Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY REVIEW ARTICLE

Conserved and variable natural killer cell receptors: diverse approaches to viral infections

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doi:10.1111/imm.13039
Received 21 November 2018; accepted 12 December 2018.
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Summary

Natural killer (NK) cells are lymphocytes of the innate immune system with essential roles during viral infections. NK cell functions are mediated through a repertoire of non-rearranging inhibitory and activating receptors that interact with major histocompatibility complex (MHC)–peptide complexes on the surface of infected cells. Recent work studying the conserved CD94–NKG2A and variable killer cell immunoglobulin-like receptor–MHC systems suggest that these two receptor families may have subtly different properties in terms of interactions with MHC class I bound peptides, and in recognition of down-regulation of MHC class I. In this review, we discuss how these properties generate diversity in the NK cell response to viruses.

Keywords: conserved; natural killer cells; polymorphic; receptor–ligand; viral infection.

Introduction

Natural killer (NK) cells are a subset of innate lymphocytes that provide an essential first line of defence against invading pathogens such as viruses, parasites and tumour cells. Innate immune responses can be highly conserved and are thought to evolve under distinct selective pressures. Whereas the adaptive immune system possesses rearranging receptors, which can adapt to different pathogens within a single host, the innate system relies on germline-encoded, non-rearranging receptors and therefore adapts over evolutionary time.[1] Hence, the genes of innate immunity may be important for dealing with current pathogens, but may also represent remnants of previous encounters as humans have evolved and migrated across the globe.

The co-evolution of hosts and their pathogens is a complex and dynamic process that requires reciprocal variation at a genomic level.[2] The population diversity of polymorphic immune response genes, such as the major histocompatibility complex (MHC) and the killer cell immunoglobulin-like receptors (KIR), implies that there is no single 'optimized' immune system, but variants that have selective advantage against specific pathogens. This has been highlighted by numerous association studies of these two gene families with infectious agents.[3–5] Genetic association studies in different populations frequently differ in their results, indicating that there are additional genetic and environmental contexts in which these genes operate to determine their role in disease outcome.[6] Furthermore, it indicates that similar selection pressures may lead to the emergence of distinct protective genes in different populations.

Viruses are thought to exert a major selective force on human NK cells. The functions of NK cells against these pathogens include the detection and elimination of infected cells by releasing cytolytic granules and by producing antiviral cytokines such as interferon-γ and tumour necrosis factor-α.[7] Viruses and NK cells are in a constant evolutionary arms race maintaining an equilibrium that preserves both host and pathogen as extant species.[8] In particular, the rapidly evolving KIR and MHC genes constitute a linked system that has been driven by these forces. This review describes the relevance of

Abbreviations: CMV, cytomegalovirus; DAP12, DNAX activation protein 12; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; ITAM, immunoreceptor tyrosine-based activation motifs; ITIM, immunoreceptor tyrosine-based inhibition motifs; KIR, killer cell immunoglobulin-like receptors; LILRB1, leucocyte immunoglobulin-like receptor B1; MHC, major histocompatibility complex; MICA, MHC class I polypeptide-related sequence A; MICB, MHC class I polypeptide-related sequence B; NK, natural killer

conserved and variable NK cell receptor–MHC interactions in the context of viral infections and explains how this receptor–ligand system has been targeted by viruses to escape from immune surveillance.

MHC class I receptors on NK cells: roles in viral infections

Natural killer cell functions are determined by a balance between inhibitory or activating signals that occur after engagement of germline-encoded, non-rearranging receptors with their ligands. The wide repertoire of NK cell receptors in humans comprises KIR, CD94–NKG2 heterodimers, LILRB1, NKG2D and natural cytotoxicity receptors such as NKp30, NKp44 and NKp46. These receptors cooperate to generate activating signals that can overcome the tonic inhibition driven by KIR and CD94–NKG2A in humans. Ligands for these receptors include molecules from the MHC class I and MHC class I-related proteins such as MHC class I polypeptide-related sequence A (MICA), MICB and UL16 binding proteins, among many others (Fig. 1).[9] In general, such receptor–ligand diversity allows NK cells to respond to multiple stimuli, and this diversity is augmented by the combinatorial manner in which NK cells can integrate signals from these multiple receptors.

