.
20. Vitale, M. *et al*. Analysis of natural killer cells in TAP2-deficient patients: expression of functional triggering receptors and evidence for the existence of inhibitory receptor(s) that prevent lysis of normal autologous cells. *Blood* **99**, 1723–1729 (2002).
This paper shows that triggering receptors in TAP2-deficient patients are functional, and the authors suggest a role for non-MHC-binding inhibitory receptors in self-tolerance.
21. Furukawa, H., Iizuka, K., Poursine-Laurent, J., Shastri, N. & Yokoyama, W. M. A ligand for the murine NK activation receptor Ly-49D: activation of tolerized NK cells from β_2-microglobulin-deficient mice. *J. Immunol.* **169**, 126–136 (2002).
22. Fernandez, N. C. *et al*. A subset of natural killer cells achieve self-tolerance without expressing inhibitory receptors specific for self MHC molecules. *Blood* 22 Feb 2005 (doi:10.1182/blood-2004-1108-3156).
23. Yu, Y. Y. *et al*. The role of Ly49A and 5E6 (Ly49C) molecules in hybrid resistance mediated by murine natural killer cells against normal T cell blasts. *Immunity* **4**, 67–76 (1996).
24. Liu, J. *et al*. Ly49I NK cell receptor transgene inhibition of rejection of H2^{b} mouse bone marrow transplants. *J. Immunol.* **164**, 1793–1799 (2000).
25. Sivakumar, P. V. *et al*. Expression of functional CD94/NKG2A inhibitory receptors on fetal NK1.1$^+$Ly-49$^-$ cells: a possible mechanism of tolerance during NK cell development. *J. Immunol.* **162**, 6976–6980 (1999).
26. Dorfman, J. R. & Raulet, D. H. Acquisition of Ly49 receptor expression by developing natural killer cells. *J. Exp. Med.* **187**, 609–618 (1998).
27. Vance, R. E., Jamieson, A. M., Cado, D. & Raulet, D. H. Implications of CD94 deficiency and monoallelic NKG2A expression for natural killer cell development and repertoire formation. *Proc. Natl Acad. Sci. USA* **99**, 868–873 (2002).
28. Sivori, S. *et al*. Early expression of triggering receptors and regulatory role of 2B4 in human natural killer cell precursors undergoing *in vitro* differentiation. *Proc. Natl Acad. Sci. USA* **99**, 4526–4531 (2002).
29. Lee, K. M. *et al*. 2B4 acts as a non-major histocompatibility complex binding inhibitory receptor on mouse natural killer cells. *J. Exp. Med.* **199**, 1245–1254 (2004).
This paper, together with references 38 and 39, characterizes 2B4-deficient mice, showing that 2B4 inhibits NK cells and that this outcome is not regulated by SAP.
30. Boles, K. S., Stepp, S. E., Bennett, M., Kumar, V. & Mathew, P. A. 2B4 (CD244) and CS1: novel members of the CD2 subset of the immunoglobulin superfamily molecules expressed on natural killer cells and other leukocytes. *Immunol. Rev.* **181**, 234–249 (2001).
31. Kubota, K. A structurally variant form of the 2B4 antigen is expressed on the cell surface of mouse mast cells. *Microbiol. Immunol.* **46**, 589–592 (2002).
32. Munitz, A. *et al*. 2B4 (CD244) is expressed and functional on human eosinophils. *J. Immunol.* **174**, 110–118 (2005).
33. McNerney, M. E., Lee, K. M. & Kumar, V. 2B4 (CD244) is a non-MHC binding receptor with multiple functions on natural killer cells and CD8$^+$ T cells. *Mol. Immunol.* **42**, 489–494 (2005).
34. Engel, P., Eck, M. J. & Terhorst, C. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nature Rev. Immunol.* **3**, 813–821 (2003).
35. Brown, M. H. *et al*. 2B4, the natural killer and T cell immunoglobulin superfamily surface protein, is a ligand for CD48. *J. Exp. Med.* **188**, 2083–2090 (1998).
36. Valiante, N. M. & Trinchieri, G. Identification of a novel signal transduction surface molecule on human cytotoxic lymphocytes. *J. Exp. Med.* **178**, 1397–1406 (1993).
37. Garni-Wagner, B. A., Purohit, A., Mathew, P. A., Bennett, M. & Kumar, V. A novel function-associated molecule related to non-MHC-restricted cytotoxicity mediated by activated natural killer cells and T cells. *J. Immunol.* **151**, 60–70 (1993).