KIR receptors

The family of KIR receptors are type 1 transmembrane glycoproteins encoded by polymorphic genes located within the leucocyte receptor complex in the long arm of chromosome 19 (19q13).[10] These receptors have two or three extracellular domains (2D or 3D) and perform inhibitory or activating functions as determined by their cytoplasmic tails. Hence, inhibitory KIRs have long (L) cytoplasmic tails and contains immunoreceptor tyrosine-based inhibition motifs (ITIMs). On the other hand, activating receptors have short (S) cytoplasmic tails and associate with adapter molecules containing immunoreceptor tyrosine-based activation motifs (ITAMs), such as DNAX activation protein 12 (DAP12).[9,11] An exception to this rule is given by KIR2DL4, which, despite having a long cytoplasmic domain, has an activating function.[12] Within an individual, NK cell clones exhibit diverse patterns of KIR expression, which is determined by gene content, copy number and allelism.[13–15] Such diversity of receptor repertoires and the high degree of KIR polymorphism allow NK cells to generate rapid responses against a broad spectrum of pathogens.

Ligands for KIR receptors are classical MHC class I molecules (human leucocyte antigens; HLA-A, -B, -C) and are considered the most polymorphic in the human genome.[16] MHC class I molecules bind intracellularly derived peptides from 8 to 10 amino acids in length, exposing them on the cell surface. Processing and presentation of endogenous peptides through MHC class I molecules are a mechanism of immune surveillance that allows host cells to alert both NK cells and $CD8^+$ T lymphocytes to infections or malignant change.[17] Engagement of inhibitory KIR receptors with their MHC class I ligands usually turns off NK cell functions. Conversely, these same interactions participate in NK cell education, which allows NK cells to become tolerant to healthy cells or 'self', while becoming fully functional.[18,19] During viral infections, expression of MHC ligands may be down-regulated or absent ('missing-self'), due to strategies employed by viruses to escape $CD8^+$ T-cell recognition (reviewed in ref. 20). The lack of an inhibitor receptor–ligand interaction results in a dominant activating response within NK cells, promoting cytotoxicity or cytokine production. Viruses that down-regulate MHC class I include herpesviruses,[21] retroviruses [22] and papillomaviruses.[23]

Genetic diversity of KIR receptors. The extensive polymorphism exhibited by KIR genes influences expression and function of KIR on individual NK cells. In humans, two KIR haplotypes have been defined: haplotypes A and B. Haplotype A displays a fixed gene content consisting of five inhibitory receptors, two activating receptors and one pseudogene.[24] Conversely, haplotype B includes variable amounts of inhibitory and activating KIR and generates population diversity at the level of the locus.[25,26]

The frequencies of the key inhibitory KIR (KIR2DL1, KIR2DL2/L3 and KIR3DL1) are maintained at a high level in most populations.[27] Conversely, frequencies of activating KIR are more variable. KIR2DS1, KIR2DS2 and KIR3DS1 frequencies are high in Papua New Guinean and Australian populations (~70–90%) but low in African or Chinese populations (< 20%). KIR2DS1 is also frequent in Latin America (~60–90%), KIR2DS2 in India (~75%) and KIR3DS1 in Brazil (80%).[27] This variability in KIR expression across populations suggests different selection pressures imposed by infectious diseases on the different KIR genes.[25]

In addition to haplotypic variability, allelism also contributes to the diversity of KIR receptors, with the most variable KIR gene being *KIR3DL2*, which has 158 alleles.[26] KIR allelic diversity also generates receptors with different binding affinities to MHC class I ligands. This phenomenon is best described for inhibitory KIR2DL2 and KIR2DL3, which segregate as alleles at a single locus but have subtly different specificities for their HLA-C ligands which, in turn, are modulated by the peptide bound by the HLA-C molecule.[28,29] Additionally, KIR3DL1 alleles exhibit different levels of cell surface expression, which alter their inhibitory potential and differentially associate with protection in human immunodeficiency virus type 1 (HIV-1) infection.[30]