38. Mooney, J. M. *et al*. The murine NK receptor 2B4 (CD244) exhibits inhibitory function independent of signaling lymphocytic activation molecule-associated protein expression. *J. Immunol.* **173**, 3953–3961 (2004).
39. Vaidya, S. V. *et al*. Targeted disruption of the *2B4* gene in mice reveals an *in vivo* role of 2B4 (CD244) in the rejection of B16 melanoma cells. *J. Immunol.* **174**, 800–807 (2005).
40. Lee, K. M. *et al*. The NK cell receptor 2B4 augments antigen-specific T cell cytotoxicity through CD48 ligation on neighboring T cells. *J. Immunol.* **170**, 4881–4885 (2003).
41. Assarsson, E. *et al*. NK cells stimulate proliferation of T and NK cells through 2B4/CD48 interactions. *J. Immunol.* **173**, 174–180 (2004).
42. Kambayashi, T., Assarsson, E., Chambers, B. J. & Ljunggren, H. G. Regulation of CD8$^+$ T cell proliferation by 2B4/CD48 interactions. *J. Immunol.* **167**, 6706–6710 (2001).
43. Tangye, S. G., Phillips, J. H., Lanier, L. L. & Nichols, K. E. Functional requirement for SAP in 2B4-mediated activation of human natural killer cells as revealed by the X-linked lymphoproliferative syndrome. *J. Immunol.* **165**, 2932–2936 (2000).
44. Tangye, S. G., Cherwinski, H., Lanier, L. L. & Phillips, J. H. 2B4-mediated activation of human natural killer cells. *Mol. Immunol.* **37**, 493–501 (2000).
45. Parolini, S. *et al*. X-linked lymphoproliferative disease. 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein–Barr virus-infected cells. *J. Exp. Med.* **192**, 337–346 (2000).
46. Glimcher, L., Shen, F. W. & Cantor, H. Identification of a cell-surface antigen selectively expressed on the natural killer cell. *J. Exp. Med.* **145**, 1–9 (1977).
47. Koo, G. C. & Peppard, J. R. Establishment of monoclonal anti-NK-1.1 antibody. *Hybridoma* **3**, 301–303 (1984).
48. Arase, N. *et al*. Association with FcRγ is essential for activation signal through NKR-P1 (CD161) in natural killer (NK) cells and NK1.1$^+$ T cells. *J. Exp. Med.* **186**, 1957–1963 (1997).
49. Carlyle, J. R. *et al*. Mouse NKR-P1B, a novel NK1.1 antigen with inhibitory function. *J. Immunol.* **162**, 5917–5923 (1999).
50. Kung, S. K., Su, R. C., Shannon, J. & Miller, R. G. The NKR-P1B gene product is an inhibitory receptor on SJL/J NK cells. *J. Immunol.* **162**, 5876–5887 (1999).
51. Iizuka, K., Naidenko, O. V., Plougastel, B. F., Fremont, D. H. & Yokoyama, W. M. Genetically linked C-type lectin-related ligands for the NKRP1 family of natural killer cell receptors. *Nature Immunol.* **4**, 801–807 (2003).
52. Carlyle, J. R. *et al*. Missing self-recognition of Ocil/Clr-b by inhibitory NKR-P1 natural killer cell receptors. *Proc. Natl Acad. Sci. USA* **101**, 3527–3532 (2004).
References 51 and 52 identify CLRs as ligands for receptors of the NKR-P1 family.
53. 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).

54. Yokoyama, W. M. & Plougastel, B. F. Immune functions encoded by the natural killer gene complex. *Nature Rev. Immunol.* **3**, 304–316 (2003).
55. Zhou, H. *et al*. A novel osteoblast-derived C-type lectin that inhibits osteoclast formation. *J. Biol. Chem.* **276**, 14916–14923 (2001).
56. Plougastel, B., Dubbelde, C. & Yokoyama, W. M. Cloning of Clr, a new family of lectin-like genes localized between mouse *Nkrp1a* and *Cd69*. *Immunogenetics* **53**, 209–214 (2001).
57. 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).
58. Mathew, P. A. *et al*. The LLT1 receptor induces IFN-γ production by human natural killer cells. *Mol. Immunol.* **40**, 1157–1163 (2004).
59. Hammarstrom, S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. *Semin. Cancer Biol.* **9**, 67–81 (1999).
60. Moller, M. J., Kammerer, R., Grunert, F. & von Kleist, S. Biliary glycoprotein (BGP) expression on T cells and on a natural-killer-cell sub-population. *Int. J. Cancer* **65**, 740–745 (1996).
61. Markel, G. *et al*. The critical role of residues 43R and 44Q of carcinoembryonic antigen cell adhesion molecules-1 in the protection from killing by human NK cells. *J. Immunol.* **173**, 3732–3739 (2004).
62. Singer, B. B. *et al*. Carcinoembryonic antigen-related cell adhesion molecule 1 expression and signaling in human, mouse, and rat leukocytes: evidence for replacement of the short cytoplasmic domain isoform by glycosylphosphatidylinositol-linked proteins in human leukocytes. *J. Immunol.* **168**, 5139–5146 (2002).
63. Kammerer, R., Stober, D., Singer, B. B., Obrink, B. & Reimann, J. Carcinoembryonic antigen-related cell adhesion molecule 1 on murine dendritic cells is a potent regulator of T cell stimulation. *J. Immunol.* **166**, 6537–6544 (2001).
64. Markel, G. *et al*. CD66a interactions between human melanoma and NK cells: a novel class I MHC-independent inhibitory mechanism of cytotoxicity. *J. Immunol.* **168**, 2803–2810 (2002).
This was one of the first reports on the inhibition of NK cells by CEACAM1, which led to much further research.
65. Markel, G. *et al*. Biological function of the soluble CEACAM1 protein and implications in TAP2-deficient patients. *Eur. J. Immunol.* **34**, 2138–2148 (2004).
66. Svenberg, T. *et al*. Serum level of biliary glycoprotein I, a determinant of cholestasis, of similar use as γ-glutamyltranspeptidase. *Scand. J. Gastroenterol.* **16**, 817–824 (1981).
67. Crocker, P. R. & Varki, A. Siglecs, sialic acids and innate immunity. *Trends Immunol.* **22**, 337–342 (2001).
68. Angata, T. & Varki, A. Chemical diversity in the sialic acids and related α-keto acids: an evolutionary perspective. *Chem. Rev.* **102**, 439–469 (2002).
69. Falco, M. *et al*. Identification and molecular cloning of p75/AIRM1, a novel member of the sialoadhesin family that functions as an inhibitory receptor in human natural killer cells. *J. Exp. Med.* **190**, 793–802 (1999).
70. Nicoll, G. *et al*. Identification and characterization of a novel siglec, siglec-7, expressed by human natural killer cells and monocytes. *J. Biol. Chem.* **274**, 34089–34095 (1999).
71. Ito, A., Handa, K., Withers, D. A., Satoh, M. & Hakomori, S. Binding specificity of siglec7 to disialogangliosides of renal cell carcinoma: possible role of disialogangliosides in tumor progression. *FEBS Lett.* **504**, 82–86 (2001).
72. Yamaji, T., Teranishi, T., Alphey, M. S., Crocker, P. R. & Hashimoto, Y. A small region of the natural killer cell receptor, Siglec-7, is responsible for its preferred binding to α2,8-disialyl and branched α2,6-sialyl residues. A comparison with Siglec-9. *J. Biol. Chem.* **277**, 6324–6332 (2002).
73. Nicoll, G. *et al*. Ganglioside GD3 expression on target cells can modulate NK cell cytotoxicity via siglec-7-dependent and -independent mechanisms. *Eur. J. Immunol.* **33**, 1642–1648 (2003).
This paper shows that GD3 expression by target cells inhibits NK cells through interaction with SIGLEC7.
74. Urmacher, C., Cordon-Cardo, C. & Houghton, A. N. Tissue distribution of GD3 ganglioside detected by mouse monoclonal antibody R24. *Am. J. Dermatopathol.* **11**, 577–581 (1989).
75. Kniep, B., Flegel, W. A., Northoff, H. & Rieber, E. P. CDw60 glycolipid antigens of human leukocytes: structural characterization and cellular distribution. *Blood* **82**, 1776–1786 (1993).
76. Ikehara, Y., Ikehara, S. K. & Paulson, J. C. Negative regulation of T cell receptor signaling by Siglec-7 (p70/AIRM) and Siglec-9. *J. Biol. Chem.* **279**, 43117–43125 (2004).
77. Avril, T., Floyd, H., Lopez, F., Vivier, E. & Crocker, P. R. The membrane-proximal immunoreceptor tyrosine-based inhibitory motif is critical for the inhibitory signaling mediated by siglecs-7 and -9, CD33-related siglecs expressed on human monocytes and NK cells. *J. Immunol.* **173**, 6841–6849 (2004).