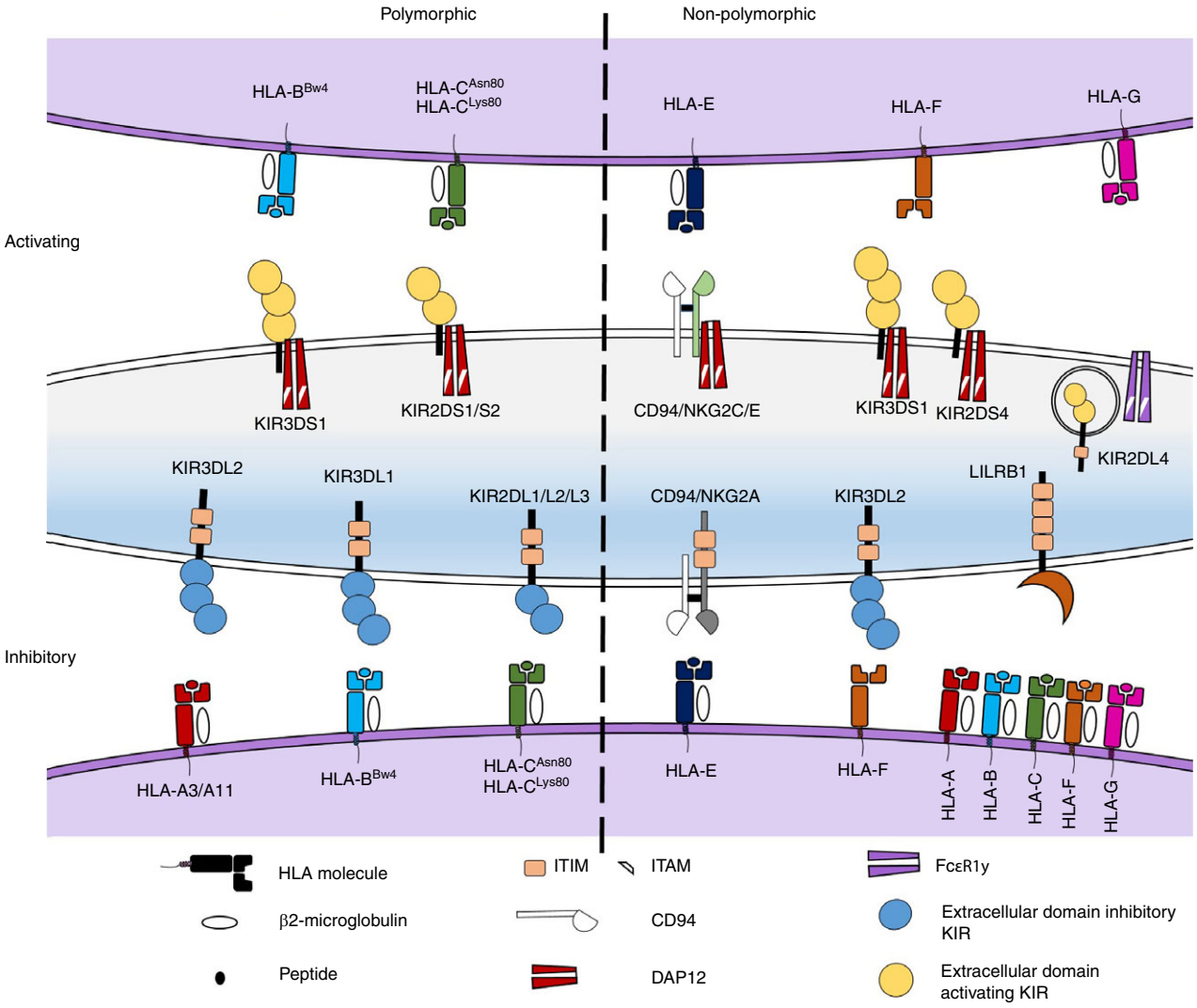

Figure 1. Activating and inhibitory natural killer (NK) cell receptors and their major histocompatibility complex (MHC) class I ligands. Shown are the different interactions between the killer cell immunoglobulin-like receptors (KIR) and CD94:NKG2 family of receptors and their MHC class I ligands. LILRB1 interacts with folded HLA class I molecules, HLA-F and HLA-G. KIR2DL4 is thought to interact with HLA-G through an endosomal compartment. HLA-F binds as an open conformer to KIR3DS1, KIR2DS4 and KIR3DL2.

Co-evolution of the KIR–MHC system. The concomitant evolution of KIR receptors and their MHC class I ligands is an intriguing topic. The rapid co-evolution of KIR receptors and their MHC ligands is subject to balancing selection. This evolutionary process favours the preservation of heterozygosity in the population, which correlates with increased diversity and host fitness.[31] Selection operates on gene segments that encode contact regions of KIR and the peptide-binding site of MHC.[32] This may be advantageous for the host because it facilitates the presentation of viral peptides that can be recognized by distinct KIR alleles, to increase the efficiency of the immune response. However, it could also favour virus persistence, because viral peptides that engage inhibitory receptors, would prevent the activation of NK cells.

CD94:NKG2 receptors

The invariant CD94 is a C-type lectin-like protein that forms heterodimers with receptors from the NKG2 family. These genes are all encoded within the NK complex, on the short arm of chromosome 12 (12p12.3-p13.1) and unlike KIRs, are non-polymorphic.[33,34] Ligands for these heterodimers are HLA-E molecules, which are conserved, non-polymorphic proteins. HLA-E binds conserved leader peptides from the N-terminus of classical and non-classical HLA molecules. Binding of leader sequences to HLA-E is therefore a mechanism to monitor normal synthesis and expression of classical MHC class I molecules (HLA-A, -B and -C), and also of the non-classical MHC molecule, HLA-G.[35,36]

The CD94:NKG2A receptor is inhibitory, containing two ITIMs, and plays an important role during viral infections.[37] Viruses may down-regulate MHC class I molecules through multiple different mechanisms, and this reduces the availability of leader peptides for presentation by HLA-E. This then decreases the expression of HLA-E on the cell surface, reducing inhibition of NKG2A$^+$ NK cells, leading to their activation.[35,38] Conversely, heterodimers formed by CD94 with NKG2C, associate with DAP12, which contains ITAMs, and this generates activating signals.[39,40] As CD94:NKG2A and CD94:NKG2C are clonally expressed on NK cells, viruses that down-regulate MHC class I, but maintain HLA-E expression through the presentation of an HLA-E binding peptide may still be recognized by this system. Consistent with this model, Gumá *et al.* have shown that individuals positive for cytomegalovirus (CMV) have higher counts of CD94:NKG2C+, and lower levels of CD94:NKG2A+ NK cells compared with non-infected controls.[41] Hence, during viral infections, NK cells can be activated through signals received from activating receptors or through the loss of expression of inhibitory ligands.

Differential roles of polymorphic and non-polymorphic receptor–ligand systems

Throughout the evolution of primates, the polymorphic KIR appear to have continuously adapted whereas CD94:NKG2A has remained relatively conserved. The evolution of these receptor families is paralleled by that of their MHC class I ligands, which can be polymorphic (HLA-A, -B, -C) or non-polymorphic (HLA-E, -F, -G) (Fig. 1). Having both receptor–ligand systems allows NK cells to sense changes in virus-infected cells using discrete mechansims, which increases diversity in the mechanisms by which NK cells can respond to the multiplicity of viruses encountered by humans.

Non-polymorphic receptor–ligand interactions

MHC-E and CD94:NKG2A receptors are well conserved across species. Functional CD94:NKG2A receptors in the common chimpanzee (*Pan troglodytes*) appear to have the same specificity for MHC-E and leader sequences as their human counterpart.[42] This suggests a common function, namely detection of down-regulation of other MHC class I molecules, which targets viruses with diverse means of down-regulating MHC class I. Such mechanisms include direct targeting of MHC class I (CMV); inhibition of antigen processing (Epstein–Barr virus); or generic cellular processes such as the inhibition of protein synthesis (vaccinia virus) and trafficking through the endoplasmic reticulum (hepatitis C virus; HCV).[43–46]

HLA-G is also relatively non-polymorphic and interacts with NKG2A through its leader sequence. However, it can also interact with the inhibitory receptor LILRB1 and the activating receptor KIR2DL4, through an endosomal pathway.[36,47,48] Although LILRB1 is not conserved between humans and the common chimpanzee, KIR2DL4 is highly conserved, a striking observation considering the rapid evolution of the KIR locus.[42] HLA-G can be up-regulated during viral infections such as CMV, hepatitis B virus (HBV) and HCV.[49–51] This raises the intriguing possibility that the HLA-G–KIR2DL4 interaction may have an important biological role that has led to their conservation between humans and chimpanzees. Such conservation may be related to beneficial effects on pregnancy, as well as, or instead of, their effects on viral infections.[47]

HLA-F has been shown to interact with KIR3DL2 and KIR2DS4 as an open conformer.[52] Like KIR2DL4, KIR3DL2 is present in all individuals whereas KIR2DS4 is present in > 90% of individuals in most populations, although it may harbour a deletion and hence be present only in a secreted form.[53] More recently, it has been shown that HLA-F also binds KIR3DS1 and importantly, it is up-regulated during both HIV-1 and HCV infections.[54,55]

Polymorphic receptor–ligand interactions

The human classical MHC class I molecules interact with NK cell receptors either by directly binding KIR or indirectly through furnishing a leader peptide for HLA-E. HLA-A is the most ancient of these molecules, and all HLA-A alleles have a methionine residue at position -21 (-21M) and so a cognate leader peptide for HLA-E to engage CD94:NKG2A. However, only a minority of HLA-A molecules interact with KIR. HLA-A*03 and HLA-A*11 interact with KIR3DL2, whereas HLA-A and HLA-B molecules with a Bw4 serological epitope bind KIR3DL1.[56] HLA-B has a dimorphism, such that HLA-B^{Bw4} alleles that bind KIR3DL1 generally have a threonine residue at position -21 (-21T) and so do not have a leader peptide cognate for HLA-E, in comparison with those with the Bw6 serological motif, which do not bind KIR3DS1 but have -21M to engage CD94:NKG2A through HLA-E.[57] The cognate leader peptide is thought to be the ancestral leader sequence, implying that the functional interaction of HLA-B^{Bw4} with KIR has developed more recently. HLA-C is the most recently evolved of the classical MHC class I molecules, and all HLA-C molecules have a functional interaction with KIR. HLA-C is expressed on the cell surface at levels of about 10% of HLA-A and HLA-B molecules and overall appears specialized to interact with NK cells, as opposed to having a primary role presenting antigens to T cells.[56,58]