78. Zhang, J. Q., Nicoll, G., Jones, C. & Crocker, P. R. Siglec-9, a novel sialic acid binding member of the immunoglobulin superfamily expressed broadly on human blood leukocytes. *J. Biol. Chem.* **275**, 22121–22126 (2000).
79. Zhang, J. Q., Biedermann, B., Nitschke, L. & Crocker, P. R. The murine inhibitory receptor mSiglec-E is expressed broadly on cells of the innate immune system whereas mSiglec-F is restricted to eosinophils. *Eur. J. Immunol.* **34**, 1175–1184 (2004).
80. Ulyanova, T., Shah, D. D. & Thomas, M. L. Molecular cloning of MIS, a myeloid inhibitory siglec, that binds protein-tyrosine phosphatases SHP-1 and SHP-2. *J. Biol. Chem.* **276**, 14451–14458 (2001).
81. van den Berg, T. K., Yoder, J. A. & Litman, G. W. On the origins of adaptive immunity: innate immune receptors join the tale. *Trends Immunol.* **25**, 11–16 (2004).
82. Piccio, L. *et al*. Adhesion of human T cells to antigen-presenting cells through SIRPβ2–CD47 interaction costimulates T cell proliferation. *Blood* **105**, 2421–2427 (2005).
83. Brooke, G., Holbrook, J. D., Brown, M. H. & Barclay, A. N. Human lymphocytes interact directly with CD47 through a novel member of the signal regulatory protein (SIRP) family. *J. Immunol.* **173**, 2562–2570 (2004).
84. Oldenborg, P.-A. *et al*. Role of CD47 as a marker of self on red blood cells. *Science* **288**, 2051–2054 (2000).
85. Blazar, B. R. *et al*. CD47 (integrin-associated protein) engagement of dendritic cell and macrophage counterreceptors is required to prevent the clearance of donor lymphohematopoietic cells. *J. Exp. Med.* **194**, 541–550 (2001).
86. Katz, H. R. Inhibitory receptors and allergy. *Curr. Opin. Immunol.* **14**, 698–704 (2002).
87. Wang, L. L., Mehta, I. K., LeBlanc, P. A. & Yokoyama, W. M. Mouse natural killer cells express gp49B1, a structural homologue of human killer inhibitory receptors. *J. Immunol.* **158**, 13–17 (1997).
88. Wang, L. L., Chu, D. T., Dokun, A. O. & Yokoyama, W. M. Inducible expression of the gp49B inhibitory receptor on NK cells. *J. Immunol.* **164**, 5215–5220 (2000).
89. Castells, M. C. *et al*. gp49B1–$\alpha_v\beta_3$ interaction inhibits antigen-induced mast cell activation. *Nature Immunol.* **2**, 436–442 (2001).
90. Wilder, R. L. Integrin $\alpha_v\beta_3$ as a target for treatment of rheumatoid arthritis and related rheumatic diseases. *Ann. Rheum. Dis.* **61**, ii96–ii99 (2002).
91. Rojo, S. *et al*. Natural killer cells and mast cells from gp49B null mutant mice are functional. *Mol. Cell. Biol.* **20**, 7178–7182 (2000).
92. Gu, X. *et al*. The gp49B1 inhibitory receptor regulates the IFN-γ responses of T cells and NK cells. *J. Immunol.* **170**, 4095–4101 (2003).
This study shows that, compared with cells from normal mice, gp49B1-deficient NK cells and T cells produce more IFN-γ *ex vivo*, following *in vivo* viral infection.
93. Abramson, J., Xu, R. & Pecht, I. An unusual inhibitory receptor — the mast cell function-associated antigen (MAFA). *Mol. Immunol.* **38**, 1307–1313 (2002).
94. Robbins, S. H. *et al*. Inhibitory functions of the killer cell lectin-like receptor G1 molecule during the activation of mouse NK cells. *J. Immunol.* **168**, 2585–2589 (2002).
95. 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).
96. Meyaard, L. *et al*. LAIR-1, a novel inhibitory receptor expressed on human mononuclear leukocytes. *Immunity* **7**, 283–290 (1997).
97. Thorley-Lawson, D. A., Schooley, R. T., Bhan, A. K. & Nadler, L. M. Epstein–Barr virus superinduces a new human B cell differentiation antigen (B-LAST 1) expressed on transformed lymphoblasts. *Cell* **30**, 415–425 (1982).