Combinatorial advantage for NK cell receptors

The KIR and CD94:NKG2A systems are strongly interlinked through their contribution to NK cell function. This is further exemplified by the diversity in leader

sequences among HLA-B alleles. Individuals with HLA-B alleles containing -21M have more CD94:NKG2A+ NK cells compared with those having HLA-B alleles with -21T.[57] Conversely, individuals with two -21T HLA-B alleles have more KIR+ NK cells. Additionally, haplotypes having HLA-B alleles with -21M contain group 1 HLA-C alleles (HLA-C^{Asn80}), rather than group 2 HLA-C alleles (HLA-C^{Lys80}).[57] Hence, genetic dimorphisms within NK cell receptors stratify the population with important consequences for the antiviral response. This has best been demonstrated for HIV-1 infection, in which -21M HLA-B alleles have a deleterious effect.[59]

The emergence of KIR and its rapid evolution suggests that this family of receptors may have subtly different functions from CD94:NKG2A. For instance, the stoichiometry of the response of NKG2A+ NK cells to down-regulation of HLA-E is different to that of KIR+ NK cells to HLA-C down-regulation.[60] Both HLA-C and HLA-E are expressed at low levels on healthy cells compared with HLA-A and HLA-B, and HLA-E is expressed at 25-fold lower levels than HLA-C.[58] However, at low levels of MHC-I, NKG2A+ NK cells are more sensitive to small changes in surface levels of MHC-I compared with KIR+ NK cells. This implies that NKG2A+ NK cells, but not KIR+ NK cells, are finely tuned to detect small changes in cell surface MHC-I, which can be driven by changes in expression of classical HLA class I molecules, in addition to a direct down-regulation of HLA-E (Fig. 2). At a functional level, this difference is evident in assays of vaccinia virus infection *in vitro*. Vaccinia virus inhibits host protein synthesis,[44] inducing down-regulation of MHC-I, such that NKG2A+, but not KIR+, NK cell clones lyse vaccinia virus-infected targets.[38] Hence, although NKG2A+ NK cells are tuned to detect MHC class I down-regulation, this begs the question as to the role of KIR, which appear to be more specialized and more recently evolved.

Peptide selectivity of NK cell receptors

Both KIR and CD94:NKG2A bind MHC class I and additionally contact the presented peptide. Therefore, both types of receptors are sensitive to peptides bound within the MHC groove.[61] Changes in the peptide repertoire can abrogate recognition of MHC class I by inhibitory receptors, which leads to NK cell activation. There appear to be two different classes of apparently 'null' peptides, with opposing actions on the KIR and CD94:NKG2A systems. These are peptides that, in isolation, do not affect NK cell reactivity, but can modulate inhibitory signals. For KIR2DL2/L3 and HLA-C, we have identified peptide antagonists.[62] In relatively small quantities, these peptides can down-modulate the inhibitory KIR signals to permit their activation against target cells (Fig. 2).[63] Mechanistically, they induce SHP-1 recruitment, but without Vav1 dephosphorylation.[64] Additionally, there are peptides that bind HLA-E, which alone do not inhibit NKG2A+ NK cells, but augment the inhibition due to canonical HLA-E-binding class I leader peptides.[60] These peptides engage CD94 in the absence of NKG2A, most likely in its homodimeric form, and include sequences derived from HIV-1 (AISPRTLNA), HCV (YLLPRRGPRL) and EBV (SQAPLPCVL).[65–67] Interestingly, these peptides do not have the canonical HLA-E binding motif, which is consistent with a broad peptide-presenting role for HLA-E.[68] Hence, peptide modulation through KIR appears beneficial to the host but modulation through NKG2A may be detrimental.

Viral peptides

A number of virus-derived peptides that augment NK cell inhibition have been described. These include the peptides for HLA-E mentioned above, but also other peptides from HIV-1 and HCV have been identified.[69–71] In general, these viral sequences act as inhibitory peptides for KIR receptors. The most compelling data come from the identification of 'KIR footprints' on the HIV-1 genome and the association of an inhibitory peptide from HIV-1 in individuals with a *KIR2DL2* gene.[72] The complex changes of the peptide repertoire that occur during viral infections may be sufficient to modulate NK cell activity.[73] For instance, in measles virus infection, quantitative

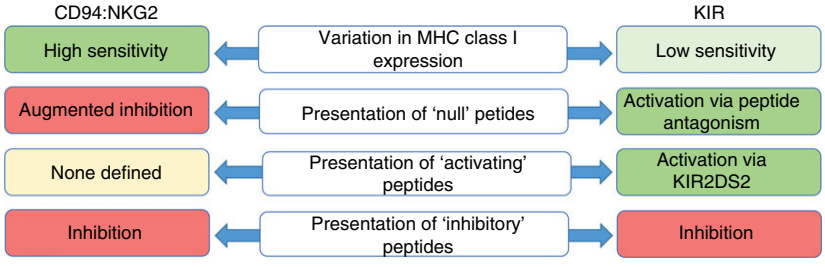

Figure 2. Comparison of how changes in major histocompatibility complex (MHC) expression and presentation of peptides differentially affect CD94:NKG2 and killer cell immunoglobulin-like receptor (KIR) interactions with MHC class I. Variations in MHC class I levels have more profound effects on inhibition through CD94:NKG2A than through inhibitory KIR whereas changes in the peptide content of MHC class I are more likely to lead through activation of natural killer (NK) cells expressing KIR.

peptide elution has shown that, although relatively few viral epitopes are presented by HLA-C, they can be of extremely high abundance.[74]

Activating receptors for MHC class I:peptide complexes

The specificity and role of activating receptors for MHC class I have been more challenging to unearth. NKG2C has a lower affinity than its inhibitory counterpart NKG2A.[75] However, NKG2C is associated with adaptive NK cells in CMV+ individuals, suggesting that they are involved in the control of CMV infection.[76] This appears to be related to the recognition of the UL40 peptide, which is similar in sequence to MHC class I leader peptides.[77] Both NKG2A and NKG2C recognize the UL40 peptide, and hence, cells expressing NKG2A may be prevented from expanding by this inhibitory signal leading to domination of the NK cell repertoire by NKG2A– cells. In TAP1-deficient patients there is an expanded NKG2C+ NK cell population, which may contribute to anti-viral immunity and in healthy blood donors, NK cells expressing activating KIR can also be expanded, implying that NK cell expansions can be driven by any receptor associating with DAP12, rather than purely through NKG2C.[76, 78]

The activating counterparts of inhibitory KIR2DL1 and KIR2DL2/L3 are KIR2DS1 and KIR2DS2, respectively. Based on their high sequence homology, these activating KIRs share similar HLA-C specificities. KIR2DS1 binds HLA-C group 2, in contrast to KIR2DL1, which binds both HLA-C group 1 and group 2 alleles.[79] KIR2DS2 appears to have a subtly different specificity from its inhibitory counterparts KIR2DL2 and KIR2DL3, having a tyrosine for phenylalanine substitution at residue 45, adjacent to residue 44 which defines the HLA-C specificity of the KIR molecule.[80] Recent work has shown that KIR2DS2 specifically recognizes peptides derived from the NS3 helicase of plus-strand RNA viruses in the context of HLA-C alleles. These include HCV and a number of related globally important flaviviruses such as dengue virus, Zika virus, Japanese encephalitis virus, tick-borne encephalitis virus and West Nile virus. The peptide in the NS3 protein from flaviviruses contains a unique 'MCHAT' motif that is not present in the human genome and is recognized by KIR2DS2. This viral motif is present in 61 out of 63 flaviviruses, which are highly divergent in other regions of their genome. This unusually high degree of conservation has a structural basis, in that it forms part of the RNA-binding motif of the helicase protein, which is critical to viral replication.[81] Flaviviruses are carried by insect vectors and cause a wide spectrum of disease across the globe. For instance, tick-borne encephalitis virus is a problem in temperate regions, whereas Zika and dengue virus infections are more common in tropical regions.[82–84] Hence, as a family, these viruses have the potential to exert a huge selection pressure on the immune system. The observation that the MCHAT sequence is present in many different flaviviruses and is absent in humans is strongly suggestive that pathogenic flaviviruses are a key driver of the evolution of KIR2DS2.

Finally, although KIR3DS1 has a high sequence homology to the inhibitory KIR3DL1 molecule, binding of KIR3DS1 to HLA-B has been largely inferred from sequence homology and disease association studies, especially in HIV-1 infection. It has been shown that KIR3DS1 can bind HIV-derived peptides presented by HLA-B, but the relevance of this for HIV-1 infection is not clear at present.[85] More recently, it has been demonstrated that KIR3DS1 recognizes open conformers of HLA-F.[54] These are up-regulated in HIV-1 and HCV infections, indicating that this may be a more general mechanism for the recognition of viruses.[55] Conversely, peptide-loaded HLA-F binds LILRB1 with high affinity.[86] As LILRB1 is expressed on both NK cells and macrophage/monocytes, this interaction may be important for the immune response generated by one or both cell types.