98. Biron, C. A., Byron, K. S. & Sullivan, J. L. Severe herpesvirus infections in an adolescent without natural killer cells. *N. Engl. J. Med.* **320**, 1731–1735 (1989).
99. 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).
100. Afonso, C. L. *et al*. The genome of fowlpox virus. *J. Virol.* **74**, 3815–3831 (2000).
101. Shchelkunov, S. N. *et al*. The genomic sequence analysis of the left and right species-specific terminal region of a cowpox virus strain reveals unique sequences and a cluster of intact ORFs for immunomodulatory and host range proteins. *Virology* **243**, 432–460 (1998).
102. Wilcock, D., Duncan, S. A., Traktman, P., Zhang, W. H. & Smith, G. L. The vaccinia virus A4OR gene product is a nonstructural, type II membrane glycoprotein that is expressed at the cell surface. *J. Gen. Virol.* **80**, 2137–2148 (1999).
103. Cameron, C. *et al*. The complete DNA sequence of myxoma virus. *Virology* **264**, 298–318 (1999).
104. Neilan, J. G. *et al*. An African swine fever virus ORF with similarity to C-type lectins is non-essential for growth in swine macrophages *in vitro* and for virus virulence in domestic swine. *J. Gen. Virol.* **80**, 2693–2697 (1999).
105. Voigt, S., Sandford, G. R., Ding, L. & Burns, W. H. Identification and characterization of a spliced C-type lectin-like gene encoded by rat cytomegalovirus. *J. Virol.* **75**, 603–611 (2001).
106. Lindberg, F. P., Gresham, H. D., Schwarz, E. & Brown, E. J. Molecular cloning of integrin-associated protein: an immunoglobulin family member with multiple membrane-spanning domains implicated in $\alpha_v\beta_3$-dependent ligand binding. *J. Cell Biol.* **123**, 485–496 (1993).
107. Campbell, I. G., Freemont, P. S., Foulkes, W. & Trowsdale, J. An ovarian tumor marker with homology to vaccinia virus contains an IgV-like region and multiple transmembrane domains. *Cancer Res.* **52**, 5416–5420 (1992).
108. Tseng, C. T. & Klimpel, G. R. Binding of the hepatitis C virus envelope protein E2 to CD81 inhibits natural killer cell functions. *J. Exp. Med.* **195**, 43–49 (2002).
109. Crotta, S. *et al*. Inhibition of natural killer cells through engagement of CD81 by the major hepatitis C virus envelope protein. *J. Exp. Med.* **195**, 35–41 (2002).
110. Razi, N. & Varki, A. Cryptic sialic acid binding lectins on human blood leukocytes can be unmasked by sialidase treatment or cellular activation. *Glycobiology* **9**, 1225–1234 (1999).
111. Razi, N. & Varki, A. Masking and unmasking of the sialic acid-binding lectin activity of CD22 (Siglec-2) on B lymphocytes. *Proc. Natl Acad. Sci. USA* **95**, 7469–7474 (1998).
112. Boulton, I. C. & Gray-Owen, S. D. Neisserial binding to CEACAM1 arrests the activation and proliferation of $CD4^+$ T lymphocytes. *Nature Immunol.* **3**, 229–236 (2002).
This paper shows that the binding of bacteria to CEACAM1 at the surface of T cells inhibits T-cell functions.
113. Dveksler, G. S. *et al*. Several members of the mouse carcinoembryonic antigen-related glycoprotein family are functional receptors for the coronavirus mouse hepatitis virus-A59. *J. Virol.* **67**, 1–8 (1993).
114. Virji, M., Watt, S. M., Barker, S., Makepeace, K. & Doyonnas, R. The N-domain of the human CD66a adhesion molecule is a target for Opa proteins of *Neisseria meningitidis* and *Neisseria gonorrhoeae*. *Mol. Microbiol.* **22**, 929–939 (1996).
115. Leusch, H. G., Drzeniek, Z., Markos-Pusztai, Z. & Wagener, C. Binding of *Escherichia coli* and *Salmonella* strains to members of the carcinoembryonic antigen family: differential binding inhibition by aromatic α-glycosides of mannose. *Infect. Immun.* **59**, 2051–2057 (1991).
116. Hill, D. J. & Virji, M. A novel cell-binding mechanism of *Moraxella catarrhalis* ubiquitous surface protein UspA: specific targeting of the N-domain of carcinoembryonic antigen-related cell adhesion molecules by UspA1. *Mol. Microbiol.* **48**, 117–129 (2003).