NK cell immune evasion by viruses: effects on MHC class I receptors

The diversity of viral pathogens for humans is enormous. They cause different types of infections including acute severe infection or more indolent chronic conditions, which may or may not be associated with disease. For example, DNA viruses such as the herpeseviruses cause long-term latent infections, with little long-term human disease in immunocompetent hosts. They can accommodate homologues of human genes to counteract immune defences, an escape mechanism not available for the much smaller RNA viruses. This allows DNA viruses to have multiple methods of modulating the immune system, targeting both T cells and NK cells. Human CMV encodes multiple proteins such as US2, US3, US6, US10, US11 and UL83, which down-regulate the expression of HLA class I molecules.[45] This mechanism allows viruses to avoid T-cell recognition, but leaves infected cells susceptible to NK cell killing. CMV express ligands for NK cell inhibitory receptors by multiple pathways, which overcomes this vulnerability (Fig. 3). In this regard, US2 and US11 proteins only target HLA-A and HLA-B, leaving HLA-C and HLA-E available for interaction with inhibitory NK receptors.[87,88] Additionally, the CMV-encoded protein UL40 expresses a sequence identical to the leader peptide of some HLA-C alleles.[89] This peptide can bind HLA-E and serve as ligand for inhibitory CD94:NKG2A receptors.[90,91] Furthermore, UL18, an MHC class I homologue expressed by CMV, can inhibit NK cells