117. Virji, M. *et al*. Carcinoembryonic antigens are targeted by diverse strains of typable and non-typable *Haemophilus influenzae*. *Mol. Microbiol.* **36**, 784–795 (2000).
118. Garrido, F. *et al*. Implications for immunosurveillance of altered HLA class I phenotypes in human tumours. *Immunol. Today* **18**, 89–95 (1997).
119. Demanet, C. *et al*. Down-regulation of HLA-A and HLA-Bw6, but not HLA-Bw4, allospecificities in leukemic cells: an escape mechanism from CTL and NK attack? *Blood* **103**, 3122–3130 (2004).
120. Saito, S. *et al*. Expression of globe-series gangliosides in human renal cell carcinoma. *Jpn J. Cancer Res.* **88**, 652–659 (1997).
121. Plunkett, T. A. & Ellis, P. A. CEACAM1: a marker with a difference or more of the same? *J. Clin. Oncol.* **20**, 4273–4275 (2002).

122. Thies, A. *et al*. CEACAM1 expression in cutaneous malignant melanoma predicts the development of metastatic disease. *J. Clin. Oncol.* **20**, 2530–2536 (2002).
123. Laack, E. *et al*. Expression of CEACAM1 in adenocarcinoma of the lung: a factor of independent prognostic significance. *J. Clin. Oncol.* **20**, 4279–4284 (2002).
124. Kammerer, R. *et al*. The tumour suppressor gene *CEACAM1* is completely but reversibly downregulated in renal cell carcinoma. *J. Pathol.* **204**, 258–267 (2004).
125. Pende, D. *et al*. Analysis of the receptor–ligand interactions in the natural killer-mediated lysis of freshly isolated myeloid or lymphoblastic leukemias: evidence for the involvement of the poliovirus receptor (CD155) and nectin-2 (CD112). *Blood* **105**, 2066–2073 (2005).
126. Ruggeri, L. *et al*. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* **295**, 2097–2100 (2002).
This study shows the clinical benefits of NK-cell alloreactivity.
127. Moffett-King, A. Natural killer cells and pregnancy. *Nature Rev. Immunol.* **2**, 656–663 (2002).
128. Markel, G. *et al*. Pivotal role of CEACAM1 protein in the inhibition of activated decidual lymphocyte functions. *J. Clin. Invest.* **110**, 943–953 (2002).
129. Blazar, B. R. *et al*. A critical role for CD48 antigen in regulating alloengraftment and lymphohematopoietic recovery after bone marrow transplantation. *Blood* **92**, 4453–4463 (1998).
130. Vitale, C. *et al*. Analysis of the activating receptors and cytolytic function of human natural killer cells undergoing *in vivo* differentiation after allogeneic bone marrow transplantation. *Eur. J. Immunol.* **34**, 455–460 (2004).
131. Smith, G. M., Biggs, J., Norris, B., Anderson-Stewart, P. & Ward, R. Detection of a soluble form of the leukocyte surface antigen CD48 in plasma and its elevation in patients with lymphoid leukemias and arthritis. *J. Clin. Immunol.* **17**, 502–509 (1997).
132. Wandstrat, A. E. *et al*. Association of extensive polymorphisms in the SLAM/CD2 gene cluster with murine lupus. *Immunity* **21**, 769–780 (2004).
133. Speckman, R. A. *et al*. Novel immunoglobulin superfamily gene cluster, mapping to a region of human chromosome 17q25, linked to psoriasis susceptibility. *Hum. Genet.* **112**, 34–41 (2003).
134. Cantoni, C. *et al*. Molecular and functional characterization of IRp60, a member of the immunoglobulin superfamily that functions as an inhibitory receptor in human NK cells. *Eur. J. Immunol.* **29**, 3148–3159 (1999).
135. Iijima, H. *et al*. Specific regulation of T helper cell 1-mediated murine colitis by CEACAM1. *J. Exp. Med.* **199**, 471–482 (2004).
136. Boulanger, L. M. & Shatz, C. J. Immune signalling in neural development, synaptic plasticity and disease. *Nature Rev. Neurosci.* **5**, 521–531 (2004).
137. Malisan, F. & Testi, R. GD3 ganglioside and apoptosis. *Biochim. Biophys. Acta* **1585**, 179–187 (2002).
138. Backstrom, E., Chambers, B. J., Kristensson, K. & Ljunggren, H. G. Direct NK cell-mediated lysis of syngenic dorsal root ganglia neurons *in vitro*. *J. Immunol.* **165**, 4895–4900 (2000).

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Competing interests statement
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