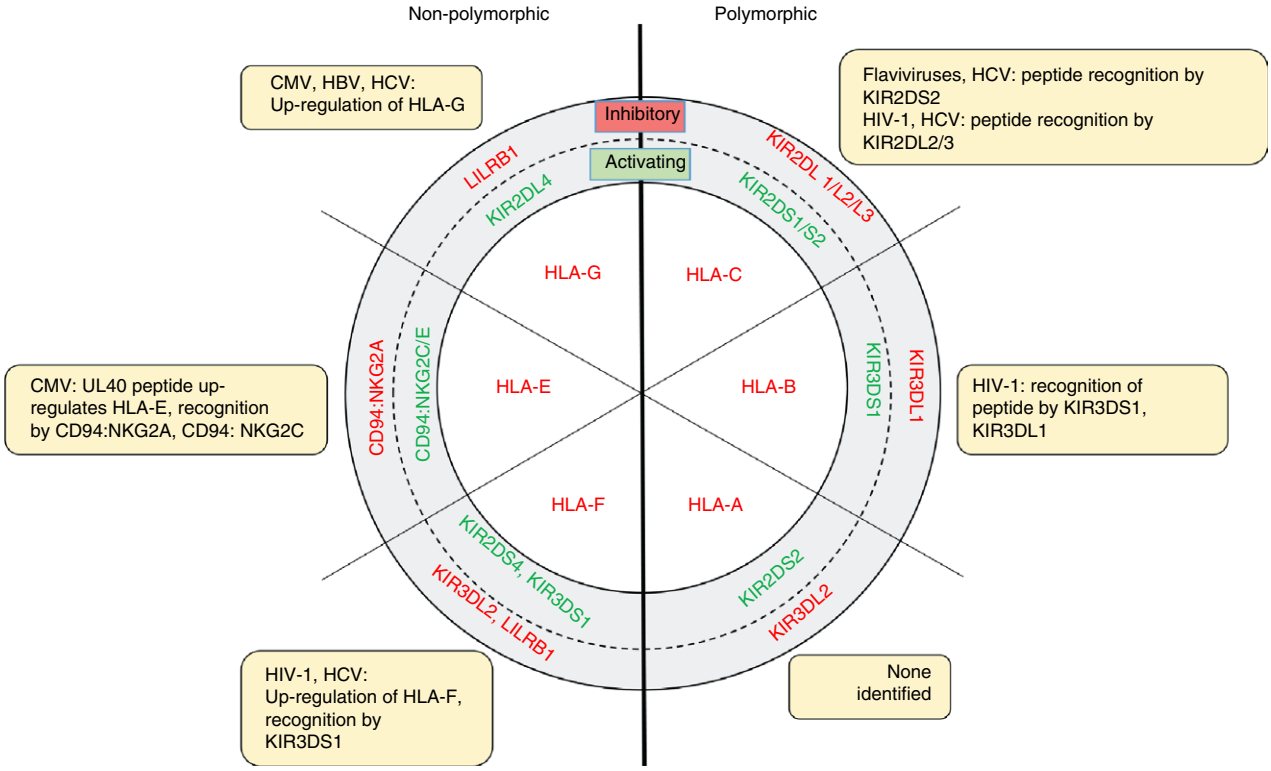

Figure 3. Effects of DNA and RNA viruses on polymorphic and non-polymorphic MHC class I molecules, demonstrating their potential for engagement of activating or inhibitory natural killer (NK) cell receptors. KIR3DS1 and KIR3DL2 are polymorphic, but bind the open conformer of HLA-F, which is up-regulated by both human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV). KIR2DS2 interacts with complexes formed by HLA-C and flaviviruses or HCV-derived peptides.

through interaction with LILRB1.[92] CMV also targets the activating NKG2D pathway through UL16, UL142, US9, US18 and US20, which prevents expression of MICA, MICB or UL16 binding protein ligands on the cell surface.[93–97] The net result of these interactions is that the virus can co-exist with its human host in the majority of individuals, in general becoming problematic to human health only following *in utero* transmission, or in the iatrogenic context of immunosuppression or transplantation.

While these multiple mechanisms have been best demonstrated for human CMV, other herpesviruses also down-regulate MHC class I and inhibit NK cell function. The K3 protein of human herpesvirus-8 targets HLA-A, -B and -C, whereas the K5 protein preferentially targets HLA-A and -B, as well as multiple ligands for NK cell receptors including MICA, MICB and activation-induced C-type lectin (reviewed in ref. 98). In contrast to DNA viruses, flaviviruses do not specifically target MHC class I. Instead, they have developed numerous mechanisms for attenuating type I interferon signalling.[99] Despite this, NK cells are activated in the acute phase of flaviviral infection and precede T-cell responses. At this phase of infection, an enhanced NK cell response may speed up clearance of the virus and so reduce transmission, providing enhanced protection on a population, as well as an individual basis.

Protective effects of KIR–MHC–peptide against RNA viruses

In general, RNA viruses cause acute infections, with some exceptions such as HIV-1 and HCV. Genetic studies of these chronic infections have informed KIR biology and demonstrate the important role that KIR diversity may have on the outcome of the infection. For instance, protection against HIV-1 has been associated with KIR3DS1, and also different alleles of KIR3DL1.[5,30] For HCV infection, different KIR genotypes have been associated with protection, with the most prominent being KIR2DL3 and HLA-C group 1. This was originally identified in both a UK Caucasian and an African American population.[4] These data illustrate the influence that inhibitory receptors can have on the outcome of viral infections, and in the absence of substantial HLA-C down-regulation by either of these viruses, its protective effect may be more related to changes in peptide content (Fig. 3).[69,70,72]

Intriguingly, HCV can also present a peptide that is recognized by KIR2DS2 in the context of HLA-C*0102,

and in the UK population, KIR2DS2 is protective against HCV infection.[81] Similar to the peptide in the flaviviruses, this peptide is also present in the NS3 helicase region of the virus and directly contacts the viral RNA. The peptide LNPSVAATL is conserved among all HCV genotypes, which in general share approximately only 70% nucleotide identity.[100] Key to its critical function in viral replication, mutation of the KIR2DS2 binding residues (alanine P7 and threonine P8) abrogate HCV replication. Interestingly, LNPSVAATL is in the Walker 1a, rather than the Walker 1b motif, showing that KIR2DS2 recognizes distinct RNA-binding viral sequences, rather than a single motif. It may be therefore that KIR2DS2 represents an adaptation of the host to RNA viruses. This begs the question as to whether the other activating KIR may have similar, as yet unrecognized functions in recognition of pathogens.

Concluding remarks

The MHC class I receptors on NK cells provide a remarkably elaborate system for controlling the functions of NK cells in the context of the diverse nature of human pathogens. Overall, there are a number of key interactions that are well established, and valuable new insights into the roles of the individual receptor–ligand pairings are now being gained. The diversity of these interactions highlights how a system of non-rearranging receptors has adapted to the various challenges of infectious diseases that can range from latent to fatal infection. Understanding the interactions between a broad range of viruses and NK cells may be the key to unravelling the complex network of receptors and ligands that determine NK cell function.

Funding

LB-L was funded by a scholarship from the Departamento administrativo de Ciencia, Tecnologia e Innovacion – Colciencias, the University of Southampton and the Newton Fund, and research in SIKs group is supported by The MRC.

Author contribution

LB-L and SIK wrote the manuscript.

Disclosures

None.

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